MNPD Crime Laboratory

Drug Identification Technical Procedures Manual

Metropolitan Government of Nashville & Davidson County
Police Department

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2 Scope

Forensic Drug Identification involves the use of various scientific procedures to examine and analyze powders, capsules, tablets, plant material, liquids, and other substances submitted by criminal justice officials to identify controlled substances and/or non- controlled substances. The results of those analyses are then compiled into an official report suitable for use in a court of law.

Due to the great diversity of drugs as well as variations in size and concentration, we have established guidelines for sample analysis. These procedures allow the individual analyst the flexibility to choose the best methods to use in performing his/her analysis.

3 Evidence Handling, Selection, and Description

3.1 Receiving Evidence from the Evidence Receiving Unit (ERU)

The Evidence Receiving Unit (ERU) has evidence vaults for drug evidence storage that are held secure.

The Forensic Scientist from Drug ID will provide a list of evidence to be retrieved for analysis to the ERU staff. Procedures for the transfer of evidence between a unit scientist and ERU staff are found in the ERU SOP.

3.2 Evidence Control During Examination (In Process)

All evidence received from the Evidence Receiving Unit will be secured in the forensic scientist's personal storage locker (PSL), long term PSL, personal storage cabinet (PSC), or Drug ID Unit bulk storage cage (UBSC) while in process.

The Drug ID Unit has an evidence storage room which has assigned PSLs for short and long term storage and two (UBSCs). Transfers to and from a PSL or PSC are not documented in the CoC and will be reflected as being in the scientist's possession.

All evidence must be stored in PSLs or in a UBSC overnight. Evidence may be left out in unit work areas while analyses are being performed, and may be temporarily stored in a PSC when the scientist is out of the lab area temporarily during work hours.

Scientists will not keep evidence in their personal custody for more than 120 days. After this time, evidence will be stored in the long term PSL, UBSC, or returned to ERU.

When evidence submission discrepancies occur on a piece of evidence (name on evidence does not correspond with information written on the request form, evidence description does not match evidence submitted, misspelled subject name, incorrect date, incorrect complaint number, etc.), documentation will be made in the case file of 1) the discrepancy and 2) the communication of the discrepancy to the submitting officer.

- Note: if prior communication has been received from the submitting officer that would

explain any evidence discrepancy (i.e. only evidence that is being requested to be tested is on 282), then no correspondence to the submitting officer is required.

No more than one item of evidence shall be opened in the scientist's work area at one time.

Work areas will be kept clean and orderly.

Scientists shall do a walkthrough of the laboratory after sampling to ensure all evidence has been secured. The walkthrough shall include checking all work areas and balances used during sampling and an inventory of evidence.

If it becomes necessary to use the entire sample, the analyst will record this in the laboratory report with a statement such as "evidence consumed in analysis."

The forensic scientist will re-seal the evidence once sampling or the examination is complete. The evidence will be sealed in such a way that it will be obvious to the scientist if it is opened at a later date. Seals will consist of MNPD Crime Lab evidence tape and additional tape if needed. Evidence can be heat-sealed in plastic with evidence tape across the seal. Additional packaging can be used as necessary to contain loose evidence samples or leaking containers but must be documented in the case notes and used in such a way as to not cover up barcodes or other important information.

The seal will, at a minimum, be marked with the forensic scientist's initials and the date sealed. The laboratory number must be clearly visible on the outer packaging.

The evidence will be stored in the scientist's PSL (short or long term, as appropriate) or UBSC until it is returned to ERU.

3.3 Evidence Description

After removing the sample from its packaging (if appropriate), a physical characterization of an exhibit will be conducted, and the observations will be noted. The observations may include the type of material, color, size, shape, amount, significant markings, odor, texture, etc.

The analyst will provide a thorough description of the evidence so that at a later date (i.e., court testimony) the analyst will be able to recognize the evidence and be able to provide specific information about the item(s) tested.

A description of the packaging and evidence received will be documented in the case file. Approved descriptive abbreviations may be used and are listed in Appendix C of this TPM.

3.4 Selection of Evidence Items for Testing

In every case, the most significant items in terms of quantity and schedule are analyzed to maximize the resources of the laboratory. Routinely, a maximum of three (3) items will be analyzed per case. Consideration will be given to the information contained within the evidence submission form. This includes information such as specific charges or types of offenses, items unique to a single suspect, the statement of facts and examinations requested, evidence

descriptions, as well as the analyst's visual inspection of the items.

If it becomes apparent that items not previously analyzed will require analysis, then upon re-submission, those items will be analyzed.

Forensic identification will only be routinely performed on controlled substances.

In general, residues in drug paraphernalia, cigarettes, or cigarette butts will not be analyzed when measurable quantities of the associated drugs are also included among the items submitted.

- Example 1: Submitted evidence includes a plastic corner bag containing solid material and a glass tube smoking device with residue. The solid material would be analyzed and the smoking device would not.

- Example 2: Submitted evidence includes three tablets containing possible oxycodone and a plastic straw containing a residue. The tablets would be analyzed, but the straw would not.

