DRUG UNIT

TEST METHODS

FOREWORD

The Drug Unit of the Indiana State Police Forensic Services Division (FSD) analyzes drug evidence submitted by criminal justice agencies in the State of Indiana. These Test Methods are designed for the guidance of Forensic Scientists who support investigations of cases involving suspected drugs. Its scope is limited to those compounds which are most frequently encountered such as narcotics, stimulants, hallucinogens, hypnotics, tranquilizers, diluents, and materials from clandestine laboratories. These Test Methods are to be used in conjunction with FSD Policies.

The Drug Unit is staffed with Forensic Scientists and technical Unit Supervisors at four Regional Laboratories in Evansville, Fort Wayne, Indianapolis, and Lowell. Each Forensic Scientist is required to have a Bachelor's Degree in Forensic Science or a Natural Science with specific course requirements in Physics, General Chemistry, Organic Chemistry, and Analytical Chemistry with laboratory classes (see Job Descriptions).

Forensic Scientists in the Drug Unit participate in an extensive formalized training program under the supervision of a Drug Unit Supervisor. The training program begins with a general laboratory and safety orientation. The Drug Unit Training Program consists of several modules covering evidence handling, drug analysis and court testimony. Each training module has a required reading list, practical exercises, and examinations. Competency test samples are used to evaluate the progress of the trainee. Mock trials are given at the end of the Cocaine module and at the completion of training, at a minimum. Upon a successful final mock trial, and the approval of the FSD Commander, the trainee will be authorized to perform casework.

These Test Methods documents provide a general approach to the examination of drug evidence. Instrumental operations are guided by the manufacturer's recommendations and standard practices in the discipline of drug analysis. All identifications made in the Drug Unit are made by direct comparison with known reference materials on the instruments in each respective Regional Laboratory under similar analytical conditions. The Drug Unit Test Methods are intended as a reference and are not necessarily all inclusive. New procedures shall be validated and approved by the FSD Commander. The degree of validation need not be exhaustive, but adequately demonstrate the purpose for which it was intended and must preclude or acknowledge false positives, false negatives, and interferences. Alterations and/or deviations to procedures may be employed with approval from a Drug Unit Supervisor.

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1. Evidence Handling

1.1. Scope: All evidence submitted for drug analysis shall be handled, stored, and sampled to preserve and protect the integrity of the evidence and to minimize the potential for cross contamination, destruction of evidence and personal exposure to drugs.

1.2. Precautions/Limitations: Forensic Scientists shall take appropriate precautions to minimize contaminating, altering, or destroying the potential for additional future examination. Specific procedures shall be used when multiple exam requests are involved on an item.

1.2.1. Drug Only Examination Requests:
If drug analysis is the only requested or anticipated examination, then general routine precautions should be taken to minimize the potential for cross-contamination and personal exposure to drugs in the evidence. Lab coats and gloves shall be worn when handling any drug evidence. When handling large quantities of powder, masks shall be worn during sampling.

1.2.2. Biohazard Items:
For drug evidence suspected or marked as “Biohazard” (i.e., cigarette butts, body cavity seizures, evidence from toilet bowls, blood contaminated containers, etc.) the following procedures shall be used:
Forensic Scientists shall wear gloves during the sampling process until the item is repackaged. After repackaging of the item, gloves shall be removed and hands washed prior to continuing with sealing the container.
Writing utensils and papers should not be handled with potentially contaminated gloved hands during the sampling process. Writing utensils handled with gloves shall be washed with alcohol or appropriate cleanser prior to handling with bare hands.

1.2.3. Examinations Involving Other Disciplines:
Items that need to be examined by another discipline, or multiple disciplines, may require consultation and/or collaboration to best process these items of evidence. With concern for dangerous drugs, collaboration and order of analysis should be considered. Routine tasks such as marking the evidence or sample collection can destroy or unnecessarily complicate evidence for those other disciplines. Consult with other Units to discuss placement of markings on items and preservation of the evidence. There may be instances where adjustments need to be made to analytical procedures and/or general evidence handling.

1.2.4. Latent Prints:
If an item is submitted for drug and fingerprint analysis, the drug evidence and the container(s) should be separated prior to submission to the Forensic Services Division (FSD) as individual items. Special precautions may be necessary to preserve fingerprints during the drug sampling process.
In the event that an item cannot be separated into different items, a Latent Print Unit Forensic Scientist should be consulted prior to the examination to determine the best

approach for the analysis and if marking of the evidence and/or its containers is appropriate. Adjustments to evidence handling procedures may be necessary to avoid the potential alteration or destruction of latent prints.
Items that have been previously examined by the Latent Print Unit may have chemical exposure hazards. Discuss what chemicals were used with the Latent Print Unit Forensic Scientist and see SDS for the pertinent items.

1.2.5. DNA Examinations:

If an item is submitted for DNA analysis on the surface of the container and drug analysis on the contents, then the drug evidence and the container should be separated prior to submission to the FSD and submitted as individual items. Forensic Scientists in the Drug Unit will not need to take any special precautions for handling these drug sub-items to prevent DNA contamination.
Precautions are required during drug sampling to minimize the potential for contamination of an item with DNA from the Forensic Scientist.

1.2.5.1. When DNA sampling occurs first, the Forensic Scientists in the Drug Unit shall still comply with precautions during drug sampling for potential DNA analysis in the future.

1.2.5.2. Writing utensils and sampling area surfaces shall be cleaned with appropriate cleaning material (e.g., methanol (MeOH), ethanol (EtOH), or a 5 - 10% bleach solution) prior to starting drug sampling, between sampling of another item with DNA analysis requested, and at the conclusion of sampling of these items.

1.2.5.3. During the drug sampling process, the Forensic Scientist shall wear a disposable face mask and disposable gloves until the item is resealed.

1.2.5.4. Gloves shall be changed after each item is sealed and before sampling another item with DNA analysis requested.

1.2.5.5. If the external surface of gloves contacts skin or un-cleansed surfaces, then the gloves shall be replaced prior to handling items of evidence.

1.2.5.6. Other personnel present in the vicinity of the sampling area shall refrain from approaching or talking to the Forensic Scientist while this evidence is open.

1.2.5.7. At the conclusion of sampling of items with DNA analysis requests, the face mask and gloves shall be removed, disposed of in the appropriate disposal receptacle, and hands washed.

1.2.6. Document Examinations:

If both drug and document analyses are requested on an item of evidence, then the handling, marking, and sampling shall be accomplished to minimize damage to paper documents. This includes creation of additional indented writing impressions or damage to existing indented writing impressions.
Care shall be exercised not to damage handwriting on a document. Do not write on the envelope or container without consulting with a Questioned Documents Forensic Scientist. Additionally, do not write on a paper that is lying on top of the evidence as this can add indented writing to the evidence.

1.3. Related Information:

- **1.3.1.** Appendix 1 – Forms and Worksheets
- **1.3.2.** Appendix 2 – Abbreviations
- **1.3.3.** Appendix 3 – Definitions
- **1.3.4.** Appendix 4 – Drug Unit Reagent Preparation Manual
- **1.3.5.** Other Test Methods
  - **1.3.5.1.** Sampling
  - **1.3.5.2.** Weight Determination

1.4. Instruments: Heat sealers, fume hoods, fume absorbers or other ventilated work area.

1.5. Reagents/Materials: The varied nature of drug samples dictates that several types of containers can be utilized. Paper sacks, paper envelopes, plastic bags and glass bottles are suitable for most drug items depending on the physical make-up of the sample. Permanent markers shall be used for marking evidence. The use of gloves, face masks, tape and cleaning materials may be necessary.

1.6. Hazards/Safety:

- **1.6.1.** Sharps, Broken Glass
- **1.6.2.** Exposure to various drugs and chemicals
- **1.6.3.** Biohazards
- **1.6.4.** FSD Bloodborne Pathogens Exposure Control Plan
- **1.6.5.** FSD Safety Manual
- **1.6.6.** FSD Chemical Hygiene Plan
- **1.6.7.** Safety Data Sheets (SDS)

1.7. Reference Materials/Controls/Calibration Checks: N/A

1.8. Procedures/Instructions: The following steps shall be accomplished during and/or after the transfer of evidence from the Evidence Specialist to the Forensic Scientist:

1.8.1. During Evidence Transfer: The identity of each item of evidence shall be verified by comparing the laboratory case number, laboratory item number, and evidence description, if possible, between the Request for Laboratory Examination Form and the actual evidence.

1.8.1.1. Verify that all evidence containers are properly sealed and labeled as per FSD guidelines and policies.
Note if any improper seals, suspected cross contamination between items or tampering has occurred. If so, the Laboratory Manager or designee shall be immediately notified and the situation documented in Laboratory Information Management System (LIMS).
When evidence is found to be improperly sealed, the procedure in FSD Evidence Policy #005 shall be followed.
When evidence is found to be improperly labeled, the procedure in FSD Evidence Policy #006 shall be followed.

1.8.1.2. The date and time of the transfer may be documented on the evidence, at the Forensic Scientists' discretion.

1.8.1.3. Numeric characters should be used as laboratory item numbers. Alpha characters should be used only as a means for identifying sub-items. (See FSD Evidence Policies.)

1.8.2. After Evidence Transfer and During Sampling:

1.8.2.1. Verify the agency case number and agency item number marked on the evidence with Request for Examination Form.

1.8.2.2. Verify that all the items of evidence are properly described on the Request for Laboratory Examination Form. Compare each item of evidence to the descriptions on the Request for Laboratory Examination Form. Significant differences or conflicting information shall be corrected on the Request for Laboratory Examination Form and the correct information shall be updated in the LIMS case information. Examples of significant differences include the incorrect number of tablets and correcting the packaging from manila envelope to plastic bag. The description on the Request for Laboratory Examination Form should not be changed for differences like off-white to tan or crystalline substance to powder. It may be necessary to contact the contributor to advise of and/or resolve discrepancies. If the Request for Laboratory Examination Form includes results of a field test or a suggestion as to what controlled substance an item may contain, that information should be crossed off and initialed.

1.8.2.3. The outer containers of evidence shall be marked with the initials of the Forensic Scientist, the laboratory case number, and laboratory item number with leading zeroes.

1.8.2.4. An Examination Worksheet shall be initiated for notes, observations, and conclusions during the analysis. (See 1.9.)

1.8.2.4.1. Each item of evidence analyzed shall be documented on an Examination Worksheet. Multiple items (or sub-items) may be combined on one worksheet or separated onto individual sheets.

1.8.2.4.2. A summary table is permitted for purposes of summarizing or clarifying analysis procedures and references. This may include summarization of multiple weights, references, observations, conclusions, extractions, etc. (See 1.9.1.1)

1.8.2.4.3. If a summary table is used, the information on the summary table does not need to be repeated on the Examination Worksheet. For each section of the Examination Worksheet in which a summary table is used, the Forensic Scientist shall record “see summary table” or similar verbiage.

1.8.2.5. After sampling each item of evidence, each container shall be resealed, and initials placed across the seal. Store the evidence in a secure temporary storage area until release of the case back to the Evidence Specialist.

1.8.2.6. Every effort should be made to avoid handling evidence repeatedly. The material should be sampled and immediately sealed. If necessary, the evidence may be closed and maintained in a secure temporary storage area until the analysis is complete.

1.8.3. Case Handling

1.8.3.1. Forensic scientists shall not have more than 75 cases in their custody or assigned to themselves at a time. This does not include cases that are in draft complete status awaiting review. Exceptions can be granted from a Drug Unit Supervisor.

1.8.3.2. In general, all items in a batch should be completed before starting a new batch. There are some exceptions to this, including holding items in order to perform a semi-quant or waiting on a reference material.

1.8.3.3. Once a case is started, it should be completed within 30 days. There are some exceptions to this, including holding items in order to perform semi-quantitation, waiting on a reference material, if the case involves a lot of test tubes, or unexpected leave. The forensic scientist should contact a Drug Unit Supervisor for approval if a case will not be finished within 60 days. A copy of the communication shall go into the case file.

1.9. Records: All evidence descriptions shall be described in detail on an Examination Worksheet. Details shall be sufficient to enable the Forensic Scientist, or other qualified individual, to identify the evidence at a later date.

1.9.1. The Examination Worksheet shall be labeled with the laboratory case number, laboratory item number with leading zeroes, and date the worksheet was initiated.

1.9.1.1. Additional sheets for documentation are permitted and may be necessary to keep records and notes in a clear, readable, and understandable form. These sheets shall be labeled with the laboratory case number, laboratory item number(s), and initialed.

1.9.2. Record a physical description for each item of the evidence on the Examination Worksheet.

1.9.3. Items that are administratively withdrawn shall be documented either on an Examination Worksheet or on the Request for Laboratory Examination Form and include the relevant Physical Evidence Bulletin (PEB) information. (See also 1.11)

1.9.4. If the outer packaging of an item has been changed, this information shall appear on the Examination Worksheet.

1.9.5. The Examination Worksheet shall be dated and initialed / signed.

1.9.6. Common abbreviations or those that are found on the approved abbreviation list are acceptable for use in analytical notes. All other abbreviations shall be defined in the case notes.

1.9.7. The date of each test or observation shall be recorded in the case notes.

1.9.8. Photographs, if taken for the purposes of analysis, shall be included in the notes or printed and attached as part of the analysis.

1.10. Interpretations of Results: Proper evidence handling is determined by an intact and sufficient seal that prevents loss, cross-contamination, or deleterious change of the evidence in a container. Markings shall be on the container and the seal for the purposes of identification and security.

1.11. Report Writing: All evidence shall be described as being “sealed” in the report, unless it is the Forensic Scientist’s opinion that there is a question regarding the integrity of a seal. In the event that a seal may be insufficient to prevent loss, cross-contamination or deleterious change, the word “sealed” shall be removed from the evidence description on the Certificate of Analysis and the Request for Laboratory Examination Form.

Items that clearly do not meet Drug Unit submission guidelines and do not have sufficient justification, or approval, for analysis may be administratively withdrawn and one of the following statements should be used:

Item XXX - the request for examination was administratively withdrawn as per Indiana State Police Physical Evidence Bulletin XX.

Or

Item XXX was withdrawn per Indiana State Police Physical Evidence Bulletin XX. If analysis is necessary, please contact the laboratory.

Any item administratively withdrawn or not examined may be resubmitted and analyzed at a later date if/when sufficient justification is given to warrant analysis. Marked pharmaceutical tablets and capsules not examined as per the PEB should be reported as reference identifications. If an exception to the PEB has been authorized, it shall be noted in the case file including who authorized the exception.

Cases that have been looked up in MyCase or DoxPop can be administratively withdrawn without contacting the contributor or prosecuting attorney, if the individual(s) has been sentenced, the charges dismissed, or the individual(s) has entered pre-trial diversion, drug court treatment, and/or conditional deferment (or other agreement that causes the case to be decided). The source of the information, cause number(s), and the date the information was looked up shall be recorded in the case file. The source of the information, cause number(s), and the date the information was looked up shall be stated on the Certificate of Analysis. The wording on the Certificate of Analysis shall read as follows: The request for laboratory examination was withdrawn per information found in MyCase or DoxPop, Cause Number(s): XXXXXXX on (date). If analysis is needed, please contact the laboratory.

1.12. References:

1.12.1. Forensic Services Division Documents

1.12.1.1. FSD Quality Assurance Manual – Technical Records
1.12.1.2. FSD Quality Assurance Manual - Handling Evidence Items
1.12.1.3. FSD Evidence Policies

1.12.2. FSD Physical Evidence Bulletins (PEBs)

1.12.2.1. PEB-19 Clandestine Laboratory Samples Submission
1.12.2.2. PEB-20 Evidence Packaging and Submission Guidelines
1.12.2.3. PEB-01 Drug Submissions

2. Sampling

2.1. Scope: This section is intended to provide procedures for the sampling of items of evidence suspected to contain controlled substances and/or other drugs.

2.2. Precautions/Limitations: The basis of sampling is that the composition found in the sample removed for analysis represents the composition of the material from which it was taken. The Forensic Scientist shall ensure that the sampled material represents the item(s) by making careful visual examinations and considering the homogeneity among drug packaging (bags, packets, etc.) and its contents.

2.2.1. General: When a single unit is to be analyzed, one sample is sufficient if the material appears to be homogeneous.

2.2.1.1. If the material is not homogeneous, additional samples may be necessary to represent the item as a whole or steps may be taken to make the sample homogeneous. A notation shall be made on the examination worksheet explaining what was sampled. Examples include, if there is a bag containing a white powder and a bag containing a brown powder in the same item, both bags shall be sampled. Or, if there is plant material mixed with a crystalline substance, both shall be sampled, separately if possible. If multiple residue bags are in the item, it is not required to sample more than one regardless of appearance. If the plant material is sampled together with another substance, GC/MS analysis shall be performed. Clandestine tablets may be treated as one population.

2.2.1.2. When multiple inner packages containing similar materials as a single item are submitted for examination, either an administrative sampling plan or statistical sampling plan shall be followed as per 2.11.2. This does not include tablets.

2.2.1.3. The sampling plan that is followed should meet the highest weight threshold for that case. It is not always necessary for weight thresholds to be met for each item (except for cases requesting aggregate charges).

2.2.1.4. If the total net weight of the contents or gross weight of the containers and the contents, within an item is less than the legal weight requirements to elevate the criminal charge, a minimum of one sample shall be taken for analysis.

2.2.1.5. It is up to the Forensic Scientist to determine if there is sufficient sample to test a specific item.

2.2.1.6. The potential presence of a controlled substance, legislative requirements, weight thresholds, and measurement uncertainty shall be considered when sampling. If a controlled substance is identified in an

item, additional items suspected to contain Cannabis plant material, waxes, vapes, and edibles should be withdrawn. For these items, probable cause, charges (dealing and possession), number of suspects, and seizure dates shall be considered.

2.2.1.7. Items containing multiple containers: For items containing 20 or more containers (foil packets, knotted plastic bags, etc.), one container should be sampled and screened for the presence of a controlled substance. If the screening of one container indicates the presence of a controlled substance, enough samples shall be taken to meet the highest weight threshold possible or a hypergeometric sampling strategy shall be used, whichever requires fewer samples (See 2.12.1 and 2.12.2). The screened container counts towards the Hypergeometric population. Hypergeometric sampling allows a portion of the containers to be analyzed and a statistical inference to be made about the entire item as a whole. When a hypergeometric sampling strategy is used, samples shall be chosen at random and a sufficient number of samples shall be examined to meet, or exceed, a 95% confidence that 90% are positive. The net weight of 90% of the population shall meet or exceed the weight threshold for that case. If the net weight of 90% of the population does not meet or exceed the weight threshold, this shall be stated in the result on the Certificate of Analysis. The date and end time of this sampling shall be recorded in the case file, at a minimum. Statistical sampling can only be used if the same controlled substance is confirmed in all analyzed samples. If the same controlled substance cannot be confirmed in all analyzed samples, non-statistical sampling shall be used and enough samples to meet and/or exceed the weight threshold(s) shall be taken.

2.2.1.8 Items containing multiple tablets and unmarked capsules: For items containing multiple illicit tablets or unmarked capsules, one tablet/capsule shall be sampled. It shall be clearly stated in the result that only one tablet/capsule was examined using this or similar verbiage. Tablets/capsules should be counted up to 100 tablets. If the tablets/capsules are not counted or there are more than 100 tablets, then the quantity can be described as “numerous”.

Item xxx – One tablet/capsule was analyzed and found to contain x, a controlled substance. The net weight of the analyzed tablet/capsule was x gram. The remaining tablets/capsules were not analyzed. The net weight of the remaining tablets/capsules was x grams. If analysis of additional tablets/capsules is necessary, please contact the laboratory.

If testing additional tablets will not meet a weight threshold, the statement “If analysis of additional tablets/capsules is necessary, please contact the laboratory” is not required.

If the lab is contacted with a request for additional tablets/capsules to be examined, the Drug Unit Supervisor shall discuss the case with the prosecutor and decide if further analysis is warranted. If further analysis is required, the analyst shall perform hypergeometric sampling or sample enough sub-items to meet the highest weight threshold, whichever requires the least amount of samples. The tablet initially sampled shall not be included in the population for hypergeometric analysis or the total weight. The description of the item shall reflect the new condition of the item at the time it is re-opened. This shall be a supplemental request. A "Remarks" statement shall be written as follows:

Remarks: Item X was re-submitted for additional sampling of the tablets/capsules. For the results of the testing of the original tablet(s)/capsule(s) and original weight(s) please refer to Case number, Request number.

2.2.2. Bulk materials: Bulk materials (e.g., bricks of compressed powder, bales of plant material) should be broken or cored to obtain a representative sample. Depending on the size of the material, samples from several locations may be required to obtain a representative sample. The locations on the bulk material from which the samples were obtained shall be described in the analysis notes. A drawing or description is sufficient.

2.2.3. Tablets and Capsules: The sampling scheme used for general unmarked drugs is not required for samples that appear to be pharmaceutical preparations, which have unique physical identifiers present and are clearly visually consistent with each other. Generally, a single sample may be taken for a given type of drug. Individual whole tablets and capsules are to be treated as separate samples and cannot be combined for analysis. It may be necessary to sample an entire tablet or capsule for low dosage preparations.

2.2.4. Residues: 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 scraping) or chemical means (e.g., rinsing with solvent). Case notes shall reflect the method by which the sample was removed. The method of sampling shall be documented on the Examination Worksheet. The evidence description should state that the item is a "residue". If no residue is observed, the item shall be described on the Certificate of Analysis as "… with no visible residue" or something similar. This does not apply to paper and windowpanes. Currency with no visible residue shall not be sampled. Currency with visible residue that cannot be removed using mechanical means shall not be rinsed to be sampled.

2.2.4.1. When possible, a sample should be removed while leaving a portion of the residue intact.

2.2.4.2. For items containing multiple sub-items with residues, a minimum of one sample shall be taken for examination unless the item can be administratively withdrawn.

2.2.5. If samples are retained for training purposes, it shall be documented on the notes along with the weight retained. A statement shall be added to the results section of the Certificate of Analysis (e.g., A portion of this item was retained for training purposes.), but the weight does not need to be reported.

2.2.6. Weighing: See Weight Determination Test Method
The uncertainty associated with the weight of individual items may affect the number of samples that need to be examined.

2.3. Related Information:

2.3.1. Appendix 1 – Forms and Worksheets
2.3.2. Appendix 2 – Abbreviations
2.3.3. Appendix 3 – Definitions
2.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
2.3.5. Other Test Methods:
2.3.5.1. Weight Determination
2.3.5.2. General Drug Analysis

2.4. Instruments: N/A

2.5. Reagents/Materials: General laboratory supplies: spatulas, scissors, scalpels, tape, pens, methanol (MeOH), chloroform ($CHCl_3$).

2.6. Hazards/Safety:

2.6.1. See Evidence Handling
2.6.2. See Safety Policies
2.6.3. Potential chemical exposure to methanol, chloroform, and clandestine laboratory chemicals.

2.7. Reference Materials/Controls/Calibration Checks: N/A

2.8. Procedures/Instructions:

2.8.1. A small representative sample shall be removed from each item to be analyzed (See 2.2 for sampling considerations). A minimal amount of sample should be removed for anticipated analysis. No more than one-half of the original material should be routinely sampled. If an entire sample is removed for analysis, the autosampler vial containing the sample shall be returned to the evidence. The date the vial was returned to the evidence shall be documented on the Examination worksheet.

2.8.2. Cigarette butts, cigarettes, and loose plant material all in one container that are potentially cross-contaminated may be treated as one item and one sample may be taken for analysis. The Forensic Scientist shall note what was weighed, sampled, and analyzed. A Drug Unit Supervisor shall approve the reporting of gross weights that are over a statutory weight limit.

2.8.3. Reference Identification: Pharmaceutical identifiers on tablets and capsules (markings, color, shape, and other characteristics) shall be compared to published references. Examples of published references are The Physicians' Desk Reference, The Logo Index (printed or computer version), Ident-A-Drug, Med Scan, Drug Identification Bible, Pill Box, Drugs.com, generic drug company lists, Poison Control, and pharmaceutical company internet sites.

2.8.3.1. If only reference identification is used, the removal and weighing of a sample is not necessary unless the tablets are marked to contain a controlled substance and a weight threshold may apply.

2.8.4. Tablets and Capsules: It is advisable to sample half or less of a tablet or capsule contents, leaving the remaining portion in the evidence for future examinations, if possible. It may be necessary to sample an entire tablet or capsule in low dosage preparations. When an entire tablet and/or capsule is sampled, the autosampler vial containing the sample shall be returned to the evidence.

2.8.4.1. Marked Pharmaceutical Tablets with weight thresholds: At a minimum, one tablet shall be fully examined. It is permissible to perform reference identification on the remaining tablets. (See 2.11.1 for reporting)

Partial Tablets with Whole Tablets: If one of the whole tablets is analyzed, the reference identification can include the partial tablets if the partial tablets are visually consistent or resemble the whole tablet(s). The partial tablets can also be grouped together and referred to as "Not examined."

Partial Tablets: Reference identification cannot be used as a second test for identification of partial tablets unless full markings are present. If the item only contains partial tablets that are visually consistent with one another, only one partial tablet needs to be examined regardless of whether or not a weight threshold applies. The remaining partial tablets can be reported as not examined or not analyzed. It is the Forensic Scientist's discretion whether or not to count the number of partial tablets.

2.8.4.2. Marked Pharmaceutical Capsules with weight thresholds: At a minimum, one capsule shall be fully examined. It is permissible to perform a reference identification on the remaining capsules, if the weights (either

taken individually or the calculated average) of the examined capsules are consistent with each other.

2.8.4.3. Marked Illicit Tablets and capsules with weight thresholds: One tablet shall be analyzed, see 2.2.1..

2.8.5. Marked Pharmaceutical Sublingual Films: If multiple sublingual films are present in an item, one film shall be fully analyzed, at a minimum. A reference identification of the remaining films should be performed if full markings are present. The weight of the examined and unexamined films shall be documented on the Examination Worksheet.

2.8.6. The sample should be transferred and stored in a disposable test tube and/or disposable analysis vial marked with the laboratory case number and laboratory item number.

2.8.7. Once the sample has been collected, the sample tube and/or vial shall be fitted with a closure, such as a stopper, cork, cap or parafilm, etc. to protect the sample from loss or contamination, except for during analysis.

2.8.8. Unused disposable sample tubes and/or vials shall be stored and handled in a manner to protect them from contamination.

2.8.9. When a hypergeometric sampling plan is used, the Forensic Scientist shall determine how many populations are present in the item. Forensic scientists should consider color, shape, and markings when identifying populations. More than one population may exist in an item. Hypergeometric sampling may be necessary on more than one population to meet or exceed a weight threshold. Refer to the table found in Appendix 7 to determine how many items to sample for a population. If population size falls in between two population sizes on the table, use the higher value for the sampling plan.

2.9. Records: The examination documentation shall be of sufficient detail to describe the contents of the item undergoing examination including all levels of interior packaging (number of inner packages, etc.), the creation of any sub-items and all weights measured.

2.9.1. Specifically define what was weighed, sampled, and tested and what, if anything, was only weighed. Tablets and capsules are to be treated as separate samples. Detail what tests were conducted on which items and/or sub-items.

2.9.2. Sample preparation shall be described in the analytical notes. This may include the method of sampling, description, or depiction of where the sample was taken and/or other steps taken to prepare the sample for analysis.

2.9.3. If a residue is removed by chemical means, and the GC/MS vial is not included, the case notes shall state that residue remains.

2.9.4. If no visible residue is observed, it is the forensic scientist's discretion whether or not to sample the item. If the forensic scientist decides sampling the item is necessary, then the possible residue shall be removed by chemical means and the GC/MS vial shall be returned to the evidence. The date the vial was returned to the evidence shall be documented on the Examination worksheet. If the item is U.S. Currency with no visible residue, then the item shall not be examined.

2.9.5. If an item is reopened to obtain an additional sample, the notes shall specify which test results are for the original sample and which tests are for the additional sample. The notes shall also indicate if the additional sample was combined with the original sample.

2.9.6. When the hypergeometric plan is utilized, the Forensic Scientist shall document the confidence level in the case notes and on the report.

2.9.7. Reference identifications shall be recorded on the analysis sheet and shall include information such as the name and version of the reference, active ingredients, size of dosage unit (mg of drug), and control status (including schedule number), at a minimum. The prescription (Rx) or over the counter (OTC) status should also be documented.

If the tablet or capsule contains multiple salt forms of the same active ingredient, each individual component does not need to be documented, and the total mg should be noted. The active ingredient(s) in the tablet or capsule can be referred to as mixed salts. For example, "mixed Amphetamine salts XXX mg".

2.10. Interpretations of Results:

2.10.1. Non-Statistical (Administrative) Sampling: A non-statistical, or administrative, approach is intended to satisfy the requirements of a specific charge. Unless all items (or containers) are weighed and individually analyzed, no inference can be made regarding the contents of any unexamined items.

Tablets and Capsules: No inference can be made regarding the contents of any unexamined tablet(s) or capsule(s). However, reference identification may be used to indicate the contents of the remaining unexamined tablet(s) or capsule(s).

2.10.2. Statistical Sampling: A statistical approach allows a specific portion of containers within an item to be examined and permits a statistical inference regarding the remaining unexamined containers (or items). This method shall be used to meet, or exceed, a 95% confidence level that at least 90% of the containers within the item are the same.

Statistical inference shall only be made if the same controlled substance is confirmed in all analyzed samples. If additional substances are confirmed or indicated in some, but not all, analyzed samples, the statistical inference shall not be made for these additional

substances. Statistical inference still applies to the substance(s) that is confirmed in all samples.

2.11. Report Writing: If an item of evidence contains several containers (example- Item XXX contains four plastic bags of vegetation), then these can be sub-itemized. If the sub-itemizing is listed in the description on the Certificate of Analysis, then the same sub-itemizing shall be in the results.

2.11.1. Non-Statistical Sampling Results: For those items where samples have been taken, examined and conclusions reached, the reports shall contain information regarding what was examined and the weight of the examined material. Additional statements containing information regarding items that were not examined and the net or gross weight of the unexamined items shall also be reported. (See also General Drug Analysis 4.11)
The report shall specify what was examined. Items that cannot be analyzed (e.g. debris, empty bags, wallet, pieces of plastic) do not need to be listed in the results section of the Certificate of Analysis as not examined.
Generally, this shall apply to the non-statistical sampling of multiple inner packages where sufficient samples are taken to meet statutory weight requirements of specific criminal charges. Statements that do not apply to the item results may be omitted.
For consistency in reporting, the below listed examples, or similar verbiage, shall be used, unless they need to be adjusted for accuracy. Descriptions and results in reports shall be clear and maintain a consistent format.

For example:

Items XXX(A-C) were found to contain X, a controlled substance.
The net weight of items XXX(A-C) was X grams.
Items XXX(D-F) were not examined and had a (net/gross) weight of X grams.

Or

Item XXX: Thirty-seven (37) packets were examined and each found to contain X, a controlled substance, and had a total net weight of X grams.

The remaining sixty-three (63) packets were (visually/not) examined and had a (net/gross) weight of X grams.

Or

If all of the samples in an item have been examined, the item may be reported as per 4.11. For example:
Item 001 was found to contain X, a controlled substance.
The net weight of item XXX was X grams

2.11.1.1. Tablet and Capsule Reporting Example:

Item XXX: One tablet (capsule) was examined and was found to contain X, a controlled substance and had a net weight of X grams.

The remaining tablets (capsules) were visually examined and had a net weight of X grams. Reference(s) indicated the presence of X, a controlled substance. No confirmatory analysis was performed on the remaining tablets (capsules).

2.11.1.2. In instances where a reference identification is performed but the active ingredient controlled substance is inconsistent with the analytical results, the remaining tablets should be reported as "not analyzed".

2.11.1.3. In instances where all the active ingredients in a marked tablet were not identified or indicated, use the following wording or similar verbiage (e.g. Oxycodone with Acetaminophen).

Item XXX: One tablet (capsule) was examined and was found to contain X, a controlled substance. Reference also indicated the presence of Y.

The net weight of the examined tablet was X grams.

The remaining tablets (capsules) were visually examined and had a net weight of X grams. Reference(s) indicated the presence of X, a controlled substance, and Y. No confirmatory analysis was performed on the remaining tablets (capsules).

2.11.2. Statistical Sampling Results: The conclusion reached shall be clearly stated with respect to what inference could be drawn from the analysis of a multiple unit population in the case notes. If a statistical sampling plan (hypergeometric) is used, it is statistically correct to infer that the results of the items examined include the unexamined items. (e.g., 29 bags out of 100 were examined and found to contain Cocaine. The results shall be reported as follows (or similar verbiage):

Item XXX was found to contain X, a controlled substance.
The net weight of item XXX was X gram(s).
This result is based on hypergeometric sampling that meets or exceeds a 95% confidence level that 90% of the containers are positive.

Hypergeometric sampling is a statistically based method which involves taking random samples of the whole population. This method allows an inference to be made about the contents of the whole population.

If hypergeometric sampling is used, even though examining 90% of the containers will not meet the statutory weight threshold the results shall be reported as follows (or similar verbiage):

Item XXX was found to contain X, a controlled substance.
The net weight of item XXX was X gram(s).
This result is based on hypergeometric sampling that meets or exceeds a 95% confidence level that 90% of the containers are positive. The weight of 90% of the containers does not exceed the statutory weight threshold.

Hypergeometric sampling is a statistically based method which involves taking random samples of the whole population. This method allows an inference to be made about the contents of the whole population.

2.12. References:

2.12.1. Methods of Analytical/Sampling Seized Drugs for Qualitative Analysis: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations 2010-Jan-29, Part IIIA.
2.12.2. Guidelines on Representative Drug Sampling, European Network of Forensic Science Institutes (ENFSI) Drugs working Group
2.12.3. Indiana Criminal Code 35-48
2.12.4. Indiana Criminal Code 35-48-1-25
2.12.5. Indiana Criminal Code 16-18-2-199
2.12.6. United States Criminal Code Title 21 Section 801
2.12.7. United States Criminal Code Title 21 Section 802 (41) (A and B)
2.12.8. United States Criminal Code Title 21 Section 812
2.12.9. United States Criminal Code Title 21 Section 813
2.12.10. United State Criminal Code Title 21 Section 353(b) (1)

3. Weight Determination

3.1. Scope: Forensic Scientists conduct examinations on evidence suspected to contain controlled substances and/or other drugs. In addition to qualitatively determining what drug or drugs are present, the Forensic Scientist routinely measures the weight of the drug or material present in the evidence submitted. (Note: Here the terms "mass" and "weight" are used interchangeably.) The weight of the evidence, whether it is plant material, powder, tablets, capsules, a rock-like substance, etc. is specifically being measured. It is recognized that in some instances the results of these measurements and their associated uncertainties have an effect on criminal charges.

3.2. Precautions/Limitations: In some situations, the mass or weight may not be recorded or reported. Items described as a residue or suspected residue do not have to be weighed.

If the sample is a food product, the weight shall be recorded as a net weight. If the sample is suspected drugs on paper (or other similar substrate such as windowpanes), the weight shall be recorded in the case notes and reported as a net weight. Only one sub-item (e.g., piece of paper, windowpane, page of a book, etc.) shall be sampled and examined per item. The sub-item shall be recorded and reported as a net weight. The report shall specify that one sub-item (e.g., piece of paper, windowpane, page of a book, etc.) was analyzed. If the item is a book or magazine with binding, the remaining book or magazine shall be recorded as a gross weight Sublingual films shall be recorded and reported as a net weight. Pharmaceutical patches do not need to be weighed and the weight shall not be reported.

Some items cannot be separated from their packaging due to their condition, such as a sticky tar-like substance. Efforts should be made to separate the sample from its packaging (e.g. – placing the sticky substance in the freezer). If the sample cannot be removed from its packaging, the Forensic Scientist shall record the measurement as a gross weight and record the condition of the material in their case notes.

It is important that balances are functioning properly prior to obtaining weight measurements.

3.3. Related Information:

- **3.3.1.** Appendix 1 – Forms and Worksheets
- **3.3.2.** Appendix 2 – Abbreviations
- **3.3.3.** Appendix 3 – Definitions
- **3.3.4.** Appendix 4 – Drug Unit Reagent Preparation Manual
- **3.3.5.** Other Test Methods
  - **3.3.5.1.** Drug Unit Measurement Uncertainty Statements
  - **3.3.5.2.** Sampling Test Method

3.4. Instruments: Measurements shall be made using the laboratory balances of various manufacturers and models, and will generally be an electronic top-loading balance with a readability of 0.01 gram. In some instances, there will be a need for a higher capacity balance for large items of evidence or an analytical balance may be used for items requiring a greater sensitivity with

different readabilities. The Forensic Scientist shall use each balance in accordance with the manufacturer recommendations found in the balance user manuals.

3.5. Reagents/Materials: Weigh boats, weigh paper or other container may be used during the weighing process.

3.6. Hazards/Safety: Forensic Scientists shall comply with the Forensic Services Division (FSD) Chemical Hygiene Plan and the FSD Safety Manual. Precautions should be taken to minimize the potential for personal exposure to drugs, hazardous chemicals, and potential biohazards. Gloves shall be worn during the weighing process of evidence handling.

3.7. Reference Materials/Controls/Calibration Checks:

3.7.1. The performance of all balances shall be verified, evaluated and their respective uncertainties calculated prior to use in case work.

3.7.2. Reference Standards (weights):

3.7.2.1. National Institute of Standards and Technology (NIST) traceable weights shall be used to verify the calibration status of the balances.

3.7.2.2. Weights used to check balance accuracy shall be re-certified by a qualified vendor every three years, at a minimum. Vendor and documentation specifications shall be maintained on a network drive.

3.7.2.2.1. Any weight found to be outside the manufacturer specified range of tolerance shall be repaired and returned to acceptable tolerances, if possible. If a weight cannot be adjusted or repaired, it shall be marked and retired from service.

3.7.2.3. Reference Standard weights of 1 gram, 5 grams and 30 grams shall be used, at a minimum, for verification of small capacity balances capable of reading 0.01 gram.

3.7.2.4. Reference Standard weights of 30 grams and 10 pounds shall be used, at a minimum, for verification of high capacity balances..

3.7.2.5. Reference Standard Weights shall be stored in a box or closed container.

3.7.2.6. Reference Standard Weights shall be handled with tweezers, or with gloves or other protective material to keep the weights from accumulating contaminants.

3.7.3. Acceptable measurements for reference standard weight sets:

3.7.3.1. High capacity balances capable of reading to 1 gram or 0.1 gram shall be within +/- 1 gram of the reference standard weight used.

3.7.3.2. Balances capable of reading to 0.01 gram shall be within +/-0.01 gram of the reference standard weight used.

3.7.3.3. Analytical balances capable of reading to 0.0001 gram shall be within +/- 0.0005 gram of the reference standard weight being used.

3.7.4. Calibration Checks:

3.7.4.1. The balance calibration shall be verified by the Forensic Scientist before and after evidence sampling using known standard weights. (See 3.9.1 and 3.9.2)

3.7.4.2. A measurement outside the acceptable limits indicates a possible problem. Re-run the verification procedure after checking the balance and weight conditions (vibration, level of balance, drafts, cleanliness of weight, etc.). If the balance does not meet acceptable measurements, then the balance shall be identified as "out of service" and the Drug Unit Supervisor and Laboratory Manager shall be notified.

3.7.4.3. Balances shall be calibrated/serviced/verified annually by a qualified external vendor, demonstrating the balance is working properly by using standard weights traceable to National Institute of Standards and Technology (NIST). The methods and specifications for the external calibration of the balances shall be determined by the vendor performing the calibration service. Vendor and documentation specifications shall be maintained on a network drive.

3.8. Procedures/Instructions:

3.8.1. Drug evidence should be weighed prior to analysis using an appropriate type of balance, except for the following examples. (See Sampling 2.2.1, 2.9.1 and 2.11.1)

3.8.1.1. Liquids: If required, the approximate volume can be recorded (e.g., clandestine laboratory samples). Weighing liquids is not an accurate measurement and this shall not be performed.

3.8.1.2. Residues: Examples include spoons, pipes, straws, bags, etc.

3.8.1.3. If evidence has not been opened (e.g., visually examined non-controlled tablets or administratively withdrawn), the items do not need to be weighed.

3.8.2. Ensure the balance is on, level and reads zero.

3.8.2.1. Tare the balance, if necessary.

3.8.3. Verify that the balance is working properly as per 3.7.

3.8.3.1. Document the satisfactory balance calibration verification on the Examination Worksheet. (see 3.9.2)

3.8.3.2. If the calibration verification check is unacceptable, see 3.7.4.2.

3.8.4. Place suitable container (see 3.5) on the pan when appropriate.

3.8.5. Re-tare the balance.

3.8.6. Place the sample in the tare container, or on the pan, as appropriate.

3.8.6.1. Record the value displayed on the balance when and where appropriate as per 3.7.3.

3.8.6.2. The uncertainty of each measurement shall be documented in the case notes. The total uncertainty for weights that are going to be combined shall also be documented in the case notes.

3.9. Records:

3.9.1. Forensic Scientists shall maintain a balance Calibration Verification log for every balance they use in casework. Measurements of the known standard weights shall be recorded once per month, at minimum, in the log to verify the calibration of the balance. Each balance is assigned a measurement assurance sample (MAS). The MAS shall be weighed once per month, at minimum, and documented in the log for the purpose of calculating and maintaining the measurement uncertainty. The only exception to this is an instance of extended leave. Once they return to work, the Forensic Scientist shall take two measurements per month until they have made up the amount of measurements that were not taken.

3.9.2. The balance calibration verification shall be documented on the Examination Worksheet with the serial number of the specific balance(s), the weight set(s) used and the date of the verification.
Balance checks shall be recorded on the Examination Worksheet before and after sampling. If verifications are performed on different dates, both dates shall be recorded on the Examination Worksheet.

3.9.3. All numerals displayed by the balance during calibration verification shall be recorded in the calibration verification log.

3.9.4. All numerals displayed by the balance during the sampling process shall be recorded on the Examination Worksheet. All weights shall be recorded as a net or gross weight and shall be recorded on the Examination Worksheet. All weights shall be taken in grams.

3.9.5. Marijuana items greater than ten (10) pounds (4535.9 grams) shall be recorded in grams in the analytical notes and reported on the Certificate of Analysis in both grams and pounds. To convert from grams to pounds, divide the gram weight by 453.6 and truncate at the tenths decimal place. The conversion shall be documented in the notes.

3.9.5.1. If a weight is reported in pounds, no more than one decimal place shall appear on the report.

3.9.6. Items that weigh less than 30 grams should not be weighed on a high capacity balance.

3.9.7. Weighing of Capsules:
Marked Pharmaceutical Capsules with reference identification: The net weight includes the capsule and the contents.
All Other Capsules: The net weight is of the contents only and the gross weight is the combination of the capsules and their contents.

3.9.8. All weights used to achieve and/or exceed weight limits to meet a particular criminal charge shall be recorded as a net weight. The remaining weight(s) may be recorded as a gross weight.

3.9.9. When adding weights of multiple packages, the total net or total gross weight shall be in the notes.

3.9.10. A record shall be kept of the calibration status of the reference standard weights and/or weight sets.

3.10. Interpretations of Results: The Drug Unit has conducted studies to estimate the uncertainty associated with weight measurements. For the Expanded Uncertainty the Drug Unit recognizes k=2 as an uncertainty window with a 95.45% confidence for a single measurement.

3.11. Report Writing:

3.11.1. All weights shall be reported as net or gross weight and to the proper decimal accuracy not to exceed the readability of the balance used.