- Example 3: Submitted evidence includes three tablets containing possible diazepam and a plastic straw with residue. The tablets would be analyzed and the straw would not unless information on the evidence form indicates that the straw was used for a different drug.

- Example 4: Submitted evidence includes a plastic bag of plant material and a glass tube smoking device with residue. Both the plant material and the smoking device may be analyzed.

Pharmaceutical preparations will be visually examined using pharmaceutical identifiers and appropriate reference material.

- No further analysis is required for intact, marked pharmaceutical preparations (e.g., tablets or untampered capsules) indicated as non-controlled or over the counter preparations. These may be reported as "Not Analyzed" or "No chemical examinations performed as item markings/logo identification are consistent with the presence of a pharmaceutically prepared [enter drug classification], a non-controlled substance." Also see QM 7.8 and TPM chapter for Reporting.

- If identical intact, marked pharmaceutical preparations (e.g., tablets or untampered capsules) are present in multiple items, analysis is required for only one item.

- Partial pharmaceutical preparations may not be analyzed when similar intact pharmaceutical preparations or measurable quantities of drugs are present.

No examinations will be performed on seeds.

No examinations will be performed on currency.

Syringes and their contents will not be routinely analyzed.

- If the nature of a case dictates that a syringe or its contents must be analyzed, a written request must be made by the District Attorney's Office explaining the importance of the syringe analysis. The request must be approved by the Drug Unit Supervisor or higher authority. The syringe must be received at the MNPD Crime Lab in a puncture resistant container.

No examinations will be performed on suspected mushroom spores.

If items are not analyzed per this procedure, the scientist's case notes shall indicate this by a notation of "not analyzed".

For cases in which the sample has been consumed in analysis, the GC vial containing the extract shall be recapped and packaged with the evidence in the event that future analysis is required. Additionally, a note will be placed in the case notes to indicate that the vial was preserved.

3.5 Returning Evidence to the Evidence Receiving Unit

When all testing or sampling is completed, the evidence is returned to the Evidence Receiving Unit and documented in the chain of custody.

4 Sampling

4.1 Introduction

Caution: New Psychoactive Substances (NPS) – See Section 27.5 for special procedures and safety precautions when sampling these substances.

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

The section's overall goal is to analyze a sufficient number of items to substantiate the highest possible charge(s) represented in the case while also minimizing the number of items tested. In the majority of cases, sample selection will be employed.

For items containing multiple specimens where it is necessary to draw a statistically valid conclusion about the presence of a drug in the whole population, sampling models (e.g., hypergeometric distribution) will allow the analyst to analyze a portion of the specimens and subsequently make statistical inferences about the population.

For cases containing multiple exhibits, the analyst working the case will select exhibits to test based on their training and experience. Items selected for testing from multi-unit populations will also normally be selected based on training and experience.

Note: Reductions in sampling procedures are acceptable with documented approval from the District Attorney General's Office. Documentation should include the date of approval, what is approved, and the attorney's name. In some instances, approval from the investigating officer/detective may be more appropriate.

4.2 General Sampling

Every effort shall be made to avoid handling evidence repeatedly. The material will be sampled and immediately sealed.

For chemical analyses, a representative sample shall be removed from the specimen. When sample size allows, testing should be applied on separate samplings of the material.

4.2.1 Sample Selection

Based on training and experience, retain items, or portion of an item, for analysis suitable to reach a conclusion. In sample selection, there is no assumption about homogeneity.

A population can consist of a single unit or multiple units. A multi-unit population has the following characteristics:

- Sub-items must exist in discrete forms (e.g., tablets, baggies, knotted plastic bag corners, etc.)
- The appearance of the sub-items is essentially the same.
- For illicit tablets, a population is defined as tablets which have the same imprint regardless of color. This definition does not apply when using the hypergeometric sampling plan.

Analysts will routinely analyze a maximum of three (3) unique exhibits per case. The exhibits selected will represent the highest schedule drugs in the case and will be tested to substantiate the highest charge (weight threshold) for each schedule. Additional exhibits may be tested at the scientist's discretion based on case information.

Multi-unit tablets or capsules: a single portion of the population will be tested.

The remaining untested specimens will be left intact in case further analysis is required.

4.2.2 Liquids

- Single layer liquids have a reasonable assumption of homogeneity. Agitate the liquid well and transfer a portion into a covered container in order to avoid evaporation of the sample.

- Multi-layer liquids require collection of a portion of each layer. Each layer must be tested and reported independently.

A net weight of the liquid will be taken as sample packaging allows. A gross weight may be reported (if there are no associated weight thresholds), as appropriate, at the scientist's

discretion.

4.2.3 Pharmaceutical preparations

Due to the unique physical identifiers present in pharmaceutical preparations, a consistent sample population can easily be determined. The thoroughness represented by the sampling scheme used for street drugs is not required for pharmaceutical preparations which are clearly visually consistent with each other.

For pharmaceutical preparations involving non-controlled substances (e.g., over-the-counter preparations, antibiotics, etc.), sampling is not normally required.

If tamperable capsules are present and/or tampering is suspected, additional testing may be required.

4.3 Hypergeometric Sampling Plan

Hypergeometric sampling is a statistically-based model involving a defined confidence level with an associated probability of finding failures in a population. It is used for a visually consistent multi-unit population that will exceed a weight threshold, but the number of fully analyzed units required to exceed that threshold would be impractical to analyze.

Hypergeometric sampling may be used when additional analysis is requested for prosecution.

The appropriate number of specimens within the population will be randomly selected to give a 95% confidence level that at least 90% of the population contains the analyte in question. See Appendix F for sampling requirements.

- Random sampling will be done by using a “black box” method. After verifying that all external characteristics are the same, the samples will be placed in a box (or something similar) so that the samples will be chosen randomly. This will reduce any bias that may be introduced by the person selecting samples.

Record the number of specimens indicated by the chart in Appendix F along with a reference to the Hypergeometric Sampling Plan in the case file.

Each specimen sampled will be analyzed separately and fully.

If a unit being analyzed demonstrates inconsistent results with other units being tested, then this statistical model is no longer valid. Testing of additional units will be required to make a complete determination of the whole population.

4.3.1 Inconsistent Results

Note: The Drug ID Supervisor and/or Technical Leader will be consulted prior to additional testing. The customer will be notified to determine the best course of action if a required sample size would result in excessive testing that could significantly affect laboratory output.

With hypergeometric sampling, negatives are defined as units that do not produce the

expected result compared to the majority of analyzed units (positives) in the same sampling event.

To use this statistical approach, the analyst must fully analyze all the units that were selected in the first sampling event **regardless of the order in which the negatives were encountered**. Appendix F outlines the correct number of additional units to sample based on the number of negatives encountered.

The analyst must meet or exceed the required number of positives out of the sample size in order to make a determination of the 90% of the whole at the 95% confidence level.

4.3.1.1 Example 1: One negative unit encountered in first sampling event

The analyst has an exhibit of 2,000 baggies containing suspected cocaine. Since the exhibit will exceed a weight threshold, he/she randomly selects 29 units to analyze. The 15th baggie tested contained heroin (negative) instead of the suspected cocaine (positive) found in the previous 14 baggies.

To use the sampling plan, the analyst must complete testing on the remaining 14 units. The analyst determined all these units to contain cocaine.

Since one negative was detected, the analyst will randomly select an additional 75 units and must detect at least 72 positives within those units. The analyst obtains the required number of positives and can report the full weight of the baggies using the hypergeometric sampling reporting guidelines.

4.3.1.2 Example 2: Two negative units encountered in first sampling event

Using the previous scenario, the analyst detects heroin in the 15th and 23rd units. After completing the remaining analyses for the first sampling, he/she must randomly select 264 units and have at least 245 positive units present in order to report the full weight.

4.3.1.3 Example 3: Failure to detect the minimum required positives for second sampling event

If the analyst could not detect the minimum of 72 positives demonstrated in Example 1, they would then be required to continue sampling units until the total number of positives would reach or exceed 1,800 units using the sampling plan. The total number of positives would be reported, and the number of negatives would be made into a separate exhibit.

If the analyst decides to test to the weight threshold, the total weight of all the positives would be reported with the remainder being included in the report. Any negative units encountered would be reported as a separate exhibit.

Note: Reductions in sampling procedures are acceptable with documented approval from the District Attorney General's Office. Documentation should include the date of approval, what is approved, and the attorney's name. In some instances, approval from

the investigating officer/detective may be more appropriate.

4.4 Multiple Specimens

If all specimens are not analyzed, this will be recorded in the case file.

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

If testing does not yield a positive result (i.e., controlled or non-controlled substance) in the sample(s) chosen, a screening test must be done either on the remaining items or using the hypergeometric sampling scheme. If additional items are screened, but not fully tested, this must be reflected on the final report.

For items consisting of specimens which are obviously non-controlled such as gum, candy or vitamins, a single representative sample may be screened.

4.5 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 scientist will record the locations from which the samples were obtained in the case notes.

4.6 Residue Samples

Residues are samples which are either too small to be weighed accurately, whose weight is below the uncertainty of measurement of the balance, or that which remains after the bulk has been removed. Residues can be sampled by mechanical means (e.g., shaking or scooping) or chemical means (e.g., rinsing with solvent).

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

4.7 References

Shark, Robert E. “Sampling Your Drugs: A User's Guide”, Commonwealth of Virginia, Bureau of Forensic Science, Technical Brief, c. 1986.

Frank, Richard S. *et. al.* "Representative Sampling of Drug Seizures in Multiple Containers." *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 2, March 1991, pp. 350-357.

Colon, Maria *et.al.* “Representative Sampling of ‘Street’ Drug Exhibits” *Journal of Forensic Sciences*, JFSCA, Vol. 38, No.3, May 1993, pp. 641-648.

SWGDRUG Recommendations, 5.1 ed. “PART IIIA - Methods of Analysis/Sampling Seized Drugs for Qualitative Analysis”, January 2011.

European Network of Forensic Science Institutes Drugs Working Group, *Guidelines on Representative Drug Sampling*, 2003.