3.11.2. Weights from balances with different readabilities shall not be combined for total weight reporting.

3.11.3. Weights of multiple items may be combined to report a total weight only if the weight types are the same. If the weights are added together, it shall be reported as a "total" weight. It is not appropriate to mix net and gross weights together for a total weight. For example: You cannot add an item with a net weight of 0.20 gram and an item with a gross weight of 0.20 gram and report a total net or gross weight of 0.40 gram. If both weights are recorded as net weights, or both are gross weights, then they can be combined for a total weight.

3.11.4. Marijuana items greater than ten (10) pounds shall be reported in both grams and pounds. If applicable, it is advisable to report the pound equivalents for individual items that are approximately one pound or more for clarity in reporting.

3.11.5. The measurement uncertainty shall be reported when the uncertainty causes the weight to drop below a statutory threshold and shall be reported as +/- the total uncertainty "at a coverage probability of 95.45%". When multiple items added together have a measurement uncertainty that causes the total weight to drop below a statutory threshold, the measurement uncertainty shall be reported for each item.

3.11.5.1. Item XXX was found to contain _____, a controlled substance. The net weight of item XXX was x.xx gram(s) +/- x.xx gram at a coverage probability of 95.45%.

3.11.6. If the weight of an item is 0.00 gram, then the weight shall be reported as "less than 0.01 gram".

3.12. References:

3.12.1. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Supplemental Document SD3 Measurement Uncertainty for Weight Determinations in Seized Drug Analysis, 2010-01-28

3.12.2. Drug Unit Measurement Uncertainty Statements (SharePoint)

4. General Drug Identification

4.1. Scope: This Test Method is intended for the guidance of Drug Unit personnel who support investigations of cases involving suspected drugs. Its scope is limited to those compounds which are most frequently encountered such as narcotics, stimulants, hallucinogens, hypnotics, tranquilizers, diluents, and materials from clandestine laboratories.

4.1.1. Techniques for analysis of drug samples are classified into three categories by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) based on their discriminating power for identification of drugs. Testing procedures selected should give useful (positive) information for suspected drugs for items being examined based upon an initial appraisal of the sample. The currently accepted analytical methods used by the Forensic Services Division are broken down into three categories:

Category A: Those that provide structural information:

Infrared Spectroscopy
Mass Spectrometry

Category B: Methods that provide a high degree of selectivity:

Gas Chromatography
Thin Layer Chromatography
Ultraviolet Spectroscopy
Macroscopic Exam (Cannabis only)
Microscopic Exam (Cannabis only)

Category C: Those that provide presumptive information:

Color Tests
Melting Point
Pharmaceutical Identifiers (Reference Identification)

4.1.2. Scientifically sound practices require the use of multiple techniques. It is the responsibility of the Forensic Scientist to identify the sample and to provide requested information about the sample. These Test Methods specify the minimum testing procedures required for the identification of seized drugs. When the term “shall” is used, the action is required unless a Drug Unit Supervisor has granted deviation from Test Methods. When the term “should” is used, the action is intended to be done unless a valid reason exists to not do it. These situations do not require supervisory approval and are at the Forensic Scientist’s discretion. For the identification of seized drugs, Forensic Scientists shall use, at a minimum, one Category A technique and one additional supporting test (Category A, B, or C). An indication on a Category A technique can be

used as a supporting test in combination with a confirmation on an additional Category A technique.

4.1.3. A validation study will be performed when a new technique is adopted by the laboratory or when a current technique is used for a new purpose.

4.1.4. Further testing procedures may be performed at the discretion of the Forensic Scientist. It is the responsibility of a Drug Unit Forensic Scientist to identify controlled substances and other drugs (e.g., potential drugs of concern or Novel Psychoactive Substances (NPS)) that may be present in evidence samples. If the result of initial testing indicates the presence of a controlled substance (even a weak indication), steps shall be taken to attempt to confirm the controlled substance. Steps should be taken to indicate non-controlled synthetic drugs. Potential drugs of concern, NPS and non-controlled synthetic drugs shall be recorded in LIMS at a minimum if a spectrum can be found for comparison.

4.1.5. When a mixture contains multiple controlled substances, at least one controlled substance shall be identified, if possible. Unless charges could be affected, other controlled substances may be indicated only, at the Forensic Scientist's discretion (e.g., – If a schedule I or II Narcotic drug, Cocaine, or Methamphetamine has been indicated in the sample, the Forensic Scientist shall make an attempt to identify at least one of the schedule I or II Narcotic drugs, Cocaine, or Methamphetamine). Attempts to identify the primary components should be made. The largest GC/MS peak of a controlled substance, potential drug of concern, NPS or common cutting agent should be, at a minimum, indicated on the report (this excludes stearic and palmitic acid, breakdown products, precursors, and similar compounds). If a substance, regardless of controlled status, is confirmed by analysis, it shall be reported as a confirmation; however, in items where there are multiple sub-items (e.g., – illicit tablets) it is not necessary to report a substance that is not confirmed in all sub-items. For non plant material items, it is not necessary to confirm or report Cannabinoids if another controlled substance is reported (e.g. Meth mixed with THCA).

4.2. Precautions/Limitations: These Test Methods do not include every possible technique or procedure. Forensic Scientists shall exercise sound analytical judgment in choosing the appropriate procedure for the circumstances. Insufficient material or concentration within submitted samples may preclude an examination and/or identification. New methods, or modification of existing methods, shall be accepted scientific techniques and apply to the individual sample.

4.3. Related Information:

4.3.1. Appendix 1 – Forms and Worksheets
4.3.2. Appendix 2 – Abbreviations
4.3.3. Appendix 3 – Definitions

4.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
4.3.5. Other Test Methods
- **4.3.5.1.** Marijuana Test Method
- **4.3.5.2.** Color (Spot) Tests
- **4.3.5.3.** Thin Layer Chromatography
- **4.3.5.4.** Ultra-Violet Spectrophotometry
- **4.3.5.5.** Fourier Transform Infrared Spectroscopy
- **4.3.5.6.** Gas Chromatography/Mass Spectrometry
- **4.3.5.7.** Gas Chromatography-Infrared Spectroscopy
- **4.3.5.8.** Polarimetry
- **4.3.5.9.** Melting Point

4.4. Instruments:

4.4.1. Ultraviolet light box
4.4.2. Thin Layer Chromatography Development Tanks
4.4.3. Ultraviolet Spectrophotometer (UV)
4.4.4. Fourier Transform Infrared Spectrometer (FTIR)
4.4.5. Gas Chromatograph/Mass Spectrometer (GC/MS)
4.4.6. Gas Chromatography-Infrared Spectroscopy (GC-IR)
4.4.7. Polarimeter

4.5. Reagents/Materials:

See Test Methods for analytical procedures (4.3.4)

4.6. Hazards/Safety:

(See appropriate Test Methods)

4.7. Reference Materials/Controls/Calibration Checks:

4.7.1. Reference materials (See Reference Materials Test Method)
4.7.2. Blanks and Controls (See appropriate Test Method)
4.7.3. Calibrations/Verifications (See appropriate Test Method)

4.8. Procedures/Instructions:

4.8.1. A minimum of one confirmatory test (category A) and one supporting test (category A, B, or C) is required to identify a substance. In some cases, additional testing may be necessary. If the confirmatory test is positive for a controlled substance and the supporting test is negative, at least one additional appropriate supporting test shall be attempted.

Dry Powder Samples: The tests which are employed can include a combination of Color Tests, Ultraviolet Spectrophotometry, Chromatography (thin-layer or gas), GC/MS, GC-IR, and/or Infrared Spectroscopy. Specialized testing techniques such as Polarimetry are used with selected drugs to determine optical activity. Since some components of drug samples can be masked or hidden during testing procedures, extraction

procedures or other analytical techniques may need to be employed in an attempt to ensure that other substances are not being missed. Substances such as Acetaminophen, Ibuprofen, Aspirin, Diphenhydramine, Quinine, and Caffeine are commonly found in combination with controlled substances, but the controlled substances may not be apparent using most screening methods. If a controlled substance is indicated by analysis (even a weak indication), steps shall be taken to attempt to identify a controlled substance that may be masked or hidden by other substances.

4.8.2. Marked Tablets and Capsules:

The Category C Pharmaceutical Identifier method is intended to be used only on tablets, capsules, and pharmaceutical packaging consistent with that from a commercial manufacturer. When conducting an examination on a dosage form, care shall be taken to ascertain that the product has not been tampered with and is of legitimate, as opposed to clandestine, origin.

Markings that cannot be located in a published reference shall be treated as a general unknown..

4.8.2.1. Legend Drugs/Non-controlled Preparations: Those marked tablets and capsules that contain drugs that do not require a prescription and/or contain non-controlled prescription drugs shall not be examined. Reference identification is sufficient unless there is evidence of tampering and/or reasons to suspect tampering. These items can be re-submitted for analysis; however, confirmation may not be possible due to instrumental limitations and/or availability of a reference material. If a reference material is not available in the Drug Unit, the reference material shall not be purchased and an indication of the drug shall be reported if sufficient supporting data is present. The report shall also state a reason why the sample could not be confirmed.

4.8.3. Manufacturer sealed packaging: If the packaging of an over-the-counter and/or prescription drug is intact; lists the weight and/or dosage information and the contents on the package, a reference identification of the packaging may be used in lieu of analysis of the item. (See 4.10.7 and 4.11.8). A separate literature reference is not required.

4.8.4. Liquid Samples: Liquid samples are generally examined using the same techniques employed to examine powder samples. Adjustments may have to be made in the sample preparation and the procedures used. Testing should be performed to determine if water may be present and the pH of the liquid prior to running it on GC/MS or GC/IR using water finding paper and pH paper. The results of the pH paper and water finding paper testing of the sample shall be documented on the worksheet along with the date the testing was performed. If a liquid sample has spilled out of its original

container into an outer container (a plastic bottle containing a glass vial), the two liquids may be combined into one sample. This shall be documented in the notes.

4.8.4.1. Blood Contaminated Liquid Samples: Drug items that are suspected to be contaminated with blood may be withdrawn according to the Drug Unit's Physical Evidence Bulletin.

4.8.5. Clandestine Laboratory Samples: Samples from clandestine laboratory reaction mixtures require unique analysis and sampling procedures. Knowledge of procedures being utilized is important. Examination and identification of precursor compounds and finished product are necessary, as well as identification of intermediate products in some cases.

4.8.6. Plant Materials and Plant Material Preparations: Plant materials are examined visually, macroscopically, and microscopically noting morphological characteristics. Additional tests such as Color Tests, Thin Layer Chromatography, Gas Chromatography Retention Time (GC-RT), GC/MS, Semi-Quantitation, and GC-IR are available to be used to identify the components of plant materials.

4.8.7. Psilocybic Mushrooms, Peyote Buttons, Opium Poppy, Khat, etc.: Mushrooms, peyote buttons, opium poppy samples, Khat and various other materials are subjected to extraction procedures to remove the drugs of interest from the bulk of the sample prior to analytical testing. These extracts are then examined using routine procedures for dry powder or residue samples.

4.8.8. In all cases, comparison with a known reference material is required for a positive identification. The unknown sample and the reference material shall have been run on the same instrument using the same or similar methodology.

4.8.9. Disposal: Sample disposal by the Forensic Scientist should be done within five working days from the completion of the analysis in order to prevent the accumulation and subsequent disposal of larger quantities of sample material. The Forensic Scientist shall maintain control of any sample waste until it is disposed. A Drug Unit Supervisor may direct the retention of samples for the use of training samples, proficiency samples, etc. At no time is the analytical waste to be allowed to accumulate without authorization.

Drug sample waste shall be disposed as per the FSD Drug Waste Management Program. A secure location shall be selected in each Regional Laboratory for the purpose of collecting post-analysis drug waste such as tablets, capsules, powders, plant materials, etc. Liquid samples may require additional procedures (example: liquid PCP) for disposal.

GC/MS vials may be placed in the broken glass disposal boxes and disposed in the regular trash.

Drug Reference materials and bulk drugs have other requirements and restrictions. Refer to Test Method 30 for disposal.

Bulk drugs are subject to DEA disposal regulations. Refer to the FSD Waste Management Program.

4.9. Records: Record in the examination documentation all notes, worksheets, data, sample preparation, detailed extraction procedures, reference identifications, and observations used to support the findings or results and opinions or conclusions. This would include:

4.9.1. All printouts of sample spectra generated for UV, GC/MS, FTIR, and GC-IR:

At a minimum the blank and sample spectrum (e.g., – Total Ion Chromatogram for GC/MS, Pages 1 and 2 of the report for GC-IR) for each sample run shall be printed and included in case file. It is not necessary to note observations on runs not being used for the final conclusion. The data shall be retained on the instrument hard drive, or external hard drive. If data cannot be stored, all data shall be printed and included in the case file. Data files shall not be over-written. Documentation of additional runs due to concentration, extractions and/or program changes shall be kept in the case notes.

4.9.2. **All analytical tests:** If an observation, data, or calculation is rejected, the reason and date shall be recorded in the case file and the data shall be marked as rejected. Observations or data are only considered rejected under the following circumstances:

- **4.9.2.1.** The instrument malfunctioned (e.g., – clogged syringe, sequence stopped running, power outage).
- **4.9.2.2.** For UV, GC/MS, or GC-IR, a peak appeared in the blank.
- **4.9.2.3.** For FTIR, the background spectrum had unidentified peaks.
- **4.9.2.4.** For color tests, the multi-step blank had an unacceptable color.
- **4.9.2.5.** For TLC, a spot was in the blank.
- **4.9.2.6.** The wrong sample was run.
- **4.9.2.7.** The reference material lot number used is unknown for TLC or GC-RT
- **4.9.2.8.** For GC/MS or GC-IR, the blank and the sample were not run on the same method.
- **4.9.2.9.** If the same solvent was not used for the blank as the sample.

No conclusions shall be drawn on rejected observations or data; however, the observations or data may be used to determine the analytical scheme.

4.9.3. The reason for any additional runs as well as the date shall be noted in the case file.

4.9.4. Reference material spectra used for comparison to the unknown.

4.9.5. Photographs, if applicable.

4.9.6. Detail what tests were conducted and the preparation method used on which items and/or sub-items.

4.9.7. The date of each test or observation shall be recorded in the case notes.

4.9.8. Category A techniques shall have data that is reviewable.

4.9.9. For Marijuana (Cannabis) a recording of detailed botanical characteristics observed is acceptable.

4.10. Interpretations of Results: For any method to be used for identification or confirmation, the test results shall be considered "positive." While "negative" test results provide useful information for ruling out the presence of a particular drug or drug class, these results have no value toward establishing the forensic identification of a drug.

4.10.1. All samples shall be compared with primary or secondary reference materials, which have been previously tested to verify their identity. (See Reference Materials Test Method)

All unknowns shall be evaluated prior to the comparison to a known.

4.10.2. Identifications: A category A technique and at least one supporting test shall be used for positive identification. This combination shall identify the drug(s) present and shall preclude a false positive identification. An indication using a category A technique can be used as a supporting test in combination with a positive result from a different category A technique.

4.10.3. Seized drugs can be conclusively identified when the results of the tests have been compared with a verified reference material of that substance and are of sufficient quality to permit identification.

4.10.4. Indications: In some instances, the results of the examination will lack acceptable analytical results to conclude that a specific substance is present. This may be a result of the item not containing a sufficient amount of material or having a concentration that prevents a positive result. Other possibilities include lack of an available reference material for comparison, and/or when an external library has been used (i.e., SWGDRUG or other library). External libraries shall be carefully evaluated, and the entry used should include the drug name. The name of the external library shall be documented in the case file.

4.10.5. Inconclusive results: In some instances, examination yields no helpful or conclusive information that support neither identifications nor indications.

4.10.6. Reference Identifications: For some items, such as marked tablets of products that contain drugs that are not controlled, samples may not be removed for testing, but simply be visually examined for purposes of reporting what the item may contain based on the markings of the tablets, capsules, packaging, etc.

A reference identification is not a valid secondary/presumptive test when analytical results are not consistent with the reference information (e.g.- M30 tablet containing Fentanyl, but markings indicate Oxycodone). The presence of another substance does not necessarily invalidate the reference identification if the peak in the TIC is less than 10% of the active ingredient, controlled substance peak. Other items present in the case should be considered.

4.10.7. Reference Identification of manufacturer sealed packaging: If the packaging of an over-the-counter and/or prescription drug is intact; lists the weight and/or dosage information and the contents on the package, a reference identification of the packaging may be used in lieu of analysis of the item. A separate literature reference is not required.

4.10.8. Drug Preparations: There are occasions where a controlled substance is part of a preparation. The controlled substance shall be identified. The other active ingredients shall be indicated, at a minimum, when the presence of the other ingredients may cause the controlled schedule to change. Analytical support of an indication is required.

4.10.9. Exempt Preparations: Exempted preparations that contain a controlled substance do not require a full examination, unless requested. See 4.11.9

4.11. Report Writing: Certificates of Analysis are generated by Forensic Scientists to report their results, opinions and interpretations following the examination of the item(s) of evidence listed on the report. The conclusions stated are a result of specifically what was tested and weighed (see Sampling 2.11). All analytical method(s) used in analysis shall be listed on the Certificate of Analysis for each item that was examined. The following are guidelines for reporting analytical results. It may be necessary to combine statements, make adjustments to accurately reflect analytical results and/or achieve consistency in reporting.

4.11.1. Analytical reports involving the examination of suspected controlled substances shall be written to offer information as to whether the materials examined are “controlled”. The report shall not list a substance as non-controlled.

In cases where an identification is made, the results shall be reported using the following or similar verbiage (see 2.11):

Item XXX was found to contain X, a controlled substance.

4.11.2. When a drug type is identified that is controlled federally but not a state controlled substance, the report shall include the drug identified as “a federally controlled substance”.

4.11.3. When an examination provides insufficient information to support an indication or identification, one or more of the following statements shall be used:

Item XXX – no controlled substance was identified (or similar wording).

Or

Item XXX contained an insufficient amount of material (or concentration, or other reason) for identification.

4.11.4. In cases where an item is examined and a non-controlled substance is indicated, but not conclusively identified, the results shall be reported using the following or similar verbiage:

Item XXX – no controlled substance was identified.

And/Or

Item XXX - Examination indicated the presence of X. .

4.11.5. In cases where an item is examined and a controlled substance is not identified within that item, but is only indicated (due to insufficient material, concentration, or degradation, etc.), the results shall reflect the reason an identification could not be made using the following or similar verbiage:

Item XXX indicated the presence of X, a controlled substance; however, there was insufficient material (or other reason) for complete identification.

Or

Item XXX indicated the presence of X, a controlled substance; however, this could not be confirmed due to insufficient material (or concentration of the sample, sample degradation, inconclusive testing results or other reason).

4.11.6. Reference Identifications without further testing: When reference identification is used and no other testing is performed, the report shall reflect the item was visually examined and what active ingredient(s) the markings of the material indicate is present.

Reference Identifications shall be reported using the following verbiage:
Item XXX was visually examined. Reference(s) and markings indicated the presence of X. No confirmatory analysis was performed.

Also acceptable:

Item XXX was visually examined. Reference(s) and markings were consistent with a preparation containing X. No confirmatory analysis was performed.

4.11.7. Reference Identifications with examination: When reference identification is used and some analysis is performed, it may be necessary to combine or alter the approved report wording to accurately reflect this in a report.

For example: Item XXX – Reference(s) and preliminary examination indicated the presence of X, a controlled substance.

4.11.8. Reference Identification of manufacturer sealed packaging: If a manufacturer sealed package is unopened, intact and the weight and contents are described in the labeling on the package, analysis of the items is not necessary. A reference identification of the packaging is sufficient. Additionally, the evidence description shall reflect the "intact/sealed" packaging and basic details of the labeling (i.e., drug name(s) and dosage(s)).

For example: Item XXX – Reference identification of the sealed packaging indicated the presence of X, a controlled substance. No confirmatory analysis of this item was performed.

4.11.9. Drug or Preparation Specific Results: There are occasions where the general result wording is insufficient to describe the test results accurately. In those instances, refer to the Test Method for the specific drug, or drug grouping.

Examples:

Item XXX was found to contain X, a controlled substance and X. Reference(s) and examination were consistent with a preparation containing X, a controlled substance.

4.11.10. Multiple drugs in one item: Many samples contain multiple substances and the results can be complex. The specified report wording may be adjusted to accurately describe the results of the examination. Multiple sentences should be used and run-on sentences should be avoided. It may be necessary to use several sentences.

4.11.11. Combining results: In a situation where there are two or more items with the same results, these may be combined to simplify the report. Weights may be reported as a total weight, as appropriate. However, it may be equally appropriate to list the individual weights. (See 3.11.3)

Example:

Items XXX and XXX were found to contain Cocaine, a controlled substance.
The total gross weight of items XXX and XXX was 2.50 grams.

4.11.12. Items not analyzed shall be reported as "not analyzed" and include a statement explaining why the item was not analyzed (e.g., unsuitable for analysis, insufficient material for analysis, etc.). This may not apply to sub-items that are not examined or items that are administratively withdrawn.

4.11.13. If examinations by another forensic discipline are deemed appropriate, the contributor should be contacted and the following or similar statement shall be added to the report: "Item XXX was transferred to the Microanalysis (or other) Unit for analysis."
This shall apply to those items (as specified in 10.8.5) that are transferred to the Indianapolis Regional Laboratory for isomer determination or confirmation by GC-IR. In that event, one of the following statements should be used:

Item XXX was transferred to the Indianapolis Regional Laboratory for further testing.

Or

If the specific isomer needs to be determined, please contact the laboratory.

Example:

Item XXX was found to contain Fluoro-PB-22. The specific isomer was not determined.

If the specific isomer needs to be identified, please contact the laboratory.

4.12. References:

4.12.1. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations , 5th Ed., 2010-01-29, Part III (Methods of Analysis)

4.12.2. BNDD Analytical Manual: Analysis of Drugs (initial issuance), United States Department of Justice Bureau of Narcotics and Dangerous Drugs

4.12.3. Indiana State Police Forensic Services Division Drug Waste Management Program in section 1 of the Laboratory Waste Management Program.

5. Color (Spot) Testing

5.1. Scope: Color (Spot) Tests are preliminary tests (SWGDRUG Category C) used to indicate the presence or absence of certain drugs found in case samples. Spots tests have the advantage of being a quick, easy, and inexpensive means to acquire information. A series of spot tests can be used to test an unknown sample in flow-chart fashion, leading to two or three possible substances out of hundreds. This Test Method is intended to provide instruction for the proper use and interpretation of Color (Spot) Tests.

5.2. Precautions/Limitations:

5.2.1. Appendix 4 contains a list of commonly used and acceptable color test reagents. This list is not necessarily all-inclusive.

5.2.2. Color Tests are suitable for use on powders, liquids, residues, tablets, and capsules. It may be necessary to make minor adaptations to perform these types of tests on liquid or plant material samples.

5.2.3. The tests are generally destructive and the sample cannot be used further in analysis.

5.2.4. These tests are non-specific and therefore cannot provide positive identification of a particular substance. In most situations, the color reaction produced is not confined to a single compound, but rather a number of related compounds in a particular class of substances (i.e., drugs with similar structures may give the same reaction). These tests can aid in narrowing the possibilities by the process of elimination.

5.2.5. Not all Color Tests (or Spot Tests) produce a color, but rather a characteristic reaction.

5.2.6. The reactions of several tests correlate with particular functional groups or other drug structures. There are reactions that are observed that are not fully understood, but have in practice been repeatable and reliable indicators of a particular substance or group of substances.

5.2.7. False positives and false negatives are possible.

5.2.8. Color Test reactions can be influenced by the concentration of the controlled substance and by interferences from diluents or other substances. It is possible to observe a combination of colors produced by the reaction.

5.2.9. It is possible to have secondary color reactions over time, however decomposition occurs rapidly. Observations should be made in a timely manner to avoid misinterpretation.

5.2.10. Forensic Scientists shall possess the visual ability to distinguish color and detect slight color changes for proper documentation and evaluation of color test reactions.

5.3. Related Information:

5.3.1. Appendix 1 – Forms and Worksheets
5.3.2. Appendix 2 – Abbreviations
5.3.3. Appendix 3 – Definitions
5.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
5.3.5. Other Test Methods
- **5.3.5.1.** General Drug Analysis
- **5.3.5.2.** Reference Materials Test Method
- **5.3.5.3.** Drug Unit Training Manual – Spot Test module

5.4. Instruments:

5.4.1. Fume Hoods/fume absorbers

5.5. Reagents/Materials:

5.5.1. Reagents: (See Reagent Preparation Manual)
5.5.2. Organic Solvents (methanol, petroleum ether, chloroform, etc.)
5.5.3. Ceramic Well plate
5.5.4. Disposable test tubes
5.5.5. Evaporating dish
5.5.6. Spatulas, pipettes

5.6. Hazards/Safety:

5.6.1. Routine use of concentrated acids and bases
5.6.2. Fumes (exposure and inhalation hazards)
5.6.3. Carcinogens
5.6.4. Poisons
5.6.5. Reproductive Hazards

5.7. Reference Materials/Controls/Calibration Checks:

5.7.1. Reagents shall be verified with a known reference material when the solution is made and on a monthly basis at a minimum. See Reagent Preparation Manual (Appendix 4) for specifics. (See also 5.9.3)

5.7.2. Infrequently used Spot Test reagents shall be verified with a reference material at the time of use. (see 5.9.2)

5.7.3. Negative Controls (Blanks) shall be run in conjunction with multi-step spot tests to demonstrate that the combination of reagents is blank or does not produce a color reaction. (5.9.4)

5.7.4. pH paper and water finding paper shall be verified on a monthly basis using acid, base, or distilled or DI water, where appropriate. All containers of testing paper currently in use shall be tested monthly and shall be documented on a Reagent Log Sheet.

5.7.5. If a color or spot test fails the verification process, it shall be discarded. The reagent shall be re-made and verified.

5.8. Procedures/Instructions: Tests can be performed directly on a portion of the sample or extract in a small test tube, spot plate or evaporating dish.

5.8.1. Place a small amount of sample, positive control, or negative control in a well of a clean, dry ceramic spot well plate or test tube.

5.8.2. Add 2-3 drops of the desired reagent to the well.

5.8.3. Observe and record reactions on the Examination Worksheet.

5.9. Records: Any color change or lack of color change (e.g. no color reaction (NCR)) shall be recorded in the casefile with the date that the test was performed. If the speed of the result is delayed, this shall also be recorded. Other reactions, including gas evolved or precipitate formed, should be recorded in the casefile with the date the test was performed.

5.9.1. Ten basic spectral colors are recommended to describe reactions. Variations in color are indicated by combining two colors (e.g., red-brown), with the second color being the dominant color.

5.9.2. Reference materials and blanks used to verify infrequently used reagents shall be recorded in the notes or on the Reagent Preparation and Verification log.

5.9.3. Reagent preparation and verifications shall be recorded on the Reagent Preparation and Verification log and shall include the lot numbers of the chemicals used, color reaction observed, date, initials of the preparer/verifier and the name, source and lot numbers of the substance(s) used to verify the reagents. An acceptable blank shall be documented as “NR” (no reaction) in the logbook. The reagent bottle shall be labeled with the date of preparation and initials of the preparer. (See 5.7.1)

5.9.4. Observations of the negative controls/blanks used for multi-step reagents shall be documented on the Examination Worksheet. A check box is sufficient to document an acceptable multi-step blank.

5.10. Interpretations of Results: Color interpretations are subject to the opinions of the Forensic Scientist performing the test. Spot tests can give a characteristic reaction if a particular substance

is present. Others can give several different reactions according to which substance is present and can be used to help distinguish between different classes of drugs, depending on which color forms.

5.10.1. A positive result shall be based upon an initial expected reaction and/or the color progression of the reaction. (See 5.12.1)

5.10.2. A positive result does not indicate that a specific drug is present. It indicates that a certain class of drugs may be present.

5.10.3. A negative test, or no reaction, indicates the absence of a substance or an insufficient amount of material.

5.11. Report Writing: N/A

5.12. References:

5.12.1. Analysis of Drugs and Poisons 3rd Edition, Clarke, E.G.C, London, Pharmaceutical Press, 2004.

5.12.2. Isolation and Identification of Drugs, Clarke, E.G.C, London, Pharmaceutical Press, 1986.

5.12.3. Spot Tests in Organic Analysis 7th Edition, Feigl, F, New York: Elsevier Scientific Publishing Company, 1966.

5.12.4. Forensic Science Handbook Volume II, Saferstein, R, Englewood Cliffs, NJ: Prentice Hall, 1988.

5.12.5. Tannic Acid as a Field Test for Caffeine, Hueske, EE, Microgram, Vol. XV, No. 9, September, 1982, p. 158.

5.12.6. The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms, Garrett, A.S., Clemens, S.R., Gaskill, J.H.SWAFS Journal, Vol. 15, No. 1, April, 1993, pp.44-45.

5.12.7. United States Department of Justice Drug Enforcement Administration, Analysis of Drugs Manual, 2nd Ed., February, 1999.

5.12.8. A New Field Test Reagent, Ferris Van Sickle, Laboratory Notes, June 4, 1974.

5.12.9. Chemical Field Tests for Narcotics and Dangerous Drugs, US Department of Justice, Bureau of Narcotics and Dangerous Drugs.

5.12.10. Color Test to Differentiate Between Cocaine and Lidocaine, Carolyn Ruybal

5.12.11. The Multiple Testing of Suspected Drugs to Minimize False Positives, Robert B. Carroll, Ph. D.

5.12.12. Color Tests-Methcathinone/Methamphetamine, Terry Dal Cason

5.12.13. Screening Test for Amphetamine, Fleischer, David (NYC Police Department, New York, NY), Microgram, Vol. VIII, No. 8 (August, 1975).

5.12.14. Kovar, K.A. and Laudszun, M. “Chemistry and Reaction Mechanisms of Rapid tests for Drugs of Abuse and Precursor Chemicals”, United Nations Scientific and Technical Notes, February 1989

6. Ultraviolet Spectrophotometry

6.1. Scope: Ultraviolet (UV) Spectrophotometry is a SWGDRUG Category B method of analysis that is widely used as a screening test in forensic drug analysis. In combination with other analytical data, this technique provides supporting data for identification of controlled substances. This Test Method is intended to give guidance and instruction for proper use and interpretation of data generated from the UV instrument.

6.2. Precautions/Limitations:

6.2.1. The UV gives limited structural information and some selectivity to allow for some distinction between similar substances. However, it does not give specific results and cannot be used as a conclusive method of identification.

6.2.2. Not all solvents are suitable for use in UV. Solvents should be selected that do not absorb in the UV region.

6.2.3. Quartz cuvettes should be used for UV analysis. Glass and plastic cuvettes may not be suitable for analysis in the UV range.

6.2.4. Compounds that lack suitable chromophores provide no absorbance pattern.

6.2.5. Different compounds may have very different absorption maxima depending on the solvent used and the solubility of the sample.

6.2.6. Highly concentrated samples and intensely absorbing compounds may shift the absorbance maxima and/or saturate the spectrum and, therefore, shall be examined in dilute solution.

6.2.7. Strong UV absorbing substances can mask the presence of other weaker UV absorbing substances. Additional testing, and/or extraction, is necessary to reveal weaker UV absorbing substances.

6.2.8. The presence of interfering substances can influence the absorption spectrum by shifting the maxima.

6.2.9. Solvent polarity and pH can affect the absorption spectrum of an organic compound.

6.2.10. Chemical composition may change during analysis.

6.2.11. It is possible to recover the sample, if necessary.

6.3. Related Information:

6.3.1. Appendix 1 – Forms and Worksheets

6.3.2. Appendix 2 – Abbreviations
6.3.3. Appendix 3 – Definitions
6.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
6.3.5. Other Test Methods
6.3.5.1. Reference Materials
6.3.5.2. General Drug Identification
6.3.5.3. Drug Unit Training Manual – UV module

6.4. Instruments: Ultraviolet Spectrophotometer capable of recording spectra in the UV range generally from 400 – 200 nanometers.

6.5. Reagents/Materials:

6.5.1. 0.5N Sulfuric Acid (H_2SO_4) (UV cutoff point 190 nm)
6.5.2. 0.45N Sodium Hydroxide (NaOH) (UV cutoff point 225 nm)
6.5.3. Methanol (MeOH) (UV cutoff point 210 nm)
6.5.4. Chloroform ($CHCl_3$) (UV cutoff 245 nm)
6.5.5. Water (UV cutoff 205 nm)
6.5.6. Quartz Cuvette (UV cutoff point 170 nm)

6.6. Hazards/Safety:

6.6.1. General Drug Exposure
6.6.2. Dilute acids and bases
6.6.3. Organic solvents

6.7. Reference Materials/Controls/Calibration Checks:

6.7.1. Reagents: Reagent acids and bases used in UV analysis shall be verified by checking the pH of the solution at the time it is prepared.

6.7.1.1. If the reagent fails verification process, then it shall be discarded and the reagent shall be re-made and verified.

6.7.2. Performance Checks: The UV Instrument shall be performance checked once per month using a Holmium Oxide reference material. The resulting absorbance maxima shall be within +/- 2 nanometers of the expected value to be considered satisfactory. (See 6.9.2 and 6.9.3)

6.7.2.1. In the event that performance checks are found to be unsatisfactory, the instrument shall be taken out-of-service and steps taken to restore the instrument to proper working order.

6.7.3. Any instrument that is out-of-service shall be visibly marked.

6.7.4. When an instrument is taken out-of-service for maintenance and/or repair, performance checks shall be performed prior to resuming casework on that instrument.

6.7.5. An infrequently used instrument may be placed in an "Inactive" status and the normal performance verification procedures may be suspended. Normal performance verification procedures shall be resumed prior to use in casework analysis.

6.8. Procedures/Instructions:

6.8.1. Samples are routinely examined in 0.5 N Sulfuric Acid, methanol (MeOH) or 0.45 N Sodium Hydroxide.

6.8.2. Samples may be run as received or extracted. Some samples, such as mushrooms, suspected LSD gelatin squares, etc. require extraction prior to UV analysis.

6.8.3. Generally, the wavelength range scanned is approximately 400 nm to 200 nm, depending on the UV cut-off of the solvent being used. (See 6.5)

6.8.4. Run a solvent blank, or background, using the same solvent that will be used to measure the sample. A solvent blank or background shall be run before each sample.

6.8.5. A small amount of sample is placed in a cuvette and dissolved in the appropriate solvent. (See 6.2.3)

6.8.6. Obtain the UV absorption spectrum by scanning the sample and solvent matrix. (See 6.8.3)

6.8.7. Print the UV spectrum. (See 6.9.4)

6.8.8. Samples can be recovered and additional tests performed, if necessary.

6.8.9. If the UV absorbance pattern and maxima indicate the presence of a non-controlled substance such as Acetaminophen, Caffeine, Ibuprofen, or Aspirin, the sample shall be extracted and analyzed via Gas Chromatography/Mass Spectrometry, Gas Chromatography-Infrared Spectroscopy, or Fourier Transform Infrared Spectroscopy. However, if the sample is analyzed unextracted using a confirmatory technique, and a controlled substance is present, the sample does not have to be extracted.

6.8.10. Preventative maintenance: The UV instrument has no routine maintenance. In the event of a source failure or malfunction, it shall be replaced. If the instrument fails its performance checks, it shall be taken out of service and repaired.

6.9. Records:

6.9.1. Reagent acid and base preparation and verification shall be documented on the Reagent Preparation and Verification log. The bottle shall bear the date of preparation and the initials of the preparer.

6.9.2. Maintenance: Each UV instrument shall have a maintenance log.

6.9.3. Calibration/verification information shall be documented in the Calibration Verification Log.

6.9.4. The status of any inoperable, in-active, or out-of-service instruments shall be reflected in the maintenance log.

6.9.5. All UV spectra printouts shall contain information regarding the observed maxima absorbance and the solvent used.

6.9.6. The solvent blank shall be printed.

6.9.7. The laboratory case number, laboratory item number, and date of the examination shall appear on the printout and may be computer generated. The Forensic Scientist shall initial any hard copy of the technical record.

6.9.8. All maxima used for identification or indication shall be marked and summarized on the Examination Worksheet, including unit of measure (nm).

6.9.9. References used for comparison shall be included and/or the source of the reference specifically documented on the Examination Worksheet, if used for identification or indication.

6.9.10. In instances where a sample is run multiple times, the existence of multiple runs shall be documented on the Examination Worksheet or table. The data from each run shall be labeled with a Run number. The reason for multiple runs shall also be documented. The Forensic Scientist shall document which blank(s) are associated with which run(s).

6.10. Interpretations of Results: UV absorption patterns can be indicative of a substance or more commonly, a particular class of substances. The Forensic Scientist should be familiar with the common spectra observed in the laboratory and published tables of UV absorbance maxima.

6.10.1. The absorbance spectra of unknowns are compared with known reference material spectra and/or with published tables of UV maxima using a +/- 2 nm uncertainty window to narrow the list of possible compounds, if applicable.

6.10.1.1. If the peak is outside the +/- 2 nm window, additional presumptive testing shall be performed.

6.10.2. The shape, position and intensity of the absorbance maxima shall be evaluated when making determinations.

6.10.3. It is recognized that mixtures result in absorbance patterns that are influenced by components with various absorptivities and concentrations. This may result in absorbance shifts or blending of absorbance maxima.

6.11. Report Writing: N/A

6.12. References:

6.12.1. Analysis of Drugs and Poisons 3rd Edition, Clarke, E.G.C, London, Pharmaceutical Press, 2004.

6.12.2. Isolation and Identification of Drugs, Clarke, E.G.C, London, Pharmaceutical Press, 1986.

6.12.3. Drug Unit's Reagent Preparation Manual (Appendix 4)

6.12.4. Resource Manual on Quantitation

6.12.5. Principles of Instrumental Analysis. 6th ed. Skoog, et al Thomson Brooks/Cole. 2007, 169-173.

6.12.6. Instrumental Data for Drug Analysis, Mills III, Terry, and Roberson, J. Conrad. 2nd Ed. New York, New York: Elsevier Science Publishing Company, 1987.

6.12.7. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 01/29/2010.

7. Thin Layer Chromatography

7.1. Scope: Thin Layer Chromatography (TLC) is a SWGDRUG Category B technique used for separation and tentative identification of controlled substances. This Test Method is intended to provide instruction for the proper use and interpretation of Thin Layer Chromatography data.

7.2. Precautions/Limitations: Thin Layer Chromatography has a number of analytical advantages. It is a relatively rapid, cost-effective method of analysis. It enables many samples to be screened simultaneously against multiple known reference materials. Samples can be recovered if non-destructive visualization techniques are used.

7.2.1. Analytes of interest should be stable in the solvent system being used.

7.2.2. TLC has a lower sensitivity and resolution than other chromatographic methods, such as gas-chromatography. A sufficient amount of material shall be available to perform this test properly. If a comparison is made, the amounts of sample and reference material should be similar.

7.2.3. Most substances dissolve readily in methanol (MeOH) and can be applied (spotted) along the origin of the TLC plate. It may be necessary to dissolve some substances in a more appropriate solvent to ensure a more concentrated sample is available for the test.

7.2.4. TLC plates are of a finite size and so it is not possible to use this method to separate the multitudes of substances in existence and provide a conclusive means of identification.

7.2.5. Irregularities in the TLC plate thickness can have an effect on separation.

7.2.6. Salt forms may have an effect on separation, spot shape and Rf values. Some salt forms produce tailing or streaking spots.

7.2.7. Chemicals used in the solvent systems shall be analytical grade equivalent, or better, and be mixed thoroughly to achieve sufficient separation of drugs in the same class.

7.2.8. Loss or evaporation of solvent can delay or skew the separation of substances. The TLC chambers should be tightly sealed to prevent solvent loss.

7.2.9. Loss of volatile samples can occur by heating the TLC plates (i.e., Methamphetamine in basic TLC systems).

7.2.10. Contamination of solvent systems can have an effect on separation by altering the polarities of the solvent system.

7.2.11. If the components of the sample are known to not separate on TLC and if the abundance of the minor component is over 10% of the larger component on GC/MS, then an alternative test shall be used (e.g., – Diphenhydramine and Heroin on the acidic system or Fentanyl and Fluorofentanyl).

7.2.12. The GC/MS vial should not routinely be used for running TLC (e.g., the GC/MS vial can be used for residue samples). A secondary aliquot shall be used, if possible. If a secondary aliquot is not used and the sample is not a residue, the analyst shall get approval for deviation from a Drug Unit Supervisor.

7.2.13. Reference materials should have one spot on TLC.

7.3. Related Information:

7.3.1. Appendix 1 – Forms and Worksheets
7.3.2. Appendix 2 – Abbreviations
7.3.3. Appendix 3 – Definitions
7.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
7.3.5. Other Test Methods
- **7.3.5.1.** Reference Materials Test Method
- **7.3.5.2.** General Drug Identification

7.4. Instruments: Long and short wave UV light box

7.5. Reagents/Materials:

7.5.1. TLC development chambers: generally rectangular glass chamber with a lid.

7.5.2. Stationary Phase: TLC plates. Silica Gel (250 µm) coated glass plates are most commonly used. Aluminum backed silica coated plates are also acceptable.

- **7.5.2.1.** The use of fluorescent indicators is recommended.

7.5.3. Mobile Phase: solvent systems depend on the compounds to be separated and stationary phase used. Systems used should be stable in air or when mixed with acids and bases. It should be easily removed from the plate after development and should not react with the substances to be separated. (See Reagent Preparation Manual)

7.5.4. Capillary tubes or micropipettes.

7.5.5. Reference Materials (as appropriate for the particular drug or drug class).

7.5.6. Reagent over-sprays/visualization reagents (See Reagent Preparation Manual).

7.5.7. Reagent pre-soak solutions (See Reagent Preparation Manual) and tray.

7.5.8. Standard 12-inch ruler for approximate Rf calculation, if applicable.

7.5.9. Pencil for marking spots.

7.6. Hazards/Safety: Hazardous Chemical Exposure, including potential carcinogen exposure.

7.6.1. Mobile phases and visualizing reagents should be prepared in the hood.

7.6.2. Any spraying of visualization reagents, or over-sprays, shall be performed with the hood on and the spray directed into a spray box.

7.6.3. TLC plates present a chemical exposure hazard after development. Plates should be viewed in a timely manner and disposed in a glass disposal box.

7.6.4. Physical Hazards: broken glass potential.

7.7. Reference Materials/Controls/Calibration Checks:

7.7.1. Primary or secondary reference materials and a solvent blank shall be used simultaneously with unknowns in all cases. All major components of the sample (any component that is greater than 10% of the size of the most abundant controlled substance peak on the TIC) shall be spotted on the TLC plates, if the reference material is available at the laboratory. This does not include substances that will not react with TLC development sprays being used.

7.7.2. In the event that spots appear in the blank(s), the TLC examination is invalid and the test shall be re-run under the same conditions. If the second blank continues to be unacceptable, steps to locate and remove the source of contamination shall be taken prior to any further TLC analysis. This may necessitate re-sampling of evidence.

7.7.3. Once the source of contamination has been eliminated, the entire test including unknowns, reference materials and blanks shall be re-run.

7.8. Procedures/Instructions:

7.8.1. Various solvent systems will be utilized depending on the material to be tested. In all cases a minimum of one solvent system shall be used. Two solvent systems are recommended for greater selectivity.