Drug Enforcement Sampling Plan. 2016.

5 Weighing Practices

5.1 Procedures

Weights for powders, marijuana, and other plant materials will be taken prior to sampling. Weighing dates will correspond to the date the sample is opened unless otherwise noted in the case record or LIMS audit record. The LIMS audit record is created when the sample weight is entered into LIMS.

Analytical, top-loading, or high-capacity electronic balances are acceptable for routine casework. The balance used and its serial number will be recorded in the case notes.

Weights will be recorded in the analytical notes as they are displayed on the balance.

Calculations involving weights will be done using the weights as they are recorded.

If the estimated uncertainty is equal to or larger than the weight, a more accurate balance shall be used or the substance shall be reported as a residue, whichever is appropriate.

Only net weights will be reported for substances which yield a positive result for controlled substances, unless sample condition precludes the ability to report a net weight. The reporting of a gross weight will be left to the discretion of the scientist.

All solid substances, plant material, mushrooms, and illicit tablets/capsules must be weighed and recorded in the case file to ensure that all statutory weight limits have been accounted for.

- The net weight of each item requiring analysis will be obtained and designated as such in the case file.

- The gross weight of the remaining items including innermost packaging will be obtained and designated as such in the case file.

- Gross weights of untested items which have bulky packaging are not required. Examples include, but are not limited to: vape pens, nasal/sublingual sprays, sublingual films, and injectable vials. The final report will reflect the number of items/units in the case which have no weight reported. The net weight of the actual item(s) tested for these types of submissions will still be required, where appropriate (i.e. net weights of nasal/sublingual sprays are not required).

- Illicit tablets/capsules will include pharmaceutical tablets/capsules that have been altered, crushed, or otherwise manipulated such that the accurate logo identification and/or proper dosage administration is compromised. This does not include tablets that have been broken for dosing purposes.

- For illicit capsules, the net weight shall include the weight of capsules and contents.

When samples are weighed and then sampled, their post-sampling weight will be recorded in the case record. Section 27.5 will be followed when analyzing possible New Psychoactive Substances (NPS).

5.1.1 Single Specimens

If a single solid substance, leafy material, or mushroom sample is received in a single submission, reporting the net weight of the total sample is sufficient clarification that only one sample was received and therefore tested.

5.1.2 Multiple Specimens

In cases containing multiple packages, it is appropriate to weigh one package, if all are the same type, and use this as an average package weight. If there are different types of packaging, at least one of each type must be weighed.

If multiple samples are received in a single submission and not all samples are tested, then both the net weight of the analyzed samples which produce a positive result and the gross weight of the remaining items will be listed on the final report.

If multiple samples are received in a single submission, and all the samples are tested, it is appropriate to only report the net weight of the total sample (since this would equal the net weight of the sample tested).

5.1.3 Significant Digits and Rounding

Weights shall be reported in the units and significant digits recorded from the weighing. Any conversion between units must be fully documented in the case file.

All significant figures will be conserved during calculation. Upon completion of a calculation, the calculated value will be rounded to the appropriate number of significant figures. The number of significant figures in the reported value must be less than or equal to the lowest number of significant figures used in the calculation. Rounding rules will be as follows:

- Round up to the next highest digit if the digit to the right of the rounding digit is 5,6,7,8, or 9.
- Do not change the rounding digit if the digit to the right of it is 0,1,2,3, or 4.

5.2 Examples

Plastic bag containing solid material has a total gross weight of 30.00 grams, consisting of 27.00 grams of solid material in a bag that weighs 3.00 grams. If the solid material is removed from the bag and weighed on a top loading balance, the weight will be reported as:

$$27.00 \text{ grams} \pm 0.08 \text{ gram}$$

However, if the weight is determined by weighing the total gross weight, then removing the solid material and weighing the empty bag, the weight will be reported as:

30.00 grams-3.00 grams= 27.00 grams (net weight)

27.00 grams ±0.08 *2

27.00 grams ±0.16 gram

5.2.1 An item consists of 30 bags containing solid material:

Total gross weight=5.00 grams

Single plastic bag weight=0.04 grams

0.04 grams * 30 bags=1.20 grams

Total net weight= 5.00 grams-1.20 grams=3.80 grams

Which is reported as:

Total net weight is 3.80 grams ±0.08 *31

3.80 grams ±2.48 grams

6 Analytical Scheme for Suspected Controlled Substances

6.1 Introduction

Controlled substance analysis typically involves the qualitative examination of suspected solid dose drug evidence to determine if the material does in fact contain a controlled substance, and if so to identify that substance. Due to the variable nature of evidence, a single approach or set of methods cannot address all possible scenarios. The technical procedures are therefore understood to be flexible, and it is the responsibility of the case scientist to use his/her knowledge, training, and experience to determine the most appropriate method of analysis.

It is the responsibility of the forensic scientist to evaluate evidence received and analyze selected items for the presence of controlled substances. The specific analytical techniques to be used in an analysis will be chosen by the scientist to be sufficiently sensitive and specific for the particular substance(s) or class(es) of substance involved. The conclusive identification of a controlled substance is accomplished by the use of at least two uncorrelated analytical techniques. At least one of these techniques must provide molecular structural data about the substance. This must be accompanied by additional testing using another analytical method or methods. These additional methods need not provide molecular structural data but will be sufficiently specific for the analyte in question as to provide a meaningful confirmation of the results of other techniques.

Category A	Category B	Category C
Fourier Transform Infrared Spectroscopy (FTIR)	Gas Chromatography (GC)	Color Test
	High Performance Liquid Chromatography (HPLC)	Pharmaceutical Identifiers[1]
Mass Spectrometry	Thin Layer Chromatography (TLC)	
	Ultraviolet Spectroscopy (UV)[2]	
	Cannabis only: Macroscopic Examination Microscopic Examination	

[1] Pharmaceutical Identifiers may provide a high degree of selectivity, but due to the potential for counterfeits, the technique has been placed in Category C.

[2] Ultraviolet Spectroscopy, when used with a wavelength range,

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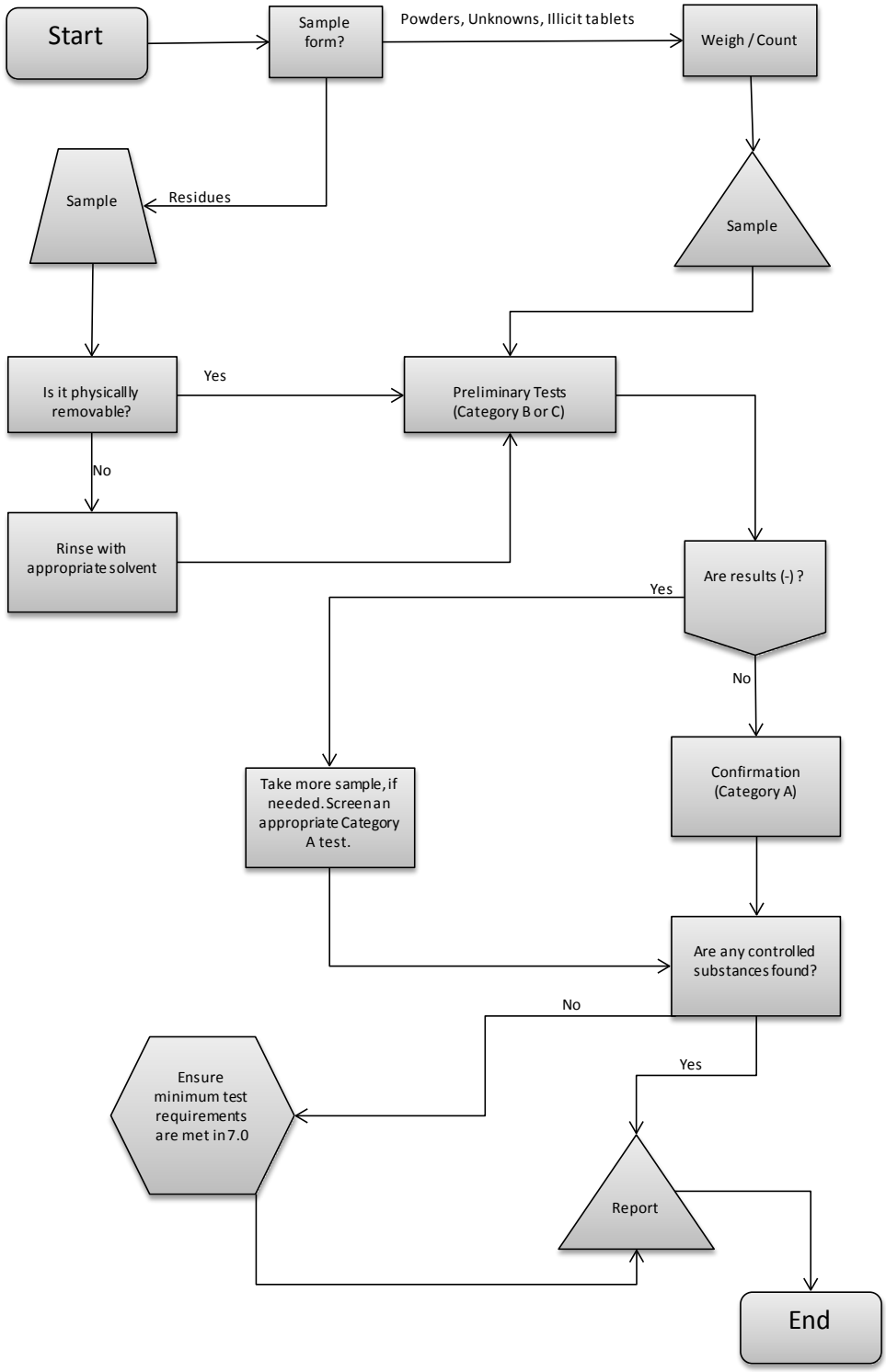
One of the structural elucidation techniques listed in Category A will be used for the confirmation of a controlled substance. At least one other technique (from either Category A, B, or C) must also be used. This combination must identify the specific controlled substance present and must preclude a false positive identification. When sample size allows, the second technique should be applied on a separate sampling, for quality assurance reasons.