7.8.2. Tank Preparation

7.8.2.1. Each Forensic Scientist is responsible for ensuring the quality, and freshness of the solvent systems. It shall be their decision to determine if

it is suitable for use or if a fresh mixture is appropriate. That person shall discard the solvent and make a “fresh” system.

7.8.2.2. Single component solvent systems shall be made up and discarded on an as needed basis.

7.8.2.3. Multi-component solvent systems have a short shelf life and should be freshly mixed just before using or daily. These systems should be changed after 2-3 full plates (20 cm X 20 cm) or the equivalent, or daily, whichever comes first.

7.8.2.4. The Forensic Scientist preparing to run multi-component TLC systems shall evaluate the existing solvent system, if any, discard and prepare the new solvent mixture if necessary. The date of this fresh mixture is to be recorded on a tag/index card/note/etc. affixed to the tank noting the tank has been prepared.

7.8.2.5. After the solvent system is mixed, add it to the tank. The developing solvent should be approximately 0.5 cm deep. Allow to equilibrate in the developing chamber.

7.8.3. Plate Preparation

7.8.3.1. Unknowns and reference materials are routinely dissolved in Methanol, Chloroform or Petroleum Ether.

7.8.3.2. Spot the sample, reference materials and solvent blanks approximately 1 cm up from the bottom edge of a dry thin layer chromatography plate.

7.8.3.3. Reference materials shall be run simultaneously with unknown samples on the same plate for comparison. The concentration of the sample and the reference material should be approximately the same.

7.8.3.4. Pre-soaking techniques are used for some plate preparations. The plate shall be soaked in the appropriate solution until saturated (See Reagent Preparation Manual) and dried with heat for 1-3 minutes or until sufficiently dried.

7.8.4. Place the plate in the tank, sealing the lid tightly. The plates should be allowed to develop approximately 10 to 20 centimeters or to the top of the plate.

7.8.5. After completion of the development, remove the TLC plates from the tank and allow them to dry. It is permissible to use a dryer to expedite the drying process.

7.8.6. View the dried plates under long (360-365 nm) and/or short (254 nm) wavelength UV light.

7.8.7. Where appropriate, mark the spots viewed under short and/or long wave UV lightly in pencil prior to proceeding with chemical visualization reagents.

7.8.8. Spray the TLC plate with appropriate visualization or color developing reagents (e.g. – Fast Blue BB, Iodoplatinate, Potassium Permanganate, p-DMAB, Ninhydrin, etc.), and mark for identification purposes, if appropriate.

7.8.9. Rf values can be calculated, if desired.

7.8.10. Preparative Thin Layer Chromatography: When samples contain other organic substances that interfere with analysis, this method can be used to clean up or remove those substances for other testing such as FTIR or GC/MS.

7.8.10.1. A neutral solvent system should be used to avoid altering the original salt form of the analyte.

7.8.10.2. Prepare TLC plates as per 7.8.3, and follow procedures in 7.8.4 - 7.8.7.

7.8.10.3. When plate is dry, scrape off the desired area and wash thoroughly with solvent (methanol) in a beaker. Filter to remove the silica gel.

7.8.10.3.1. It may be necessary to use extraction procedures from an aqueous acidic or basic solution to separate the substance from the silica gel.

7.9. Records:

7.9.1. Plate pre-soaking preparation (if used), Solvent system(s) and method(s) of visualization used shall be documented on the Examination Worksheet.

7.9.2. Observations of the solvent blank and any spot(s) in the unknown sample and the reference materials used shall be recorded on the Examination Worksheet. Observations shall include the final color of the spot and the distance traveled from the baseline to the center of the spot. A photocopy, photograph, or diagram is permitted to be used instead of a written description of color and distance traveled. A check box is sufficient to document an acceptable blank.

7.9.3. The source and lot numbers of reference materials used for tentative identifications/indications shall be in the case notes. An attached sheet may be appropriate.

7.9.4. It is recommended, but not required, to list all reference materials used in the TLC comparison.

7.9.5. If no spots are visualized in the unknown, the use of at least one reference material shall be documented.

7.9.6. Photographs may be taken of TLC plates, if desired. TLC plates shall be contained in a plastic sleeve or plastic bag before placing on photocopier. (See 7.6.3)

7.10. Interpretations of Results:

7.10.1. If a spot is in the blank, no conclusions shall be made from the plate.

7.10.2. Positive indication of the unknown sample shall be based on comparable color and location of the sample spot(s) on the plate relative to the reference material. If the spot is seen on UV only, it cannot be used as the only supporting test for confirmation. Example: +Caffeine, UV only, or similar documentation.

7.10.3. If a spot does not move from the baseline, no conclusions shall be made from that spot.

7.10.4. A positive result recorded refers to the drug reference material that was used in the comparison.

7.11. Report Writing: N/A

7.12. References:

7.12.1. Clarke's Isolation and Identification of Drugs. 2nd Edition; Clarke, E. G.C., The Pharmaceutical Press, 1986.

7.12.2. Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G.C., The Pharmaceutical Press, 2004

7.12.3. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 01/29/2010.

7.12.4. Instrumental Applications in Forensic Drug Chemistry – Proceedings of the International Symposium; USDOJ Office of Science and Technology, May 29-30, 1978.

8. Fourier Transform Infrared Spectroscopy

8.1. Scope: Fourier Transform Infrared Spectroscopy (FTIR) is a SWGDRUG Category A method of analysis. This method uses reflected, absorbed, or transmitted radiant energy in the mid-infrared region of the electromagnetic spectrum to produce data capable of providing specific chemical and structural information of a substance. It is particularly useful in determining salt forms of controlled substances, differences between closely related compounds and identification of small or low molecular weight compounds. This Test Method is intended to give guidance for proper use and interpretation of FTIR data.

8.2. Precautions/Limitations:

8.2.1. FTIR can be used as a confirmatory technique when the substance being analyzed is in a relatively pure form or when the sample is mixed with substances that do not absorb in the mid-IR region. Most casework samples are not pure enough to permit identification as received. It is usually necessary to perform extraction procedures, which can result in a substantial loss of sample.

During separation procedures it is possible that chemical changes in the material (i.e., salt form conversions) may occur.

8.2.2. FTIR requires a larger sample than most other techniques to perform the test. However, the sample is usually recoverable and could be used for other testing procedures.

8.2.3. Optical isomers cannot be distinguished using this method of analysis.

8.2.4. Gas Samples - Gas phase spectra differ from condensed-phase spectra because the molecules are free to rotate in a gas which minimizes intermolecular interaction. This results in more fine structure and fewer peaks.

Samples that must be analyzed as a gas shall be stable in that form at room temperature. An air-tight gas cell shall be used for this type of analysis.

8.2.5. Pure samples may give different spectra due to polymorphism.

8.2.6. Environmental conditions, such as high humidity, can complicate the spectrum by adding additional peaks (typically CO_2 and H_2O).

8.2.7. Attenuated Total Reflectance (ATR) spectra are similar to transmission spectra; however, the absorbance bands are shifted.

8.3. Related Information:

8.3.1. Appendix 1 – Forms and Worksheets

8.3.2. Appendix 2 – Abbreviations
8.3.3. Appendix 3 – Definitions
8.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
8.3.5. Other Test Methods
8.3.5.1. General Drug Analysis
8.3.5.2. Reference Materials

8.4. Instruments:

8.4.1. Fourier Transform Infrared Spectrometers (FTIR) of various makes and models capable of recording spectra in the mid-IR range of approximately 4000-450 cm^{-1}.
8.4.2. Attenuated Total Reflectance Apparatus (ATR)
8.4.3. Diffuse Reflectance Apparatus
8.4.4. Hydraulic press and Pellet dies
8.4.5. Desiccators with desiccants
8.4.6. Mechanical shaker/grinder/mixer

8.5. Reagents/Materials:

8.5.1. Methanol
8.5.2. Polystyrene Reference Material and/or other known Reference Materials.
8.5.3. Infrared Grade Potassium Bromide (KBr)
8.5.4. Salt plates Sodium Chloride (NaCl) or Potassium Bromide (KBr)
8.5.5. Gas cell and syringe
8.5.6. Mechanical shaker vials and caps
8.5.7. Solvent cover
8.5.8. Glass mixing beads

8.6. Hazards/Safety:

8.6.1. Exposure to chemicals and drugs during analysis
8.6.1.1. MeOH
8.6.1.2. Acetone
8.6.1.3. $CHCl_3$
8.6.1.4. Drugs – See individual SDS for specifics

8.6.2. Maintenance Hazards: IR light/radiation exposure. Optical hazards due to laser exposure.

8.7. Reference Materials/Controls/Calibration Checks:

8.7.1. Calibration and/or performance checks shall be run weekly using a known reference material and documented in the maintenance log. (See 8.9.2)

8.7.2. Instruments configured for transmission mode shall be verified each week using a polystyrene reference material and a background.

8.7.3. Instruments configured for ATR mode shall be verified each week using a known Cocaine HCl reference material and a background.

8.7.4. Performance checks: The most intense peak in each of three regions (4000-2000 cm^{-1}, 2000-1200 cm^{-1}, and 1200-650 cm^{-1}) shall be within +/- 4 cm^{-1} of the expected value.

8.7.4.1. The expected value means the wavenumber for the most intense peak in each of the three regions (4000-2000 cm^{-1}, 2000-1200 cm^{-1}, and 1200-650 cm^{-1}) for the spectra of Cocaine HCl reference material, when it was originally run on the crystal, source, detector, and laser currently installed in the FTIR instrument. If that data is not accessible, the expected value means the oldest data available with the most intense peaks in each of the three regions labeled.

8.7.4.2. When service is performed and the crystal, source, detector, or laser is changed, the Cocaine HCl reference material shall be re-run. The values for the most intense peak in each of the three regions (4000-2000 cm^{-1}, 2000-1200 cm^{-1}, and 1200-650 cm^{-1}) for the spectra of Cocaine HCl reference material shall be evaluated and compared to the expected values. If they fall inside +/- 4 cm^{-1}, these newly obtained values will become the new expected values, and a new log sheet shall be started with the newly obtained values recorded at the top of the log sheet.

8.7.5. In the event that calibration or performance checks are found to be unsatisfactory, the instrument shall be taken out-of-service and measures taken to restore the instrument to proper working order.

8.7.6. Any instrument that is out-of-service shall be visibly marked and the maintenance log shall reflect the inoperable status.

8.7.7. When an instrument is taken out-of-service for maintenance and/or repair, performance and/or calibration checks shall be performed prior to resuming casework on that instrument.

8.7.8. Performance check procedure:

8.7.8.1. Run a background spectrum.
8.7.8.2. Scan the reference material and print the spectrum.
8.7.8.3. Record and store the performance verification spectra in the instrument calibration and maintenance log.

8.7.9. If unexpected peaks appear in the background, steps shall be taken to restore the instrument to proper operating status (e.g., the ATR crystal and anvil shall be cleaned, etc.) and the background shall be repeated.

8.7.10. If the background continues to be unacceptable, steps shall be taken to resolve the issue prior to further analysis.

8.7.11. The baseline should also be monitored for increased noise level.

8.8. Procedures/Instructions:

8.8.1. All instruments shall be operated according to their respective operations manuals.

8.8.2. Solid Samples: Solid samples, including powders as received, should be analyzed using the ATR accessory, KBr Pellets, Diffuse Reflectance or FTIR microscope.

8.8.3. Liquid Samples: Examination of liquid samples may be accomplished by use of a liquid cell, a headspace sample in a gas cell, liquid between salt plates (NaCl or KBr) or examination by using Diffuse Reflectance or ATR. It may be necessary to use a solvent cap to prevent loss by evaporation.

8.8.4. Gas Samples: Gas or Headspace samples can be examined by using a gas cell. Clean the gas cell by evacuating it with an air stream. Seal the cell and scan the background. Introduce the sample into the cell and scan the sample.

8.8.5. Salt plates: Run a background spectrum of the salt plate(s) being used. Spread a solution of the sample (or neat sample) onto a salt plate in a thin layer. Let the solvent evaporate and, if necessary, place a second salt plate of like material on top of the first plate, creating a “sandwich”. The sample is then placed in the sample holder and the spectrum is scanned.

8.8.6. When Diffuse Reflectance or Attenuated Total Reflectance is used, it is possible that little or no sample preparation is required.

8.8.7. The surfaces of accessories shall be cleaned with methanol or acetone prior to the collection of each sample and when sample acquisition is complete.

8.8.8. A background shall be run before the collection of each sample. (See 8.7.9 – 8.7.10)

8.8.9. Drugs are examined using a wavelength range of approximately 4000 cm^{-1} to 450 cm^{-1}. The wavelength range used with an ATR accessory shall be 4000 cm^{-1} to 650 cm^{-1}.

8.8.10. Samples and reference material spectra should be evaluated in % Transmittance units.

8.8.11. Sample Analysis Procedure:

8.8.11.1. Run and print a background spectrum. (See 8.7.9 – 8.7.10)
Place the sample on/in the accessory or in the sample holder.

Run and print the unknown sample spectrum.

Clean the accessory, if applicable.

8.8.12. Maintenance: the desiccant shall be changed, as necessary. Source and laser replacement shall be replaced on an as needed basis. Operations such as mirror alignment shall be performed as necessary to keep the instrument in optimal working order.

8.9. Records:

8.9.1. Maintenance: Each FTIR instrument shall have a maintenance log.

8.9.2. The status of any instrument that is out of service shall be recorded in the maintenance log as "out of service". The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

8.9.3. All FTIR spectral data shall be labeled with the laboratory case number and laboratory item number, the date, initials of the Forensic Scientist, and unique identifier of the instrument.

8.9.4. In instances where a sample is run multiple times, the existence of multiple runs shall be documented on the Examination Worksheet. The data from each run shall be labeled with a Run number. The reason for multiple runs shall also be documented.

8.9.5. Unless otherwise noted, the sample form is ATR. If using a different sample form, e.g., KBr pellet or gas cell, this shall be documented on the spectral data or on the Examination Worksheet.

8.9.6. If performed, extraction procedures shall be noted on the Examination Worksheet.

8.9.7. For identification, all spectral data shall be compared to a known reference material and/or a user generated spectral library that has been generated on that instrument (regardless of wavelength range used at the time the reference material was run).

8.9.8. The source and lot numbers of reference materials used for comparison shall be included in the case file.

8.9.9. All data generated from FTIR analysis, including backgrounds and reference material spectra used for comparison, shall be printed, labeled appropriately, and documented in the case file.

8.9.10. When literature references or reference spectral data are used in analysis, the source of the spectral data shall be documented in the case file.

8.9.11. The conclusion or results from the analysis of the infrared spectrum shall be documented on the Examination Worksheet as positive or similar verbiage, or indication of a specific drug.

8.9.12. In cases where neither identification, nor a sufficient indication, can be made based on the spectral data, the results shall be labeled with an evaluation such as unidentified or other similar verbiage.

8.9.13. All reference material spectra shall be maintained electronically on the instrument that generated it, at a minimum. This data shall be backed up either in hard copy form, on an external hard drive or other storage medium.

8.10. Interpretations of Results:

8.10.1. The spectrum shall be well resolved and of a sufficient intensity.

8.10.2. Identifications shall be made by direct comparison to a known reference material of the substance being analyzed, and/or an entry from a user generated spectral library, generated on the same instrument.

8.10.3. The comparison can be accomplished by comparing the position and relative intensity of each peak. The overall appearance and location of major peaks in the sample should correspond with the reference spectrum.

8.10.4. Literature Matches: In the event that the Forensic Services Division does not possess a known reference material or that a reference material is commercially unavailable, a recognized literature reference may suffice as supporting data for indications. The source of the spectral data shall be documented in the case file.

8.10.5. Computer aided searches: A search of possible compounds can be conducted using the computer search algorithms in the instrument software. The results of a computer search are to be used only for the purpose of narrowing the number of possible compounds. Computer searches are just survey tools, limited by library, resolution difference, spectra quality, and sample preparation. The Forensic Scientist evaluates and interprets the comparison of two spectra.

8.10.6. ATR or Diffuse Reflectance: Spectra obtained using an accessory, such as ATR, shall be compared to spectra also obtained using that accessory. For unknowns, a

correction factor may be used to aid in searching a transmission library for spectral comparison. The uncorrected spectra should be compared to that of an uncorrected ATR spectrum of a known reference material, if available.

8.10.7. A positive identification, or indication, recorded refers to the drug reference material used in the comparison.

8.10.8. Mixed FTIR spectra can be used for indications if sufficient spectral details are strong and clearly indicate the drug(s) present. A third test should be used to support identification.

8.11. Report Writing: N/A

8.12. References:

8.12.1. Instrument Software

8.12.2. Forensic Services Division Quality Assurance Manual

8.12.3. Clarke's Isolation and Identification of Drugs, 2nd Ed.; Clarke, E. G. C., The Pharmaceutical Press, 1986.

8.12.4. Instrumental Data for Drug Analysis. 2nd Ed, Mills III, Terry, and Roberson, J. Conrad. New York, New York: Elsevier Science Publishing Company, 1987.

8.12.5. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 5th Edition, Supplemental Document SD-2, 02/09/2006.

9. Gas Chromatography/Mass Spectrometry

9.1. Scope: Gas Chromatography/Mass Spectrometry (GC/MS) is a combination technique of Gas Chromatography (GC), SWGDRUG category B technique, which is a separation technique used to introduce the sample into the Mass Spectrometer. Mass Spectrometry (MS), a SWGDRUG Category A technique, is a specific method of identification for most controlled substances. It can be used for qualitative and quantitative analyses. The instrument can be used to perform individual analysis or in conjunction with an autosampler for automated batch analysis. This Test Method is intended to give guidance for proper use and interpretation of GC/MS data.

9.2. Precautions/Limitations:

9.2.1. The GC/MS is capable of generating electron ionization spectra in the range of 0-700 amu. The sampling range used for most drug analysis is generally 40-400 amu.

9.2.2. GC/MS has the capability of separating the components of a mixture and providing spectral data for each component. However, it cannot directly distinguish between optical isomers, or salt forms.

9.2.3. Low molecular weight compounds produce few ions and are not easily analyzed using this method. Additional data may be needed to support identification.

9.2.4. Compounds shall be volatile and thermally stable for GC/MS analysis. Some common substances degrade upon introduction to the injection port and give spectral information of related substances rather than the compound originally injected.

9.2.5. It is not always possible to identify the molecular ion in a spectrum. There are some classes of compounds that do not give a molecular ion.

9.2.6. It may be necessary to convert some drugs to their free acid or free base form to achieve good chromatographic results.

9.2.7. Periodic maintenance and inspection of the GC/MS will ensure accurate analytical results. Simple issues, such as a dirty injection port liner can have a significant effect on sample analysis.

9.3. Related Information:

9.3.1. Appendix 1 – Forms and Worksheets
9.3.2. Appendix 2 – Abbreviations
9.3.3. Appendix 3 – Definitions
9.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
9.3.5. Appendix 6 – Instrument Maintenance
9.3.6. Other Test Methods

9.4. Instruments:

9.4.1. Agilent quadrupole GC/MS or equivalent
9.4.2. Autosampler

9.5. Reagents/Materials:

9.5.1. Capillary GC column.
9.5.2. Carrier Gas Ultra High Purity grade compressed helium (99.999% purity)
9.5.3. ACS Certified Solvents or equivalent
9.5.4. Consumables for the instrument
9.5.5. Autosampler syringes/manual syringes
9.5.6. Autosampler vials and caps
9.5.7. PFTBA (Perfluorotributylamine)
9.5.8. Restek Standard Test Mix (Reference Material Test Mix) or other approved mixture of Reference Materials

9.6. Hazards/Safety:

9.6.1. Solvent/chemical exposure
9.6.1.1. Wash solvents
9.6.1.2. PFTBA
9.6.2. Burns – hot injection port, oven, transfer line, etc.
9.6.3. High pressure carrier gas
9.6.4. Gas cylinder safety concerns
9.6.5. Electrical/Shock hazards

9.7. Reference Materials/Controls/Calibration Checks:

9.7.1. Each instrument shall be autotuned on a weekly basis at a minimum using Perfluorotributylamine (PFTBA) as an autotune reference standard.

9.7.2. A full autotune or equivalent shall be performed. Full autotunes shall be an “A-tune” or an “S-tune”.

9.7.3. A satisfactory autotune shall be when:

9.7.3.1. Mass assignments of m/z 69, 219, and 502 shall be +/- 0.2 amu.

9.7.3.2. Peaks are symmetrical, smooth and the widths shall be between 0.45 and 0.65 amu.

9.7.3.3. There should be less than 200 peaks in the autotune and the mass abundance of the 69 peak should be between 200,000 and 400,000.

9.7.3.4. The tune shall be evaluated for leaks. Peaks at 18, 28 and 32 amu indicate that there may be leaks in the system. Manufacturers' recommendation is that the nitrogen (28) peak be 10% or less.

9.7.3.5. The EM volts should be monitored for increases and decreases.

9.7.3.6. Ion ratios should be in the below listed ranges:

m/z 69	should be the base peak
70/69	≥0.5 but ≤1.6
219/69	≥40% but ≤85%
220/219	≥3.2 but ≤5.4
502/69	≥2.0% but ≤5%
503/502	≥7.9 but ≤12.3

9.7.4. To monitor the instrument performance, a weekly check using a blank run prior to the test mixture and a test mixture consisting of three or more drug reference materials (Reference Material Test Mix) shall be run using a temperature program. The chromatogram shall be examined for peak shape, height, and retention time reproducibility as compared to another performance check of the same mixture that was previously run. The retention time of each component of the test mix shall be recorded and compared to the retention times of each component of the previously run test mix and shall fall within +/- 0.050 min unless maintenance has just been performed. If the retention time of one or more component of the test mix is out of range attempt to re-run one time. If the retention time of one or more component is still out of range, consult a Drug Unit Supervisor. Spectra shall be compared against the mass spectrum of a verified reference material or published reference material. Documentation of "Test Mix Ok" in the log indicates that all of these criteria were satisfactory.

9.7.5. When a new batch of a test mixture is used, it first shall be run twice to demonstrate repeatability for that test mixture. It then can be used weekly to monitor the instrument performance. A blank shall be run before each test mixture run.

9.7.5.1. The test mixture and blank before the test mix shall be evaluated for each individual instrument. The blank does not need to be printed but shall be saved electronically.

9.7.6. Performance checks shall be considered satisfactory upon the distinct separation of the components in the Reference Material test mix and the concentration is consistent with the last acceptable performance check of that test mixture.

9.7.7. If a performance check or calibration is unsatisfactory, the instrument shall be clearly marked and placed out-of-service until acceptable instrument performance has been restored.

9.7.8. When the instrument has undergone repair or has been out of the control of the Regional Laboratory for any reason (i.e., shipped out for repair), performance checks shall be run to ensure proper operation before analysis resumes on that instrument.

9.7.9. Additional maintenance may be performed as needed. Manufacturer's recommendations and/or laboratory practices may specify the frequency of maintenance procedures. See the Instrument Manuals and the Instrument Preventative Maintenance Appendix for more information.

9.8. Procedures/Instructions:

9.8.1. Instrument Set-up: The instrument conditions (column conditions, carrier gas flow, split or split-less injection mode) should be set to maximize the chromatographic and mass spectral data to be derived from the sample run.

9.8.1.1. The temperature range used is dependent on the length of the column, column flow, and the temperature limits of the column.

9.8.1.2. Capillary GC/MS analysis can be used in either split or split-less injection modes.

9.8.1.3. Either constant pressure or constant column flow of the carrier gas may be used.

9.8.1.4. Isothermal temperature conditions will suffice for most single component drug samples.

9.8.1.5. When dealing with unknowns with no supporting analytical data use one of the following methods:

9.8.1.5.1. Run a low temperature program and if no drugs are indicated, then a high temperature screening program (a program that starts at 180 ^{O}C or above).

9.8.1.5.2. Run a general screening temperature program that will elute both Dimethyl sulfone and Buprenorphine.

9.8.2. Sample Preparation:

9.8.2.1. Solid or liquid samples should be dissolved or diluted in methanol or chloroform, as appropriate.

9.8.2.2. Liquid samples may be run as headspace samples.

9.8.2.3. Samples may be placed in autosampler vials, capped, and run on the autosampler.

9.8.2.4. Autosampler vials shall be labeled with the appropriate identifiers.

9.8.3. Procedure:

9.8.3.1. Blanks (negative controls) shall be run before and/or between each sample. Reference materials are to be treated as samples and are required to have their own blanks. Any time a sample needs to be re-run, it is not necessary to re-run the sample blank if the same sample is re-run immediately. No other injections can be made between back-to-back sample injections.

9.8.3.2. A solvent blank consisting of the solvent used to dissolve the sample shall be run using the same temperature parameters as the sample, including temperature range, ramp rate and hold times. (See 9.9.5.3)

9.8.3.3. Dissolve the unknown sample and/or reference material in a suitable solvent (usually methanol or chloroform) and inject 1-2 µl into the gas chromatograph/mass spectrometer.

9.8.4. If GC/MS is used as a confirmatory test, then GC retention time can be used as a second test if:

9.8.4.1. Two separate aliquots should be prepared.

9.8.4.2. The two vials are run on different instruments or if not possible, the two runs cannot be run consecutively on the same instrument. If the vials are run on the same instrument, documentation shall be included to demonstrate that the vials were not run consecutively.

9.8.4.3. If a separate sample vial cannot be made, then a Drug Unit Supervisor approval is required for use of retention time as a second test. If the sample is a residue, Drug Unit Supervisor approval is not required.

9.9. Records:

9.9.1. Methods: All general GC/MS Methods shall be archived and maintained by the Regional Laboratory. Archived GC/MS methods shall include the column type, column length, and temperature program.

9.9.1.1. If a GC/MS method is created or modified and saved, a new printout shall be generated listing the parameters, dated, and maintained in a Methods binder or saved to the computer and backed up on an external hard drive.

9.9.1.2. Old methods that are not being used shall be maintained either in a Methods binder or in an archive binder or saved to the computer and backed up on an external hard drive.

9.9.1.3 Any modification to an existing acquisition method requires printing/archiving, logging and updating the method log with initials and date by two analysts. No changes shall be made to methods without documenting the change. Each method shall have its own log in the methods logbook to track method changes. If the method changes result in a new naming convention, then the method shall be saved under that new name and a new log sheet shall be initiated.

9.9.1.3.1 All methods (except the semi-quant methods and maintenance methods) shall follow the standard method naming convention. The injection volume shall also be added to the end of the name if the injection volume is anything other than 1 microliter (e.g. 2 microliters shall be _2uL). The solvent delay shall also be added to the end of the name if the solvent delay is more than 2.5 min (e.g. 6 minute solvent delay shall be _6sd.) If the method is a general method that elutes Dimethyl sulfone to Buprenorphine, it shall be indicated at the end of the naming as _gen.

Injection Type_ Temperature Range_Split Ratio_End Hold Time
e.g. A_90-280_ 50-1_10min

9.9.2. Maintenance: Each GC/MS instrument shall have a maintenance log.

9.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

9.9.4. Autotunes and Performance Checks: All calibration and performance check data shall be recorded in the instrument maintenance log for each respective instrument. (See Appendix 1)

9.9.4.1. Performance checks and calibration evaluation results shall be indicated in the maintenance log and initialed.

9.9.5. GC/MS Data: GC/MS data, including sample data, solvent blanks, and reference material spectra used for comparison shall be printed or electronically transferred, appropriately labeled, and included in the case notes. It is permissible to use the library search information with the comparison of the unknown and known reference spectra.

All data shall be retained on the instrument hard drive and/or external hard drive. If data cannot be stored, a Drug Unit Supervisor shall be contacted to discuss alternative methods for storage. Data files and blank files shall not be over-written. Existence of multiple sample runs, as well as the reasons for the multiple runs, shall be noted in the case notes.

9.9.5.1. The GC/MS spectra shall be labeled with the name of the instrument. The instrument shall be named with the Lab Location Mass Spectrometer Model and a Numerical value if more than one instruments of that model are at that lab location (e.g. FWRL 5977B1). The Intuvo instruments are not required to include the laboratory location as part of the instrument name. The GC/MS spectra shall be labeled with the program (method) name and/or general parameters. Multiple runs of blanks and samples shall be identified as such, on the Total Ion Chromatogram, at a minimum. If a sample is run multiple times, the Forensic Scientist shall document which blank(s) are associated with which run(s) and the data from each run shall be labeled with a Run number.

9.9.5.2. The data file should be named with the Case number, Item number, and Run number (e.g. 25F-01234 Item 002 Run 1). Each page of the GC/MS data shall be labeled with the laboratory case number, laboratory item number, and the initials of the Forensic Scientist. Labeling the first run (Run 1) is optional unless there are multiple runs.

9.9.5.3. The solvent used for the blank and to dissolve the sample shall be documented on their respective Total Ion Chromatograms (TIC).

9.9.5.4. The Examination Worksheet shall indicate how the samples were prepared if more preparation was done to the sample than dissolving in a solvent.

9.9.5.5. All significant peaks in the TIC (all peaks greater than 10% of the most abundant peak in the chromatogram that contain ions consistent with a controlled substance, potential drug of concern, or known cutting agent) should be printed and labeled with an evaluation such as identified or unidentified or other similar verbiage unless recorded on the Examination worksheet. If the peaks greater than 10% are not printed because they are not deemed relevant, a note should be made that all other peaks were evaluated. If more than one peak is printed, each peak should be labeled in the technical record with an identifier (e.g., letters, numbers, arrows, etc.).

9.9.5.6. Peaks less than 10% shall be evaluated and printed if deemed relevant (e.g. – indication of a controlled substance).

9.9.5.7. If a sample is found to contain Marijuana, based on semi-quantitative analysis, it is only necessary to print the Tribenzylamine (TBA) and delta-9 Tetrahydrocannabinol (THC) peaks. Other peaks are not required to be printed unless there is a controlled substance present that is not typically found in Marijuana, or the peak is approaching the same abundance as the delta-9 THC peak. For Marijuana samples, it is not necessary to include a statement about the other peaks being evaluated and not printed.

9.9.5.8. A positive identification or indication recorded refers to the drug reference material used in the comparison.

9.9.5.9. Results of GC/MS data comparison that are being reported (either confirmed or indicated) shall be recorded on the Examination Worksheet.

9.9.5.10. The reason for the additional sample runs shall be noted in the case file.

9.9.5.11. At a minimum, the blank and Total Ion Chromatogram (TIC) from all runs shall be included. This shall also include data for runs with an instrument malfunction or problem with the method if the file can be printed. If the file is corrupted and cannot be printed, then a statement shall be added to the worksheet including the run number, date of acquisition, and reason the data could not be printed.

9.9.5.11.1. If a sample is rerun due to concentration or poor chromatography, a note about the concentration or chromatography shall be documented on the worksheet or summary table and any changes made to the sample shall be noted on the worksheet or summary table. For example, if the concentration is too weak, and the sample was evaporated down, it shall be noted that the sample was concentrated on the worksheet or summary table. If sample is added to increase the concentration, the addition of sample shall be noted. If the sample is rerun due to concentration, then the mass spectrum of the peak of interest shall be printed with the TIC and included in the case file.

9.9.5.11.2. If there is a peak in the blank, then the mass spectrum of the peak shall be printed with the TIC and included in the case file.

9.9.5.12. If a sample is rerun due to the presence of a peak in the blank following the sample run, that blank shall be included in the case notes. If applicable, the different laboratory case number on the following blank does not need to be omitted.

9.9.5.13. If there is a peak in the blank following the sample run, and no controlled substance was identified in the original run, the sample shall be rerun with a temperature program appropriate to fully elute the peak in the following blank.

9.9.5.14. If the sample changes between runs, then the TIC and MS with any comparisons shall be included in the notes. (e.g. – Run 1 in Methanol – Indicates Acetaminophen and Hydrocodone, Run 2 – extracted – only Hydrocodone peak remains. Both runs shall be included.) Binders and additives (e.g. Stearic Acid) do not need to be included for both runs.

9.9.5.15. If a sample vial needs to be run again, the vial should be rerun on GC/MS and/or GC-RT within 10 days of initial run. If there is breakdown or there is a significant loss of concentration, then a new portion of the sample shall be prepared and the preparation shall be recorded in the notes.

9.9.5.16. The source and lot number of the reference materials used for identification shall be documented in the case file.

9.9.5.17. Each reference material spectra in the user generated spectral library shall be labeled with the source and lot number of the reference material.

9.9.5.18. Retention time data and conclusions shall be recorded in the analysis notes. The acceptable range (+/- 0.050 minute) shall be recorded in the case notes. The retention time of the reference material and the sample shall be documented in the case notes.

9.10. Interpretations of Results:

9.10.1. The blank shall be evaluated.

9.10.1.1. The blank shall be free of discernible peaks to be acceptable. If a minor peak (a peak at a greater abundance than two times the highest baseline abundance in the TIC) is observed in a blank, the spectrum of the minor peak shall be printed to show that the peak is an expected solvent peak, a result of column bleed, or septa bleed. If the peak is not expected solvent peak, column bleed, or septa bleed, the blank shall be rejected.

9.10.1.2. If a minor artifact (a sharp raised portion of the total ion chromatogram that is less than two times the largest level of background noise) is present, then the blank is acceptable (see 9.10.1.3 for the exception). Multiple minor artifacts should not be printed.

9.10.1.3. If the blank has a minor artifact at the same retention time as a controlled substance or non-controlled substance (e.g. common cutting agent or drug of concern) in the following sample run, it shall be evaluated. If the spectrum of a minor artifact at the same retention time as that substance that contains the base peak ion and the second highest in abundance ion found in the target compound, it shall be rejected.

9.10.1.4. Raised gas chromatograph baselines do not invalidate the blank.

9.10.2. The sample spectra shall be compared against reference material spectra and/or searched against a user-generated reference material library. The library comparison shall be printed or electronically transferred to show the file path and library name. The user-generated library shall contain both controlled substances and non-controlled common cutting agents. If the compound is in the user-generated library, then that library entry shall be used for comparison. If the compound is not in the user-generated library and the lab has that primary reference material (whether controlled or not), it should be added to the user-generated library. If no primary reference material is available to run on the user-generated library, then an external library can be used for comparison. All library entries shall be named Drug Name Source Lot (Lab location Instrument Name "RM/Ref Mat") (Example Methamphetamine Sigma 14F02753 (IRL 5977C1 RM)). If an analyst adds a new reference material or updates an older entry in the user generated library on the instrument computer, then that analyst shall also update the library version saved on the shared drive.

9.10.3. Spectra shall be evaluated by comparing the molecular ion and base peak, and significant ions in the spectrum to that of the known reference material.

9.10.4. Spectra being used for confirmation that have significant ions, above the molecular weight of the compound being analyzed are not acceptable, except if:

9.10.4.1. The ions are normally expected isotopic ions or

9.10.4.2. The ions are known column and septa ions:

147, 177, 191, 193, 197, 207, 221, 253, 281, 295, 315, 325, 327, 331, 341,346, 355, 369, 377, 383, 387, 389, 399, 401, 405, 415, 429, 439, 447, 451, 461, 470, 475, 479, 489, 491, 497, and 499.

The ions shall be identified and labeled as "column ions" or similar verbiage.

9.10.5. Spectral subtraction is also acceptable if the following data is included: the original spectrum, the spectrum being subtracted, and the resulting spectrum. These shall be clearly labeled on the data.

9.10.6. Positive identification requires comparison of the sample spectrum to a mass spectrum of a verified reference material that has been run on the same instrument.

9.10.7. Samples that degrade in the instrument shall have other supporting data or information to support identification.

9.10.8. Some compounds degrade or lose water during GC/MS analysis and may not have a molecular ion present. Degradation peaks may appear early in the gas chromatogram.

9.10.9. Baseline resolution is not required unless retention time is being used. Baseline resolution should be attempted if multiple controlled substances are co-eluting.

9.10.10. If the peak is not fully eluted within the time window of the Total Ion Chromatogram and the peak appears to be a controlled substance or drug of concern, the sample shall be run under a different method that fully elutes the peak.

9.10.11. Retention time: The retention time of the unknown peak shall be within (+/-) 0.050 minute of the retention time of the drug reference material.

9.10.11.1. A solvent blank shall be run prior to the reference material being used for comparison. The blank shall be the same solvent as the reference material. The blank shall be included in the case notes.

9.10.11.2. Peak heights of the reference material and unknown sample should be approximately of equal abundance. The abundance of the reference material shall not be more than ten times the abundance of the unknown sample and the abundance of the reference material shall not be less than one tenth the abundance of the unknown sample.

9.10.11.3. The reference material and sample shall be run using the same temperature parameters as the sample, including temperature range, ramp rate, and hold times. The split ratio does not have to be the same in the GC/MS methods used.

9.10.11.4. The retention time of the peak should be determined by either selecting the apex of the peak or by performing integration. The retention time of the reference material being used for the comparison and the case sample peaks shall be determined using the same method.

9.10.11.5. Reference material retention time data may be used for comparison to an unknown sample from the date the reference material is run until maintenance has been performed. If maintenance performed on the instrument affects the retention time (e.g., column changed, column trimmed, preventative maintenance), then the reference materials shall be

re-run. If a reference material is rerun on an instrument then the older reference material ran at that temperature method shall be archived. Reference material retention time data shall be stored in a way it is easily accessible to each Forensic Scientist in the Regional Laboratory and can be used by all Forensic Scientists.

9.10.11.6. The manufacturer and lot number of the reference material used for retention time comparison shall be documented in the analytical notes or on the data.

9.10.11.7. If structural report wording is available (e.g. fentanyl related substance or 2-aminopropan-1-one), then it is only necessary to run one isomer.

9.10.11.8. If using GC retention time for isomer determination and structural report wording does not apply, all possible isomers that are commercially available and cannot be distinguished by Mass Spectrum, shall be run for comparison.

9.10.11.8.1. The calculated retention time ranges shall not overlap under the instrument conditions used.

9.10.11.9. A multi-component reference material solution is permitted to be used for retention time reference material data (e.g. Fluorofentanyl mix or GC/MS Test mix). The same restrictions apply to the multi-component reference material solution as a single component reference material. The multi-component reference material mix shall be verified using reference materials of each of the components contained within the mix. The peaks do not have to have complete baseline resolution in this instance as long as the retention time ranges do not overlap.

9.11. Report Writing: Substances that degrade and have additional testing procedures to support identifications, or indications, may require alternative wording.

9.12. References:

9.12.1. GC/MS Operator Manuals – Agilent Technologies
9.12.2. Forensic Services Division Quality Assurance Manual
9.12.3. Clarke's Isolation and Identification of Drugs. 2nd Ed. Clarke, E. G. C., King of Prussia, Pennsylvania, The Pharmaceutical Press, 1986.
9.12.4. Clarke's Analysis of Drug and Poisons, 3rd Edition; Clarke, E. G. C., London, Pharmaceutical Press, 2004.
9.12.5. Instrumental Data for Drug Analysis. 2nd Ed., Mills III, Terry, and Roberson, J. Conrad. New York, New York: Elsevier Science Publishing Company, 1987.

9.12.6. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 02/09/2006.

10. Gas Chromatography-Infrared Spectroscopy

10.1. Scope: Gas Chromatography-Infrared Spectroscopy (GC-IR) is a combination technique that utilizes the separation capability of the gas chromatograph and the specificity of Fourier Transform Infrared Spectroscopy for the purpose of identifying controlled substances. The DiscovIR GC-IR separates components of complex mixtures in the gas chromatograph (GC), then deposits and freezes each component onto a ZnSe (Zinc Selenide) disk. The instrument then uses an infrared microscope to focus radiant energy through the frozen material to produce solid phase transmission spectral data capable of providing specific chemical and structural information of each substance. It is particularly useful for determining and differentiating between structural isomers of substances. This Test Method is intended to give guidance for proper use and interpretation of GC-IR data.

10.2. Precautions/Limitations:

10.2.1. GC-IR requires liquid nitrogen to cool the detectors. Safety precautions shall be taken while handling liquid nitrogen.

10.2.2. A warming detector may lead to poor spectral quality or loss of data.

10.2.3. Disk temperatures may need to be set at lower points for substances such as methamphetamine to improve retention of material on the disk and overall chromatographic quality.

10.2.4. Disk speeds can affect the quality of peak chromatography. Fast speeds cause peak broadening. Slower speeds can cause peaks to co-elute.

10.2.5. The position of the GC column tip may need to be adjusted routinely. Misalignment of the tip will affect chromatography and retention time.

10.2.6. The disk in the GC-IR can accommodate approximately 72 hours of run time before it will need to be cleaned.

10.2.7. As with the GC/MS, the injection port liner, o-ring, etc. will need periodic maintenance to ensure good analytical results.

10.2.8. Compounds shall be volatile and thermally stable for GC-IR analysis. The same substances that degrade upon introduction to the injection port on a GC/MS will do the same on the GC-IR. (See also 9.2.4)

10.2.9. Sample concentrations of approximately 1.5-2 mg/ml are necessary to give quality spectral data. This technique may not be appropriate for most residues.

10.2.10. The spectra generated from the DiscovIR are transmittance infrared spectra and can be directly compared with published transmittance data. Care should be taken to note that some spectra produced by GC-IR may not be comparable to published FTIR data

because the sample is no longer in a salt form (e.g., Cocaine HCl and Cocaine base will both give the same spectrum (Cocaine base)).

10.2.11. The GC-IR can distinguish between most positional and structural isomers.

10.2.12. This technique cannot distinguish between salt forms of substances, or optical isomers.

10.3. Related Information:

10.3.1. Appendix 1 – Forms and Worksheets
10.3.2. Appendix 2 - Abbreviations
10.3.3. Appendix 3 - Definitions
10.3.4. Appendix 4 - Drug Unit Reagent Preparation Manual
10.3.5. Appendix 6 – Instrument Maintenance
10.3.6. Other Test Methods

10.4. Instruments:

10.4.1. Gas Chromatograph
10.4.2. Spectra Analysis DiscovIR direct deposition and detection system
10.4.3. Autosampler

10.5. Reagents/Materials:

10.5.1. Capillary GC Column
10.5.2. Carrier Gas: Ultra High Purity compressed Helium (99.999% purity)
10.5.3. Liquid Nitrogen
10.5.4. ACS grade solvents
10.5.5. Consumables for the instrument
10.5.6. Autosampler syringes
10.5.7. Autosampler vials and caps
10.5.8. Polystyrene (internal to the IR)
10.5.9. Approved mixture of Reference Materials.

10.6. Hazards/Safety:

10.6.1. Solvent/chemical exposure
  10.6.1.1. Liquid nitrogen
  10.6.1.2. Wash solvents
10.6.2. High pressure carrier gas
10.6.3. Gas Cylinder safety concerns
10.6.4. Burns- hot injector port, oven, etc.
10.6.5. Electrical/Shock hazards

10.7. Reference Materials/Controls/Calibration Checks:

10.7.1. Performance checks shall be performed every week the instrument is used for casework:

10.7.1.1. A voltage check,

10.7.1.2. A noise check,

10.7.1.3. A polystyrene Reference Material check,

10.7.1.4. A Test Mix/Reference Material Mix check

10.7.2. Satisfactory checks shall be when:

10.7.2.1. Voltage checks are between 3 and 7 volts.

10.7.2.2. Noise checks are between 0.4 and 1.0 mABS.

10.7.2.3. Polystyrene checks are +/- 2.5 $cm^{-1}$of the following bands:

Band 1	3060.2 cm^{-1}
Band 2	1601.5 cm^{-1}
Band 3	1583.2 cm^{-1}
Band 4	1028.5 cm^{-1}
Band 5	906.7 cm^{-1}

10.7.3. Test Mix check shall be examined for chromatographic peak shape, height and retention time reproducibility as compared to another performance check of the same mixture that was previously run. (See also 9.7.5 and 9.7.6)

10.7.3.1. Retention times shall be within (+/-) 0.15 min for satisfactory reproducibility of the Test Mix.

10.7.3.2. Additionally, the IR spectra of each component of the Test Mix shall be compared against the infrared spectrum of a verified reference material or published reference material. Documentation of “Test Mix Ok” in the log indicates that all of these criteria were satisfactory.

10.7.4. If any of the performance checks are found to be outside their designated ranges or otherwise found to be unsatisfactory, the instrument shall be clearly marked and placed out of service until satisfactory performance of the instrument has been restored.

10.8. Procedures/Instructions:

10.8.1. Check Performance/Operating Procedure:

10.8.1.1. Cool Instrument by adding liquid nitrogen to the detector and the disk.

10.8.1.2. Settings should be:

10.8.1.2.1. Vacuum pressure should be below 9 X 10^{-3} torr.

10.8.1.2.2. Transfer Line* and Restrictor Temperatures should be set at the same temperature.

These values should be (+/-) 10 degrees of the start point.

*This should be set at low temperatures during periods of down time to extend the life of the transfer line.

10.8.1.2.3. Disk Temp: below -30 °C

10.8.1.2.4. Resolution: 4 cm^{-1} (Manually set/permanent setting)

10.8.1.2.5. Split Ratio: 10:1 (recommended)

10.8.2. Voltage and Noise Check Procedures: Record these values on the Calibration Verification log.

10.8.3. Polystyrene Check: Print the spectrum and record the check on the Calibration Verification log.

10.8.4. Test Mix Performance Check: Print the background, the Test Mix blank, and chromatogram of the Test Mix. These shall be kept in the instrument Calibration Verification log.

10.8.5. Sample Preparation:

10.8.5.1. Samples should be dissolved in a suitable solvent, such as $CHCl_3$, MeOH and/or Acetone.

10.8.5.2. Samples may be placed in autosampler vials, capped, and run on the autosampler. The GC/MS vial should not routinely be used for running GC-IR. If a secondary aliquot is not used, Drug Unit Supervisor approval must be obtained unless the sample is a residue.

10.8.5.3. Autosampler vials shall be labeled with the appropriate identifiers.

10.8.6. General Operating Procedure:

10.8.6.1. A solvent blank consisting of the solvent used to dissolve the sample shall be run within the same temperature range of the sample.

10.8.6.2. Solvent blanks shall be run before each sample in the same location where the sample is to be deposited on the ZnSe disk.

10.8.6.3. Approximately 1-2 µL of sample is to be injected using an autosampler. Recommended sample concentrations should be approximately 1.5-2.0 mg/mL.

10.9. Records:

10.9.1. Methods: All methods shall be archived and maintained in the Regional Laboratory.

10.9.1.1. If a GC-IR method has been modified and saved, a new printout shall be generated listing the parameters, dated, and maintained in a Methods binder or saved to the computer and backed up on an external hard drive.

10.9.1.2. Old methods that are not being used shall be maintained either in a Methods binder or in an Archive binder or saved to the computer and backed up on an external hard drive.

10.9.2. Maintenance: Each instrument shall have a maintenance log.

10.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

10.9.4. Performance Checks: All calibration and performance check data shall be recorded on the instrument Calibration Verification log.

10.9.4.1. Performance checks and calibration verification evaluation results shall be indicated on the Calibration Verification log and initialed.

10.9.4.2. The evaluation and acceptance of the FTIR spectral data associated with the Test Mix shall be documented on the Calibration Verification log.

10.9.5. GC-IR Data: GC-IR data, including sample data, solvent blanks, and reference material spectra used for comparison shall be printed, appropriately labeled, and included in case notes. It is permissible to use the library search information with the comparison of the unknown and known reference spectra.

Additional sample and blanks runs that are not used in comparison shall be retained in hardcopy form in the notes and/or stored electronically. If stored electronically, the data

shall be retained on the instrument hard drive and/or external hard drive. If data cannot be stored, a Unit Drug Unit Supervisor shall be contacted to discuss alternative methods for storage. Data files are not to be over-written.
Existence of multiple runs and the reasons shall be included in the case file.

10.9.6. GC-IR data shall be labeled with the name of the instrument (or other unique identifier), the program (method) name and/or general parameters. Multiple runs of blanks and samples shall be identified as such, on the Absorbance Chromatogram, at a minimum (e.g., Run 1, Run 2, etc.). The data from each run shall be labeled with a Run number on the blank and TIC at a minimum.

10.9.7. GC conditions such as column type, length and temperature program shall be indicated on the Examination Worksheet, unless this information is specified on the printed GC-IR data.

10.9.8. The Examination Worksheet shall indicate how the samples were prepared if more preparation was done to the sample than dissolving in a solvent. The solvent used for the blank and to dissolve the sample shall be documented on their respective spectral data.

10.9.9. Each page of the GC-IR data shall be labeled with the laboratory case number and laboratory item number, and the initials of the Forensic Scientist.

10.9.10. All significant peaks in the Absorbance Chromatogram (all peaks greater than 10% of the most abundant peak in the chromatogram) should be printed and either marked as identified or unidentified. Any peak below 10% of the most abundant peak in the chromatogram should be evaluated and labeled with an evaluation such as identified or unidentified or other similar verbiage unless recorded on the Examination Worksheet.

10.9.11. A positive identification or indication recorded refers to the drug reference material used in the comparison.

10.9.12. Results of GC-IR data comparisons shall be recorded on the Examination Worksheet. The reasons for additional sample and blank runs shall be noted in the case file.

10.9.13. The source and lot number of the reference material(s) used for identification shall be documented in the case file.

10.9.14. Each reference material in the user generated library shall be labeled with the name of the material, the source and lot number of the reference material.

10.9.15. All reference material spectra, and/or user generated library spectra, shall be maintained electronically on the instrument that generated it, in addition to being stored and/or backed up on an external hard drive.

10.10. Interpretation of Results:

10.10.1. Spectra shall be well resolved and of a sufficient intensity to permit identification.

10.10.2. Identifications shall be made by direct comparison to a known reference material of the substance being analyzed, and/or an entry from a user-generated library, generated on the same instrument.

10.10.3. Spectral comparison shall be accomplished by evaluating the overall appearance of the sample spectrum and position of major peaks as it compares with a known reference material.

10.10.4. Literature Matches: In the event that the Forensic Services Division does not possess a known reference material or that a reference material is commercially unavailable, a recognized literature reference may suffice as supporting data for indications. The name of the literature source shall be included in the case file.

10.10.5. In the absence of published literature, spectral data from another accredited laboratory may be used as supporting data for indications of identity.

10.10.6. Verification of reference material spectra. If published spectra are not available, spectral data from two other accredited laboratories may be used as verification of reference material spectra, or another method of verification of the reference material may be used in combination with spectral data from one independent accredited lab.

10.11. Report Writing: N/A

10.12. References:

10.12.1. DiscovIR Operating Manual, Spectra Analysis, Rev B., June 2011
10.12.2. Gas-Chromatography-Infrared Spectroscopy Validation, (Indiana State Police), Roskowski, Newton and Yovanovich, 2013.
10.12.3. GC-IR Operators Instructions, (Indiana State Police) Yovanovich, 2013

11. Polarimetry

11.1. Scope: Polarimetry is used to determine the optical isomer of optically active compounds. Optical activity of submitted samples is measured by passing plane polarized light through a solution containing the sample. There are some substances that have only one optical isomer or racemic mixture that is controlled, whereas the remaining isomer is not. This Test Method is intended to provide instruction for the proper use and interpretation of Polarimetry data.

11.2. Precautions/Limitations: The magnitude of rotation is dependent on several factors:

11.2.1. The temperature of the solution. Experimental values will vary due to inability to maintain temperatures specified in literature.

11.2.2. The concentration of the solution will affect the magnitude of the rotation. Sample and reference materials should be compared at similar concentrations.

11.2.3. Wavelength of the light used in the analysis.

11.2.4. The path length (of the cell) the light travels through the sample.

11.2.5. It is essential to have optically pure reference materials and optically purified unknown samples. Mixtures of optically active substances will lead to incorrect results. It may be necessary to extract the sample.

11.2.6. Nature of the solvent is important. This information is specified in literature.

11.2.7. The properties of the compound being subjected to analysis. The correct salt or free base form is necessary for polarimetry analysis and comparison with literature values. Mixtures with optically inactive substances do not interfere with polarimetry analysis.

11.3. Related Information:

11.3.1. Appendix 1 – Forms and Worksheets
11.3.2. Appendix 2 – Abbreviations
11.3.3. Appendix 3 – Definitions
11.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
11.3.5. Other Test Methods
11.3.5.1. General Drug Analysis
11.3.5.2. Reference Materials

11.4. Instruments: Polarimeter

11.5. Reagents/Materials:

11.5.1. Dextropropoxyphene Reference Material
11.5.2. Dextromethorphan Reference Material
11.5.3. Other reference materials, as procedures are validated.
11.5.4. Chloroform ($CHCl_3$)
11.5.5. Distilled or deionized water
11.5.6. 10ml volumetric flask
11.5.7. Pipettes
11.5.8. Polarimetry cell

11.6. Hazards/Safety:

11.6.1. Chemical exposure to $CHCl_3$, Dextropropoxyphene, and other drugs.

11.7. Reference Materials/Controls/Calibration Checks:

11.7.1. The dextropropoxyphene (base) performance check solution shall be made according to the following specifications: 0.6 gram Dextropropoxyphene base in 100 ml of chloroform.

11.7.2. The performance of the polarimeter is verified on the day of analysis using dextropropoxyphene (base).

11.7.3. A 1 dm cell and sodium lamp shall be used. The calculated rotation of dextropropoxyphene would be ± 0.404.

11.7.4. Observed rotation shall be ± 0.1 from the calculated rotation.

11.7.5. If the observed rotation of the performance check solution is outside the acceptable limits, it shall be discarded. The solution shall be re-made and verified.

11.7.6. A solvent blank shall be run before and after each reference material. This provides verification that the polarimetry cell and solvent are not contaminated.

11.7.6.1. If the solvent blank is not satisfactory, the cell shall be cleaned and the blank re-run. If the cell cannot be cleaned, it shall be replaced.

11.7.6.2. Maintenance: The polarimeter has no routine maintenance. In the event of a source failure or malfunction, it shall be replaced. If the instrument repeatedly fails its performance checks, it shall be taken out of service and repaired.

11.8. Procedures/Instructions:

11.8.1. Polarimetry measurements shall be determined for the optically pure drug reference material (dextro, levo, or both) and the purified unknown drug.

11.8.2. A solvent blank shall be run before and after each sample or reference material. This provides verification that the polarimetry cell and solvent are not contaminated.

11.8.3. Run the performance check solution.

11.8.4. Dissolve extracted sample in $CHCl_3$, or suitable solvent.

11.8.5. Place sample solution in the polarimetry cell and obtain sample rotation value.

Absolute rotation and specific operating conditions for optical isomer determination of optically active drugs are available in references such as The Merck Index.

11.9. Records:

11.9.1. Maintenance: Each polarimeter instrument shall have a maintenance log.

11.9.2. The performance checks, including observed rotation of the reference material and solvent blank, shall be documented in the instrument maintenance log. The source and lot number of the reference material used and date shall be noted.

11.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

11.9.4. The observed degree of rotation for the reference material, unknown, and a solvent blank, as well as a conclusion as to dextro, levo or racemic isomer form for the unknown drug shall be recorded in the notes.

11.10. Interpretations of Results:

11.10.1. A determination for optical isomeric form (dextro or levo) shall be based on a positive or negative rotation of plane polarized light by the unknown sample.

Rotation in the positive direction (+) identifies the dextrorotatory isomer.

Rotation in the negative direction (-) identifies the levorotatory isomer.

No rotation indicates an optically inactive compound or a racemic mixture.

11.10.2. The solvent blank should show no rotation of plane polarized light.

11.11. Report Writing:

11.11.1. If the observed rotation is determined to be the dextrorotatory isomer, it shall be reported as the dextro or d- isomer.

11.11.2. If the levorotatory isomer is identified, it shall be reported as the levo or l- isomer.

11.11.3. If the optical isomer has not been determined, the report shall reflect the drug name without reference to its isomeric form (e.g. Propoxyphene or Methorphan) and "the specific isomer was not determined" statement shall be included in the report. If the difference in isomeric form (dextro- vs levo-) results in a change of control status, the control status shall be omitted from the report.

11.12. References:

11.12.1. Merck Index

11.12.2. Drug Unit Training Manual

11.12.3. Forensic Services Division Quality Assurance Manual

12. Mixed Melting Point Determination

12.1. Scope: Melting Point Determination is a SWGDRUG Category C Test and another method of optical isomer determination. This method is capable of determining the isomeric form of a wide range of compounds.

12.2. Precautions/Limitations:

12.2.1. As of 2025, Melting Point analysis is no longer in the Laboratory's scope.

12.3. Report Writing:

12.3.1. If the optical isomer has not been determined, the report shall reflect the drug name without reference to its isomeric form (e.g. Propoxyphene or Methorphan) or its control status, and "the specific isomer was not determined" statement shall be included in the report. If the difference in isomeric form (dextro- vs levo-) results in a change of control status, the control status shall be omitted from the report.

13. Separation and Extraction Procedures

13.1. Scope: Most drugs are often complex mixtures of substances and contain large amounts of diluents and/or adulterants. These additives can interfere with analysis and it is frequently necessary to separate them so that the drug(s) of interest can be identified by analytical methods. A variety of separation or purification procedures can be used to purify drugs including, but not limited to: liquid-liquid extractions, preparative thin layer chromatography, Alternate Non-Aqueous Organic Ratio (ANOR) extractions, and solvent dry extractions. This Test Method is not an all-inclusive list of the acceptable extractions, but rather a guide for such procedures.

13.2. Precautions/Limitations:

13.2.1. Extraction procedures require a sufficient sample size to perform the test. This may result in a significant loss of sample.

13.2.2. Clean glassware shall be used. Dirty glassware can be a source of contamination.

13.2.3. Extraction procedures may convert the sample form of the analyte to its free base or acid, or cause sample decomposition.

13.2.4. Some extracted drugs are volatile and will evaporate unless converted to a stable form.

13.2.5. Some drug preparations (e.g., Pregabalin) require a physical extraction along with chemical extraction. The Pregabalin capsule shall be emptied and the crystals shall be physically separated from the included powder. The crystals shall then be washed with acetone, allowed to dry, and ran on FTIR for analysis. Multiple washes with acetone may be necessary for confirmation. If after multiple washes confirmation on FTIR is not possible, then GC/MS may be used for confirmation (the resulting GC/MS will be 4-isobutyl-2-pyrrolidinone) .

13.3. Related Information:

13.3.1. Appendix 1 – Forms and Worksheets
13.3.2. Appendix 2 – Abbreviations
13.3.3. Appendix 3 – Definitions
13.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
13.3.5. Other Test Methods

13.4. Instruments: Centrifuge and/or vortex, if necessary.

13.5. Reagents/Materials:

13.5.1. Organic Solvents: $CHCl_3$, Petroleum Ether, Methanol, Hexane
13.5.2. Acids: HCl, H_2SO_4
13.5.3. Bases: NaOH, NH_4OH, Sodium Bicarbonate

13.5.4. Filter Paper
13.5.5. Pipettes
13.5.6. Separatory funnels
13.5.7. Beakers
13.5.8. Culture Tubes
13.5.9. Prep Thin Layer Supplies (See TLC method)

13.6. Hazards/Safety:

13.6.1. Inhalation Hazards
13.6.2. Exposure Hazards
13.6.3. Sharps Hazard

13.7. Reference Materials/Controls/Calibration Checks: When verifying an extraction procedure, the procedure shall be verified by using a known reference material or preparation to demonstrate the performance of the extraction.

13.8. Procedures/Instructions:

13.8.1. The selection of a purification or separation procedure shall be based upon the components of the sample.

13.8.2. Recommended extraction procedures are listed in each drug Test Method.

13.8.3. For compounds not individually listed, extraction and solubility information may be found in references, such as, Clarke's Isolation and Identification of Drugs, Clarke's Analysis of Drugs and Poisons, The Merck Index, and the Physician's Desk Reference. The manufacturer may also supply this information.

13.8.4. Samples shall be dissolved in and extracted with the appropriate solvents.

13.8.5. It may be necessary to add HCl fumes to convert volatile samples to a more stable form.

13.8.6. Fume hoods shall be used when evaporating solvent extracts.

13.8.7. Glassware used to collect extracted samples should be covered while in storage to protect from loss or contamination. Parafilm is sufficient for this purpose.

13.9. Records:

13.9.1. A description of the extraction or purification procedure shall be recorded in the case notes in sufficient detail to be understood and replicated by a trained Forensic Scientist.

13.9.2. If a sample preparation or extraction procedure is detailed in the relevant Test Method, it is permissible to cite that portion of the Test Method that contains the details of the extraction. Optional steps shall be noted if they were or were not used in the procedure. (e.g., mushroom sample preparation and extraction)

13.9.3. The tests that have been run using an extracted sample shall be identified in the case notes.

13.10. Interpretations of Results: N/A

13.11. Report Writing: N/A

13.12. References:

13.12.1. Drug Unit Resource Manual(s)

13.12.2. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure, Mary A. Rhodes, Criminalist, Birmingham, Alabama, April 1982.

13.12.3. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure, Allen R. Adair, B.S., F. Taylor Noggle, Jr., B.S., Martha S. Odom, B.S., Mary A. Rhodes, B.S., Microgram, Vol. XVI, No.1, 1 January 1983.

13.12.4. Extraction Procedures, William S. Bowles, Memo to J. Forbes, March 1981

13.12.5. Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

13.12.6. Clarke's Analysis of Drugs and Poisons, 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

13.12.7. The Merck Index, 8th Edition; Merck and Company, Inc. 1968

13.12.8. Occurrence of Excipient Materials in Illicit Tablet Manufacture, Rhodes, and Thornton (University of California – Berkeley), Microgram, Vol. XII, No. 5, (May 1979).

14. Marijuana Examination

14.1. Scope: Suspected Marijuana and/or Marijuana preparations are examined visually, macroscopically, and microscopically noting morphological characteristics. Additional tests such as thin layer chromatography, gas chromatography, and mass spectrometry are available to be used to identify the components of plant material, waxes, oils, food products, and residues.

14.2. Precautions/Limitations:

14.2.1. Immature plants may not have enough developed plant features to permit microscopic identification. Additionally, they may not be mature enough to produce enough cannabinoids to detect.

14.2.2. Burnt plant material may not have enough identifiable plant features remaining for microscopic identification.

14.2.3. Finely pulverized material, compressed, and/or extracted plant material preparations (i.e., baked goods, residues, etc.) pose difficulties in identifying botanical characteristics due to the small size of the material, and the matrices involved.

14.2.4. Cannabis Oil is very concentrated and should be diluted for analysis.

14.2.5. Wet plant material shall not be accepted for analysis. It is the submitting officer's responsibility to dry plant material. Mold not only presents a health and fire hazard, it also obscures the plant features. Long term exposure to moisture contributes to severe degradation of the plant material.

14.2.6. Mature stalks of Marijuana are exempt from the Indiana Criminal Code. If a sample includes plant stalks, and the weight of the plant material is needed, it shall be stripped from the stalks. It is the responsibility of the submitting officer to strip the plant material from the stalks.

14.2.7. The acid form of cannabinoids converts to cannabinoids in the injection port of the GC.

14.2.8. Delta-9 THC and its isomers can be very close in retention time and the spectra shall be carefully evaluated in order to determine which isomer is present.

14.3. Related Information:

14.3.1. Appendix 1 – Forms and Worksheets
14.3.2. Appendix 2 – Abbreviations
14.3.3. Appendix 3 – Definitions
14.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
14.3.5. Appendix 5 - Semi-Quantitative Analysis
14.3.6. Other Test Methods

14.3.6.1. General Drug Identification
14.3.6.2. Weighing Determinations
14.3.6.3. Evidence Handling
14.3.6.4. Sampling
14.3.6.5. Thin Layer Chromatography
14.3.6.6. Gas Chromatography Mass Spectrometry
14.3.6.7. Gas Chromatography-Infrared Spectroscopy

14.4. Instruments/Equipment:

14.4.1. Stereomicroscope
14.4.2. GC/MS
14.4.3. GC-IR
14.4.4. Calibrated Mechanical Pipettes
14.4.5. Calibrated Balance
14.4.6. Calibrated Repeater Pipettes
14.4.7. Volumetric Flasks

14.5. Reagents/Materials:

14.5.1. TLC supplies (See Thin Layer Chromatography Test Method)
14.5.2. Culture tubes
14.5.3. Tribenzylamine Internal Standard Solution
14.5.4. Delta-9 THC Reference Material
14.5.5. Tetrahydrocannabinolic Acid Reference Material
14.5.6. Delta-8 THC Reference Material

14.6. Hazards/Safety:

14.6.1. Moldy plant material presents both health and fire hazards. The Aspergillus fungus can cause a condition known as Farmer's Lung, which can be fatal. Wet and moldy plant material generates its own heat and can start a fire if left unattended.

14.6.2. Insects and bugs are commonly found in plant material evidence. Improper packaging can lead to an infestation of the evidence storage facilities.

14.6.3. Chemical Exposures/Inhalation Hazards including potential carcinogens.

14.7. Reference Materials/Controls/Calibration Checks:

14.7.1. Thin Layer Chromatography (TLC): Appropriate reference materials shall be run with the blanks and samples.

14.7.2. For semi-quantitative analysis, a positive control, negative control, and a decision point shall be run. CBD reference material shall be run at the beginning and end of each semi-quantitation batch (See Appendix 5).

14.7.2.1. The decision point may be used for 48 hours from the time it is initially injected in the GC/MS. After 48 hours a new decision point shall be prepared.

14.8. Procedures/Instructions:

14.8.1. Items of plant material should be administratively withdrawn if no additional weight thresholds will be met by the analysis of these items. The unexamined evidence shall be visually examined. It shall be noted if the visual exam is consistent with Cannabis. If the plant material can be seen through the outermost packaging, the evidence does not need to be opened and a gross weight including outermost packaging should be taken. If Marijuana or a synthetic drug is not confirmed in the analyzed item, the unexamined evidence shall be analyzed.

14.8.2. If Marijuana is confirmed in a case, items that appear to contain THC (e.g., edibles, vapes, etc.) for that same person of interest may be withdrawn per PEB-01. If an item of plant material is examined and is found to contain delta-9 THC, but does not qualify for semi-quant due to weight, then any items that appear to contain THC for that same person of interest may be withdrawn per PEB-01. If there is another person of interest associated with the suspected THC item or it is the probable cause item, that item shall be analyzed.

14.8.3. Microscopic Identification: Suspected Marijuana shall be examined using a stereomicroscope with approximately 7x to 30x powers of magnification for the presence of botanical characteristics such as leaf fragments with both cystolithic and fine hairs, veins on leaves, seeds, multi-cellular hairs, stems, stalk, and flowering tops that are consistent with Marijuana. Microscopic examination shall be performed prior to Thin Layer Chromatography (TLC) and if applicable, Semi-Quantitation.

14.8.3.1. Finely ground Cannabis samples should be examined microscopically for the presence of botanical characteristics such as cystolithic hairs, simple hairs, etc. The presence of detached hairs should be noted for finely ground Cannabis samples, if present.

14.8.4. Thin Layer Chromatography:

14.8.4.1. TLC is sufficient to resolve the three major cannabinoids (delta-9-Tetrahydrocannabinol (THC), Cannabinol, and Cannabidiol) using the following system. (See TLC Test Method).

14.8.4.1.1. Unknown samples and cannabinoid reference materials are routinely dissolved in Methanol, Petroleum Ether, Chloroform, or Acetonitrile.

14.8.4.1.2. Plates should be sprayed with Diethylamine to improve separation.

14.8.4.1.3. Toluene shall be used as the development solvent.

14.8.4.1.4. Fast Blue BB shall be used as the visualizing spray.

14.8.4.1.5. If an isomer of THC was indicated or confirmed on GC/MS but no delta-9 THC is indicated or confirmed on GC/MS then, TLC shall be attempted with the THC isomer that was seen on GC/MS, if the reference material is available.

14.8.4.2. TLC is sufficient to resolve delta-9-Tetrahydrocannabinol, delta-8-Tetrahydrocannabinol, and THCA using the following system.

14.8.4.2.1. Plates shall not be sprayed with Diethylamine.

14.8.4.2.2. Hexane: Acetone (43:7) shall be used as the development solvent.

14.8.4.2.3. High heat shall be applied to plate after development to visualize THCA.

14.8.4.2.4. Fast Blue BB shall be used as the visualizing spray.

14.8.4.2.5. This TLC system shall not be used as the only test to differentiate between THC and THCA.

14.8.5. FTIR:

14.8.5.1. This technique may be used to analyze items in a powder form that contain cannabinoids.

14.8.5.2. FTIR can be used as a confirmatory test for cannabinoids.

14.8.5.3. FTIR can be used as a supporting test if THC can be identified or indicated, then this result in combination with a positive THC spectrum, GC/MS or GC-IR, can be used to confirm the presence of THC.

14.8.5.4. FTIR can be used as a supporting test if THCA can be identified or indicated, then this result in combination with a positive THC spectrum, GC/MS or GC-IR, can be used to confirm the presence of THCA.

Gas Chromatography/Mass Spectrometry: If a sample does not qualify for semi-quantitative analysis, GC/MS shall be performed to identify Cannabinoids present in the sample. If confirming an isomer of Tetrahydrocannabinol, the sample shall be derivatized. To confirm the Tetrahydrocannabinol, a positive derivatized sample and a positive additional test are required. If a Tetrahydrocannabinol is mixed with another confirmed controlled substance (e.g. Methamphetamine), it is not necessary to distinguish between Tetrahydrocannabinol and its acid form.

14.8.5.5. Semi-Quantitative analysis should be performed if the sample meets the following criteria:

14.8.5.6. The net weight of the item is greater than or equal to 0.20 gram.

14.8.5.7. Cystolithic and fine hairs are identified during microscopic examination.

14.8.6. Gas Chromatography-Infrared Spectroscopy: This technique may be used to analyze plant materials for synthetic and other drugs, as necessary, or to distinguish cannabinoid isomers.

14.8.7. THC Derivatization Procedure:

When the term “derivatized” or its abbreviation “der” is used in case notes, the term is referring to the THC derivatization procedure listed below.

1. Extract sample with a suitable solvent (do not use MeOH)
2. Add 10 drops 99%BSTFA w/1%TMCS to 10 drops of the dissolved or extracted sample in suitable solvent.
3. Cork and parafilm test tube, heat to 60 °C for 10 minutes.
4. Inject onto GC/MS (or GC-IR).

14.9. Records:

14.9.1. All weights used to meet or exceed weight limits of a particular criminal charge shall be recorded as net weight.

14.9.2. Observations of microscopic botanical characteristics shall be recorded on the analysis notes.

14.9.3. Thin Layer Chromatography: Observations of the solvent blank and spots in the unknown sample shall be recorded in the analysis notes. Weak or intense reactions should be particularly noted.

14.9.4. Gas Chromatography/Mass Spectrometry: See GC/MS Test Method.

14.9.5. If Semi-Quantitative Analysis is performed:

14.9.5.1. The negative control blank and the negative control shall be evaluated and printed. The negative control should have one peak matching Tribenzylamine (TBA) and the TIC and spectrum for TBA shall be printed. The negative control data shall not have any controlled substance peaks or peaks for any non-controlled substance over 5% by height of the abundance of the TBA peak.

14.9.5.1.1. If the negative control is run multiple times on the same instrument, the reason for the rerun/rejection and the date of the rerun/rejection shall be documented in the case notes.

14.9.5.2. The decision point blank and the decision point shall be evaluated and printed. The decision point shall only have a peak for TBA and delta-9-THC. The TICs and spectra for TBA and delta-9-THC shall be printed. The quantitation report generated shall be printed.

14.9.5.2.1. If the decision point is run multiple times on the same instrument, the reason for the rerun/rejection shall be documented in the decision point log. If the decision point is used for casework, the reason shall also be documented in all affected case notes.

14.9.5.3. For each sample, the sample blank and sample shall be evaluated and printed. The quantitation report generated by ChemStation shall be printed.

14.9.5.4. The positive control blank and positive control shall be evaluated, printed, and retained in the Drug Unit DP Preparation and Verification Log. The quantitation report generated by ChemStation shall be printed and retained in the Drug Unit DP Preparation and Verification Log.

14.9.5.4.1. If the positive control is run multiple times on the same instrument, the reason for the rerun/rejection and the date of the rerun/rejection shall be documented in the Drug Unit DP Preparation and Verification Log.

14.9.5.5. The forensic scientist shall ensure that the correct peak is being integrated and that the entire peak is integrated.

14.10. Interpretations of Results:

14.10.1. Microscopic examination: A positive microscopic examination is required to identify the plant material as Marijuana. A positive microscopic examination shall be when cystolithic hair(s) and fine hair(s) are observed in the sample. Cystolithic hairs and fine hairs should be observed on opposite sides of the same leaf, or leaf fragment. The observations of additional features are supportive.

14.10.2. Thin Layer Chromatography: Positive indication of the unknown sample shall be based on color and location of spots on the plate relative to the cannabinoid reference material(s) and indicate the cannabinoid(s) present. Additional testing shall be required to confirm the presence of other cannabinoids.

14.10.3. Gas Chromatography/Mass Spectrometry: (See GC/MS Test Method).

14.10.4. Gas Chromatography-Infrared Spectroscopy: (See GC-IR Test Method).

14.10.5. A sample analyzed using Semi-Quantitation is identified as Marijuana if it meets the following criteria:

14.10.5.1. Positive microscopic examination

14.10.5.2. Positive delta-9-THC or delta-9-THCA on TLC

14.10.5.3. MS confirmation of delta-9 THC

14.10.5.4. A concentration ratio ≥1 (See Appendix 5)

14.10.6. A sample analyzed using Semi-Quantitation is inconclusive if it meets the following criteria:

14.10.6.1. Positive microscopic examination

14.10.6.2. MS confirmation or indication of cannabinoid(s)

14.10.6.3. A concentration ratio <1 (See Appendix 5)

14.11. Report Writing:

14.11.1. Suspected marijuana items with a total weight greater than ten (10) pounds shall be recorded in both grams and converted to pounds in the notes and reported as both grams and pounds (regardless of the semi-quantitation result).

14.11.2. Cannabinoids shall not have a control status listed on the Certificate of Analysis.

14.11.3. If the sample analyzed using Semi-Quantitation is positive for Marijuana, it shall be reported as follows:

14.11.3.1. Item XXX was found to contain Marijuana, a controlled substance, as determined by comparison with a 1% delta-9-Tetrahydrocannabinol (THC) reference material.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.

14.11.4. If the sample analyzed using Semi-Quantitation is inconclusive and the net weight was ≥0.50 gram, it shall be reported as follows:

14.11.4.1. Item XXX was found to contain/indicated the presence of Tetrahydrocannabinol (THC)/ Cannabidiol (CBD)/ Cannabinol (CBN). When compared to a 1% delta-9-Tetrahydrocannabinol (THC) reference material, Hemp/Marijuana could not be determined. If quantitative analysis is necessary, please contact the laboratory.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.

Or

Similar verbiage for cannabinoids identified

14.11.5. If the sample analyzed using Semi-Quantitation is inconclusive and the net weight was <0.50gram, it shall be reported as follows:

14.11.5.1. Item XXX was found to contain/indicated the presence of Tetrahydrocannabinol (THC)/ Cannabidiol (CBD)/ Cannabinol (CBN). When compared to a 1% delta-9-Tetrahydrocannabinol (THC) reference material, Hemp/Marijuana could not be determined. However, the sample size is insufficient for quantitative analysis.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.

Or

Similar verbiage for cannabinoids identified

14.11.6. Regardless of the weight of the sample, if it was analyzed using Semi-Quantitation and THC cannot be indicated in the sample, it shall be reported as follows:

14.11.6.1. Item XXX was found to contain/indicated the presence of Cannabidiol (CBD)/ Cannabinol (CBN). Hemp/Marijuana could not be determined.

14.11.7. Plant material that weighs under 0.20 gram shall be visually examined either microscopically or macroscopically to ensure the item is consistent with Cannabis.

14.11.7.1. If the plant material appears to be consistent with synthetic cannabinoids or other plant material containing potential controlled substances (Salvia, Khat, Mushrooms), the analyst should continue with analysis.

14.11.7.2. If the plant material has a positive microscopic exam for Cannabis/Marijuana (cystolithic hairs and fine hairs) or has a general appearance microscopically that is consistent with Cannabis (e.g. – detached hairs, burnt hairs, stems) or is too burnt to see any microscopic characteristics (ash material), then the item should be withdrawn. The burnt characteristics or if the appearance is not consistent with Cannabis shall be noted in the case notes. This shall be reported as follows:

14.11.7.2.1. Item XXX was visually examined. The net weight of item XXX was XXX gram. This item did not qualify for determination of Hemp/Marijuana. No confirmatory testing was performed.

14.11.8. If an item contains Cannabis and other substances (e.g powder, crystal, wax, tobacco) and can be separated from each other, analyze the other substance. If the two substances can be separated, the items should be sub-itemized and weighed separately.

14.11.8.1. If the other substance is delta-9-THC or delta-9-THCA, semi-quant analysis shall not be performed on the Cannabis plant material. Analyze cannabinoids using GC/MS and TLC.

14.11.8.2. If the other substance is not delta-9-THC or delta-9-THCA, conduct semi-quant analysis on the Cannabis plant material. Report out the other substance, if necessary.

14.11.9. If the Cannabis plant material cannot be separated from the other substance, a representative sample shall be taken. GC/MS shall be performed on the representative sample. Analyze cannabinoids using GC/MS and TLC.

14.11.9.1. Item XXX was found to contain/indicated the presence of Tetrahydrocannabinol (THC)/Cannabidiol (CBD)/Cannabinol (CBN). This item did not qualify for determination of Hemp/Marijuana.

14.11.10. If a cigarette is coated in powder, crystal, or other plant material and the cigarette wrapper containing the coating can be separated from the plant material, analyze the coating substance. If the two substances can be separated, the items should be sub-itemized and weighed separately.

14.11.10.1. If the coating substance is delta-9-THC or delta-9-THCA, semi-quant analysis shall not be performed on the Cannabis plant material. Analyze cannabinoids using GC/MS and TLC.

14.11.10.2. If the coating substance is not delta-9-THC or delta-9-THCA, conduct semi-quant analysis on the Cannabis plant material. The other substance should be reported.

14.11.11. If a cigarette is coated in powder, crystal, or other plant material and the cigarette wrapper containing the coating cannot be separated from the plant material, a representative sample shall be taken. GC/MS shall be performed on the representative sample. Analyze cannabinoids using GC/MS and TLC.

14.11.12. If there was not a positive microscopic examination, but cannabinoid(s) were identified or indicated, the cannabinoids shall be reported without a control status, and the results shall be reported as follows:

14.11.12.1. Item XXX was found to contain/indicated the presence of Tetrahydrocannabinol (THC)/ Cannabidiol (CBD)/ Cannabinol (CBN). This item did not qualify for the determination of Hemp/Marijuana.

14.11.12.2. Items reported as delta-9-Tetrahydrocannabinol (delta-9-THC) or delta-8-Tetrahydrocannabinol (delta-8-THC) shall be derivatized to differentiate between the cannabinoid and its acidic form, THCA.

14.11.12.3. In some cases, items of plant material can be administratively withdrawn because no additional weight thresholds can be met. Weigh the item but

do not report the weight. If the item has been visually examined through the packaging, take a gross weight of the item.

14.11.12.3.1. Item XXX was visually examined. The item was withdrawn per Indiana State Police Physical Evidence Bulletin 01. If analysis is necessary, contact the laboratory.

14.11.13. Oils, edibles, waxes, powders, and plant material samples that do not qualify for semi-quantitation shall be reported as follows. For plant material samples, it may be necessary to add additional statements to the report (See 14.11.9). Items reported as delta-9-Tetrahydrocannabinol (delta-9-THC) or delta-8-Tetrahydrocannabinol (delta-8-THC) shall be derivatized to differentiate between the cannabinoid and its acidic form, THCA, unless confirmed using FTIR.

14.11.13.1. If TLC is run with Δ9-THC, CBN, and CBD reference materials, a spot for Δ9-THC is observed for TLC, and GC/MS that has been derivatized is positive for the derivatized form of Δ9-THC, the results shall be reported as follows:

14.11.13.1.1. Item XXX was found to contain delta-9-Tetrahydrocannabinol (delta-9-THC).

14.11.13.2. If TLC is run with Δ9-THCA, CBN, and CBD reference materials, a spot for Δ9-THCA is observed for TLC, and GC/MS that has been derivatized is positive for the derivatized form of Δ9-THCA, the results shall be reported as follows:

14.11.13.2.1. Item XXX was found to contain/indicates delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA).

14.11.13.3. If TLC is run with Δ9-THC, CBN, and CBD reference materials, a spot for Δ9-THC is observed for TLC, and GC/MS that has been derivatized is positive for the derivatized form of Δ8-THC, further testing shall be performed as second test for Δ8-THC.

TLC shall be re-run using both Δ9-THC and Δ8-THC reference materials on the plate and observing separation between the two isomers.

If no separation is observed between the Δ9-THC and Δ8-THC, then the item shall be reported as an indication based on the derivatized Mass Spectrum.

If the TLC is positive for Δ8-THC, the results shall be reported as follows:

14.11.13.3.1. Item XXX was found to contain delta-8-Tetrahydrocannabinol (delta-8-THC).

14.11.13.4. If TLC is run with Δ9-THC, CBN, and CBD reference materials, a spot for Δ9-THC is observed for TLC, and GC/MS that has been derivatized is positive for the derivatized form of both Δ8-THC and Δ9-THC, further testing shall be performed as second test for Δ8-THC and Δ9-THC.

TLC shall be re-run using both Δ9-THC and Δ8-THC reference materials on the plate and observing separation between the two isomers.

If no separation is observed between the Δ9-THC and Δ8-THC, then the item shall be reported as an indication based on the derivatized Mass Spectrum.

If separation is observed on TLC and is positive for Δ8-THC and/or Δ9-THC, the isomers that were identified/indicated shall be reported.

14.11.13.4.1. Possible wording options:

Item XXX was found to contain/indicated the presence of delta-8-Tetrahydrocannabinol (delta-8-THC) and delta-9-Tetrahydrocannabinol (delta-9-THC).

Item XXX was found to contain delta-8-Tetrahydrocannabinol (delta-8-THC) and indicated the presence of delta-9-Tetrahydrocannabinol (delta-9-THC).

14.11.14. If only indicating Tetrahydrocannabinol and/or Tetrahydrocannabinolic acid, the following wording shall be used or similar verbiage:

14.11.14.1. Item XXX indicated the presence of delta-9-Tetrahydrocannabinol (delta-9-THC) and/or delta-9-Tetrahydrocannabinolic acid (delta-9-THCA).

14.12. References:

14.12.1. Drug Unit Resource Manual – Marijuana

14.12.2. The Botany and Ecology of Cannabis, Robert Connell Clark

14.12.3. The Botany and Chemistry of Cannabis, Joyce & Curry, Chapters 1,2,6 pages 93-99, 111-115, 120-121

14.12.4. Basic Training Program for Forensic Drug Chemists, Canaff, US Department of Justice Bureau of Narcotics and Dangerous Drugs, May 1972.

14.12.5. Controlled Substance Act, pertaining to Marijuana and Hashish

14.12.6. Indiana Criminal Code, IC 35-48-1-19: Definition of Marijuana

14.12.7. Marijuana Thin Layer Chromatography Systems, Memo to J. Forbes, Huttsell, F. (ISP), February, 1991

14.12.8. Visual Characteristics of Cannabis Sativa (Marijuana) Seeds; Fussel, Thornton, and Whitehurst, Journal of Forensic Identification 59(5), 2009.

15. Synthetic Drugs

15.1. Scope: Synthetic drugs, such as synthetic cannabinoids, substituted cathinones, etc. are generally found on plant materials. Tests such as Thin Layer Chromatography (TLC), Gas Chromatography (GC), Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography-Infrared Spectroscopy (GC-IR) are available to be used to identify the components of these drug-laced plant materials. Gas Chromatography-Infrared Spectroscopy is available through the Indianapolis Regional Laboratory.

15.2. Precautions/Limitations:

15.2.1. Synthetic drugs include several different types of substances. Many positional isomers are possible. Forensic Scientists shall be prepared to acknowledge the existence of other positional isomers, particularly when the specific isomeric form has not been identified.

15.2.2. Some synthetic drugs convert to other drugs in the injection port of the GC (example – 25I-NBOH converts to 2C-I, 25E-NBOH converts to 2C-E, 25B-NBOH converts to 2C-B, 25H-NBOH converts to 2C-H and 25C-NBOH converts to 2C-C in the GC/MS). TLC or IR shall be needed to identify the compound present.

15.2.3. Many are not commercially available and therefore reference materials may not be available.

15.2.4. Finely pulverized material, compressed, and/or extracted plant material preparations (i.e., baked goods, residues, etc.) pose difficulties due to the small size of the material, the matrices involved, and potential for complex mixtures.

15.2.5. Synthetic drugs are found in/on paraphernalia similar to those commonly found with Marijuana evidence.

15.2.6. Synthetic cannabinoids are chemically different than traditional “cannabinoids”.

15.2.7. Items commonly have multiple drugs present. Additional screening may be necessary to detect other components of the samples.

15.2.8. Substances in synthetic drug mixtures may not resolve sufficiently by Thin Layer Chromatography and therefore TLC may not be a good second test for identification.

15.2.9. Generally, the material is not in sufficient quantity or condition for Fourier Transform Infrared Spectroscopy (FTIR) to be possible or practical. If GC-IR is available and practical, it may be used for identification.

15.2.10. GC retention time is generally necessary for identification.

15.3. Related Information:

15.3.1. Appendix 1 – Forms and Worksheets
15.3.2. Appendix 2 – Abbreviations
15.3.3. Appendix 3 – Definitions
15.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
15.3.5. Other Test Methods
- **15.3.5.1.** General Drug Identification
- **15.3.5.2.** Weighing Determinations
- **15.3.5.3.** Evidence Handling
- **15.3.5.4.** Sampling
- **15.3.5.5.** TLC
- **15.3.5.6.** FTIR
- **15.3.5.7.** GC/MS
- **15.3.5.8.** GC-IR

15.4. Instruments:

15.4.1. GC/MS, with retention time
15.4.2. FTIR or GC-IR

15.5. Reagents/Materials:

15.5.1. TLC supplies (See TLC Test Method)
15.5.2. GC/MS Supplies (See GC/MS Test Method)
15.5.3. GC-IR Supplies (See GC-IR Test Method)

15.6. Hazards/Safety:

15.6.1. Like all plant materials, moldy plant material presents both health and fire hazards. Wet and moldy plant material generates its own heat and can start a fire if left unattended.

15.6.2. Chemical exposures/inhalation hazards including potential carcinogens may exist.

15.6.3. Some synthetic cannabinoids are more potent than traditional cannabinoids. No known toxicity studies have been performed on humans.