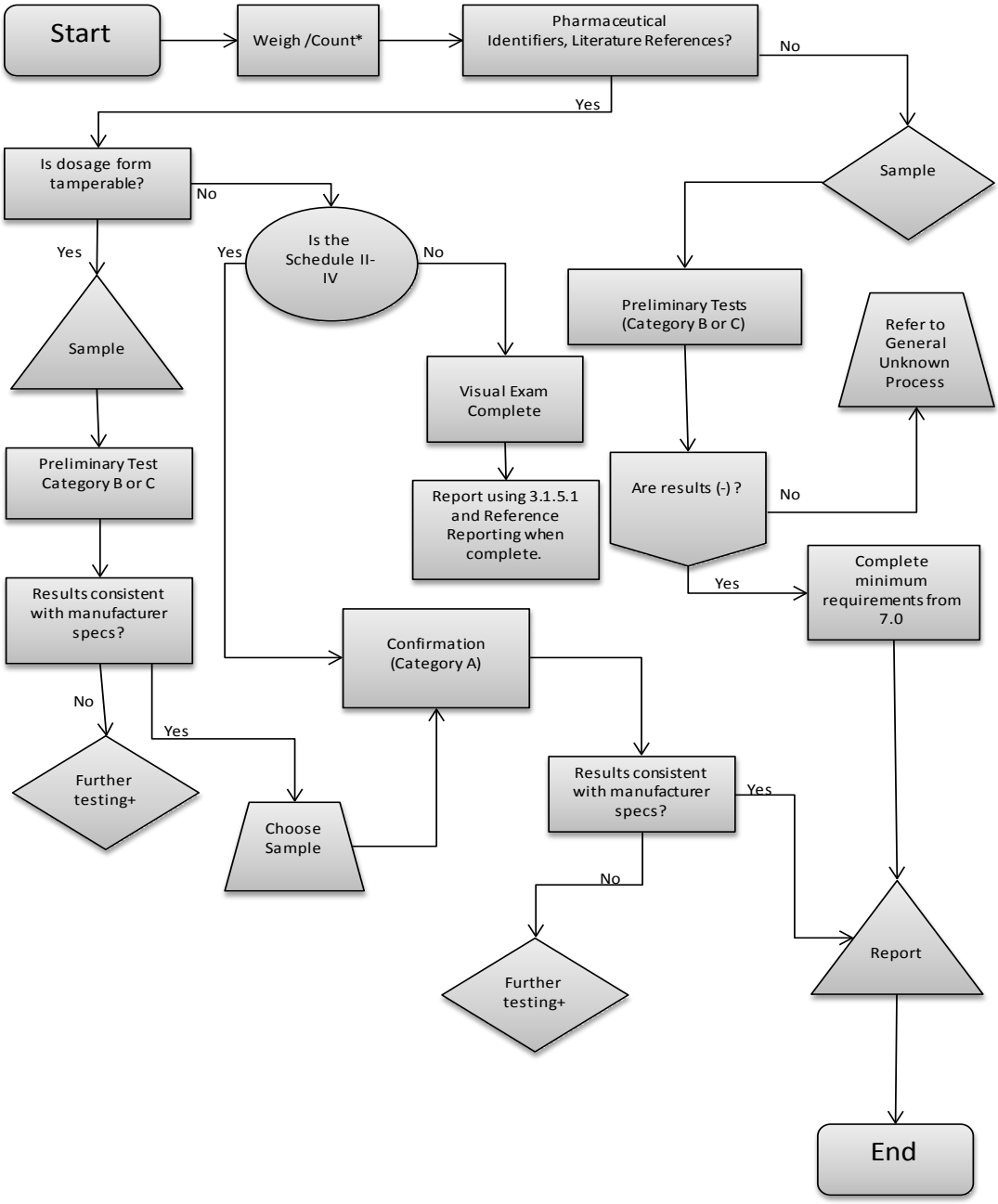
A hyphenated technique (e.g., gas chromatography-mass spectrometry, gas chromatography-infrared spectroscopy) may be considered as two separate techniques within the analytical scheme provided the criteria for positive results are fulfilled for both techniques.

- When using a hyphenated technique, the analyst is not precluded from using only one of the two results produced, as long as the results are not inconsistent.
- When only two tests are performed and the results originate from the use of a hyphenated technique, quality practices such as those described below are required.
    1. removing two aliquots from the sample and testing them independently
    2. for GC/MS, analyzing the aliquots on two separate instruments