15.7. Reference Materials/Controls/Calibration Checks:

15.7.1. TLC: Appropriate reference materials shall be run with the blanks and samples. (See TLC Test Method.)

15.7.2. GC/MS: Appropriate reference materials shall be used for comparison. (See GC/MS Test Method.)

15.7.3. FTIR: Appropriate reference materials shall be used for comparison. (See FTIR Test Method)

15.7.4. GC-IR: Appropriate reference materials shall be used for comparison. (See GC-IR Test Method)

15.8. Procedures/Instructions:

15.8.1. Items of plant material can be administratively withdrawn if no additional weight thresholds will be met by the analysis of these items. The unexamined evidence shall be weighed. If the plant material can be seen through the outermost packaging, the evidence does not need to be opened and a gross weight including outermost packaging can be taken.

15.8.2. Microscopic Examination: Suspected synthetic drugs are found on a variety of plant materials. All plant materials should be examined microscopically.

15.8.3. Unknown samples and reference materials are routinely dissolved in methanol or chloroform. Other solvents may be suitable.

15.8.4. Thin Layer Chromatography: TLC may be useful to analyze single component samples. There may not be sufficient resolution using general chromatography systems for mixtures.

General TLC systems may help screen synthetic cannabinoid samples for the presence of other drugs that may not be readily apparent when using the normal GC/MS program

15.8.5. Fourier Transform Infrared Spectroscopy: This method gives the most specific structural information available, if and when the sample is in a sufficient quantity to permit the test.

15.8.6. Gas Chromatography/Mass Spectrometry: Generally, the synthetic cannabinoids elute at higher temperatures. A general high temperature program may be sufficient, however if no peaks are identified, then a general screening temperature program shall be used.

15.8.7. GC Retention Time: Due to the possibility of multiple positional isomers, GC retention time comparison is necessary when identifying controlled synthetic cannabinoids and their respective isomeric forms. Additionally, GC retention time may be necessary as a second test for identification of the components in a mixture.

15.8.8. Gas Chromatography-Infrared Spectroscopy: This is a complementary technique that can distinguish between most positional isomers. It is particularly useful with mixtures or when GC retention time comparisons are not sufficient to determine a specific form or isomer of a drug.

15.9. Records:

15.9.1. All weights used to meet or exceed weight limits of a particular criminal charge shall be recorded as net weight.

15.9.2. Thin Layer Chromatography: See TLC Test Method.

15.9.3. Gas Chromatography/Mass Spectrometry: See GC/MS Test Method.

15.9.4. Gas Chromatography Retention Time: See GC/MS Test Method.

15.9.5. Gas Chromatography-Infrared Spectroscopy: See GC-IR Test Method.

15.10. Interpretations of Results:

15.10.1. Microscopic examination: If used, see Marijuana Test Method. There are no helpful botanical features associated with synthetic cannabinoids or other synthetic drugs.

15.10.2. Thin Layer Chromatography: Positive indication of the substances present in the unknown sample shall be based on color and location of spots on the plate relative to the reference material(s). This is best used for single component samples. Components in mixtures may not be resolved enough to use this method as a second test. TLC may help rule out the presence of drugs other than synthetic drugs.

15.10.3. Fourier Transform Infrared Spectroscopy: See FTIR Test Method.

15.10.4. Gas Chromatography- Mass Spectrometry: Higher temperature programs are generally sufficient for identification; however, some substances may be missed if other screening techniques are not employed (e.g., TLC) to rule out the presence of other drugs. Retention time can be used as a second test for identification when necessary.

15.10.5. Gas Chromatography-Infrared Spectroscopy: (See GC-IR Test Method) Higher temperature programs are necessary for most synthetic drugs. As with GC/MS, substances may be missed if other screening techniques are not employed (e.g., TLC, general GC/MS temperature program) to rule out the presence of other drugs.

15.10.6. If multiple controlled synthetics or potential isomers are present in the item, at a minimum, one shall be confirmed. The remaining substances shall be indicated in the analytical notes or on the data (at a minimum).

15.11. Report Writing:

15.11.1. Weights obtained to meet or exceed charges shall be reported as a net weight.

15.11.2. If the date of seizure is before the effective date that a drug became controlled, the control status shall be omitted, and an additional statement shall be included into the report indicating the date of control.

Example: Item XXX was found to contain X. X was controlled in the State of Indiana as of date. The specific isomer was not determined. If the specific isomer needs to be determined, please contact the laboratory.

Or

Item XXX indicated the presence of X. X was controlled in the State of Indiana as of date.

If there is reasonable doubt as to the control status of a drug, the control status should be omitted. The Forensic Scientist shall discuss this with their immediate Drug Unit Supervisor.

15.11.3. If the date of seizure is before the effective date that a drug became state controlled, but after the date of federal control, the report shall indicate that it is a federally controlled substance. The date of control in the State of Indiana shall also be included on the report. If the date of seizure is after the date it was controlled in the State of Indiana, the date of federal control is not required to be on the Certificate of Analysis.

15.11.4. If the specific isomeric form has not been determined, the following statement shall be included in the results:

“The specific isomer was not determined.”

If the isomeric form has been determined, this statement shall be omitted. The specific isomer can only be determined by GC retention time if the reference materials for potential positional isomers have been run on the instrument, or if IR spectral data has been obtained (e.g., Eutylone).

If the substance is only indicated, then the specific isomer statement shall be omitted.

15.11.5. Substances listed in a section in the Indiana Criminal Code that does not specifically say “including their isomers” (or similar) of the substance shall be reported without a control status or numeric value of the position of the isomer (e.g. MDMB-en-PINACA) if

the isomer has not been determined (for example - “Synthetic drugs” section of the Indiana Criminal Code IC 35-31.5-2-321 or 4-ANPP).

If the substance is only indicated, then the specific isomer statement shall be omitted.

Example: Item XXX was found to contain ANPP. 4-ANPP is a controlled substance. The specific isomer was not determined. If the specific isomer needs to be determined, please contact the laboratory.

15.11.6. Substances controlled by structure should be reported by name and a reference to the grouping to which it belongs in the Criminal Code. Isomer determination is not necessary. If the isomer is determined, the statement “or an isomer thereof” does not need to be included.

Example 1:

Item XXX was found to contain α-Pyrrolidinoisohexanophenone (α-PiHP) (or an isomer thereof), a controlled substance structurally derived from 2-aminopropan-1-one.

Or

Item XXX was found to contain α-Pyrrolidinoisohexanophenone (α-PiHP) (or an isomer thereof), a controlled substance. α-Pyrrolidinoisohexanophenone (α-PiHP) and its isomers are structurally derived from 2-aminopropan-1-one.

Example 2:

Item XXX was found to contain Fluorofentanyl, a controlled substance structurally related to Fentanyl, a controlled substance.

15.11.7. If a synthetic drug is substantially similar to a controlled substance, the report shall state the name of the controlled substance that the synthetic drug is substantially similar to. Substances that are only substantially similar to a controlled substance do not need to be confirmed and weight thresholds do not need to be met unless re-submitted by the agency.

Example:

Item xxx was found to contain/indicated the presence of ADB-INACA, which has a substantially similar structure to ADB-PINACA, a controlled substance.

15.11.8. If a substance is run on FTIR or GC-IR, and the drug can be confirmed (or a good indication on either IR instrument), it can be called controlled and the isomer sentences can be omitted.

15.11.9. Substances shall not be reported as “analogs” unless authorized by the Forensic Scientist’s Drug Unit Supervisor.

15.11.10. Indications: If a synthetic cannabinoid/drug is indicated, but not identified, the structure based wording and isomer statements may be omitted from the results.

15.11.11. See General Drug Identification.

15.12. References:

15.12.1. Indiana Criminal Code, IC 35-48-2-4

15.12.2. Indiana Criminal Code, IC 35-31.5-2-321

16. Cocaine

16.1. Scope: Cocaine is a naturally occurring alkaloid that is extracted from the Erythroxylum coca plant.

16.2. Precautions/Limitations:

16.2.1. Cis and trans cinnamoyl cocaines are frequently present in cocaine samples. These are natural products of the coca plant.

16.2.2. Ecgonine, methylecgonine and benzoylecgonine may be present in sample as a result of the purification process, or may be produced by the high temperatures in the GC/MS.

16.2.3. Illicit samples may contain a large variety of substances such as Procaine, Lidocaine, Benzocaine, PTHIT, and/or other drugs.

16.2.4. Cocaine mixtures containing alkaline substances, such as sodium bicarbonate, may convert the form of the Cocaine when water or aqueous solutions are added.

16.2.5. Salt or base form determination may be necessary for Federal charges and/or sentencing requirements.

16.2.6. The condition of the sample may prohibit salt form determination.

16.2.7. Cocaine samples are soluble in methanol and chloroform. Chloroform may be preferable due to possible degradation.

16.3. Related Information:

16.3.1. Appendix 1 – Forms and Worksheets
16.3.2. Appendix 2 – Abbreviations
16.3.3. Appendix 3 – Definitions
16.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
16.3.5. Other Test Methods
- **16.3.5.1.** General Drug Identification
- **16.3.5.2.** Color (Spot) Tests
- **16.3.5.3.** UV
- **16.3.5.4.** TLC
- **16.3.5.5.** FTIR
- **16.3.5.6.** GC/MS
- **16.3.5.7.** GC-IR
- **16.3.5.8.** Separations and Extractions

16.4. Instruments:

16.4.1. UV

16.4.2. FTIR
16.4.3. GC/MS
16.4.4. GC-IR

16.5. Reagents/Materials:

16.5.1. See Separations and Extraction Test Method
16.5.2. See Color (Spot) Test Reagent Preparation Guide

16.6. Hazards/Safety:

16.6.1. Exposure: numbness of fingers or areas that have been in direct contact with the drug.

16.6.2. Chemical Exposure hazards

16.6.3. See SDS for Cocaine and related substances.

16.7. Reference Materials/Controls/Calibration Checks:

16.7.1. Appropriate Reference Materials for Cocaine, related materials, excipients, and diluents.

16.8. Procedures/Instructions:

16.8.1. See General Drug Identification Test Method.

16.8.2. Color (Spot) Tests: The recommended color tests for Cocaine are the Cobalt Thiocyanate or Scott Tests.

16.8.3. UV: Generally, Cocaine type samples are analyzed in 0.5 N H_2SO_4

16.8.4. TLC: Recommended TLC Recommended Systems

16.8.4.1. General TLC solvent systems:
MeOH :NH_4OH (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)

16.8.5. Extraction: Cocaine is very soluble in CHCl3, Pet. Ether and Methanol. It has a low solubility in water in the base form. Generally, Cocaine is extracted with organic solvents from aqueous alkaline solutions.

The following are common extractions used for purifying street samples containing Cocaine:

16.8.5.1. Cocaine Base extraction: Pet. Ether dry extract, or dissolve in Pet. Ether and wash with distilled or deionized water.

16.8.5.2. General Cocaine Extraction: Pet. Ether or $CHCl_3$ from base (0.45N NaOH)

16.8.6. Due to the known false positives of the Cobalt Thiocyanate color test, at a minimum a combination of any of the following two tests shall be used for confirmation of Cocaine: UV, TLC, Gas Chromatography – Retention time, Gas Chromatography/Mass Spectrometry, Gas Chromatography-Infrared Spectroscopy*, and FTIR. (*Note: salt forms cannot be determined by using GC-IR)

16.9. Records:

16.9.1. See General Drug Identification
16.9.2. See Other Test Methods

16.10. Interpretations of Results:

16.10.1. Color Tests:

16.10.1.1. Cobalt Thiocyanate = Cocaine HCl, Procaine, Lidocaine, forms a blue precipitate; Cocaine Base turns a slow blue and forms a blue precipitate with the addition of HCl (if HCl is added, this is considered a multi-part test and the blank shall be checked). Observations of both steps of the multi-part test shall be documented on the worksheet. There are many other substances that react similarly to Cocaine with this test.

16.10.1.2. Scott's Test = Cocaine turns blue in the first step. The blue should disappear with addition of HCl to give a pink solution. The mixture should turn blue again when $CHCl_3$ is added and the mixture shaken.

16.10.2. UV See references for expected wavelengths. Shifts occur when mixed with other substances. The degree, direction and shape of the shift may indicate the identity of the interfering substance.

16.10.3. Thin Layer Chromatography/Over-sprays:

16.10.3.1. Ninhydrin – turns Procaine and Benzocaine pink

16.10.3.2. p-DMAB – turns Procaine and Benzocaine yellow

16.10.3.3. Iodoplatinate

16.10.3.4. Potassium Permanganate ($KMnO_4$)

16.10.4. FTIR: See FTIR Test Method and Reference Material Test Method.

16.10.5. GC/MS: See GC/MS Test Method.

16.10.6. GC-IR: See GC-IR Test Method.

16.11. Report Writing:

16.11.1. The base form shall only be reported if requested by the Prosecutor and with Drug Unit Supervisor approval.

16.12. References:

16.12.1. Analytical Profiles of Cocaine, Local Anesthetics and Common Diluents Found with Cocaine, CND Analytical, Inc. 1990

16.12.2. Cocaine, Marijuana, Designer Drugs: Chemistry, Pharmacology and Behavior, K. Redda, C. Walker, G. Barnett, CRC Press, 2000.

16.12.3. The Analysis of Controlled Substances, Cole, Michael D., Wiley, 2003

16.12.4. Drug Unit Cocaine Resource Manual

17. Tryptamines/Indoles (General)

17.1. Scope: This Test Method covers substances that contain the indole nucleus and may be classified as hallucinogens. This group includes drugs such as Tryptamines, Psilocybic Mushrooms and Lysergic Acid Diethylamide (LSD). Psilocybic mushrooms and LSD are covered in detail in separate Test Methods due to their complex analytical requirements.

17.2. Precautions/Limitations:

17.2.1. Hallucinogenic
17.2.2. Typically, small dosages, but potent.
17.2.3. The media in which the drug resides usually comprises the majority of the weight of the exhibit.

17.3. Related Information:

17.3.1. Appendix 1 – Forms and Worksheets
17.3.2. Appendix 2 – Abbreviations
17.3.3. Appendix 3 – Definitions
17.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
17.3.5. Other Test Methods
- **17.3.5.1.** General Drug Identification
- **17.3.5.2.** Color Tests
- **17.3.5.3.** UV
- **17.3.5.4.** TLC
- **17.3.5.5.** FTIR
- **17.3.5.6.** GC/MS
- **17.3.5.7.** GC-IR
- **17.3.5.8.** Separation and Extractions Procedures

17.4. Instruments:

17.4.1. UV
17.4.2. FTIR
17.4.3. GC/MS
17.4.4. GC-IR

17.5. Reagents/Materials:

17.5.1. See Color (Spot) Tests Test Method
17.5.2. See Thin Layer Chromatography Test Method
17.5.3. Methanol (MeOH)
17.5.4. Chloroform ($CHCl_3$)

17.6. Hazards/Safety: Exposure - skin absorption of hallucinogenic drugs.

17.7. Reference Materials/Controls/Calibration Checks:

17.7.1. Reference materials as appropriate.

17.8. Procedures/Instructions:

17.8.1. Extraction: See Separation and Extractions Test Method and Reference Materials.
17.8.2. Color (Spot) Tests: Marquis, p-DMAB, Mecke's
17.8.3. UV (in acid)
17.8.4. TLC Systems: MeOH :NH_4OH (100:1.5), $CHCl_3$:MeOH:HOAc (75:20:5)
17.8.5. FTIR – extracted
17.8.6. GC/MS – extracted
17.8.7. GC-IR - extracted

17.9. Records: See Other Test Methods.

17.10. Interpretations of Results:

17.10.1. Color Tests:

17.10.1.1. Marquis: strong blues, Substituted tryptamines – some olive green

17.10.1.2. p-DMAB: purple with LSD, grey/violet with indole alkaloids, also various pink colors are possible.

17.10.1.3. Mecke's: strong reactions – blues, purples, grey-black

17.10.2. UV: Generally strong UV absorbers with absorbance patterns that are characteristic of the group.

17.10.3. TLC: See TLC Test Method

17.10.4. FTIR: See FTIR Test Method

17.10.5. GC/MS: See GC/MS Test Method

17.10.6. GC-IR: See GC-IR Test Method

17.11. Report Writing: See General Drug Identification.

17.12. References:

17.12.1. Tryptamines Volume 1: Synthesis, Analog Synthesis and Precursor Synthesis, Clandestine Laboratory Investigating Chemists, 2001

17.12.2. Tryptamines Volume 2: Analytical Data and Natural Product Synthesis, Clandestine Laboratory Investigating Chemists, 2001

17.12.3. Analytical Profiles for Five “Designer” Tryptamines, Spratley, et. al. (US Department of Justice, DEA), Microgram Journal, Vol. 1, Jan-Jun 2003.

18. Lysergic Acid Diethylamide (LSD)

18.1. Scope: LSD is a synthetic hallucinogen commonly found in liquid form, on blotter paper, tablets (microdots), windowpanes and sugar cubes. This Test Method is intended to outline the procedures for identification of LSD that is usually present in very small quantities and/or concentrations.

18.2. Precautions/Limitations:

18.2.1. A structural isomer exists [Lysergic Acid Methyl Propylamide (LAMPA)], which produces similar GC/MS spectral data, but can be differentiated by Thin Layer Chromatography and GC retention time.

18.2.2. GC-IR analysis may be used; however, limited concentrations of sample material may not produce IR data of sufficient quality to permit identification.

18.2.3. LSD also has a stereoisomer, Iso-LSD, which has different physical and chemical properties than LSD. It can be easily separated from LSD by using Thin Layer Chromatography. However, a reference material may not be available.

18.2.4. Small amount of drug per dosage unit.

18.2.5. Presence of dyes and/or other complex media can interfere with analysis.

18.2.6. LSD has an affinity for filter papers, and the resulting extraction yields will be very low, if anything at all.

18.3. Related Information:

18.3.1. Appendix 1 – Forms and Worksheets
18.3.2. Appendix 2 – Abbreviations
18.3.3. Appendix 3 – Definitions
18.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
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- **18.3.5.1.** General Drug Identification
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- **18.3.5.3.** UV
- **18.3.5.4.** TLC
- **18.3.5.5.** FTIR
- **18.3.5.6.** GC/MS
- **18.3.5.7.** GC-IR
- **18.3.5.8.** Separations

18.4. Instruments:

18.4.1. UV
18.4.2. UV light box or other UV light source
18.4.3. FTIR, possibly, but not common due to insufficient sample size
18.4.4. GC/MS
18.4.5. GC-IR, possibly, if enough sample exists.

18.5. Reagents/Materials:

18.5.1. p-DMAB Color Test Reagent
18.5.2. Concentrated Hydrochloric acid (HCl)
18.5.3. Methanol
18.5.4. Chloroform
18.5.5. Extraction chemicals
18.5.6. TLC system chemicals
18.5.7. Chemical over-sprays
18.5.8. Laboratory glassware

18.6. Hazards/Safety:

18.6.1. Exposure – through skin contact, solvent exposure.
18.6.2. See SDS for drugs and chemicals used in analysis.

18.7. Reference Materials/Controls/Calibration Checks:

18.7.1. Reference Materials of LSD and LAMPA.

18.8. Procedures/Instructions:

18.8.1. See General Drug Identification

18.8.2. Suggested Extractions:

18.8.2.1. Methanol,

Or

18.8.2.2. Filter Methanol through a glass pipette (with a glass wool plug and filled with alumina),

Or

18.8.2.3. Sugar Cube Extraction:

1. In a separatory funnel containing a crushed sugar cube, add 10-15 ml of a 1% tartaric acid solution.

2. Add ~20 ml $CHCl_3$ and shake. Drain. Repeat one time.

3. Make solution basic with a NaOH pellet.

4. Add ~30 ml $CHCl_3$ and shake for several minutes.

5. Drain $CHCl_3$ into a ~50 ml beaker using NO FILTER PAPER.

6. Evaporate to dryness.

7. Add two drops of MeOH for use in TLC or GC/MS. GC-IR may also be used if enough sample exists and this technique is available.

18.8.2.4. Windowpanes: Cut or crush the windowpane.

Soak the crushed windowpane in MeOH for an extended time period; or

Soak the crushed windowpane in 0.45 N NaOH for several hours to dissolve the windowpane. Extract with $CHCl_3$ and evaporate to dryness.

18.8.3. Color Test: p-DMAB

18.8.4. UV in Methanol

18.8.5. TLC Systems (Suggested):

18.8.5.1. Acetone

18.8.5.2. Acetone: NH_4OH sat'd $CHCl_3$ (9:1)

18.8.5.3. Over-spray with p-DMAB. It may be necessary to heat the plate to get good results with the overspray.

18.8.6. TLC with degradation (optional):

18.8.6.1. Prepare TLC plates by spotting samples and reference materials.

18.8.6.2. Expose to short wave UV light for approximately 30 minutes.

18.8.6.3. Place the plate in the TLC tank and develop.

18.8.6.4. After plate is removed and dried, look at the plate under short and long wave UV light. Mark spots with pencil.

18.8.6.5. Spray the plate with p-DMAB.

18.8.6.6. Observe and compare degradation spots in samples and reference materials.

18.8.7. GC/MS: Temperature programs from approximately 240 to 280 °C.

18.8.8. GC-IR: Similar temperature programs used in GC/MS may be appropriate.

18.9. Records: See General Drug Identification Test Method

18.10. Interpretations of Results:

18.10.1. Color Test: p-DMAB Positive = purple with LSD

18.10.2. UV in Methanol. See references for expected wavelengths.

18.10.3. UV light box: Both LSD and LAMPA fluoresce blue under long wave UV light

18.10.4. TLC: LSD and LAMPA should separate and turn purple/blue with p-DMAB over-spray.

18.10.5. TLC with degradation: compare sample degradation spot locations and reactions to over-spray with the degradation spots of the reference material.

18.10.6. GC/MS: See GC/MS Test Method. Care should be taken to evaluate the spectrum closely when comparing LSD and LAMPA.

18.10.7. GC-IR: See GC-IR Test Method. LSD and LAMPA can be clearly distinguished using this method. Concentration and chromatography quality may not be sufficient for identification.

18.11. Report Writing: See General Drug Identification

18.12. References:

18.12.1. Lysergic Acid Amide Workshop, Rosenthal, J. Midwestern Association of Forensic Scientists (MAFS) , Oct 1998

18.12.2. LSD Analysis, Robison, Mary; 1983

18.12.3. Differentiation of LSD and LAMPA, Kebabjian, Dennis; Microgram, Vol. VIII, No. 4 (April, 1975) pp 53-54

18.12.4. A Technique for the Infrared Identification of LSD, Clodfelter, Ronald; Microgram, Vol. VIII, No. 9, (Sept 1975) pp 137-138.

18.12.5. Micro-Infrared Analysis of LSD, Morgan and Francois, Microgram, Vol. IX, No. 9 (Sept 1976) pp 130-135.

18.12.6. Basic Training Program for Forensic Drug Chemists, US Department of Justice Bureau of Narcotic and Dangerous Drugs; Canaff, May 1972.

19. Psilocybin Mushrooms

19.1. Scope: Mushrooms encountered in routine case work often contain Psilocyn and/or Psilocybin. The mushroom (genus Psilocybe) itself is not controlled, but rather the hallucinogens found within it.

19.2. Precautions/Limitations:

19.2.1. Extraction is necessary for identification. Mushrooms contain large amounts of alkaloids, fats and sugars that complicate analysis and shall be removed.

19.2.2. Psilocybin cannot be identified by GC/MS or GC-IR alone, since it breaks down into Psilocyn in the injection port. Thin Layer Chromatography is required for information to support identification. If Psilocybin identification is needed, derivatization may be necessary.

19.2.3. Psilocybin is the phosphorylated ester of Psilocyn and easily converts to Psilocyn with heat and during extraction with acid or alkaline solutions.

19.2.4. TLC shall be performed on a solvent extract prior to further extraction to determine the presence of Psilocyn or Psilocybin, or both.

19.2.5. FTIR is not generally performed due to insufficient sample size and extraction is not sufficient to isolate Psilocyn from Psilocybin.

19.2.6. Acetylpsilocyn can convert to Psilocyn in the injection port and derivatization may be required.

19.2.7. The extraction process may result in the generation of Phenethylamine and/or Tryptamine. Phenethylamine and/or Tryptamine shall not be reported on the Certificate of Analysis if Psilocyn or Psilocybin are being reported.

19.3. Related Information:

19.3.1. Appendix 1 – Forms and Worksheets
19.3.2. Appendix 2 – Abbreviations
19.3.3. Appendix 3 – Definitions
19.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
19.3.5. Other Test Methods
- **19.3.5.1.** General Drug Identification
- **19.3.5.2.** Color Tests
- **19.3.5.3.** UV
- **19.3.5.4.** TLC
- **19.3.5.5.** Separations
- **19.3.5.6.** GC/MS
- **19.3.5.7.** GC-IR

19.4. Instruments:

19.4.1. UV
19.4.2. GC/MS
19.4.3. GC-IR

19.5. Reagents/Materials:

19.5.1. Color (Spot) Test Reagents
19.5.2. pH paper
19.5.3. TLC Solvent Systems and supplies
19.5.4. Chemical over-sprays/visualization reagents
19.5.5. Methanol
19.5.6. Acetone
19.5.7. $CHCl_3$
19.5.8. Acetic Acid
19.5.9. Ammonium hydroxide
19.5.10. Extraction chemicals

19.6. Hazards/Safety: See SDS.

19.6.1. Exposure to hallucinogenic drugs
19.6.2. Exposure to hazardous chemicals

19.7. Reference Materials/Controls/Calibration Checks:

19.7.1. Psilocybin and Psilocyn Reference Materials.

19.8. Procedures/Instructions:

19.8.1. Visual examination of mushroom, note presence of blue bruises on stems and odor, if present. If a case has multiple mushroom items only enough items to exceed the weight threshold needs to be examined.

19.8.2. Color Tests: (i.e., p-DMAB or Weber) if desired, direct on a portion of the mushroom or on an extract.

19.8.3. Recommended Sample Preparation:

1. Optional: Pulverize the mushroom.
2. Soak in Methanol.
3. Optional: Heat MeOH/Mushroom mixture (@40 °C max) for two hours.

4. Pour off MeOH into a clean beaker and filter. Repeat MeOH soak, if desired.

5. Optional Step: Add 10 ml Acetone, and place in freezer for 30 minutes to freeze out the fats. Filter.

6. Evaporate to dryness in a beaker without heat.

19.8.4. Ultraviolet Spectroscopy in MeOH; can be run before or after extraction.

19.8.5. ***Reconstitute in MeOH to run TLC***

19.8.6. Recommended TLC Systems:

19.8.6.1. MeOH: NH_4OH (100:1.5)
19.8.6.2. $CHCl_3$:MeOH:HOAc (75:20:5)
19.8.6.3. n-butanol:dH_2O:HOAc (2:1:1)
19.8.6.4. Overspray with acidified p-DMAB

19.8.7. Recommended Extraction for GC/MS and GC-IR:

1. Dissolve sample (from MeOH extract) with 1% – 5% acetic acid and pour into separatory funnel.

2. Rinse sample beaker with 1% – 5% acetic acid and pour into the separatory funnel.

3. Rinse beaker again with $CHCl_3$ and pour into the same separatory funnel.

4. Add more $CHCl_3$ and extract.

5. Discard the $CHCl_3$.

6. Make the aqueous layer basic with NH_4OH, extract with $CHCl_3$.

7. Evaporate to dryness.

8. Reconstitute in MeOH and run on GC/MS.

19.8.8. GC/MS: See GC/MS Test Method.

19.8.9. GC-IR: See GC-IR Test Method.

19.9. Records: See General Drug Identification.

19.10. Interpretations of Results:

19.10.1. Visual Examination: blue-grey bruising is indicative of oxidation of indole-containing compounds.

19.10.2. Odors associated with Psilocybic Mushrooms are generally unpleasant, but are characteristic of these types of mushrooms.

19.10.3. Color Tests:

19.10.3.1. p-DMAB (Ehrlich's) = purple-black

19.10.3.2. Weber's = Fast Blue B = red, addition of HCl = blue

19.10.3.3. Mecke's – green color

19.10.3.4. UV: See references for expected wavelengths

19.10.4. TLC: See TLC Test Method (7.10). It is essential that separation occurs between Psilocyn and Psilocybin reference materials.

19.10.5. GC/MS without derivatization will identify only Psilocyn since Psilocybin breaks down in the injection port of the GC.

Since Psilocybin converts to Psilocyn, TLC is essential when determining which substances are present. If no Psilocybin is present on TLC, it can be concluded that the GC/MS is that of Psilocyn and only Psilocyn.

If TLC reveals the presence of both Psilocyn and Psilocybin, the resulting GC/MS spectrum can be concluded to be some combination of both Psilocyn and converted Psilocybin.

If TLC reveals the presence of only Psilocybin, the resulting GC/MS will result in the spectrum of Psilocyn. The combinations of TLC and GC/MS results are sufficient to make the conclusion that the sample contained Psilocybin if the TLC was performed prior to the acid/base extraction procedures.

19.10.6. GC-IR would not be able to differentiate between Psilocyn and Psilocybin. The same breakdown issues that occur with GC/MS also exist with this technique.

19.11. Report Writing:

19.11.1. If Psilocyn is the only substance indicated on TLC and identified by GC/MS (or GC-IR), it shall be reported as “Psilocyn”.

19.11.2. If Psilocybin is the only substance identified on TLC and supported by GC/MS (or GC-IR) spectral data for Psilocyn, it may be identified and reported as “Psilocybin”.

19.11.3. If Psilocyn and Psilocybin are indicated on TLC and Psilocyn was identified by GC/MS (or GC-IR), the item shall be reported using the following verbiage or similar verbiage: “was found to contain Psilocyn, a controlled substance. Examination also indicated the presence of Psilocybin, a controlled substance.”

19.12. References:

19.12.1. Isolation and Identification of Psilocybin and Psilocin, M.A. Bonin (US Army Criminal Investigation Laboratory, Fort Gordon, GA), Microgram Vol. XVI, No. 6, June 1983

19.12.1.1. Hallucinogenic Mushrooms, Oliveria and Medeiros de Silva (translated by Morris Grodsky); Microgram, Vol XI, No.2 (February 1978)

19.12.1.2. The Identification of Psilocyn and Psilocybin in Mushrooms Using High Resolution Gas Chromatography/Mass Spectrometry, Timmons, James E. (Arizona Department of Public Safety, Phoenix, AZ), Microgram, Vol. XVII, No. 2, February 1984.

19.12.1.3. Identification of Psilocybin in Mushrooms, Miller Daniel S. (Florida Department of Law Enforcement)

19.12.1.4. The Assay of Psilocybe Mushrooms for Hallucinogens, The Drug Chromatographer, Volume 1992.2, Bulletin 244 Alltech Applied Science Labs.

19.12.1.5. An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms, Casale, John F., Journal of Forensic Sciences, Vol 30, No. 1, Jan 1985, pp 247-250.

19.12.1.6. Psilocybin Mushroom Workshop, Penabraker, Scott; Midwestern Association of Forensic Scientists (MAFS), Oct 1998.

19.12.2. Tryptamines Volume 1: Synthesis, Analog Synthesis and Precursor Synthesis, Clandestine Laboratory Investigating Chemists, 2001

19.12.3. Tryptamines Volume 2: Analytical Data and Natural Product Synthesis, Clandestine Laboratory Investigating Chemists, 2001

19.12.3.1. Quantitative Analysis of Psilocybin and Psilocin in Psilocybe Baecystis (Singer and Smith) by High Performance Liquid Chromatography and by Thin Layer Chromatography, Beug, M. and Bigwood, J., Journal of Chromatography, 207 (1981) P 370-385

19.12.3.2. Botanical and Chemical Characterization of Forensic Mushroom Specimen of the Genus *Psilocybe*, Heim, Genest, Hughes, and Belec; Journal of Forensic Science Society, Vol 6, No. 4, 1966

19.12.3.3. Weber Test; Garrett, Allen; Clemens, Steven and Gaskill, James. Weber State College, Laboratory of Criminalistics, Ogden, Utah.

19.12.3.4. Blueing on Conocybe, Psilocybe and a Stropharia and the Detection of Psilocybin., Benedict, Tyler, and Watling; Lloydia, Vol. 30, No.2 ,2, June 1967

19.12.4. TiHKAL The Continuation, Shulgin, Alexander and Shulgin, Ann; Transform Press, Berkeley, CA, 1997

19.12.5. Analysis and Characterization of Psilocybin and Psilocyn Using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC ESI MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID), Rodriguez, S. (US Dept. Of Justice DEA, Vista, CA), Microgram Journal, Vol 3, No. 34, July- Dec 2005.

20. Khat

20.1. Scope: Catha Edulis (Khat) is a plant native to east Africa and southern Arabia that contains two naturally occurring central nervous system (CNS) stimulants, Cathine and Cathinone. Cathinone, the primary active component that is structurally related to amphetamine. Cathine (d-norpseudoephedrine) is related to Pseudoephedrine.

20.2. Precautions/Limitations:

20.2.1. Cathinone levels are highest in freshly cut khat plants. Once cut, levels of Cathinone start to decline.

Research indicates enzyme action in the plant material causes the Cathinone (Schedule I) to break down to Cathine (Schedule IV). When the plant material is in a dried state or the Cathinone and Cathine have been removed from the leaves, the enzyme action appears to be slowed down significantly.

It is recommended that the plant material be refrigerated (or frozen if it is to be in storage for a period of time) to reduce the rate of degradation of the Cathinone.

20.2.2. Needs to be carefully extracted to avoid converting Cathinone to Cathine during extraction.

20.2.3. The botanical identification of the Khat plant is beyond the scope of the ISP Drug Unit analysis.

20.3. Related Information:

20.3.1. Appendix 1 – Forms and Worksheets
20.3.2. Appendix 2 – Abbreviations
20.3.3. Appendix 3 – Definitions
20.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
20.3.5. Other Test Methods
- **20.3.5.1.** Color (Spot) Tests
- **20.3.5.2.** UV
- **20.3.5.3.** TLC
- **20.3.5.4.** FTIR
- **20.3.5.5.** GC/MS
- **20.3.5.6.** GC-IR
- **20.3.5.7.** Separation and Extraction
- **20.3.5.8.** General Drug Identification

20.4. Instruments:

20.4.1. UV

20.4.2. FTIR
20.4.3. GC/MS
20.4.4. GC-IR

20.5. Reagents/Materials: See Other Test Methods

20.6. Hazards/Safety: See SDS for Cathinone, and Cathine

20.7. Reference Materials/Controls/Calibration Checks:

20.7.1. Reference Materials for Cathinone, and Cathine.

20.8. Procedures/Instructions:

20.8.1. Extraction:

1. Weigh out at least 5 g of plant material (do not crush).

2. Soak in MeOH (enough to cover plant material) overnight.

3. Filter and evaporate to dryness.

4. Dissolve residue in 0.02 N H_2SO_4.

5. Wash with $CHCl_3$.

6. Make aqueous layer basic (pH 8 – 9) with sat. $NaHCO_3/H_2O$.

7. Extract with $CHCl_3$.

8. Evaporate down to use for TLC and GC/MS (DO NOT evaporate to dryness as oxidation may occur or substances may evaporate – run in $CHCl_3$).

20.8.2. UV: See UV Test Method

20.8.3. TLC: See TLC Test Method

20.8.4. FTIR: See FTIR Test Method

20.8.5. GC/MS: See GC/MS Test Method

20.8.6. GC-IR: See GC-IR Test Method

20.9. Records: See General Drug Identification Test Method.

20.10. Interpretations of Results: The results of the analysis would conclude the presence of Cathinone and/or Cathine. While these results would indicate that the material is consistent with the Khat plant, the botanical identification of the Khat plant is beyond the scope of the ISP Drug Unit analysis.

20.11. Report Writing: See General Drug Identification Reporting.

20.12. References:

20.12.1. Khat Fact Sheet (December 1992), US Department of Justice, Drug Enforcement Administration, Microgram, Vol. XXVI, No. 3 March 1993

20.12.2. The Identification of Cathinone and Methcathinone, Dal Cason, Terry A. (DEA Central Laboratory, Chicago, IL), Microgram, Vol. XXV, No. 12, December 1992.

20.12.3. Drugs and Chemicals of Concern: Khat., Office of Diversion Control Information and Legal Resources, June 2009

20.12.4. The Identification of Cathinone in Khat (Catha Edulis): A Time Study, Lee, M.M. Journal of Forensic Sciences, Vol. 40, No.1, January 1995, pp116-121.

20.12.5. ISP Khat Extraction Validation of 20.12.4

21. Methoxyamphetamines

21.1. Scope: Methoxyamphetamines are frequently called “designer drugs” and are closely related to the indoles and phenethylamines. These compounds may be synthetic, semi-synthetic or naturally occurring. Most commonly they tend to be found in club drugs and sold as hallucinogens.

21.2. Precautions/Limitations:

21.2.1. Hallucinogenic in nature.

21.2.2. Frequently found with other controlled substances and a variety of adulterants.

21.2.3. Many regioisomeric forms exist and may be difficult to differentiate.

21.2.4. Peyote: buttons shall be dry and finely ground before extraction in order to isolate mescaline.

21.3. Related Information:

21.3.1. Appendix 1 – Forms and Worksheets
21.3.2. Appendix 2 – Abbreviations
21.3.3. Appendix 3 – Definitions
21.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
21.3.5. Other Test Methods
- **21.3.5.1.** General Drug Identification
- **21.3.5.2.** Color Tests
- **21.3.5.3.** UV
- **21.3.5.4.** FTIR
- **21.3.5.5.** GC/MS
- **21.3.5.6.** GC-IR
- **21.3.5.7.** Separations
- **21.3.5.8.** Clandestine Laboratory Sample Analysis

21.4. Instruments:

21.4.1. UV
21.4.2. FTIR
21.4.3. GC/MS
21.4.4. GC-IR

21.5. Reagents/Materials:

21.5.1. See Color (Spot) Tests Test Method
21.5.2. See General Test Methods

21.6. Hazards/Safety: Chemical Exposure – See SDS

21.7. Reference Materials/Controls/Calibration Checks:

21.7.1. Appropriate Reference Materials for drugs of interest.

21.8. Procedures/Instructions:

21.8.1. Extractions: Generally organic solvents from aqueous alkaline solutions are used and are the same as most Phenethylamines. Some may require special considerations and procedures. These may require HCl fumes to keep from evaporating.

21.8.1.1. Peyote (Mescaline): Dry and Crush peyote buttons.

21.8.1.1.1. Soak in MeOH.

Or

21.8.1.1.2. Mix approximately 0.5 g sample with 0.1 N HCl, make basic with 2.0 N NaOH, extract with hexanes. HCl fume. Let evaporate. Run on GC/MS in methanol.

Or

21.8.1.1.3. Add 2.0N NaOH to approximately 0.5 g sample and extract with $CHCl_3$. Run on GC/MS in $CHCl_3$.

21.8.2. General analytical procedures are sufficient.

21.8.2.1. Color Tests

21.8.2.2. UV in acid (0.5 N H_2SO_4)

21.8.2.3. TLC systems:

21.8.2.3.1. MeOH:NH_4OH (100:1.5)

21.8.2.3.2. $CHCl_3$:MeOH:HOAc (75:20:5)

21.8.2.4. FTIR

21.8.2.5. GC/MS

21.8.2.6. GC-IR

21.9. Records: See Other Test Methods

21.10. Interpretations of Results:

21.10.1. Color Tests: Generally strong Marquis and Mecke's reactions, ranging from intense blues to grey and black. Some pink and purples are possible as well.

21.10.1.1. MDMA:
Marquis: intense purple to black
Mecke's: fast and intense yellow-green to dark blue to black

21.10.1.2. Peyote (mescaline):
Marquis: orange
Mecke's: orange-brown

21.10.2. UV in acid: Generally, one or two intense peaks for most methoxyamphetamines.

21.10.3. TLC: See TLC Test Method; General Drug Identification

21.10.4. FTIR: See FTIR Test Method, General Drug Identification

21.10.5. GC/MS: See GC/MS Test Method, General Drug Identification

21.10.6. GC-IR: See GC-IR Test Method, General Drug Identification

21.10.7. Peyote (Mescaline): Results of the analysis would conclude the presence of Mescaline. While these results would indicate that the material is consistent with the Peyote Cactus, or Peyote Buttons, the botanical identification of Peyote is beyond the scope of the ISP Drug Unit analysis.

21.11. Report Writing:

21.11.1. See General Drug Identification.

21.11.2. Peyote: Results shall be reported as "found to contain Mescaline, a controlled substance", if appropriate.

21.12. References:

21.12.1.1. Extraction of Mescaline from Peyote, Maloney, David (Jefferson County Sheriff's Office, Golden, Colorado), Microgram, Vol. XXXIV, No. 8, (August 2001).

21.12.1.2. Extraction of Mescaline from Peyote and Subsequent Instrumental Analysis, Barbara, John (State of Tennessee Forensic Laboratory, Knoxville, TN), Microgram, Vol. VIII, No. 12 (December, 1975) p 182-187

21.12.1.3. Extraction of Mescaline from Peyote Buttons, Dal Cason, Terry A., Microgram, Vol. VI, No.3 (March, 1973) p 43

21.12.1.4. Peyote: Interpretation under Federal Law, Drug Enforcement, (Summer 1975) p 40-41.

21.12.1.5. Isolation and Identification of Drugs, Clarke, E.G.C., Vol. 1 and II.

21.12.1.6. The Identification of Methoxyamphetamine, Methoxy-N-Methylamphetamine and Methylenedioxymethamphetamine, Bailey, K., Legault, D., and Verner, D. (Drug Research Laboratories Health Protection Branch, Ottawa, Canada)

21.12.1.7. Methods of Differentiation for Regioisomeric 2,3- and 3,4-Methylenedioxyphenalkylamines by Liquid Chromatography and Mass Spectrometry, Clark, C. Randall, Noggle, F. Taylor, Holston, Pamela L., and DeRuiter, Jack (Auburn University, Auburn, Alabama), Microgram, Vol. XXXI, No. 9, September 1998.

21.12.2. PiHKAL: A Chemical Love Story, Shulgin, Alexander and Shulgin, Ann, Transform Press, 1991.

21.12.3. A Discussion of 2C-I and Acetylated 2C-T-7, Shanks, Kathy; Koresch, Sandra and Oehldrich, James (Wisconsin State Crime Laboratory Milwaukee, WI)

21.12.4. The Identification of 2,5-Dimethoxy-4-(N)-Propylthiophenenethylamine (2C-T-7), Zimmerman, Michelle M.(Wisconsin State Crime Laboratory, Wausau, WI),Microgram, Vol. XXXIV, No. 7,July 2001.

21.12.5. Analytical Profiles of 4-Bromo-2,5-Dimethoxyphenethylamine ("Nexus") and Related Precursor Chemicals, Noggle, DeRuiter, and Clark (Alabama Department of Forensic Sciences, Auburn, AL), Microgram, Vol. XXVII, No. 10, October 1994.

22. Phenethylamines

22.1. Scope: Phenethylamines are central nervous system stimulants and appetite suppressants. Some of the more commonly analyzed substances within this group are Amphetamine, Methamphetamine, Phentermine, Phendimetrazine, Methcathinone, Ephedrine, Pseudoephedrine, and Methylphenidate.

22.2. Precautions/Limitations:

22.2.1. Phenethylamines are typically mixed with a variety of adulterants, diluents, impurities and/or precursors.

22.2.2. Generally, phenethylamines are soluble in methanol.

22.2.3. Alkaline extracts of these types of samples may be volatile and are prone to loss if not converted to a stable salt form.