6.2 Analytical Scheme for General Unknowns

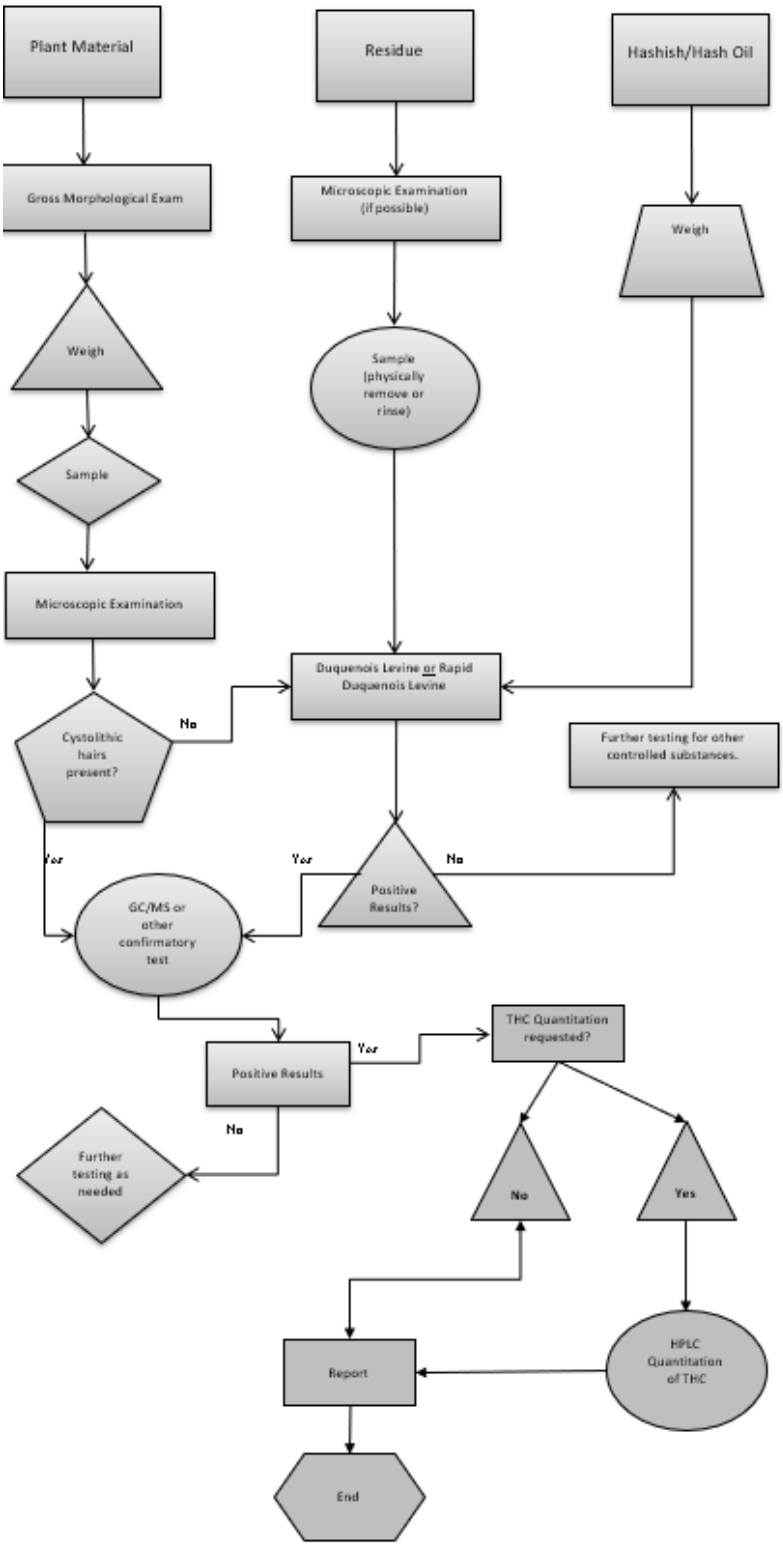


6.3 Analytical Scheme for Tablets/Capsules



+ As needed

6.4 Analytical Scheme for Plant Material and Hash/Hash Oil



6.5 References

SWGDRUG Recommendations, Version 8., June 13, 2019

7 Analytical Scheme for Non-Controlled Substances

Additional testing to determine the specific non-controlled substance in a sample can be performed at the analyst discretion when necessary. If additional testing is requested by a criminal justice official, and the nature of the case dictates that additional testing is needed, a written request must be made by the District Attorney's Office, and then be approved by the Drug Identification Supervisor or higher.

7.1 Interpretation of Data

Spots on a thin layer plate or absorbance on the UV may be acceptable tests for non-controlled substances confirmed if they can be explained through other tests and if those tests prove collectively that there are no controlled substances present.

If GC/MS is one of the techniques used to report no controlled substances detected, then that sample must be run on the GC/MS Screen Method. If GC/IR is used, then the sample must be run on the MNPD Crime Lab GCIR method.

Identification of a pharmaceutical preparation, containing a controlled substance, through logo identification and confirmation through a structural elucidation technique is sufficient evidence that it is negative for any other compound unless objective evidence suggests otherwise.

8 Color Tests

8.1 Scope

Chemical color tests produce characteristic color reactions that provide information regarding the nature of the substance being tested. Certain compounds or classes of compounds produce distinct colors when brought into contact with various chemical reagents. These simple reactions can indicate the presence of a particular functional group or molecular moiety, or class of drugs. This is a Category C analytical technique and cannot be used to specifically identify a compound.