22.2.4. Pharmaceutical preparations may contain Phenethylamines that are contained within resin beads or time release formulations that shall be crushed prior to analysis.

22.2.5. Members of this drug grouping are typically very small molecules, which can make GC/MS analysis difficult. Care shall be taken when making comparisons due to the limited spectral information available. FTIR may be a better method of confirmation if the sample quantity permits.

22.2.6. Gas chromatography of salt forms is usually poor. It is advisable to run these in their free base form.

22.2.7. Methamphetamine and Phentermine have similar GC/MS spectra. GC/MS and GC-RT are not sufficient to confirm Methamphetamine or Phentermine unless both are run for GC-RT. Phentermine is not required to be run for GC-RT if the Sodium Nitroprusside test is positive or FTIR test is positive/indication.

22.3. Related Information:

22.3.1. Appendix 1 – Forms and Worksheets
22.3.2. Appendix 2 – Abbreviations
22.3.3. Appendix 3 – Definitions
22.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
22.3.5. Other Test Methods
- **22.3.5.1.** Clandestine Laboratory Sample Analysis
- **22.3.5.2.** Methoxyamphetamines
- **22.3.5.3.** Color (Spot) Tests
- **22.3.5.4.** UV
- **22.3.5.5.** FTIR

22.3.5.6. GC/MS
22.3.5.7. GC-IR
22.3.5.8. Separations

22.4. Instruments:

22.4.1. UV
22.4.2. FTIR
22.4.3. GC/MS
22.4.4. GC-IR

22.5. Reagents/Materials:

22.5.1. See General Drug Identification Test Method
22.5.2. See Separations Test Method

22.6. Hazards/Safety:

22.6.1. Chemical Exposure: See SDS for individual drug hazards.

22.7. Reference Materials/Controls/Calibration Checks:

22.7.1. Appropriate Reference Materials of drug of interest, common excipients, and diluents.

22.8. Procedures/Instructions:

22.8.1. Extraction from aqueous alkaline solutions with organic solvents is routinely necessary to obtain good results. Generally, petroleum ether or $CHCl_3$ from 0.45 N NaOH works well. HCl fumes may be needed to stabilize the drug, depending on the type of analysis to be performed.

22.8.2. Spot Tests: Marquis, Mecke's and Sodium Nitroprusside.

22.8.3. UV in acid (0.5 N H_2SO_4)

22.8.4. Thin Layer Chromatography: general drug systems. (See 24.10.3)

22.8.5. FTIR: using ATR or transmittance.

22.8.6. GC/MS: general temperature programs with low starting temperatures are sufficient. The addition of sodium bicarbonate ($NaHCO_3$) to a methanolic extraction of phenethylamines improves chromatographic response.

22.8.7. GC-IR: general temperature programs similar to those used in GC/MS are sufficient. The addition of sodium bicarbonate does not improve chromatography with this technique. Full extraction is needed.

22.9. Records: See General Drug Identification Test Method

22.10. Interpretations of Results:

22.10.1. Spot Tests

22.10.1.1. Marquis turns Amphetamine-like substances orange → brown.

22.10.1.2. Phendimetrazine, Phenmetrazine, Ephedrine, Pseudoephedrine, and Propylhexedrine do not give an orange on the Marquis test.

22.10.1.3. Sodium Nitroprusside turns secondary amines, such as Methamphetamine, blue.

22.10.2. UV: Phenethylamines generally give a triplet UV Spectrum in acid (0.5 N H_2SO_4).

22.10.3. Thin Layer Chromatography:

22.10.3.1. General TLC solvent systems:
MeOH:NH_4OH (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)

22.10.3.2. Over-sprays:

22.10.3.2.1. Ninhydrin turns primary and secondary amines pink

22.10.3.2.2. Iodoplatinate (If no spots are visible, then Potassium Permanganate shall also be used.)

22.10.3.2.3. Potassium Permanganate ($KMnO_4$)

22.10.3.2.4. Marquis Reagent

22.10.4. FTIR:

22.10.4.1. Extraction may be necessary to obtain a good spectrum for comparison.

22.10.4.2. See FTIR Test Method

22.10.5. GC/MS:

22.10.5.1. Methamphetamine shall have m/z 148 ion.

22.10.5.2. See GC/MS Test Method.

22.10.6. GC-IR:

22.10.6.1. Extraction may be necessary to obtain good chromatography

22.10.6.2. See GC-IR Test Method

22.11. Report Writing:

22.11.1. Reference identification of marked Pseudoephedrine or Ephedrine tablets is sufficient unless the charges are manufacturing.

22.11.2. See General Drug Identification Test Method.

22.12. References:

22.12.1. Indiana Criminal Code (scheduling)

22.12.2. Amphetamine CLIC Monographs

22.12.3. Validation of Pseudoephedrine/Ephedrine Quantitation Method, Early, K. (Indiana State Police, Evansville, IN). October 2004.

22.12.4. Isolation and Identification of Drugs, Clarke, E.G.C., The Pharmaceutical Press, London. 1969.

22.12.5. Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

22.12.6. Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

22.12.7. The Merck Index, 8th Edition; Merck and Company, Inc. 1968

22.12.8. Spot Tests in Organic Analysis, Fiegl, F. and Anger, V., Elsevier Publishing, New York. 1966.

22.12.9. Separation and Identification of Amphetamine or Methamphetamine in combination with Ephedrine or Caffeine, Stinson, Samuel and Berry, Michael; Microgram, Vol. VII, No. 4 (April, 1974) p. 51.

22.12.10. The Identification of Propylhexedrine, Dal Cason, Terry A. (Drug Enforcement Administration), Microgram, Vol. XV, No. 4 (April 1982).

22.12.11. Extractions of Methamphetamine from Vick's Inhalers, O'Neil, Quinn, Kern, and Finley (Commonwealth of Virginia), Microgram, Vol. XII, No. 7 (July 1979).

22.12.12. Separation and Identification of Methamphetamine in Phentermine, Methamphetamine, Ephedrine and Caffeine "Mini-Bennies", Anderson, Gundy and Lorch (Michigan Department of Public Health, Lansing, MI), Microgram, Vol. IX, No. 7 (July 1976).

22.12.13. Separation of Caffeine, Ephedrine and Phentermine, Stall, Walter (US Army Criminal Investigation Laboratory, Fort Gordon, GA). Microgram, Vol. X, No. 1 (January, 1977)

22.12.14. Screening Test for Amphetamine, Fleischer, David (NYC Police Department, New York, NY), Microgram, Vol. VIII, No. 8 (August, 1975).

22.12.15. Identification of Cathinone and Methcathinone, Dal Cason, Terry A., Microgram, Vol. XXV, No. 12, (December 1992).

22.12.16. Analysis of Phentermine/ Methamphetamine/ Ephedrine/ Caffeine Mixtures by GC/MS, Smith, R. Martin (Wisconsin Department of Justice, Madison, WI), Microgram, Vol. IX, No. 4, (April, 1976)

23. Phencyclidines and Ketamine

23.1. Scope: Phencyclidine (PCP) and Ketamine are animal tranquilizers. These are frequently found in powder, crystal, or liquid form. Both have been found in tablets and capsules and in Marijuana cigarettes. Several analogs of PCP exist and have been found in casework.

23.2. Precautions/Limitations:

23.2.1. PCP does not visualize well under UV for Thin Layer Chromatography.

23.2.2. Several phencyclidine analogs exist.

23.2.3. Multiple peaks may be present in GC/MS analysis. These peaks may be from precursors or breakdown products of the phencyclidines. The Forensic Scientist should be aware of these substances and extend GC runs to allow for the parent compounds to elute from the GC column.

23.2.4. The same GC issues in 23.2.3 apply for GC-IR as well.

23.3. Related Information:

23.3.1. Appendix 1 – Forms and Worksheets
23.3.2. Appendix 2 – Abbreviations
23.3.3. Appendix 3 – Definitions
23.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
23.3.5. Other Test Methods
- **23.3.5.1.** General Drug Identification
- **23.3.5.2.** Color Tests
- **23.3.5.3.** UV
- **23.3.5.4.** TLC
- **23.3.5.5.** Separations
- **23.3.5.6.** FTIR
- **23.3.5.7.** GC/MS
- **23.3.5.8.** GC-IR

23.4. Instruments:

23.4.1. UV
23.4.2. FTIR
23.4.3. GC/MS
23.4.4. GC-IR

23.5. Reagents/Materials:

23.5.1. Color Test Reagents

23.5.2. TLC Solvent Systems
23.5.3. Methanol
23.5.4. $CHCl_3$

23.6. Hazards/Safety:

23.6.1. Inhalation/Exposure hazards: Ether (liquid form)
23.6.2. Chemical Hazard: Cyanide Precursor, Use of acids with PCP may potentially release cyanide gas.

23.7. Reference Materials/Controls/Calibration Checks:

23.7.1. Appropriate Reference Materials for Phencyclidine, Ketamine, or other drug of interest.

23.8. Procedures/Instructions:

23.8.1. Color Tests:
23.8.1.1. $Co(SCN)_2$ (See Reagent Preparation Guide)

23.8.2. Extractions:
23.8.2.1. PCP may be extracted with organic solvents from aqueous alkaline solutions.

23.8.2.2. PCP may be extracted from plant materials by washing the plant material with a suitable solvent (hexane, methanol, etc.) and filtered. It may be necessary to extract further to remove color from the sample.

23.8.2.3. Ketamine may be extracted with organic solvents from aqueous alkaline solutions.

23.8.3. TLC Systems: General Acid and Base systems are sufficient:
$MeOH:NH_4OH$ (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)
Oversprays: Ninhydrin (if desired), iodoplatinate.

23.8.4. FTIR, if possible. Extraction may be necessary.

23.8.5. GC/MS: PCP analogs can be separated at appropriate temperatures. Ketamine generally chromatographs well. A general temperature program may be appropriate. See GC/MS Test Method.

23.8.6. GC-IR: General temperature programs may be appropriate. See GC-IR Test Method.

23.9. Records: See General Drug Identification Test Method.

23.10. Interpretations of Results:

23.10.1. Color Tests:

23.10.1.1. $Co(SCN)_2$ turns blue with Phencyclidine, but this cannot be used as the only supporting test for confirmation due to known false positive reactions.

23.10.1.2. (Morris Test) Basified $Co(SCN)_2$ turns lavender with Ketamine HCl.

23.10.2. UV (in acid): strong UV absorbers.

23.10.2.1. PCP: triplet. See references for expected wavelengths..

23.10.2.2. Ketamine near triplet. See references for expected wavelengths.

23.10.3. TLC: Ninhydrin over-spray is good for detecting PCP and Ketamine.

23.10.4. FTIR: See FTIR Test Method

23.10.5. GC/MS: See GC/MS Test Method.

23.10.6. GC-IR: See GC-IR Test Method.

23.11. Report Writing: See General Drug Identification.

23.12. References:

23.12.1. Indiana Criminal Code

23.12.2. PCP: The Threat Remains, DEA Intelligence Division, Microgram, Vol. XXXVI, No.8, August 2003.

23.12.3. The Identification of N-ethyl-1-phenylcyclohexylamine Hydrochloride (Cyclohexamine), Barron, R.P. (DEA – Special Testing Laboratory), Sept.1973

23.12.4. 1-Pyrrolodinocyclohexane Carbonitrile and Intermediate to the Pyrrolidine Analog of Phencyclidine, Teets, Barbara S. (Virginia Department of General Services, Bureau of Forensic Sciences, Merrifield, Virginia), Microgram, Vol. XIX, No. 8., August 1986

23.12.5. 1-Piperidinocyclohexane Carbonitrile A Phencyclidine Precursor, Siefert, John. H. (Michigan State Police Crime Detection Laboratory, Madison Heights, MI), Microgram Vol. X, No. 7, July 1977.

23.12.6. Thiophene Analog of Phencyclidine, Alvarez, Jose (DEA Laboratory Notes), Microgram, Vol. X, No. 9, September 1977.

23.12.7. Thiophene Analog of PCP, Heagy, James (San Francisco Regional Laboratory, San Francisco, CA), November 1972.

23.12.8. Differentiation of PCP, TCP, and a Contaminating Precursor PCC, by Thin Layer Chromatography, Shulgin, Alexander.

23.12.9. Analysis and Identification of Phencyclidine Hydrochloride (PCP, Sernyl), DeZan, Paul and Bianchi, Robert (US Food and Drug Administration, New York)

23.12.10. PCP Purification, Huttsell , Fred L. (Indiana State Police Laboratory, Indianapolis, IN)

23.12.11. A Spectroscopic and Chromatographic Study of Phencyclidine (PCP) and Its Analogs, Rao, Soni, and Mullen (Baltimore Police Department, Baltimore, MD), Microgram, Vol. XIII, No. 4 , April 1980.

23.12.12. Purification and Identification of Phencyclidine, Johns, Susan Hart and Bubonic, John (Illinois Bureau of Identification, Perkin, IL), Microgram, Vol. X, No.7, July 1977.

23.12.13. Analysis and Identification of 1-[1-(2-thienyl) cyclohexyl]piperidine (TCP), Picard, David R. (Wisconsin Crime Laboratory Bureau, Madison, WI)

23.12.14. The Identification of a New Analog of PCP, 1-(1-phenylcyclohexyl)pyrrolidine (PCPy), Morris, Wayne (Florida Department of Criminal Law Enforcement, Jacksonville, FL), Microgram, Vol. X, No. 11, November 1977.

24. Clandestine Laboratory Sample Examinations

24.1. Scope: Samples from clandestine laboratory reaction mixtures require unique analysis and sampling procedures. Knowledge of procedures being utilized is important. Examination and identification of precursor compounds and finished product are necessary, as well as identification of intermediate products in some cases. Analysis and subsequent identification of inorganic compounds, including acids and bases, may require the transfer of certain items to the Microanalysis Unit.

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 primary product, precursors should be identified when present.

24.2. Precautions/Limitations: Items of evidence submitted from clandestine labs are often liquids containing volatile, flammable, and toxic chemicals as well as suspected drugs, volatile samples, complex media, intermediates, small amounts of materials, hazardous chemicals, and potential reactions.

24.3. Related Information:

- **24.3.1.** Appendix 1 –Forms and Worksheets
- **24.3.2.** Appendix 2 – Abbreviations
- **24.3.3.** Appendix 3 – Definitions
- **24.3.4.** Appendix 4 – Drug Unit Reagent Preparation Manual
- **24.3.5.** Other Test Methods
  - **24.3.5.1.** Phenethylamines
  - **24.3.5.2.** Methoxyamphetamines
  - **24.3.5.3.** Phencyclidines and Ketamine
  - **24.3.5.4.** General Drug Identification
  - **24.3.5.5.** Separations and Extractions
  - **24.3.5.6.** Evidence Handling
  - **24.3.5.7.** Sampling
  - **24.3.5.8.** UV
  - **24.3.5.9.** FTIR
  - **24.3.5.10.** GC/MS
  - **24.3.5.11.** GC-IR

24.4. Instruments:

- **24.4.1.** UV
- **24.4.2.** FTIR
- **24.4.3.** GC/MS
- **24.4.4.** GC-IR

24.5. Reagents/Materials:

24.5.1. Color Test Reagents
24.5.2. TLC Solvent Systems
24.5.3. Water finding paper
24.5.4. pH paper
24.5.5. Methanol
24.5.6. $CHCl_3$

24.6. Hazards/Safety: This type of evidence can pose significant health hazards that are not commonly encountered with routine controlled substance examinations. These hazards may include but are not limited to: corrosives, caustic materials, explosives, toxic gases, and flammable solvents.

Caution should be exercised when opening and examining evidence of this nature by utilizing appropriate personal protective equipment and sampling in a fume hood. Every effort should be made to prevent exposure to potentially hazardous materials. Special storage precautions may be necessary.

See General Drug Identification Test Method

24.7. Reference Materials/Controls/Calibration Checks:

24.7.1. Appropriate Reference Materials for drugs of interest.

24.8. Procedures/Instructions:

24.8.1. These items of evidence can consist of multiple layers of liquid. Determine if the liquids are aqueous or organic in nature. Check the pH of the aqueous layer prior to proceeding.

When the aqueous layer is acidic, then basic drugs will be in the aqueous layer and not in the organic layer. If the aqueous layer is basic, then basic drugs will be in the organic layer. The liquid layer suspected of containing the drug of interest shall be examined, and the two-layer liquids shall not routinely be examined as separate sub-items.

24.8.2. After sampling organic liquids from clandestine lab evidence, the Forensic Scientists should fume organic liquids with hydrochloric acid to convert free base amines (amphetamine and methamphetamine) to the stable hydrochloride salt. Organic liquids should then be evaporated in a fume hood prior to any examinations. This process is to minimize the hazards to laboratory personnel from ether and other solvents from clandestine labs.

24.8.3. After evaporation of organic liquids, the examination of the resulting residue should use the same procedures as “general unknown” solid or residue drug evidence.

24.8.4. Due to the nature of clandestine labs and the need to identify precursors, Forensic Scientists may be required to confirm the identity of Ephedrine or Pseudoephedrine in clan lab samples. It is sufficient to identify items containing either Ephedrine or Pseudoephedrine, without the requirement of purification for specific drug identification by infrared spectroscopy. Consideration should be made for weight thresholds for precursors. If methamphetamine and ephedrine/pseudoephedrine are present in a mixture, it is not necessary to confirm or indicate the ephedrine/pseudoephedrine.

24.9. Records: See General Drug Identification.

24.9.1. Results of pH and water finding paper testing on the sample, as well as the dates of these tests, shall be documented in the analytical notes.

24.9.2. Extraction and sample preparation procedures used shall be documented in the analytical notes.

24.10. Interpretations of Results: See General Drug Identification Test Method

24.11. Report Writing: See General Drug Identification Test Method

24.11.1. Results in the report can be stated as "found to contain Ephedrine and/or Pseudoephedrine" if the specific drug has not been identified.

24.12. References:

24.12.1. Impurities In Methamphetamine Manufactured From Over-The-Counter Pseudoephedrine Tablet Preparation, Melgoza, Lynn (California Department of Justice, Riverside, CA) Journal of the Clandestine Laboratory Investigating Chemists Association, Vol. 9, No. 2-3, April-July, 1999.

24.12.2. A Field Test for Phenyl-2-Propanone, Kiser, Wilmer (DEA, Southeast Laboratory), Microgram Vol. XV, No. 9 (August, 1982).

24.12.3. Some Information Regarding Phenyl-2-Propanone, Dal Cason, Terry A. (DEA Central Laboratory, Chicago, IL), Journal of The Clandestine Laboratory Investigating Chemists Association (CLIC), Vol. 4, No. 1, January 1994.

24.12.4. Isolation and Identification of Drugs, Clarke, E.G.C., The Pharmaceutical Press, London. 1969.

24.12.5. Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

24.12.6. Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

24.12.7. Basic Training Program for Forensic Drug Chemists, Canaff, BNDD

24.12.8. Analytical Profiles of Amphetamines and Related Phenethylamines, CND Analytical

24.12.9. Forensic Investigation of Clandestine Laboratories, Donnell R. Christian, CRC Press

24.12.10. CLIC Journal (past issues)

25. Opiates

25.1. Scope: Members of this drug class are naturally occurring alkaloids of the Papaver somniferum poppy, and their semi-synthetic derivatives. These include, but are not limited to, Morphine, Heroin, Codeine, Hydrocodone, Oxycodone, Dextropropoxyphene, Dextromethorphan and Methadone.

25.2. Precautions/Limitations:

25.2.1. While most members of this group are easily extracted using organic solvents from aqueous alkaline solutions, Morphine extraction is pH sensitive and requires a weak basic solution.

25.2.2. It is necessary to determine the optical isomer of Propoxyphene and Methorphan, when practical, since one isomer of these substances is not controlled. (See Polarimetry Test Methods)

25.2.3. The UV absorbances of some members of this group resemble Phenethylamines more than the rest of the opiates.

25.2.4. Certain members of this group appear in more than one controlled substance schedule. The presence of other substances may dictate the scheduling of the preparation.

25.2.5. Opium is a naturally occurring material that contains a variety of alkaloids.

25.2.6. Special considerations may be needed when aggregate weight thresholds apply. For GC/MS, in the TIC, if the Heroin and/or Fentanyl peak is less than 10% of the peak with the highest abundance, no further attempt to confirm Heroin and/or Fentanyl needs to be made. If it is greater than 10%, at least one additional attempt should be made to confirm Heroin and/or Fentanyl, depending on the complexity of the mixture.

25.2.7. Fentanyl and NPS can be in small quantities in relation to cutting agents due to their strength. Special consideration should be taken in an attempt to identify low level Fentanyl and NPS.

25.2.8. Tianeptine undergoes thermal degradation in the GC/MS and the resulting mass spectrum will be that of a breakdown product. Analysis requires esterification or derivatization.

25.3. Related Information:

25.3.1. Appendix 1 – Forms and Worksheets
25.3.2. Appendix 2 – Abbreviations
25.3.3. Appendix 3 – Definitions
25.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
25.3.5. Other Test Methods

25.3.5.1. General Drug Identification
25.3.5.2. Color Tests
25.3.5.3. UV
25.3.5.4. TLC
25.3.5.5. Separations
25.3.5.6. FTIR
25.3.5.7. GC/MS
25.3.5.8. GC-IR
25.3.5.9. Polarimetry

25.4. Instruments:

25.4.1. UV
25.4.2. FTIR
25.4.3. GC/MS
25.4.4. GC-IR
25.4.5. Polarimeter

25.5. Reagents/Materials:

25.5.1. Color Test Reagents
25.5.2. See Other Test Methods

25.6. Hazards/Safety: See individual drug and chemical SDS.

25.7. Reference Materials/Controls/Calibration Checks:

25.7.1. Appropriate Reference materials for drugs of interest.

25.8. Procedures/Instructions:

25.8.1. Extraction: Most opiates can be extracted from aqueous alkaline solutions with organic solvents.

25.8.1.1. Morphine and some derivatives of Morphine are an exception. It is pH sensitive and its sulfate form is not soluble in $CHCl_3$. Morphine can also be run direct in MeOH. See Separation and Extractions Test Method and Drug Unit Resource Manual(s).

25.8.1.2. Heroin can be extracted with $CHCl_3$ from 1 N HCl or from aqueous alkaline solutions.

25.8.2. Optical Isomer Determination: The optical isomer of Propoxyphene should be determined. The optical isomer of Methorphan, or other opiate, should be determined, if necessary. (See Polarimetry Test Methods).

25.9. Records: See Other Test Methods.

25.10. Interpretations of Results:

25.10.1. Color Tests:

25.10.1.1. Marquis: turns purple for opiates

25.10.1.2. Mecke's: yellow green- turquoise for most opiates

25.10.2. UV: Propoxyphene resembles the UV spectra of phenethylamines. See references for expected wavelengths.

25.10.3. TLC: General Acid and base systems

25.10.3.1. MeOH: NH_4OH (100:1.5)

25.10.3.2. $CHCl_3$: MeOH: HOAc (75:20:5)

25.10.3.3. Cyclohexane: Toluene: Diethylamine (75:15:10)

25.10.3.4. Over-sprays: Ninhydrin, Iodoplatinate, Potassium Permanganate

25.10.4. FTIR: See FTIR Test Method

25.10.5. GC/MS: See GC/MS Test Method

25.10.6. GC-IR: See GC-IR Test Method

25.10.7. Polarimetry: See Polarimetry Test Method

25.11. Report Writing:

25.11.1. The presence of non-controlled substances in a pharmaceutical preparation that contains a controlled substance may change the schedule of the controlled substance. Examples are Dextropropoxyphene and Acetaminophen, and Codeine with Aspirin or Acetaminophen. In this situation, both drugs shall be listed in the report. Non-

controlled substances can be reported as indications and are not required to be confirmed.

25.11.2. Optical Isomer Determination: See Polarimetry

25.11.3. All others, See General Drug Identification Test Method

25.12. References:

25.12.1. Identification of Dextropropoxyphene and its Diastereomers, Newby, N.R and Hughes, Journal of Forensic Sciences, JFSCA, Vol. 25, No. 3, July, 1980, pp.646-654.

25.12.2. Extraction of Dextropropoxyphene from Pharmaceutical Mixtures, Gundy, E., Kemppainen, A. (Michigan State Police), Microgram, Vol. XII, No.6, June 1979.

25.12.3. Determination of Codeine in Cough Syrups, Van Sickle, Department of Justice Drug Enforcement Administration, Chicago, IL

25.12.4. Chromatographic and Electrochemical Investigations of Codeine, Meinsma and Kissinger, Purdue University, 1985.

25.12.5. Quantitation of Codeine in Cough Syrup, Netsch, S. (Indiana State Police), January 1986.

25.12.6. The Synthetic Drug 3-methylfentanyl: Identification and Quantitation of Powdered Samples, Esposito Samples, Esposito and Winek, Journal of Forensic Sciences, Vol 36, No 1, Jan 1991, p86-92

25.12.7. BNDD Analytical Manual: Analysis of Drugs (initial issuance), United States Department of Justice Bureau of Narcotics and Dangerous Drugs.

26. Barbiturates and Hypnotics

26.1. Scope: Barbiturates are substituted derivatives of Barbituric acid. Examples include, but are not limited to Barbital, Butalbital, Pentobarbital, Secobarbital, Amobarbital, Butabarbital and Phenobarbital. These substances are most commonly found in pharmaceutical preparations. Hypnotics generally include a variety of substances such as Methaqualone, Gamma Hydroxybutyric Acid (GHB), Choral Hydrate and Ethchlorvynol.

26.2. Precautions and Limitations:

26.2.1. Reproducibility of infrared spectral data may be difficult due to the presence of multiple crystalline forms (polymorphism).

26.2.2. Barbiturates generally do not absorb during UV analysis when run in aqueous acid solutions. UV analysis of barbiturates should be run in aqueous alkaline solutions.

26.2.3. GHB and its lactone, Gamma Hydroxy butyrolactone (GBL) exist in equilibrium with each other. It is very easy to convert one to the other depending on the pH of the sample or application of heat.

26.2.4. GHB is very hygroscopic and may need to be carefully dried prior to IR analysis.

26.2.5. Some drugs may be suspended in oils, or other viscous liquid. Extraction is necessary for analysis.

26.2.6. It may be necessary to extract the sample before proceeding with instrumental analysis.

26.3. Related Information:

26.3.1. Appendix 1 – Forms and Worksheets
26.3.2. Appendix 2 – Abbreviations
26.3.3. Appendix 3 – Definitions
26.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
26.3.5. Other Test Methods
- **26.3.5.1.** Color Tests
- **26.3.5.2.** UV
- **26.3.5.3.** TLC
- **26.3.5.4.** FTIR
- **26.3.5.5.** GC/MS
- **26.3.5.6.** GC-IR
- **26.3.5.7.** General Drug Identification
- **26.3.5.8.** Separation and Extractions

26.4. Instruments:

26.4.1. UV
26.4.2. FTIR
26.4.3. GC/MS
26.4.4. GC-IR

26.5. Reagents/Materials:

26.5.1. Color Test Reagents: See Color Test Method
26.5.2. TLC Systems: See TLC Test Methods

26.6. Hazards/Safety: See SDS for individual drugs and chemicals.

26.7. Reference Materials/Controls/Calibration Checks:

26.7.1. Appropriate reference materials for drug(s) of interest.

26.8. Procedures/Instructions: See General Drug Identification

26.8.1. Extraction: Barbiturates are extracted from either acidic or weak basic aqueous solutions with organic solvents.

26.8.2. Complex mixtures suspected to contain GHB may need to be derivatized to preserve the form of the substance.

26.8.3. GHB Derivatization Procedure:

1. Sample extraction: Add HCl to pH ~2;

2. Add NaCl to form GHB sodium salt if GHB present.

3. Extract with Ethyl acetate and evaporate to dryness.

4. Reconstitute dried extract with a suitable solvent (do not use MeOH)

5. Add 10 drops 99%BSTFA w/1%TMCS to 10 drops of extracted sample in a suitable solvent.

6. Cork and parafilm test tube, heat to 60 °C for 10 minutes.

7. Inject onto GC/MS (or GC-IR) using a low starting temperature program.

26.8.4. Exempt Preparations: See General Drug Identification 4.10.10.

26.8.5. pH of GHB should be neutral, approximately pH 6-7

26.8.6. Color Tests:

26.8.6.1. Barbiturates: Dille-Koppanyi

26.8.6.2. GHB: 5% Ferric Chloride or 1% Cobalt Nitrate

26.8.7. UV:

26.8.7.1. Barbiturates: 0.45 N NaOH.

26.8.7.2. GHB: MeOH

26.8.7.3. Methaqualone: 0.5 N H_2SO4

26.8.8. TLC Systems:

26.8.8.1. Barbiturates: $CHCl_3$:Acetone (9:1); Over-sprays: saturated mercurous nitrate, potassium permanganate ($KMnO_4$), Diphenylcarbazone

26.8.8.2. GHB: Water: MeOH (1:1); Over-spray with 5% Ferric Chloride

26.8.9. FTIR: Difficulties with polymorphism of barbiturates can be circumvented by subjecting the drug reference material and the unknown sample to the same extraction procedures.

The hygroscopic nature of GHB may make IR analysis difficult. Dry the sample with low heat to drive off residual water.

26.8.10. GC/MS: GHB converts to the lactone in the injection port. Derivatization with BSTFA or BSTFA-TMCS may be required to confirm the presence of GHB.

26.8.11. GC-IR: This technique has the same limitations as with GC/MS.

26.9. Records: See General Drug Identification.

26.10. Interpretations of Results:

26.10.1. Color Tests:

Dille-Koppanyi turns purple/violet in the presence of barbiturates.

The Ferric Chloride test turns rust-red in the presence of GHB. GBL and the butanediols do not react to this test.

26.10.2. UV:

26.10.2.1. Barbiturates: (in base) See references for wavelengths.

26.10.2.2. GHB: (in MeOH) See references for wavelengths

26.10.2.3. GBL: (in MeOH) See references for wavelengths

26.10.3. TLC Systems: See TLC Test Method/ Reagent Prep Manual

26.10.4. Over-sprays:

26.10.4.1. Barbiturates:
$KMnO_4$ – reacts with barbiturates, give yellow spots on a purple background.

$HgNO_3$ – spray heavily to give light spots on an off-white background

26.10.4.2. GHB: Over-spray with 5% Ferric Chloride

26.10.5. FTIR may require extraction to be performed prior to this type of analysis. Acceptable FTIR spectral comparisons may be difficult due to the polymorphism of barbiturate samples.

Excess water may affect GHB FTIR spectral comparisons and may necessitate drying the sample under low heat prior to performing this type of analysis. See FTIR Test Method.

26.10.6. Analysis by GC/MS and GC-IR may require extraction. Derivatization may be necessary for GHB samples. See GC/MS and /or GC-IR Test Methods.

26.11. Report Writing: See General Drug Identification Reporting – 4.11

26.11.1. It may be necessary to use a combination of statements to accurately describe the analysis results for exempt preparations.

26.12. References:

26.12.1. Analytical Profiles of Barbiturates and Other Depressants, CND Analytical, Inc., Auburn , AL 1991

26.12.2. Separation and Identification of the Components of a Common Barbituric Acid Preparation, Stall, Walter (US Army Laboratory – San Francisco, CA); Microgram, Vol. XI, No. 12, December, 1978.

26.12.3. A Scheme for the Separation of Sandoptal (Butalbital) from “Fiorinal”, Krautman, K. and Nanneman, D. (Missouri State Highway Patrol); Microgram, Vol. XIII, No. 12, December, 1980.

26.12.4. Validation of GHB Color Test Method, Nickless, R. (Indiana State Police), 2004.

26.12.5. Purification and Identification of Clandestinely Synthesized Mecloqualone, Dal Cason, T., Microgram, Vol. IX, No. 12, December, 1976.

26.12.6. GC-MS Identification of Methaqualone, Nowicki, H., Microgram, Vol. IX, No. 9, September, 1976.

26.12.7. A Color Test for the Detection of Methaqualone, Medina, F. and Goldson, B., Microgram, Vol. XIV, No. 4, April, 1981.

27. Benzodiazepines

27.1. Scope: Benzodiazepines are usually found in tablet or capsule preparations that have been diverted from legitimate sources. Analysis of a marked dosage unit generally consists of a reference identification and subsequent confirmation of the active ingredient(s).

27.2. Precautions/Limitations:

27.2.1. Presumptive color (spot) tests do not react with benzodiazepines.

27.2.2. Multiple TLC systems are suggested due to the variety of benzodiazepines.

27.2.3. There is not a specific visualization reagent for benzodiazepines.

27.2.4. Clorazepate decarboxylizes to Desmethyldiazepam in the GC/MS. FTIR is the recommended method of confirmation, if possible.

27.2.5. Clonazepam can break down to 7-aminoclonazepam and may possibly co-elute as indicated by an elevated 256 m/z ion. Steps should be taken to address this including different temperature programs, different columns, and changing the liner.

27.2.6. Counterfeit tablets marked as containing benzodiazepines have been encountered in casework.

27.3. Related Information:

27.3.1. Appendix 1 – Forms and Worksheets
27.3.2. Appendix 2 – Abbreviations
27.3.3. Appendix 3 – Definitions
27.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
27.3.5. Other Test Methods
- **27.3.5.1.** General Drug Identification
- **27.3.5.2.** UV
- **27.3.5.3.** TLC
- **27.3.5.4.** FTIR
- **27.3.5.5.** GC/MS
- **27.3.5.6.** GC-IR
- **27.3.5.7.** Separations

27.4. Instruments:

27.4.1. UV
27.4.2. FTIR
27.4.3. GC/MS
27.4.4. GC-IR

27.5. Reagents/Materials: See Other Test Methods

27.6. Hazards/Safety: See Other Test Methods and SDS.

27.7. Reference Materials/Controls/Calibration Checks:

27.7.1. Appropriate reference materials for drug(s) of interest.

27.8. Procedures/Instructions: See General Drug Identification Test Method.

27.8.1. Extraction: Most benzodiazepines are soluble in Methanol. However, some extractions work better using $CHCl_3$. Dry extractions with $CHCl_3$ generally work well.

27.8.2. Clorazepate: Extraction options will yield the monopotassium form of the drug or Desmethyldiazepam. FTIR is recommended for confirmation.

27.8.3. Recommended TLC Systems:

MeOH: NH_4OH (100:1.5)
$CHCl_3$: Acetone (80:20) or (9:1)
Cyclohexane: Toluene: Diethylamine (75:15:10)

Over-sprays: Iodoplatinate overspray

27.9. Records: See General Drug Identification Test Method.

27.10. Interpretations of Results:

27.10.1. General Benzodiazepines: See General Drug Identification Test Method.

27.10.2. Clorazepate:

27.10.2.1. FTIR analysis can be used to confirm the presence of the monopotassium salt, rather than the original dipotassium salt.

27.10.2.2. GC/MS will give the spectrum of Desmethyldiazepam.

27.10.2.3. GC-IR will give a Desmethyldiazepam IR spectrum.

27.10.2.4. Markings can be used as an indicator of the drug contained in a capsule or tablet.

27.10.3. Ketazolam:

27.10.3.1. Ketazolam degrades to Diazepam in the GC inlet.

27.10.3.2. Ketazolam and Diazepam can be distinguished by UV according to reference literature.

27.10.3.3. If a sample is not a legitimate pharmaceutical preparation and the data indicates the presence of Diazepam, additional testing shall be performed.

27.11. Report Writing: See General Drug Identification Test Method

27.11.1. If FTIR is the confirmatory technique for identification of a salt form of Clorazepate, it shall be reported as "found to contain Clorazepate, a controlled substance".

27.11.2. If FTIR analysis is sufficient for either an indication or identification, and GC/MS is used for confirmation resulting in Desmethyldiazepam, the item may be reported as "Clorazepate, a controlled substance".

27.11.3. If the item is a marked tablet or capsule, FTIR analysis is not possible or is of insufficient quality for identification, and GC/MS is used for confirmation resulting in Desmethyldiazepam, it may be reported as "Clorazepate, a controlled substance."

Or

Either situation in 27.11.2 and 27.11.3 may also be reported as "Markings and examination were consistent with a preparation containing Clorazepate, a controlled substance."

27.12. References:

27.12.1. Analytical Profiles of the Benzodiazepines, CND Analytical, Auburn, AL, 1989.

27.12.2. The Analysis of Controlled Substances, Cole, Michael D., Wiley, 2003.

27.12.3. Identification of Some Interferences in the Analysis of Clorazepate, Suzuki, E.M. and Gresham, W.R., JFS Vol 28., No. 3, July 1983, pp 655-682.

27.12.4. Isolation and Identification of Clorazepate, Suzuki, and Gresham (Washington State Patrol, Crime Laboratory Division, Seattle, WA), Microgram, Vol. XVII, No. 4, April 1984.

27.12.5. The Extraction and Analysis of Salts of Clorazepate, Siefert, John (Michigan Department of Public Health, Warren, MI), Microgram, Vol. X, No. 10, October 1977.

27.12.6. Extraction and Identification of Clorazepate Monopotassium from Clorazepate Dipotassium, Smith, George E. (Indiana State Police Laboratory, Indianapolis, IN)

27.12.7. Mass Spectra of Benzodiazepines, Huttsell, Fred L. (Memo from J. Forbes), July 1984.

28. Anabolic Steroids

28.1. Scope: "Anabolic steroids" is the name given to a series of natural and synthetic substances whose primary effects are to promote skeletal muscle growth. Most of these substances also have varying "androgenic" effects; which increase male sexual characteristics. Anabolic steroids are controlled within Schedule III of the Federal Drug Code in the United States and are defined and listed as "any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth". The Indiana Controlled Substance schedule III also includes Anabolic Steroids (as defined in 21 U.S.C.802 (41)(A) and 21 U.S.C. 802(41)(B).

28.2. Precautions/Limitations:

28.2.1. Many are manufactured in foreign countries with minimal quality control and may not contain the substances listed as ingredients on the label.

28.2.2. Complex mixtures are common and present difficulties in separation.

28.2.3. Steroids for intramuscular injection are frequently found in oils, such as cottonseed, sesame, or soybean oils, and need to be extracted prior to analysis to avoid contaminating instrumentation.

28.2.4. Steroids have many synonyms and confusing nomenclature.

28.2.5. GC/MS analysis time may be lengthy due to the large size of the molecules. It is not uncommon to have a 30-minute GC run.

28.2.6. GC-IR analysis has the same limitations as GC/MS.

28.2.7. Oxymetholone reacts with Methanol and it is recommended to be run in $CHCl_3$ for the correct GC/MS data to be obtained.

28.3. Related Information:

28.3.1. Appendix 1 – Forms and Worksheets
28.3.2. Appendix 2 – Abbreviations
28.3.3. Appendix 3 – Definitions
28.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
28.3.5. Other Test Methods
- **28.3.5.1.** General Drug Identification
- **28.3.5.2.** Separation and Extractions
- **28.3.5.3.** Color Tests
- **28.3.5.4.** UV
- **28.3.5.5.** TLC
- **28.3.5.6.** FTIR

28.3.5.7. GC/MS
28.3.5.8. GC-IR

28.4. Instruments:

28.4.1. UV
28.4.2. FTIR
28.4.3. GC/MS
28.4.4. GC-IR

28.5. Reagents/Materials: See Other Test Methods

28.6. Hazards/Safety: See Other Test Methods

28.7. Reference Materials/Controls/Calibration Checks:

28.7.1. Appropriate reference materials for drug(s) of interest.

28.8. Procedures/Instructions:

28.8.1. Suggested Extraction:

28.8.1.1. Tablets: generally, a filtered methanol extract will suffice.

28.8.1.2. Injectables: 1:1 ml mix with MeOH to the sample. Vortex. If top layer is not clear, cool in freezer for one hour and filter, while cold, into a clear beaker. Concentrate sample and perform testing procedures.

A methanol: distilled or deionized water (9:1) may work as well.

28.8.1.3. Immiscible Oils: add sodium bicarbonate/distilled or deionized H_2O to pH of approximately 8. Extract with $CHCl_3$, evaporate and run on GC/MS.

28.8.2. Thin Layer Chromatography:

28.8.2.1. Chloroform: Ethyl Acetate (40:10), UV light box, with EtOH: H_2SO_4 (4:1) over spray

28.8.2.2. Chloroform: Acetone (9:1), UV light box, with Iodoplatinate, then $KMnO_4$; or EtOH: H_2SO_4 (4:1) over spray.

28.8.3. UV – See UV Test Method

28.8.4. FTIR – See FTIR Test Method

28.8.5. GC/MS – Long, high temperature programs, See GC/MS Test Method.

28.8.6. GC-IR – Long, high temperature programs would be necessary. See GC-IR Test Method.

28.9. Records: See General Drug Identification Method.

28.10. Interpretations of Results:

28.10.1. TLC: steroids show up well under short wave UV light.

28.10.2. UV spectrophotometry: See references for wavelengths.

28.10.3. FTIR: See FTIR Test Method

28.10.4. GC/MS: See GC/MS Test Method

28.10.5. GC-IR: See GC-IR Test Method

28.11. Report Writing: See General Drug Identification Test Method.

28.12. References:

28.12.1. 21 Code of Federal Regulations – Chapter 11 – Food and Drugs, National Archives and Records Administration, Section 1308.02 Definitions, April 1, 1994.

28.12.2. Indiana Criminal Code 35-48-2-8(f).

28.12.3. United States Criminal Code, 21 USC 802(41)(A) and (41)(B).

28.12.4. Analysis of Anabolic Steroids, Koverman, Gary (Colorado Bureau of Investigation), Microgram, Vol. XXVI, No. 11, November 1993.

28.12.5. Screening of Steroids by Thin Layer Chromatography, Morley Chromatography, Morley, M. and Matkovich, C. (DEA Mid-Atlantic Laboratory)

28.12.6. Analytical Profiles of the Anabolic Steroids and Related Substances (Vol. I), CND Analytical, Inc. Auburn, AL, 1989.

28.12.7. Analytical Profiles of the Anabolic Steroids and Related Substances (Vol. II), CND Analytical, Inc. Auburn, AL, 1991.

28.12.8. TLC Screen for Anabolic Steroids, FDA San Francisco Laboratory.

28.12.9. Black Market in Anabolic Steroids – Analysis of Illegally Distributed Products, Musshoff, Daldrup and Ritsch, Journal of Forensic Sciences 1997; 42(6):1119-1125.

29. Proficiency Testing

29.1. Scope: Each Forensic Scientist conducting analysis in the Drug Unit shall participate in the Forensic Services Division's (FSD) proficiency testing program. Participation, evaluation, documentation, and any necessary corrective actions shall comply with procedures listed in the FSD Quality Assurance Manual. Procedures used for analysis of proficiency samples shall be similar to the procedures used for casework analysis and shall follow Drug Unit Test Methods. The following are guidelines for compliance with the proficiency testing program.

29.2. Precautions/Limitations: N/A

29.3. Related Information:

29.3.1. Appendix 1 – Forms and Worksheets
29.3.2. Appendix 2 – Abbreviations
29.3.3. Appendix 3 – Definitions
29.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
29.3.5. Other Test Methods

29.4. Instruments: N/A

29.5. Reagents/Materials:

29.5.1. External Proficiency Samples

29.5.2. Internal Proficiency Samples

29.6. Hazards/Safety: N/A

29.7. Reference Materials/Controls/Calibration Checks:

29.7.1. External Proficiency Testing: This sample shall be obtained from an approved test provider.

29.7.2. Open Internal Proficiency Testing: This sample can be a proficiency sample prepared and distributed within the FSD.

All internal proficiency samples shall be prepared using known primary or secondary drug reference materials and may be mixed with various diluents and/or in various combinations of drugs to simulate street-type drug items.

29.7.3. Blind Proficiency Testing: If blind proficiency testing is to be conducted, a Drug Unit Supervisor(s) or the Chemistry Section Supervisor shall prepare the blind proficiency samples using known primary or secondary drug reference materials mixed with various diluents and in various combinations of drugs to simulate street-type drug items.

29.8. Procedures/Instructions:

29.8.1. Proficiency Testing:

Forensic Scientists in the Drug Unit shall participate in one open proficiency test in drug analysis annually. Exceptions to this procedure include trainees released for casework after all proficiencies have been distributed and Forensic Scientists who are unavailable during the proficiency timeframe.

The Drug Unit Supervisors shall assign the annual external proficiency sample to a Forensic Scientist at each Regional Laboratory. The Drug Unit Supervisors shall prepare and distribute internal proficiency samples to Forensic Scientists within their respective Regional Laboratories as deemed necessary.

The Forensic Scientists are to complete the examination and forward the results, all notes and documentation to a Drug Unit Supervisor prior to the completion deadline.

29.8.2. Blind Internal Proficiency Testing:

Each Forensic Scientist conducting drug analysis in the Drug Unit may be assigned drug items for blind proficiency testing.

Blind proficiency samples shall be submitted for analysis without the knowledge of the Forensic Scientists, Evidence Specialists, or Laboratory Managers at the respective Regional Laboratory. Blind proficiency samples shall be submitted as normal drug cases by police agencies, and shall be assigned to Forensic Scientists for analysis by the Drug Unit Supervisors.

29.9. Records:

29.9.1. External and Internal Proficiencies: All notes, documentation, and results are to be returned to the Drug Unit Supervisor by the assigned deadline. The Quality Assurance Manager is responsible to ensure the completed necessary documentation has been submitted to the external vendor for evaluation.

29.9.2. Blind Proficiency: Since Blind proficiency tests are treated as regular casework, records shall be maintained in the laboratory case file as if it were a normal case.

29.9.3. A Proficiency Test Log shall be completed upon evaluation of the test results by the Drug Unit Supervisor responsible for administering the proficiency. The appropriate Forensic Scientist shall be notified of the results as per the FSD Quality Assurance Manual.

29.10. Interpretations of Results:

29.10.1. External Proficiency: A Drug Unit Supervisor shall review and evaluate the analysis by comparing to the manufacturer's report when it becomes available.

29.10.2. Open Internal Proficiency: A Drug Unit Supervisor shall review and evaluate the analysis of the known material in the sample(s).

29.10.3. Blind Proficiency: A Drug Unit Supervisor shall review and evaluate the analysis of the known material in the sample(s).

29.11. References:

29.11.1. Forensic Services Quality Assurance Manual
29.11.2. SWGDRUG Guidelines

30. Drug Reference Materials

30.1. Scope: This Test Method is intended as a guide to the proper acquisition, verification, use, and storage of Drug Reference Materials (formerly known as Drug Standards) used for drug identification.

30.2. Precautions/Limitations:

30.2.1. Reference Materials may not be commercially available for comparison.

30.2.2. Analytical data may not be available for verification or authentication of identity.

30.3. Related Information:

30.3.1. Appendix 1 – Forms and Worksheets
30.3.2. Appendix 2 – Abbreviations
30.3.3. Appendix 3 – Definitions
30.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
30.3.5. Other Test Methods

30.4. Instruments:

30.4.1. UV
30.4.2. FTIR
30.4.3. GC/MS
30.4.4. GC-IR
30.4.5. Polarimetry

30.5. Reagents/Materials: See Other Test Methods.

30.6. Hazards/Safety: See SDS for individual Reference Materials and chemicals.

30.7. Reference Materials/Controls/Calibration Checks:

30.7.1. Drug Reference Materials: All Drug Reference materials shall be identified by a source and lot number, and/or other assigned identifier.

30.7.2. Drug Reference Material Libraries: All reference collections of drug or other materials used for identification purposes, comparison and/or interpretation shall be documented, uniquely identified, and controlled. This includes, but is not limited to, user generated libraries, purchased data libraries or libraries obtained from reputable sources.

30.7.3. All Drug Reference Materials shall be secured in a locked cabinet, refrigerator, or freezer accessible only to Drug Unit Forensic Scientists and Unit Supervisors assigned

to that Regional Laboratory. It is the responsibility of the Drug Unit personnel to maintain the security and integrity of the Drug Reference Materials.

30.7.3.1. If a refrigerator or freezer is not available in the Drug Unit, the Drug Reference Materials shall be stored in a Regional Laboratory's evidence storage refrigerator or freezer.

30.7.3.1.1. The Forensic Scientist shall place the Drug Reference Materials in an appropriate container and seal it with initials on the seal(s), in such a way that it cannot be opened without obvious signs of tampering. The container shall be clearly labeled as Drug Reference Materials and that it is not evidence.

30.7.3.1.2. The Forensic Scientist shall transfer the sealed and labeled container to an Evidence Specialist, who shall place it in an appropriate Regional Laboratory's refrigerator or freezer storage location.

30.7.3.1.3. When Drug Reference Materials are needed from a Regional Laboratory's evidence storage refrigerator or freezer, the Forensic Scientists shall request an Evidence Specialist to obtain the sealed container for them.

30.7.3.1.4. Only a Forensic Scientist shall open the sealed containers with the Drug Reference Materials.

30.7.4. Drug Reference Material Transfers Between Laboratories:

30.7.4.1. The package shall be properly sealed.

30.7.4.2. The package shall be transferred from one Regional Laboratory to another via an ISP Forensic Services Division employee, or through a commercial delivery service with traceable shipping.

30.7.4.3. If the substance is controlled in Federal Schedules I or II, a DEA 222 form shall be completed for the transfer.

30.7.4.4. If the substance is controlled in Federal Schedules III, IV, V or by the State of Indiana, and/or is not a controlled substance, the Indiana State Police Drug Transfer form shall be completed.

30.7.4.5. If the transfer involves non-Drug Unit personnel, then a Drug Transfer form shall be completed and uploaded onto SharePoint. The completed form shall be uploaded to SharePoint by the final receiving lab. The files shall

be named using the following nomenclature: Originating Lab_to_Destination Lab_Date. Example: FWRL_to_IRL_9-24-17

30.8. Procedures/Instructions:

30.8.1. All Drug Reference Materials shall be verified prior to use in casework.

30.8.1.1. It is not necessary to reverify a Reference Material if it has been verified at a different Regional Laboratory. A copy of the Reference Material verification paperwork shall be maintained at both labs or electronically on Cheminventory.

30.8.2. Multiple containers of a Drug Reference Material that have the same lot number may share a Reference Material Testing Record regardless of when they were received by the Regional Laboratory. At least one container shall be verified prior to use in casework.

30.8.2.1. Each vial needs to be uniquely identified even if the vials are intended to be transferred to a different Regional Laboratory (A, B, C or 1, 2, 3).

30.8.2.2. Data generated to verify or re-verify the reference material needs to specify which vial was used (A, B, C or 1, 2, 3).

30.8.2.3. A Reference Material Testing Record does not need to be completed for each individual vial. The form is used to document the verification of the specific lot number.

30.8.2.4. Dates of receipt need to be documented on both the Reference Material Testing Record and the vials.

30.8.3. Expired Drug Reference Materials:

30.8.3.1. An expired reference material can still be used if verified prior to use. The new expiration date shall be two years from the date of verification. The documented reverification date refers to the date the reference material was run.

30.8.3.2. Recommended retest dates that appear on the vendor's Certificate of Analysis shall be treated as expiration dates.

30.8.3.3. If it is discovered that an expired Reference Material has been used in casework, the Forensic Scientist shall immediately notify a Drug Unit Supervisor and reverify the Reference Material. The Drug Unit Supervisor shall notify the Chemistry Section Supervisor and the Accreditation and Quality Assurance Manager.

30.8.4. A Drug Reference Material Testing Record shall be initiated for each Reference Material when it has been received by the Regional Laboratory.

30.8.4.1. An additional Forensic Scientist shall review the initial verification data, and initial both the printout of the data and the Testing Record.

30.8.5. It is only necessary to verify a reference material one time on one instrument. It is not necessary to verify or document the verification of Reference Materials in the Reference Material Testing Record every time they are used in an analytical procedure.

30.8.6. Access to the Drug Reference Materials shall be restricted to the members of each respective Regional Laboratory Drug Unit.

30.8.7. All verifications or authentications shall be made by spectral comparison to a known literature reference in the following order:

30.8.7.1. Peer reviewed articles, libraries, and data (SWGDRUG monographs and libraries, Forendex, etc.).

30.8.7.2. Manufacturers data (ex. Cayman spectral data)

30.8.7.3. Data from other accredited laboratories with sources and lot numbers

30.8.7.4. Data from other ISP Regional Laboratories (requires Drug Unit Supervisor approval).

Whenever possible, the literature source should have data from traceable materials and the source and lot number of the reference material being verified should be different from that of the literature source.

30.8.8. Comparisons of mass spectral or infrared data shall constitute the minimum requirements for verification of primary Reference Materials.

30.8.9. Secondary Reference Materials shall be authenticated by a relevant preliminary test and confirmed by either GC/MS or FTIR at a minimum.

30.8.10. Primary Reference Materials are preferred for case material identification when available. In the absence of a primary Reference Material, secondary Reference Materials may be used for identification purposes.

30.8.11. Spectral data may be entered into the user generated libraries after verification or authentication.

30.8.12. When a Drug Reference Material has been consumed, the bottle shall be discarded after a notation has been made on the Reference Material Testing Record. The procedure shall be observed by an additional Forensic Scientist. Both Forensic Scientists shall initial the Reference Material Testing Record once the bottle has been discarded. If sub-vials still exist for TLC, GC-RT, or other testing, then a notation shall be placed on the Reference Material Testing Record. The sub-vials are ok to use until the next expiration date (if one exists) or until they start to show signs of breakdown. If a sub-vial is still in use, then the Reference Material shall not be "archived/disposed" in ChemInventory until all sub-vials are no longer in use.

30.8.13. When a Drug Reference Material shows signs of breakdown that inhibit the use as a Reference Material, the Drug Reference Material shall be discarded as in 30.8.12. with documentation of the reason the reference material was discarded notated on the Reference Material testing record. If the Drug Reference Material can still be used for training purposes, the storage bottle or secondary container (e.g. bag or envelope containing the bottle), testing record, and ChemInventory entry shall be marked "For Training Purposes Only" and the physical location of the Reference Material shall be moved to the training material storage. An email dissemination to all of the analysts and supervisor at that laboratory location shall be sent when a reference material is discarded due to signs of breakdown and/or when something is marked "For Training Purposes Only". All sub-vials of that reference material shall also be discarded at that time.

30.8.14. When a Drug Reference Material has been spilled, the following actions shall be taken:

1. Take a picture(s) of the spillage.

2. If possible, scoop the spilled reference material into a plastic bag and seal it (with initials). This is not required if the reference material is a liquid.

3. Take a picture of the plastic bag containing the spilled reference material.

4. Return the reference material and the sealed plastic bag to its designated storage area.

5. Send an email to the immediate Drug Unit Supervisor and Laboratory Manager detailing what happened and include the picture(s) of the spill.

6. The Drug Unit Supervisor shall dispose of the plastic bag containing the spilled reference material in the same way as a consumed reference material (with documentation in the logbook and observed by another Forensic Scientist).

7. The Drug Unit Supervisor shall write a memo about the spill. All of the documentation regarding the spill shall be uploaded onto SharePoint and the hardcopy shall be kept with the DEA paperwork.

30.9. Records:

30.9.1. Drug Reference Material Testing Record: Documentation of the receipt, identity, source and lot number, verification or authentication record(s) of each Drug Reference Material, the initials of the Forensic Scientist that performed the verification or authentication and the initials of the reviewer of the data. This record shall be available for each Drug Reference Material used for identification and maintained by each respective Regional Laboratory Drug Unit.

30.9.2. Instrumental data (original or copies) supporting verification and/or authentication of the Reference Material shall be attached to the Drug Reference Material Testing Record. It is not necessary to attach the blank for the reference material verification. The solvent used for verification needs to be documented on the data.

30.9.2.1. Re-verification or extension of expiration date information such as manufacturer re-certification date and/or analytical data shall be kept in the Drug Reference Material Testing Record.

30.9.2.2. Re-verification is only necessary when a Reference Material has expired, or is about to expire.

30.9.2.3. Reference Materials that do not have supporting verification data shall be reverified prior to use in casework. This data shall be attached to the Drug Reference Material Testing Record. It is not necessary to reverify the Reference Material if it is not being used in casework.

30.9.3. Manufacturer's analytical data (or copies) shall be attached to the Drug Reference Material Testing Record, if available.

30.9.4. Literature references or sources used for verification or authentication of Drug Reference materials shall be documented in the Drug Reference Testing Record. If applicable and reasonable, attach copies of the spectra to the Testing Record.

30.9.5. An evaluation of the spectra by two Forensic Scientists shall be performed before a Drug Reference Material is entered into the user generated library.

30.9.6. All entries in user libraries shall have the source and lot number of the Drug Reference Material included as part of the data file and printed on the spectrum. This source and lot number shall be reviewed by both Forensic Scientists evaluating the Drug Reference Material. Both Forensic Scientists shall check the source and lot number of both the printed spectra and the library entry on the instrument for accuracy when compared to

Cheminventory and the Reference Material Testing Record. This shall be documented by check marks and initials near the source and lot number on the printed spectra or electronically documented in ChemStation.

30.9.7. Purchased spectral libraries may not have source and lot number information available. This is beyond the control of the ISP FSD and these entries cannot be changed.

30.9.8. When a Drug Reference Material has been consumed and the bottle discarded, two Forensic Scientists shall be present, and both shall initial and record the date the material was consumed, and the container discarded on the Reference Material Testing Record.

30.9.9. The DEA 222 forms shall be filed in an accessible location Internal transfer forms shall be uploaded to a network drive (e.g., SharePoint).

30.9.10. A Reference Material inventory shall be completed annually. The inventory shall include the name of the Reference Material, the manufacturer, the lot number, the location in the Drug Unit laboratory, and expiration date, if applicable. Completed inventories shall be initialed by two analysts and uploaded to SharePoint.

30.9.11. Each Regional Laboratory shall designate a primary and secondary Forensic Scientist who is in charge of the inventory and organization of Reference Materials. The Forensic Scientist shall notify the Unit Drug Supervisor who has been designated for this assignment.

30.10. Interpretations of Results:

30.10.1. A Drug Reference Material is acceptable for use in case material identification after it has been analyzed in the Forensic Services Division and its analytical data has been compared to literature and found to be satisfactory. It may be included in the user generated spectral libraries.

30.10.2. If the literature spectral comparison is unsatisfactory, the Drug Reference Material cannot be used for casework identification, the spectrum shall not be included in the user generated libraries, and the Drug Unit Supervisor shall be notified.

30.11. Report Writing: N/A

30.12. References:

30.12.1. Methods of Analytical/Sampling Seized Drugs for Qualitative Analysis: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations.

30.12.2. Indiana State Police Forensic Services Division Quality Assurance Manual.

31. Drug Unit Case Reviews

31.1. Scope: This Test Method is intended as a guide for the proper review of Drug Unit case records (examination and administrative documentation).

31.2. General:

31.2.1. Technical Reviews: A technical review is an evaluation of examination records after the examination has been performed. This review shall take place before the report has been released. This review consists of determining whether the appropriate examinations have been performed, the conclusions are consistent with the recorded data and are within the scope of the discipline or category of testing. Technical reviews are not required for cases when no examinations were performed.

31.2.2. Administrative Reviews: Administrative Reviews are reviews of administrative records and a cursory review of analytical information prior to the approval of the report. This is not intended to be a technical review, but may include Test Method compliance and technical matters.

31.3. Related Information:

31.3.1. Technical Review Form
31.3.2. LIMS Module information

31.4. Procedures/Instructions:

31.4.1. The Forensic Scientist is responsible for preparing accurate, complete, and organized examination records (case notes). The Forensic Scientist shall review documentation constituting the case file (examination and administrative records) for compliance with Forensic Services Division Quality Assurance Manual and technical accuracy prior to submitting for administrative or technical review.

31.4.2. When the Forensic Scientist submits the case for administrative or technical review, it shall be considered completed. The Drug Unit Supervisor shall be notified by the Forensic Scientist and the reviewer if additional analysis is performed after the case is submitted for administrative or technical review.

31.4.3. Administrative Reviews: At a minimum, this review shall include:

31.4.3.1. A review of the Certificate of Analysis including:

31.4.3.1.1. Spelling and grammatical accuracy.

31.4.3.1.2. A review of the Certificate of Analysis to ensure that all key information is included.

31.4.3.1.3. Results are properly reported as per Drug Unit Test Methods

31.4.3.2. A review of all administrative and examination records associated with the Certificate of Analysis to ensure that the records are uniquely identified.

31.4.3.2.1. Laboratory Case number

31.4.3.2.2. Laboratory Item number(s)

31.4.3.2.3. Forensic Scientists' initials or signature (does not apply to administrative documents)

31.4.3.3. At the completion of a case, an administrative review shall be conducted on all Certificates of Analysis prior to release to the contributing agency and/or officer.

31.4.4. Technical Reviews: At a minimum, all examination documentation shall be reviewed for the following:

31.4.4.1. All of the requirements for an Administrative review, AND

31.4.4.2. Records of Weight and Balance checks,

31.4.4.3. Weight measurements,

31.4.4.4. Measurement Uncertainty,

31.4.4.5. Testing procedures used,

31.4.4.6. Quality of data (same conclusion would have been reached given the information provided),

31.4.4.7. Test Method compliance,

31.4.4.8. Compliance with applicable Forensic Services Division policies,

31.4.4.9. Use of appropriate reference materials, blanks, and controls

31.4.5. Additional items that may be reviewed:

31.4.5.1. Evidence Seals and markings

31.4.5.2. Evidence receipts

31.4.5.3. Chain of Custody records

31.4.6. Technical reviews shall be conducted by individuals authorized by the Forensic Services Division Commander that have expertise gained through training and experience in the category of testing being reviewed and shall have knowledge of the Drug Unit's Test Methods.

31.4.7. Technical and Administrative reviews shall not be conducted by the author of the Certificate of Analysis.

31.4.8. Technical reviews shall be performed on new Forensic Scientist cases as per the Drug Unit Training Manual.

31.4.9. As of 2025 all cases shall be technically reviewed unless no analysis was performed on the case.

31.5. Records:

31.5.1. Administrative Reviews: The individual completing administrative reviews shall mark the case as Administratively Reviewed in the LIMS module to affix their Permanent Employee (PE) number to the final report.

31.5.2. Technical Reviews: The individual completing the review shall complete a Drug Unit Technical Case Review form, noting any problem(s) found, and corrections or action(s) taken. The case shall be marked Technically Reviewed in the LIMS module.

31.5.3. An electronic copy of the form used for the Technical Review shall be appropriately named and saved in the respective case record in the LIMS system by the reviewer.

31.6. Interpretations of Results:

31.6.1. Changes to notes: If the correction requires change(s) to the case record, the Forensic Scientist shall make the corrections in the electronic version of the examination record and upload the corrected electronic version of the examination record to LIMS using the appropriate nomenclature. All pages of the original electronic examination record shall be retained. If possible, the corrected file should be sent back to the original reviewer.

31.6.2. Changes to Reports:

31.6.2.1. Prior to release: If the correction requires a change to the report prior to the approval and release of the original report, the Forensic Scientist shall make the correction, and send the corrected file back to the original reviewer, if possible.

31.6.2.2. After release: If the correction requires a change(s) to the Certificate of Analysis after the original report has been released, the Forensic Scientist shall make the necessary correction(s) and issue an Amended Report. All items in the original Certificate of Analysis shall be included on the Amended Report. The case reviewer shall perform an Administrative Review on the Amended Report and shall ensure all necessary corrections were made. A remarks section shall be added and include reference to the original Certificate of Analysis and reason for the Amended Report.

31.6.3. Supplemental Report: If additional testing of an item is requested after a Certificate of Analysis has been issued, then the report shall be a supplemental report and the request shall be a related request (0001_0001). Only the item being requested for testing needs to be included on the Supplemental Report. A remarks section shall be added and include a reference to the original Certificate of Analysis and previous testing performed.

31.6.4. If testing is requested on an item that has been previously withdrawn (no testing was performed), then a new request shall be generated (R0002). Only the item being requested for testing needs to be included on the new Certificate of Analysis.

31.6.5. If testing is done at multiple laboratory locations, the results shall be on separate reports that reflect those locations.

31.6.6. Resolution of Conflicts of Opinion: (See Forensic Services Division Quality Assurance Manual)

31.6.6.1. Once a formal case review has begun, the same reviewer, if available, shall complete the process.

31.6.6.2. If the Forensic Scientist and reviewer disagree, the Forensic Scientist shall not seek a second reviewer. Both Forensic Scientist and reviewer shall consult a Drug Unit Supervisor to attempt to resolve the disagreement.

31.6.6.3. The Drug Unit Supervisor shall be notified of substantive variations of opinions. The Forensic Scientist, the reviewer, and Drug Unit Supervisor

shall discuss the examination results, interpretations/opinions, and conclusions.

31.6.6.4. If the difference of opinion cannot be resolved, Drug Unit Supervisor(s) shall notify the Chemistry Section Supervisor and Quality Assurance Manager to pursue resolution as per Forensic Services Division Quality Assurance Manual.

31.6.6.4.1. After consultation with the Quality Assurance Manager, Chemistry Section Supervisor, Drug Unit Supervisor, and other staff as necessary, the situation shall be evaluated and a determination shall be made if a corrective action is to be required.

APPENDIX 1 FORMS AND WORKSHEETS

The following Drug Unit worksheets and log sheets as found on SharePoint. Forensic Scientists shall use an Examination Worksheet when documenting their analysis. However, the Forensic Scientist may customize these worksheets with the specifics for their analysis and respective laboratories. Additional sheets are acceptable when appropriately marked as per Forensic Services Division Quality Assurance Manual requirements.

1. Balance Verification Log
2. Drug Transfer Form
3. Drug Technical Case Review Form
4. Drug Unit Personal Review Form
5. Drug Unit Examination Worksheet 1
6. Drug Unit Examination Worksheet 2
7. Drug Unit Examination Worksheet 3
8. Drug Unit Examination Worksheet 4
9. Drug Unit Examination Worksheet 5
10. GC-IR Verification Log
11. Multiple GC-IR Runs Worksheet
12. Multiple GC/MS Runs Worksheet
13. GC/MS Preventative Maintenance Checklist
14. GC/MS Weekly Checklist
15. GC/MS Method Log
16. IRL Polarimeter Verification Log
17. FTIR Performance Verification Log
18. UV Performance Verification Log
19. Drug Reagent Preparation and Verification Log I
20. Drug Reagent Preparation and Verification Log II
21. pH and Water Finding Paper Verification Log
22. Drug Reference Material Testing Record
23. Reference Material with Multiple Vials
24. Drug Waste Label
25. Decision Point Log
26. Internal Standard Solution Logs
27. DP Pipette Logs
28. GC/MS Maintenance Log

APPENDIX 2 ABBREVIATIONS

~ or approx	**approximate**
+ or pos	positive
AC, Ac-Cod	Acetylcodeine
acet, APAP	Acetaminophen
ACN	acetonitrile
amph	Amphetamine
AMT	amount
ANOR	Alternate Non-aqueous Organic Ratio
aq	aqueous
asv	autosampler vial
bkg	background
Blk	Blank
Bupr	Buprenorphine
cap(s)	capsule(s)
ć, c/, cont, or :	containing
CBC	Cannabichromene
CBD	Cannabidiol
CBDV	Cannabidivarin
CBG	Cannabigerol
CBGM	Cannabigerol monomethyl ether
CBL	Cannabicyclol
CBN	Cannabinol
CBV	Cannabivarin
cig	cigarette
coc	Cocaine
CMP	1-(1’,4’-cyclohexadienyl)-2-methyl Aminopropane
Conc, con	Concentrated/concentration
CS or cs	controlled substance
CT or ct	color test(s)
cwc	Consistent with Cannabis
d or D or Δ	delta
der	derivatized
DIB	Drug Identification Bible
dis	dismissed
diss.	dissolved
DMS	Dimethyl sulfone
DOS	date of seizure
DP	Decision Point
DPH	Diphenhydramine
Duq. Lev or D-L	Duquenois-Levine
DXM	Dextromethorphan

eff, ◊	effervescence, gassing
ENP or enp	Evaluated, not printed
env	envelope
eph or ephed	Ephedrine
equiv	equivalent
evap	evaporated
evid	evidence
ext or extr	extraction
gpm	green plant material
gwt	gross weight
HC	Hydrocodone
HER	Heroin
HM	Hydromorphone
hrc	hand rolled cigarette
IDDA or Mills	Instrumental Data for Drug Analysis
incon	inconclusive
ind	indicates
ingred.	ingredients
init.	initials/initialed
inj.	injection
ISS	Internal standard solution
liq.	liquid
MAM	Monoacetylmorphine
man.	manual
mat or mat'l	material
MC	Moisture Content
Meth, Methamp	Methamphetamine
mfr	manufacturer
MJ or mj	Marijuana
MTD	Methadone
MU	Measurement uncertainty
N/E	not examined
NCR or NR	no color reaction or no reaction
NCS	no controlled substance
neg	negative
nwt	net weight
nid	not identified
OC	Oxycodone
pb	plastic bag
pbs	plastic bags
PE, Pet E, Pet ether	petroleum ether
pharm.	pharmaceutical(s)
pm	plant material / plant-like material

PPX	Propoxyphene
precip, ppt	precipitate
prog	program
pros	prosecutor
pse, pseudo	pseudoephedrine
ptd	Pre-trial diversion
PTHIT	Phenyltetrahydroimidazothiazole, Levamisole, Tetramisole
pwd	powder
quant	quantitation
rec'd	received
ref	reference
rej	rejected
Reppb	Repackaged in lab provided plastic bag
ret	retention
Rm, ref mat	reference material
RT	retention time
rxn	reaction
sap	secondary aliquot prepared
sat, or sat'd	saturated
SQ	Semi-Quant
sen or sent	sentenced
sch	schedule
sl	slight
sme	sealed manila envelope
soln, or 'ol'n	solution
sp	sealed plastic
spb(s)	sealed plastic bag(s)
szpb(s)	sealed ziplocked plastic bag(s)
sub, subs	substance
syn	synthetic
t	time
tab(s)	tablet(s)
TBA	Tribenzylamine
tgw	total gross weight
THCA or THCA-A	Tetrahydrocannabinolic Acid
THCV	Tetrahydrocannabivarin
TIC	Total Ion Chromatogram
tnw	total net weight
v	very
V/E	visually examined
veg	vegetation
ẃ, w/	with
Wt	Weight
zpb(s)	zip-locked plastic bag(s)

APPENDIX 3 DEFINITIONS

1. Atomic Mass Unit (amu): A unit of mass equal to 1/12 the mass of the most abundant isotope of carbon, carbon 12 (carbon which is assigned a mass of 12).

2. BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide; a derivatization compound. It is the preferred reagent for trimethylsilylation of alcohols, alkaloids, amines and biogenic amines, carboxylic acids, phenols, and steroids.

3. BSTFA-TMCS: N,O-Bis(trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane; a derivatization compound; used for amides, secondary amines, especially good for analyzing drugs of abuse – THC, morphine, PCP, etc. is the preferred reagent for trimethylsilylation of alcohols, alkaloids, amines, biogenic amines, carboxylic acids, phenols, and steroids.

4. Calibration: Adjusting a piece of equipment to a certain set of performance standards.

5. Chromophores: the molecular grouping that is responsible for UV absorption, usually a conjugated system (double bonds) where the electron density is spread out over the molecule.

6. Confidence level: the extent or likelihood that an assumption or number is true; the statistical likelihood (probability) that a random variable lays within the confidence interval of an estimate.

7. Coverage factor: the number that is multiplied by the standard uncertainty to produce an uncertainty estimate that will contain a large fraction of all values that might be obtained on a test. The coverage factor is commonly denoted as k=2 is used for 95.45% coverage and k=3 for 99.7% coverage. The Drug Unit uses a coverage factor (k=2) to estimate a 95.45% coverage probability that the weights measured fall within our uncertainty window. This window varies depending on the type and readability of the respective balances.

8. Cystolithic trichomes: the claw shaped hairs found on the top side of the Cannabis leaf; the simultaneous presence of these bear claw-shaped trichomes on the upper surface and the fine, slender non-cystolithic trichomes on the lower surface of the leaves is a characteristic of Cannabis.

9. Expanded Uncertainty: The expanded uncertainty is the combined standard uncertainty (or standard uncertainty, if there is only one component), multiplied by the coverage factor.

10. General operating parameters: the general specifications for the method to indicate the procedure used. Should be enough so that a reviewer can locate the method file in the archive.

11. Generic name: A name that is not or does not include a trademark or brand name. The official nonproprietary name of a drug, under which it is licensed and identified by the manufacturer.

12. Homogeneous: of the same nature or kind; uniform in structure or composition.

13. Marijuana seeds: The fruit of the marijuana plant is an achene; a single seed with a hard shell, ellipsoid, slightly compressed, smooth, about 2-5 mm long, generally brownish and mottled. The fruit is commonly regarded as a seed.

14. Mass-to-charge ratio (m/z): the mass number of an ion divided by its charge, a dimensionless quantity used in mass spectrometry; the measurement of the sample mass as a ratio to its ionic charge.

15. Minor Artifact: a sharp raised portion of the total ion chromatogram that is less than two times the largest level of background noise.

16. Multiple unit population: a group of items that are similar in appearance, size, and composition.

17. National Institute of Standards and Technology (NIST): A national bureau of standards and testing that sets guidelines for standards and measurement.

18. Nonproprietary name: The chemical or generic name of a drug, chemical, or device, as distinguished from a brand name or trademark.

19. Perfluorotributylamine (PFTBA): the calibration material used for tuning the GC/MS instruments.

20. Polymorphism: when a substance can exist in multiple crystalline forms.

21. Primary Reference Material: a verified reference material used in the identification of substances from a verifiable source.

22. Proper/appropriate identifiers: laboratory case number, laboratory item number, etc. some means of identifying the sample and keeps it distinguishable from other items.

23. Proper scales for identification: rulers, some way of relating size (scale) to the viewer.

24. Readability: the level to which a balance can read accurately.

25. Reference Material Testing Record: The records of the authentication and/or verification of reference materials used for drug identification. See also "Standard Testing Record".

26. Regioisomeric: isomeric forms of a substance where the substances have the same molecular weight, but the atoms are attached at different places. Some spectra will be very similar.

27. Representative sample: a sample taken from an item of evidence that represents the contents of the evidence exhibit.

28. Residue: if any item cannot be weighed or the uncertainty results in a measurement that is zero or negative, the item may be described and reported as a residue.

29. Secondary Reference Material: a verified, or previously analyzed, material that can be used in the identification of substances, but whose source may not be verifiable. This may include samples taken for demonstration purposes, i.e., previously identified case materials, etc.

30. Standard Testing Record: After January 1, 2011, these are named "Reference Material Testing Records".

31. Ten Basic Spectral Colors suggested for use in Spot Tests: red, orange, yellow, green, blue, violet, pink, brown, gray, and black are suggested for color (spot) test interpretation.

32. Trade name: A name used to identify a commercial product or service, which may or may not be registered as a trademark. Also called brand name.

33. Uncertainty: The estimated amount or percentage by which an observed or calculated value may differ from the true value.

34. Validation: A process that ensures new or substantially modified methods provide accurate and reliable results prior to being used to analyze and evaluate physical evidence.

35. Verification: A process that checks a piece of equipment, method, or reagent to confirm that it is working correctly.

APPENDIX 4
REAGENT PREPARATION MANUAL

Reagents, such as those used on chemical color (spot) testing, are used directly in testing procedures and are subject to quality control testing procedures. Reagents may be purchased, but more commonly are prepared by combining chemicals. Chemicals and/or materials used to make reagents are generally purchased from reputable chemical supply companies.

Reagents shall be verified with a known reference material at the time of preparation and subsequently on a monthly basis at a minimum. Exceptions are infrequently used Spot Test reagents that shall be verified with a reference material at the time of use. The preparation and monthly verification shall be recorded on the Reagent Preparation and Verification log. Bottles containing the reagents shall be labeled with the date of preparation and the initials of the Forensic Scientist who prepared the reagent. The Reagent Preparation and Verification log shall include the date of preparation (and subsequent monthly verification), the initials of the Forensic Scientist who prepared and verified the reagent, the method of verification, and the source and lot number of the reference material used to verify the reagent. The source and lot number of the chemicals used to make the reagents shall be recorded on the Reagent Preparation and Verification Log.

Chemicals are not used directly in testing procedures and are not subjected to the same quality control testing procedures as are reagents. Chemicals may be in dry (e.g., sodium bicarbonate), or liquid (e.g., chloroform) form. Chemicals may be used to make chemical solutions (e.g., sodium hydroxide is used to make 0.45 N sodium hydroxide solution). Generally, these materials can be concluded to be free from drug contamination through means such as TLC solvent blanks, GC/MS solvent blanks or even the recognition that the same source of chemical was used indirectly for separate and independent case samples resulting in unlike drug types identified or indicated.

Chemical and reagent containers shall be dated and initialed when received and also when first opened.

Chemical solutions, such as 0.5 N H_2SO_4 shall be marked with the date of preparation and the initials of the Forensic Scientist who prepared the solution. The preparation date and initials of the Forensic Scientist shall be documented on the Reagent Preparation and Verification Log. The source and lot number of the chemicals used to make the reagents shall be recorded on the Reagent Preparation and Verification Log. At the time of preparation, acid and base solutions shall be verified as either acidic or basic with the use of pH paper.

The method of verification for chemical color (spot) testing reagents requires combining the reagent with a known reference material and observing the resulting chemical color reaction. A reagent that produces the expected color reaction when combined with the known reference material is considered verified and the entry recorded on the Reagent Preparation and Verification Log documents the satisfactory performance for the reagent. Multi-step chemical color testing reagent verification shall include a negative control (blank) to demonstrate that the combination of reagents is blank or does not produce a color and

shall be recorded on the Reagent Preparation and Verification Log. If a color or spot test fails the verification process, it shall be discarded. The reagent shall be re-made and verified.

TLC spray reagents, such as Fast Blue BB and iodoplatinate spray are exempt from the monthly verification entry requirement on the Reagent Preparation and Verification Log. These spray reagents are in effect verified during each use based upon their satisfactory reaction to known reference materials included on the TLC plate.

The following color test reagents, TLC reagent sprays and chemical solutions are prepared using the following or appropriately proportional procedures. A recommended reference material is included to verify the color (spot) testing reagents' performance; however, any reference material that reacts with the reagent to produce a known and expected reaction may be substituted.

Color (spot) Test Reagents

1% Cobalt Nitrate Reagent (for GHB)

1 gram of cobalt nitrate dissolved in 100 ml of distilled or deionized water

Verify using GHB (produces a pink-to-violet color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

Cobalt Thiocyanate Reagent (for Cocaine)

Cobalt Thiocyanate 2% by weight in water

Or

6.8 grams of cobalt chloride
4.3 grams of ammonium thiocyanate
Dissolve in 100 ml of distilled or deionized water.

Optional: Step 2: Concentrated HCl

Verify using Cocaine HCl (produces a blue precipitate).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 294, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 136, editor Richard Saferstein, 2002.

Cobalt Thiocyanate, Modified (for Ketamine HCl)

Add 0.1 N sodium hydroxide to sample,
Add Cobalt thiocyanate reagent

Verify with Ketamine HCl (produces a violet color reaction)

Reference: Modified Cobalt Thiocyanate Presumptive Test for Ketamine Hydrochloride, Morris, J., J. Forensic Sci, January 2007 Vol. 52, No.1.

Validation of Modified Cobalt Thiocyanate Test for Ketamine HCl, Curry, A. (Indiana State Police), August 2007

Supplemental Validation of Modified Cobalt Thiocyanate Test for Ketamine, Ballard, T. and Roskowski, D. (Indiana State Police), June 2009.

p-Dimethylaminobenzaldehyde (p-DMAB) Reagent (for Indoles, LSD)

Solution A: 5 grams of p-Dimethylaminobenzaldehyde in 500 ml of Methanol.
Solution B: Concentrated HCl

Verify using LSD (produces a violet color)

Or

Verify using Procaine or Benzocaine (produces an intense yellow color)

Solutions A and B can also be combined prior to use.

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 284, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 144, editor Richard Saferstein, 2002.

Dille-Koppanyi Reagent (for Barbiturates)

Solution A: 0.1 gram of Cobaltous Acetate
0.2 ml of glacial Acetic Acid
100 ml of Methanol

Solution B: 5% Isopropylamine (base) in Methanol by volume
(5 ml Isopropylamine base and 95 ml Methanol)

Verify using Phenobarbital or known barbiturate (produces blue-violet color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 284, London, Pharmaceutical Press, 2004.

Duquenois-Levine Reagent (Modified) (for Cannabinoids)

Solution A: 5.0 ml of Acetaldehyde
8.0 grams of Vanillin
400 ml of Methanol

Solution B: Concentrated Hydrochloric Acid

Solution C: Chloroform

Add 2-3 drops of solution A and 2-3 drops of solution B (concentrated Hydrochloric Acid). After purple/violet color develops, add 3-5 drops of solution C (Chloroform).

Verify with THC, CBD, or secondary Marijuana reference material (produces a purple/violet color that extracts into the Chloroform layer)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 285, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 168, editor Richard Saferstein, 2002.

The Identification of Marijuana, Thornton (University of California, Berkeley) and Nakamura (Bureau of Narcotics and Dangerous Drugs), J. Forensic Sci. Soc, (1972), 12, 461.

5% Ferric Chloride Reagent (for GHB, phenolic compounds)

5 grams of Ferric Chloride
100 ml of distilled or deionized water

Verify with GHB (produces a rust-red color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 285, London, Pharmaceutical Press, 2004.

Froehde's Reagent (for opiates)

0.25 grams of Molybdic Acid or Sodium Molybdate
50 ml of concentrated Sulfuric Acid (Hot)

Verify with Codeine or Morphine (produces green color reaction)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 286, London, Pharmaceutical Press, 2004.

GHB Test # 3:

Bromocresol green: 0.03 gram bromocresol green in 100 ml 4:1 methanol: water, pH adjusted to 7.0 with 0.1 N NaOH using pH meter.

Methyl orange: 0.01 gram methyl orange in 100 ml methanol pH adjusted to 7.0 with 0.1 N NaOH using pH meter.

Bromocresol green and methyl orange are mixed in a 1:1 ratio, then the combined reagent is mixed with modified Schweppes reagent in a 3:1 ratio.

Verify with GHB (produces an immediate green color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

GHB validation study 12/6/04, Nickless, R. (Indiana State Police)

Mandelin's Reagent (for aromatics with saturated ring with one N-atom)

1 gram of Ammonium Vanadate in 100 ml of concentrated Sulfuric Acid

Verify with Morphine (produces blue-gray) or Amphetamine (green to dark green)

Reference: Forensic Science Handbook, Volume II, 2nd edition, page 166-168, editor Richard Saferstein, 2002.

Marquis Reagent (for amphetamines, opiates)

5.0 ml of 37% Formaldehyde

Dilute to 100 ml with concentrated Sulfuric Acid

Verify with an amphetamine-like substance (Methamphetamine or Amphetamine) (produces orange color reaction)

Or

Verify with opiate (Codeine, Heroin, or Morphine) (produces violet color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 289-291, London, Pharmaceutical Press, 2004.
Forensic Science Handbook, Volume II, 2nd edition, page 166-169, editor Richard Saferstein, 2002.

Mecke's Reagent (for Opiates, etc.)

0.25 gram of Selenious Acid

25 ml of concentrated Sulfuric Acid

Verify with an opiate (Codeine, Morphine, or Heroin) (produces an immediate blue or green color).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 289-292, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 167-169, editor Richard Saferstein, 2002.

Schweppes reagent: (Modified) (for GHB)

Solution A: 2 grams dextrose in 20 ml water
Solution B: 2.4 grams aniline hydrochloride in 20 ml ethanol.

Mix both solutions together and dilute to 80 ml total volume with methanol.

Verify with GHB (a dark green color indicates GHB) (GBL gives a yellow-orange)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

GHB validation study 12/6/04, Nickless, R. (Indiana State Police)

Scott (Ruybal) Test (for Cocaine)

Cobalt Thiocyanate Reagent + glycerine (1:1) (turns blue with Cocaine)
Add HCl, blue color disappears & pink solution develops
Add Chloroform, Cocaine produces intense blue color

Verify using Cocaine HCl

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 294, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 136, editor Richard Saferstein, 2002.

Silver Ammonium-Nitrate Reagent (for Ascorbic Acid – Vitamin C)

Dissolve 2.5 gram of silver nitrate in 80 ml of distilled or deionized water

Cautiously add dilute ammonium solution until the precipitate first formed is nearly dissolved.

Allow to stand, decant the clear liquid, and add it to sufficient water to produce 100ml.

Verify with Ascorbic Acid (produces a silver-colored metallic looking reaction)

Reference: Isolation & Identification of Drugs, E.G.C. Clarke, Volume 1, page 805, Pharmaceutical Press, London, 1974.

Sodium Nitroprusside (Modified) (for secondary amines)
(aka Sodium Nitroferricyanide) (Simon's test)

Solution A: 0.25 grams Sodium Nitroprusside (Sodium Nitroferricyanide)
25 ml of distilled or deionized Water
2.5 ml of Acetaldehyde

Solution B: 0.5 gram of Sodium Carbonate
25 ml of distilled or deionized Water

Verify with Methamphetamine for secondary amine (produces a dark blue color).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 295, London, Pharmaceutical Press, 2004

Tannic Acid Test (for Caffeine)

Dissolve small amount of Tannic Acid in 1-3 ml of distilled or deionized Water
(or 0.2 grams of Tannic Acid in 30 ml of distilled or deionized Water).

Drop small amount of pulverized sample onto top of solution.

Verify with Caffeine (produces white trails as sample falls through solution)

Reference: Analysis of Phentermine/Methamphetamine/Ephedrine/Caffeine Mixtures by GC/MS, R. Martin Smith, Wisconsin Department of Justice Crime Laboratory Bureau, Microgram, Volume IX, No. 4, April 1976.

Tannic Acid as a Field Test for Caffeine, Hueske, EE.; Microgram, Vol. XV, No. 9, September, 1982, p. 158.

Weber Test (for Psilocyn)

Dissolve 0.01 gram of Fast Blue B (o-dianisidine, tetrazotized) in 10 ml of Water.

Add 2 to 3 drops of reagent to sample of mushrooms
(the solution will turn red in the presence of Psilocyn)

Add 1 to 2 drops of concentrated HCl to solution
(turns from red to blue if Psilocyn is present.)

Verify with Psilocyn or a confirmed Psilocybic mushroom sample. (See above reactions.)

THC or cannabinoids can also be used if other sufficient material is not available. The reaction produced by THC or other cannabinoids may be red, purple, or orange, depending on the substance used.

Reference: The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms, Garrett, A.S., Clemens, S.R., Gaskill, J.H. SWAFS Journal, Vol. 15, No. 1, April, 1993, pp.44-45.

Weber Test; Garrett, Allen; Clemens, Steven and Gaskill, James. Weber State College, Laboratory of Criminalistics, Ogden, Utah. (Found in Drug Unit Resource Manual – Tryptamines Vol 2)

Weber Color Test, Koppenhaver, D., ISP Filter Paper (in-house publication), (circa 1996).

Thin Layer Chromatography Solvent Systems

Thin Layer Chromatography is generally conducted using covered glass chambers with a variety of solvents making up the mobile phase. Generally, the chamber can support approximately 50 ml of solvent. The following solvent systems are routinely used and have been found over several years to provide suitable separation of components in mixtures to allow for indications of drugs present in samples. In addition, references such as the Isolation and Identification of Drugs by E.G. C. Clarke Volume 1, Clarke's Analysis of Drugs and Poisons 3rd edition and other related references provide an extensive listing of potential TLC solvent systems and visualization reagents. The list below includes, but is not limited to, the recommended and commonly used solvent systems. Several of these solvent systems and visualization reagents have been in use by the Indiana State Police Drug Unit for over 25 years.

Suspected Marijuana TLC system:

Toluene (Plates should be sprayed with Diethylamine prior to development to improve separation)

Separation of delta-9-Tetrahydrocannabinol from delta-8-Tetrahydrocannabinol:

Toluene (Plates should not be sprayed with Diethylamine prior to development to improve separation)

Toluene w/ Preparative Pre-soak in a 5% Silver Nitrate ($AgNO3$) in Acetonitrile solution

Hexane: Acetone (43:7) (Plates shall not be sprayed with Diethylamine prior to development to improve separation)

General Unknowns:

Methanol: NH_4OH (100:1.5) (This system is commonly referred to in Clarke's references as T1 and TA systems)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5) (This system has been in use prior to January 1974)

Reference: Thin Layer Chromatography, 2nd Edition, Randerath, K., Academic Press, New York and London, 1962, p 101.

Suspected LSD Unknowns:

Acetone

Acetone: NH_4OH saturated $CHCl_3$ (9:1)

Suspected Psilocyn/Psilocybin

Methanol: NH_4OH (100:1.5)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5)

Chloroform: Methanol (4:1)

N-Butanol: Water: Acetic Acid (Glacial) (2:1:1)

Suspected Barbiturates and Hypnotics

Chloroform: Acetone (9:1)

Water: Methanol (1:1)

Suspected Benzodiazepines

Methanol: NH_4OH (100:1.5)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5)

Chloroform: Acetone (80:20) or (9:1)

Cyclohexane: Toluene: Diethylamine (75:15:10)

Suspected Steroids

Chloroform: Ethyl Acetate (4:1)

Chloroform: Acetone (9:1)

Suspected Opiates

Methanol: NH_4OH (100:1.5)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5)

Cyclohexane: Toluene: Diethylamine (75:15:10)

TLC Spray Reagents

p-Dimethylaminobenzaldehyde Spray Reagent

5 grams of p-Dimethylaminobenzaldehyde
500 ml of Methanol
50 ml of concentrated Hydrochloric Acid

Or

p-DMAB color test stock solution (Solution A): HCl (Approximately 10:1)

Dragendorff Spray Reagent

Solution A: 0.57 gram of Bismuth Subnitrate
78.6 ml of glacial Acetic Acid
100 ml of distilled or deionized Water

Solution B: 14.29 grams of Potassium Iodide
312.2 ml of Distilled or deionized Water

Mix Solution A and B to prepare 500 ml of reagent

Or

Solution A: 2.0 grams of Bismuth Subnitrate
25 ml of glacial Acetic Acid
100 ml of distilled or deionized Water

Solution B: 40 grams of Potassium Iodide
100 ml of distilled or deionized Water

Mix 10 ml of each of Solutions A and B, add 20 ml of glacial Acetic Acid, and add 100 ml of distilled or deionized Water. (Prepare mixture fresh as needed)

Ethanol/Sulfuric Acid Spray Reagent

20 ml of Ethanol
5 ml of concentrated Sulfuric Acid

Fast Blue B Spray Reagent or Fast Blue BB Spray Reagent

Small amount of powder dissolved in distilled or deionized Water
(Approximately 1% Fast Blue BB solution in distilled or deionized water)

Iodoplatinate Spray Reagent (Acidified)

0.25 grams of Platinic Chloride (Chloroplatinic Acid)
5.0 grams of Potassium Iodide

Dilute to 100 ml with distilled or deionized Water
Add 2.0 ml of concentrated Hydrochloric Acid

Mercurous Nitrate Spray Reagent

Saturated solution of Mercurous Nitrate in distilled or deionized Water

Ninhydrin Spray Reagent

0.5 gram of Ninhydrin
1.0 ml of glacial Acetic Acid
100 ml of Isopropyl Alcohol

Or

2% Ninhydrin in Acetone
(2 grams of Ninhydrin in 100 ml of Acetone)

Potassium Permanganate Spray Reagent

2.0 grams of Potassium Permanganate
5 drops of Phosphoric Acid
Dilute to 100 ml with distilled or deionized Water

Or

1 gram of Potassium Permanganate
Dilute to 100 ml with distilled or deionized Water

Acid and Base Solutions

Concentrated Hydrochloric Acid = 12 Normal = 12 Molar

2.8 N Hydrochloric Acid (Check with pH paper)

116.5 milliliters of concentrated Hydrochloric Acid
Add to distilled or deionized Water for final volume of 500 ml.

Saturated Sodium Hydroxide = 17.3 Molar = 17.3 Normal

0.45 N Sodium Hydroxide (Check with pH paper)

26.01 ml of saturated Sodium Hydroxide (saturated in distilled or deionized Water)
Dilute to 1.0 liter with distilled or deionized Water

Or

18 grams of Sodium Hydroxide
Dilute with 1000 ml (1.0 liter) of distilled or deionized Water

Concentrated Sulfuric Acid = 36 Normal = 18 Molar

0.5 N Sulfuric Acid (Check with pH paper)

13.9 ml of concentrated Sulfuric Acid
Add to distilled or deionized Water for final volume of 1.0 liter

APPENDIX 5
SEMI-QUANTITATIVE ANALYSIS

Semi-Quantitative Analysis: This procedure includes sample preparation and instrumental analysis. The following procedure is used to determine if a Cannabis plant material submission is Marijuana by applying a semi-quantitative threshold of 1% Δ9-THC. The total Δ9-THC content of plant material will be compared to the response of a 1% Δ9-THC decision point (DP), using Tribenzylamine (TBA) as the internal standard.

Definitions:

1. Internal Standard Solution (ISS): 5 mg/mL Tribenzylamine (TBA) in MeOH
   - The internal standard is added in a constant amount to all samples, the decision point, and the positive control. The ratio of the internal standard peak to the delta-9 THC peak is used to determine whether a sample is Marijuana.

   - Semi-Quant ISS: 5mg/mL Tribenzylamine (TBA) in MeOH

2. Decision Point (DP): The DP is manufactured to be equivalent to a 1% total THC yield in a theoretical 100 mg sample of plant material extracted with 2 mL of solvent.

   - 100 mg of theoretical 1% THC plant material = 1 mg of THC
   - An extracted theoretical 1% THC plant material sample should contain 1mg THC, 50 µl ISS and 1950 µl MeOH.

     ○ Established ratio= $\frac{1 \text{ mg THC}}{50 \text{ µl ISS} + 1950 \text{ µl MeOH}}$

   - Δ9-THC Reference Material (1 mg/mL in MeOH)
   - In order to conserve reference material, the ratio was maintained and reduced in half.

     ○ Reduced ratio= $\frac{500 \text{ µg THC}}{25 \text{ µl ISS} + 975 \text{ µl MeOH}}$

3. Total THC: delta-9-Tetrahydrocannabinol and delta-9-Tetrahydrocannabinolic Acid (THCA)
   - Total Potential THC = (Δ9-THCA% weight *0.877) + Δ9-THC% weight

4. Concentration Ratio (Units): Measurement of unknown THC to TBA response compared to response of known 1% decision point.

   - When combined with supporting tests, a sample with a total Δ9-THC concentration ratio greater than or equal to 1 will be reported as Marijuana.

   - If the total Δ9-THC concentration ratio is less than 1, the cannabinoids, if present, will be reported.

- Concentration ratio= $\frac{\left(\frac{\text{Unknown THC Response}}{\text{TBA Response}}\right)}{\left(\frac{\text{Known THC Response}}{\text{TBA Response}}\right)}$

5. Negative Control: An extracted blank will be run with each sequence to evaluate the materials used in the extraction for contamination. This sample comprises only the materials used in the extraction, with no plant material. There shall be only TBA present and no reportable cannabinoid peak in this negative control for the sequence results to be acceptable.

6. Positive Control: An extracted known total THC concentration will be run with each sequence to evaluate the Decision Point (DP). The concentration ratio shall produce the correct finding (Marijuana/Inconclusive).

   - Finding: Inconclusive

     - A sample is considered inconclusive if the concentration ratio is < 1.

   - Finding: Marijuana

     - A sample is considered Marijuana if the concentration ratio is ≥ 1.

Instruments/Equipment/Reagents:

- Gas Chromatograph/Mass Spectrometer with the semi-quant method on an approved column
- Calibrated mechanical pipettes
- Calibrated balance
- Volumetric flask
- Glass pipettes filled with glass wool for filtering
- Methanol (MeOH)
- Tribenzylamine (TBA)
- Delta-9-THC Reference Material (1 mg/mL)

Procedure:

In order to perform semi-quantitative analysis, a minimum of 0.20 gram of plant material is required. Two separate samples of plant material shall be placed in separate test tubes. One test tube will be only for semi-quantitative analysis. Any plant material sample that undergoes semi-quantitative analysis should be analyzed in the following order:

- Microscopic examination
- Thin-layer chromatography
- Semi-quantitative analysis on GC/MS

Tribenzylamine Internal Standard Solution (ISS) Preparation:

1. 5.0 mg/mL Tribenzylamine (TBA) in MeOH

2. Add 0.5 g TBA to a class A 100 mL volumetric flask
3. Fill to volume with MeOH
4. Agitate for several minutes until TBA is fully dissolved into solution.
5. Assign lot # to ISS. (ISSmmddyy)
6. Example: ISS made on August 7, 2020 would have the lot # ISS080720
7. Parafilm solution and store in refrigerator

Decision Point (DP) Preparation:

1. Prepare the same day as unknown samples that the Semi-Quant sequence will begin running. The DP can be used for 48 hours from the time it is initially injected on the GC/MS.
2. Add 500 µl of ∆9-THC reference material to an autosampler vial with a calibrated mechanical pipette
3. Add 25 µl of ISS to the autosampler vial with a calibrated mechanical pipette
4. Add 475 µl of MeOH to the autosampler vial with a calibrated mechanical pipette
5. Cap vial and agitate
6. Assign lot # to DP. (DPmmddyy)
7. Example: DP made on August 7, 2020 would have the lot # DP080720

Semi-quantitation Sample Preparation:

1. Weigh out 90-110 mg of plant material and add to a test tube
2. Add 50 µl of ISS to test tube with a calibrated mechanical pipette
3. Add 1950 µl of MeOH to test tube with a calibrated mechanical pipette
4. Vortex for 10 seconds and allow extract to sit for 10 minutes
5. Filter extract using glass pipette filled with glass wool into autosampler vial

Semi-quantitation Negative Control Preparation:

Negative control shall be prepared at the same time as sample. The same lot number of ISS and MeOH shall be used for the negative control and case samples.

1. Add 50 µl of ISS to test tube with a calibrated mechanical pipette
2. Add 1950 µl of MeOH to test tube with a calibrated mechanical pipette
3. Vortex for 10 seconds and allow extract to sit for 10 minutes
4. Filter extract using glass pipette filled with glass wool into autosampler vial

Semi-quantitation Positive Control Preparation:

Positive control shall be prepared on the same day as the Decision Point. The same lot number of ISS and MeOH shall be used for the positive control and the decision point.

1. Weigh out 100 mg of positive control of known THC concentration from Purdue University
2. Add 50 µl of ISS to test tube with a calibrated mechanical pipette
3. Add 1950 µl of MeOH to test tube with a calibrated mechanical pipette

4. Vortex for 10 seconds and allow extract to sit for 10 minutes
5. Filter extract using glass pipette filled with glass wool into autosampler vial

Semi-quantitation GC/MS Sequence Preparation:

A negative control and a decision point shall be run prior to the samples. If the blank for the decision point or the negative control is unacceptable, the blank for the decision point and/or negative control and the decision point and/or negative control can be re-run after the samples within a 48-hour window. A blank shall be run before each sample. A positive control shall be run as part of the GC/MS sequence.

GC/MS Method Parameters:

An approved semi-quantitative GC/MS method shall be used.

Data Analysis

All data analysis is performed using ChemStation software. The decision point (DP) is used to set the expected response ratio for a 1% sample, and it is assigned a value of 1. Each of the unknowns is compared to that ratio. The software calculates a value, using the decision point, which is either greater than, equal to, or less than 1.

$$\textbf{Concentration ratio} = \frac{\left(\frac{\textbf{Unknown THC Response}}{\textbf{TBA Response}}\right)}{\left(\frac{\textbf{Known THC Response}}{\textbf{TBA Response}}\right)}$$

If a # symbol is observed on the Quantitation Report generated for a sample, the Forensic Scientist shall evaluate the integration of the peak to ensure that it was properly integrated. Documentation that this evaluation was performed shall be documented in the case notes; a note, checkmark, or initials next to the # symbol is sufficient documentation. If the peak was not properly integrated, the peak shall be manually integrated.

Decision Point Response Ratio

The response ratio of the TBA and the Δ9-THC shall be evaluated to determine if the decision point (DP) response ratio falls within 20-50%. The DP response ratio shall be calculated using the following formula:

$$\boldsymbol{DP\ Response\ Ratio} = \frac{\boldsymbol{DP\ TBA\ Response}}{\boldsymbol{DP\ \Delta 9-THC\ Response}}\ \boldsymbol{x\ 100}$$

If the DP response ratio falls within this range and the positive control result is acceptable, semi-quantitation may continue as usual. If the DP response is outside of this range, semi-quantitation shall not continue until the problem is resolved.

Sample/Positive Control TBA Response

The same concentration of TBA is present in the DP and all samples; therefore, the TBA response in the DP and all samples run in a semi-quantitation batch should be similar. Forensic Scientists shall check the TBA responses for the DP, positive control, and all samples, for consistent TBA responses. An acceptable TBA response for the positive control and all case samples shall be no greater than twice the TBA response of the DP.

$$\boldsymbol{Maximum\ TBA\ Response\ in\ Sample = TBA\ Response\ in\ DP\ x\ 2}$$

If the TBA response of the positive control falls outside of this acceptable limit, semi-quantitation shall not continue until the problem is resolved. If the positive control fails, do the following:

- Re-run the decision point and positive control on a different instrument with an appropriate column for semi-quantitation.
- If the TBA response of the positive control now falls within the acceptable range and the DP response ratio of the new run of the DP also falls between 20-50%, semi-quant may be run on the new instrument.
  - Document the failure of the original positive control on the Decision Point Log.
- If the TBA response of the positive control is still outside of the acceptable range, the forensic scientist shall re-prep the positive control and re-run. If the DP is still within the 48-hour window, the DP does not need to be re-run. If the DP is outside of the 48-hour window, a new DP shall be prepared.

If the TBA response of a sample falls outside of this acceptable limit, the data from that run shall be rejected. If a sample fails, do the following:

- Re-run the decision point, positive control, negative control, and sample on a different instrument with an appropriate column for semi-quantitation.
  - If the TBA response of the sample now falls within the acceptable range and the DP response ratio of the new run of the DP also falls between 20-50%, the semi-quantitation result for this new run may be used.
    - Document the data for both runs on the Excel spreadsheet on SharePoint.
  - If the TBA response of the sample is still outside of the acceptable range, the forensic scientist shall re-sample the item and re-extract for semi-quantitation (a new negative control shall also be prepared). If the DP is still within the 48-hour window, the DP does not need to be re-run. If the DP is outside of the 48-hour window, a new DP shall be prepared.
    - If there isn't enough of the item to re-sample for semi-quantitation, contact a Drug Unit Supervisor.

APPENDIX 6
INSTRUMENT PREVENTATIVE MAINTENANCE

Preventive maintenance is performed on analytical equipment to ensure the system continues to perform properly. This is accomplished by inspecting, testing, and/or cleaning the equipment at specific intervals. All preventive maintenance shall be documented in the appropriate instrument's maintenance log or checklist. The maintenance schedules below represent the maximum maintenance intervals.

Each instrument has a primary operator that is responsible for the scheduling of calibration, conducting verifications and maintenance of the instrument. In the absence of the primary operator, another Forensic Scientist shall be assigned these duties.

The maintenance schedules for balances, ultraviolet spectrophotometers, polarimeters, and FTIR instruments are located within their individual Test Methods section.

Microscopes:

Microscopes shall be cleaned as needed. If a microscope fails to perform properly or is in need of repair, the appropriate personnel shall be notified.

Gas Chromatograph/Mass Spectrometer (GC/MS) and Gas Chromatograph-Infrared Spectrophotometer (GC-IR):

The GC/MS and GC-IR instruments shall be serviced as needed. When one of the instruments cannot pass the calibration and quality control checks as per Drug Unit Test Methods, it shall be serviced by a qualified Forensic Scientist or certified technician. The service shall be documented in the maintenance log for the instrument. Before being placed back into service, the instrument shall pass all calibration and quality control checks.

The instrument shall be cleaned as needed. The column, chips, filaments, gaskets, and seals as applicable to the instrument shall be replaced as needed. The GC liner, syringe and septum shall be replaced as needed.

For GC/MS, if maintenance is performed on the GC, which necessitates the instrument being vented, a blank and test mix shall be run before putting the instrument back into service. If maintenance is performed on the MS, then a tune, blank, and test mix shall be run before the instrument is placed back into service. If a filament is switched without venting the instrument, only a tune is required to put the instrument back into service. When the column is replaced or the column is trimmed at the transfer line nut and ferrule during any maintenance, the column length shall be calibrated. All methods on that instrument shall be updated with the new column length. All updates to the methods shall be verified by a secondary analyst. Both analysts shall initial and date method log sheet and update the binder. The methods shall be updated, printed/archived, and verified by both the first and second analyst before the

instrument can be placed back in service. Calibration is not required when a minimal amount (less than 6 inches) is trimmed to replace the inlet nut and ferrule.

For GC-IR if maintenance is performed on the GC that requires removing the column from the inlet or butt connector, a blank and test mix shall be run before putting the instrument back into service. If maintenance is performed on the IR, then a voltage check, noise check, and polystyrene reference material check shall be run before the instrument is placed back into service.

APPENDIX 7
HYPERGEOMETRIC TABLE

Population size N	95% confidence	99% confidence
	k=0.9	k=0.9
10	8	9
20	12	15
30	15	20
40	18	23
50	19	26
60	20	28
70	21	30
80	22	31
90	23	32
100	23	33
200	26	38
300	27	40
400	27	41
500	28	41
600	28	42
700	28	42
800	28	42
900	28	43
1000	28	43
5000	29	44
10000	29	44

APPENDIX 8
WASTE DISPOSAL PROCEDURES

Non-hazardous Chemical Waste:

Not all waste materials and chemicals in a laboratory are hazardous waste. For the Indiana State Police Forensic Services Division, these include paper and plastic trash, empty containers, broken glass, non-hazardous liquid and solid wastes, GC/MS vials, and color/spot testing waste from spot plates.

Solvents used in extractions to recover drugs for further analysis are evaporated in a fume hood.

> Example: Chloroform used in extraction from 0.45 N Sodium Hydroxide to purify Hydrocodone from a mixture with acetaminophen is evaporated to recover Hydrocodone for further analysis.

Chemicals from color/spot testing and clean-up of spot plate may be disposed of through flushing into a sink drain connected to a sanitary sewer with at least twenty (20) volumes of water for each volume of waste.

GC/MS vials containing small amounts of solvent can be disposed of in the “broken glass” box for final disposal in “normal” trash.

Empty glass containers and broken glass are collected in “broken glass” boxes. Empty containers as defined by EPA and IDEM are described in the Laboratory Waste Management Program. After the “broken glass” boxes are full, they shall be sealed for final disposal in “normal” trash.

Empty aerosol cans may be disposed of in “normal” trash. To be considered empty, aerosol cans shall contain no propellant and no product, and shall be at atmospheric pressure.

Non-hazardous liquid and solid wastes may be processed for disposal down a sink drain or in “normal” trash as outlined in the Laboratory Waste Management Program. A list of non-hazardous chemicals suitable for drain or trash disposal is included as an appendix in the Laboratory Waste Management Program. You may dispose these types of solid chemicals in normal trash if the containers are tightly capped and of good integrity.

If you are unsure whether or not you should dispose of a material as a non-hazardous waste, then it should be handled as a hazardous chemical for waste disposal.

Acid and Base Disposal:

Acidic and alkaline (basic) chemical wastes are classified as hazardous waste if the pH is less than or equal to 2 or greater than or equal to 12.5. If the acid or alkaline waste ONLY has characteristics of corrosivity and is NOT a listed waste, it may be neutralized to within a pH range of 5 to 9 before disposal to a sanitary sewer. Neutralization can be incorporated in the analysis procedure.

Neutralized acid and alkaline waste shall be flushed with at least twenty (20) volumes of water for each volume of waste.

Acid and Base Neutralization Procedures:

These procedures explain the disposal of concentrated solutions of acids, such as hydrochloric, nitric, and sulfuric acid, and bases such as ammonium hydroxide and sodium hydroxide.

Caution: vapors and heat are generated during neutralization.

You are not required to neutralize any wastes yourself. If you choose to neutralize and dispose of these materials yourself, please adhere to the following.

- Perform all steps slowly.
- Keep containers cool while neutralizing.
- Acid neutralization: While stirring, add acids to large amounts of a cold solution of aqueous base (sodium carbonate, calcium hydroxide, or 8 M sodium hydroxide).
- Base neutralization: First add the base to a large vessel containing cold water. Slowly add a 1 M solution of HCl.
- Neutralize concentrated acid and base solutions to within a pH range of 5 to 9, and then flush them into the sanitary sewer with at least 20 volumes of water for each volume of waste.
- If necessary, allow the contents to react for at least twenty-four hours to obtain a stable pH and to dissipate any heat associated with the neutralization reaction. The container should not be hot, and the contents should not be smoking.

Hazardous Chemical Waste:

Forensic Services Division personnel are not responsible for final classification of waste chemicals for hazardous waste manifests, yet shall be generally aware of waste classification criteria to determine if a chemical is hazardous or non-hazardous for disposal. The classifications for hazardous wastes are: F-list, K-list, U-list, P-list, and characteristic wastes. Information is included in the Laboratory Waste Management Program to classify potentially hazardous chemical wastes.

F-list waste: These are non-specified source waste. This includes all spent solvent mixture/blends containing, before use, a total of 10% or more (by volume) of one or more of the solvents listed in F001, F002, F003, F004, and F005

K-list waste: **This list does not apply to Indiana State Police Regional Laboratories.** It includes certain waste from specific industries, such as petroleum refining or pesticide manufacturing.

U- and P-list waste: (discarded and unused commercial chemical products) U and P list waste include specific commercial chemical products in an unused or "virgin" form.

- Virgin chemicals: a chemical that has not been previously used or consumed, or subjected to processing other than for its original production.

- P-list chemicals are classified as acutely hazardous waste, and are subject to a 1 kg limit for accumulation quantity.

Characteristic hazardous waste groups are classified by characteristics of ignitability, corrosivity, reactivity, and toxicity.

Drug Unit procedures for collection, storage, and disposal of hazardous waste:

The proper way to collect and store hazardous waste is through use of hazardous waste containers in a Satellite Hazardous Waste Accumulation area until full. Transfer the full container to a Central Hazardous Waste Accumulation area for disposal by a contracted chemical waste disposal vendor.

A minimum of three satellite hazardous waste collection containers shall be available for use in the Drug Unit.

1. Chlorinated waste including chloroform, chloroform mixtures (e.g., chloroform, methanol, and acetic acid thin layer chromatography system mixture), etc.

2. Flammable wastes including methanol, acetone, pentane, hexane, petroleum ether, toluene, flammable organic chemical mixtures, etc.

3. Oxidizers including iodoplatinate and potassium dichromate

Organic chemicals used as a rinse in cleaning glassware shall be collected as either chlorinated wastes or flammable waste.

> Example: Chloroform rinse of glassware shall be collected as chlorinated hazardous wastes. Methanol rinse of glassware shall be collected as flammable hazardous wastes.

Solvents used in extractions that are not evaporated to recover drugs for further analysis shall be processed as hazardous waste.

> Example: Mushroom extractions using chloroform from acid, followed by making the aqueous solution basic and extracting with chloroform to extract psilocin. Chloroform from the acidic extraction shall be handled as chlorinated hazardous waste. Chloroform from the basic extraction will be evaporated to recover psilocin.

Chemicals in color tests (i.e., Duquenois Levine) conducted in a test tube shall be processed as chlorinated hazardous waste.

Full or partially empty aerosol cans shall be collected for disposal as hazardous waste in a satellite container labeled as "Aerosol Cans" for hazardous waste disposal. IDEM and EPA regulate all partially empty spray cans as hazardous waste because they may still contain chlorinated solvents, flammable material, or toxic substances. **Do Not** discard partially empty spray cans in the trash. **Do Not** puncture any aerosol cans.

Dilution of hazardous chemical wastes and disposal in the sink drain is not the proper way to dispose of hazardous waste.

If a spill occurs, the chemical in the spill and the materials used to clean up the spill are considered to have the same hazard classification. Spill clean-up materials are not to be thrown in the "normal" trash. These materials are to be properly disposed of as hazardous waste. Procedures in the Laboratory Chemical Spill Management Program shall be used for spill clean-up and disposal.

Hazardous Waste Containers:

- Each Regional Laboratory and/or Drug Unit shall supply their own containers
- For liquid wastes, the amber 4-liter solvent bottles are preferred because they are non-recyclable and are compatible with most types of waste.
- All containers shall be in good condition and compatible with their waste contents. The original container the chemical came in is usually the best container for chemical waste.
- All containers shall have securely fitting lids or caps.
- Funnels shall be removed and not left in waste containers.
- Hazardous waste container shall be marked "hazardous waste."
- The chemical waste contents shall be listed on the label or an attached tag.
- Containers shall be stored with a closed lid or cap.

Liquid Hazardous Waste Containers:

- Leave 10% headspace (volume left at top of container) in case of expansion due to temperature.
- Do not pour hot liquids into hazardous waste bottles.
- Do not combine or comingle incompatible wastes (i.e., acids and bases)

- Provide secondary containment.
- Any container with a capacity of less than or equal to 4 liters shall have secondary containment.

Solid Hazardous Waste Containers:

- The original container is generally the “best” waste container for solid hazardous waste.
- If original containers are not available – double bag the material and place in a sturdy cardboard box for support.
- Do not use Biohazard bags.
- Bags used should be trash bags.

Satellite Hazardous Waste Storage:

- Hazardous waste regulations require that the generator accumulate hazardous chemical waste in containers at or near the point of generation where waste initially accumulates until full and which is under the control of the operator who generated the waste.
- Under no circumstances shall waste be stored down the hall and/or out of your control.

Central Hazardous Waste Storage:

- Full hazardous waste containers shall be marked with the accumulation date (date the satellite waste container was completely filled with the hazardous waste, not the date the collection of hazardous waste began in the satellite container).
- Move the full waste container to the Central Hazardous Waste Accumulation Storage area within three days after being filled to capacity.
- All waste containers shall have securely fitting lids or caps.
- Provide secondary containment, as necessary to contain spills.

Secondary Containment to Minimize Spills of Hazardous Wastes:

- Secondary containment shall be used to minimize the potential for breakage, spillage, and the comingling of incompatible materials (i.e., acids and bases).
- Plastic trays, pans, or tubs may be used.
- Without exception, secondary containment is required for the following:

- All glass containers of liquid hazardous waste stored on the floor.
- All containers with capacity less than or equal to 4 -liters of liquid hazardous waste, regardless of storage location.

- Hazardous materials shall be segregated by hazard class and stored in separate cabinets, trays, or pans.

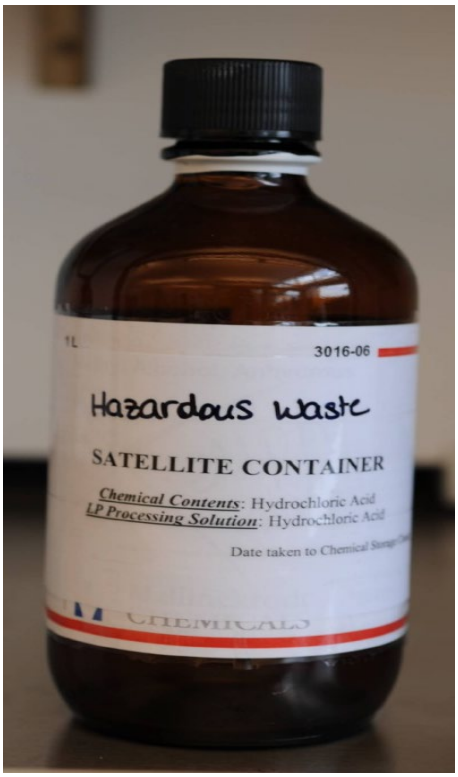
Example – Leaks with Spill Contained in Tray:

Lids or Caps on Hazardous Waste Containers:

- Lids shall be securely in-place except when material is being removed or added to the container.
- A funnel resting on the mouth of a bottle does not constitute a lid
- Lids on waste containers shall be on tight (Note: Be sure that gas producing reactions have worked to completion before transferring the material to a hazardous waste container).
- A closed container, when tipped over, will not leak!

Example - Improper Lids [Open Containers]:

1. Lid open when not in use.
2. A funnel is not a lid.



Labels on Hazardous Waste Containers:

- If a chemical container is reused, the original label shall be defaced, removed, or completely covered.

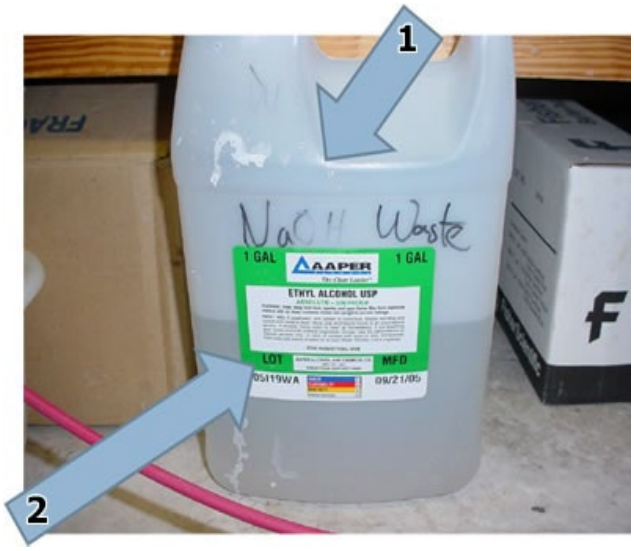
- EPA and IDEM regulations require that the name of waste chemicals be clearly identified on the label or attached tag.

- Chemical formulas and abbreviations such as H_2SO_4, HCl, NaOH, HOAc, and MeOH are **NOT** accepted by EPA and IDEM. Use the chemical name such as sulfuric acid, hydrochloric acid, sodium hydroxide, acetic acid, and methanol.

- Hazardous waste regulations require the words "Hazardous Waste", or words which clearly identify the contents such as "Acetone Waste", be on each waste container.

- The satellite container label shall have an area where the accumulation date (the date that the container is full – NOT the date that collection began in the container) can be documented.

Example – Proper Label: **Example - Improper Label:**



1. Chemical formulas or abbreviations are not allowed.

2. If you re-use a container for collecting hazardous waste, you shall deface, cover, or remove the original label.

APPENDIX 9
WORKSHEET AND DOCUMENTATION GUIDELINES

Electronic Notes

- Electronic worksheets shall be in portable document format (pdf).
- The controlled text shall be in black font color (e.g. names of tests, balances, weights).
- The fillable portions of version 1 of the worksheets shall be in blue font color.
- Any text added to the worksheet shall be in Verdana 9pt font. Any text added to your notes shall be Verdana 9-12pt font.
- Observations shall be typed contemporaneously, and any non-contemporaneous changes needed should be corrected by a single line, initial, and date.
- Any corrections/changes made to subsequent versions of the worksheet shall be done in red or purple font color.
- Any corrections/changes made to subsequent versions of the worksheet should be made electronically by a single line, initial, and date with the addition of any new/updated text.
- Any corrections made to instrumental data shall be single lined and initialed in all but the file name. The file name shall not be changed on the data since it refers to the storage location of the electronic copy of that data file.
- The worksheet shall be printed to pdf before being uploaded to LIMS.
- All pages of the technical record should be initialed (or signed) electronically or by hand.

Case Note Organization

- Supporting data shall be in the same order as the worksheet.
- Data shall be in sequential order for each test (e.g. GC/MS blank 1, run 1, GC-RT blank 1, run 1)
- The TIC should only be included once per run.
- The mass spectrum of the low concentration drug that you are rerunning shall be printed at the bottom of the TIC for a low concentration run.
- The mass spectrum for the peak in the blank shall be printed at the bottom of the blank.
- For GC/MS, GC-RT, and GC/IR the peaks shall be in order by retention time.
- For semi-quantitation analysis of multiple plant material items in one case, the NC and DP shall only be included once.
- Semi-quantitation data shall be in the following order:
  DP blank, DP, DP report, NC blank, NC, Sample blank, sample, sample report

APPENDIX 10
REPORT WORDING

The following are report wording templates for most situations. These wording guidelines or similar verbiage shall be used.

General Non-Statistical Results (single item or all samples within an item have been examined)

Item XXX was found to contain X, a controlled substance.
The net weight of item XXX was X grams.

General Non-Statistical Results (with sub-items)

Items XXX(A-C) were found to contain X, a controlled substance.
The net weight of items XXX(A-C) was X grams.
Items XXX(D-F) were not examined and had a (net/gross) weight of X grams.

Or

Item XXX: Thirty-seven (37) packets were examined, and each found to contain X, a controlled substance, and had a total net weight of X grams.
The remaining sixty-three (63) packets were (visually/not) examined and had a (net/gross) weight of X grams.

General Reporting- Insufficient for Confirmation

Item XXX indicated the presence of X, a controlled substance; however, there was insufficient material (or other reason) for complete identification.

Or

Item XXX indicated the presence of X, a controlled substance; however, this could not be confirmed due to insufficient material (or concentration of the sample, sample degradation, inconclusive testing results or other reason).

Items where MU causes the weight to fall below a Weight Threshold

Item XXX was found to contain X, a controlled substance. The net weight of item XXX was x.xx gram(s) +/- x.xx gram at a coverage probability of 95.45%.

Reference ID (No Further Testing)

Item XXX was visually examined. Reference(s) and markings indicated the presence of X. No confirmatory analysis was performed.

Also acceptable:

Item XXX was visually examined. Reference(s) and markings were consistent with a preparation containing _____. No confirmatory analysis was performed.

Reference ID of Manufacturer Sealed Packaging

Item XXX – Reference identification of the sealed packaging indicated the presence of X, a controlled substance. No confirmatory analysis of this item was performed.

Tablet and Capsule Reporting (with Reference ID for Remaining)

Item XXX: One tablet (capsule) was examined and was found to contain X, a controlled substance, and had a net weight of X grams.

The remaining tablets (capsules) were visually examined and had a net weight of X grams. Reference(s) indicated the presence of Y, a controlled substance. No confirmatory analysis was performed on the remaining tablets (capsules).

Also acceptable:

Item XXX: One tablet (capsule) was examined and was found to contain X, a controlled substance. Refence also indicated the presence of X.

The net weight of the examined tablet was X grams.

The remaining tablets (capsules) were visually examined and had a net weight of X grams. Reference(s) indicated the presence of X, a controlled substance. No confirmatory analysis was performed on the remaining tablets (capsules).

Tablet and Capsule Reporting (No Reference ID for Remaining)

Item XXX: One tablet (capsule) was examined and was found to contain X, a controlled substance and had a net weight of X grams.

The remaining tablets (capsules) were not examined/analyzed and had a net weight of X.

Illicit Tablet/Capsule Reporting

Item XXX: One tablet/capsule was examined and was found to contain X, a controlled substance, and had a net weight of X grams.

The remaining tablets/capsules were not examined/analyzed and had a net weight of X. If analysis of additional tablets/capsules is necessary, please contact the laboratory.

Statistical (Hypergeometric) Sampling

Item XXX was found to contain X, a controlled substance. The net weight of item XXX was X gram(s). This result is based on hypergeometric sampling that meets or exceeds a 95% confidence level that 90% of the containers are positive.

Hypergeometric sampling is a statistically based method which involves taking random samples of the whole population. This method allows an inference to be made about the contents of the whole population.

Or

Item XXX was found to contain X, a controlled substance.
The net weight of item XXX was X gram(s).
This result is based on hypergeometric sampling that meets or exceeds a 95% confidence level that 90% of the containers are positive. The weight of 90% of the containers does not exceed the statutory weight threshold.

Hypergeometric sampling is a statistically based method which involves taking random samples of the whole population. This method allows an inference to be made about the contents of the whole population.

Items Where Specific Isomer Cannot Be Determined at Lab of Submission

Item XXX was found to contain Fluoro-PB-22. The specific isomer was not determined.

If the specific isomer needs to be identified, please contact the laboratory.

Plant Material Reporting

Marijuana Reporting (positive on Semi-Quant)

Item XXX was found to contain Marijuana, a controlled substance, as determined by comparison with a 1% delta-9-Tetrahydrocannabinol (THC) reference material.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.The net weight of item XXX was XXX grams.

Plant Material Reporting (Semi-Quant Result of <1, Weight ≥0.50g)

Item XXX was found to contain/indicated the presence of THC/CBD/CBN. When compared to a 1% delta-9-Tetrahydrocannabinol (THC) reference material, Hemp/Marijuana could not be determined. If quantitative analysis is necessary, please contact the laboratory.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.**Plant Material Reporting (Semi-Quant Result of <1, Weight <0.50g)**

Item XXX was found to contain/indicated the presence of THC/CBD/CBN. When compared to a 1% delta-9-Tetrahydrocannabinol (THC) reference material, Hemp/Marijuana could not be determined. However, the sample size was insufficient for quantitative analysis.

The analysis did not differentiate between delta-9-Tetrahydrocannabinol (delta-9-THC) and delta-9-Tetrahydrocannabinolic Acid (delta-9-THCA). Delta-9-THCA is a precursor of delta-9-THC.

Semi-Quant Analysis, No THC Identified

Item XXX was found to contain/indicated the presence of Cannabidiol (CBD)/ Cannabinol (CBN). Hemp/Marijuana could not be determined.

No Positive Microscopic and/or No THC on TLC

Item XXX was found to contain Tetrahydrocannabinol (THC)/ Cannabidiol (CBD)/ Cannabinol (CBN). This item did not qualify for determination of Hemp/Marijuana.

Or

Item XXX indicated the presence of delta-9-Tetrahydrocannabinol (delta-9-THC)/delta-9-Tetrahydrocannabinolic acid (delta-9-THCA).

Plant Material Reporting (Less than 0.20g, Microscopically Consistent with Marijuana, add Microscopic to Tests Used)

If the plant material has a positive microscopic exam for Cannabis/Marijuana (cystolithic hairs and fine hairs) or has a general appearance microscopically that is consistent with Cannabis (e.g. – detached hairs, burnt hairs, stems) or is too burnt to see any microscopic characteristics (ash material), then the item should be withdrawn. The burnt characteristics or if the appearance is not consistent with Cannabis shall be noted in the case notes. Use the following wording:

Item XXX was visually examined. The net weight of item XXX was XXX gram. This item did not qualify for determination of Hemp/Marijuana. No confirmatory testing was performed.

Cannabis mixed with other substances

If an item contains Cannabis and other substances (e.g powder, crystal, wax, tobacco) and can be separated from each other, analyze the other substance. If the two substances can be separated, the items should be sub-itemized and weighed separately.

If the other substance is delta-9-THC or delta-9-THCA, semi-quant analysis shall not be performed on the Cannabis plant material. Analyze cannabinoids using GC/MS and TLC. If an isomer of Tetrahydrocannabinol is present in the GC/MS, derivatize the sample.

If the other substance is not delta-9-THC or delta-9-THCA, conduct semi-quant analysis on the Cannabis plant material. Report out the other substance, if necessary.

If the Cannabis plant material cannot be separated from the other substance, a representative sample shall be taken. GC/MS shall be performed on the representative sample. Analyze cannabinoids using GC/MS and TLC. If an isomer of Tetrahydrocannabinol is present in the GC/MS, derivatize the sample.

Item XXX was found to contain/indicated the presence of Tetrahydrocannabinol (THC)/Cannabidiol (CBD)/Cannabinol (CBN). This item did not qualify for determination of Hemp/Marijuana.

Withdrawn Additional Plant Material (Cannabis and Synthetics that will not meet additional weight thresholds)

Item XXX was visually examined. The item was withdrawn per Indiana State Police Physical Evidence Bulletin 01. If analysis is necessary, contact the laboratory.

Sample Sent to Outsourcing Lab

The results/opinions/interpretations for Laboratory Case Number XXXX Item XXX can be found in the Drug Analysis Request 0001.

A portion of Item XXX (sub-item XXXA) has been outsourced to another laboratory to determine the percent concentration of total potential THC, the result of which will be the subject of a separate report by the outsourcing laboratory.

See TM Section 14 for additional wording options for items concerning Δ9-THC and Δ8-THC.

<u>Synthetic Drugs Reporting</u>

Date of Seizure before Control Date

Item XXX was found to contain X. X was controlled in the State of Indiana as of date. The specific isomer was not determined. If the specific isomer needs to be determined, please contact the laboratory.

Or

Item XXX indicated the presence of X. X was controlled in the State of Indiana as of date.

Specific Isomer Not Determined

If the specific isomeric form has not been determined, the following statement shall be included in the results:

“The specific isomer was not determined.”

This statement shall be omitted if the specific isomer has been determined.

If the substance is only indicated, then the specific isomer statement shall be omitted.

Substances listed in a section in the Indiana Criminal Code that does not specifically say "including their isomers"(for example - "Synthetic drugs" section of the Indiana Criminal Code IC 35-31.5-2-321 or 4-ANPP)

Item XXX was found to contain ANPP. 4-ANPP is a controlled substance. The specific isomer was not determined. If the specific isomer needs to be determined, please contact the laboratory.

If the substance is only indicated, then the control status and the specific isomer statement shall be omitted.

Structurally Controlled Substances

Item XXX was found to contain α-Pyrrolidinoisohexanophenone (α-PiHP) (or an isomer thereof), a controlled substance structurally derived from 2-aminopropan-1-one.

Or

Item XXX was found to contain α-Pyrrolidinoisohexanophenone (α-PiHP) (or an isomer thereof), a controlled substance. α-Pyrrolidinoisohexanophenone (α-PiHP) is (a compound) structurally derived from 2-aminopropan-1-one.

Item XXX was found to contain Fluorofentanyl, a controlled substance structurally related to Fentanyl, a controlled substance.

Substantially Similar Substances (Confirmation not Required)

Item XXX was found to contain/indicated the presence of ADB-INACA, which has a substantially similar structure to ADB-PINACA, a controlled substance.

Withdrawn Items (Backlog Reduction Plan)

Item XXX was withdrawn per Indiana State Police Physical Evidence Bulletin 01. If analysis is necessary, please contact the laboratory.