8.2 Preparation of Reagents, Standards, and Controls

Alternate color test reagent procedures can be found in scientific literature. Analysts may use appropriate color test reagents found in published reference texts or peer-reviewed scientific journals. Examples of reference texts include, but are not limited to: Clarke's Analysis of Drugs and Poisons (Moffat et al.). Peer-reviewed journals are those such as the Journal of Forensic Sciences, the CLIC Journal, and Forensic Science International. Preparation of the alternate color test reagent(s) will be documented in the appropriate logbook. When using alternate color test reagent procedures, case notes will include a citation of the reference as well as

documentation of positive controls (with associated lot numbers of standards) and negative controls (if applicable).

8.2.1 Cobalt Thiocyanate

Cobalt Thiocyanate reacts with tertiary and quaternary amines to form a blue precipitate and is used for general screening.

8.2.1.1 Recipes

- (Primary) 2 g cobalt thiocyanate in 100 mL H2O or methanol

8.2.1.2 Procedure

Place reagent in well and add sample.

8.2.1.3 Results

- Cocaine HCl – blue precipitate forms, cocaine base may be initially negative or faintly blue, but blue intensifies upon the addition of dilute HCl.
- PCP - blue
- Amitriptyline / doxepin - blue
- Barbiturates with unsaturated side chain (i.e., butalbital) - faint blue

8.2.2 Duquenois-Levine Test

Reacts with cannabinoids.

8.2.2.1 Recipe

- 4 grams vanillin and 2.5 mL fresh acetaldehyde per 200 mL 95% ethanol

8.2.2.2 Procedure

See Plant Material Section

8.2.2.3 Results

Cannabinoids-blue/purple, pink/purple extracts into chloroform.

8.2.3 Marquis Test

Reacts with opiates and phenethylamines and is used for general screening.

8.2.3.1 Recipes

- 10 mL 37% formaldehyde in 90 mL H2SO4 (conc.)
- 2 mL 37% formaldehyde in 75 mL H2SO4 (conc.)

WARNING! 37% formaldehyde is a “particularly hazardous substance”, for which OSHA

has established very low permissible airborne concentrations and prohibited skin or eye contact. Therefore, it must be handled in an exhaust hood using appropriate PPE

8.2.3.2 Procedure

Place reagent in well and add sample.

8.2.3.3 Results

- opiates - purple
- amphetamine/methamphetamine - orange/brown
- aspirin – pink/deep red on standing
- phenoxymethylpenicillin - red
- MDA/MDMA - black

8.3 References

Johns, S. H. et. al., "Spot Tests: A Color Chart Reference for Forensic Chemists," *Journal of Forensic Sciences,* Volume 24, No.3, July 1979, pp. 631-649.

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Butler, W. P., *Methods of Analysis*, Internal Revenue Service, Reprinted by BNDD, June 1967.

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Connors, K. A., *A Textbook of Pharmaceutical Analysis,* 2nd Edition, John Wiley & Sons, New York, 1975.

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United States Pharmacopeia National Formulary, United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, p. 1722.

9 Ultraviolet Spectroscopy

9.1 Scope

Ultraviolet spectrophotometry is used as a screening tool in drug analysis procedures. Although some compounds exhibit unique UV spectra, the screening of samples by UV usually allows for general compound classification. Ultraviolet analysis is not to be used as a confirmatory test. Concentration of sample(s) can affect the spectra and may need to be adjusted to improve the quality of the data.

9.2 Reporting Results

At a minimum, the data shall include:

- MNPD Case number and Item (exhibit) number
- Date performed
- Instrument name and serial number
- Sampling information (e.g., acid, base, extract, etc.)
- Method used
- Analyst initials

9.2.1 Positive Results

UV spectrum of case sample matches standard or reference spectrum located in *Instrumental Data for Drug Analysis (IDDA)*.

Any reference spectrum used to support a positive conclusion from an approved reference source (including the IDDA) must be referenced in the case file.

The negative control for acid/base analysis must be above 80% T and exhibit no absorbance.

The negative control for EtOH analysis must be between 96%T and 104% T and exhibit no absorbance.

Wavelength values of any discrete peaks (less than 20 nm wide) present must match within +/- 4 nm. The remaining spectrum must closely match the shape of the reference spectrum. All portions of the spectrum used for comparison must be between 3% T and 80% T.

9.2.2 Negative Results

The spectrum obtained will exhibit no absorbance.

The negative control for acid/base analysis must be above 80% T and exhibit no absorbance.

The negative control for EtOH analysis must be between 96%T and 104% T and exhibit no absorbance

Spectra exhibiting absorbance may be used to support negative findings if the absorbance can

be explained through other tests and if those tests prove collectively that there are no common drugs of abuse present.

9.2.3 Inconclusive Results

A spectrum exhibiting absorbance with no match to any standard will be considered inconclusive.

10 Pharmaceutical Identifiers

10.1 Scope

Pharmaceutical preparations possess unique identifying information both in the general appearance of the preparation and the inscriptions or markings. These inscriptions and markings are used to identify the chemical compounds contained within these preparations using approved reference materials. They can also be used for informational purposes when selecting samples for analysis.

10.2 Materials

Approved pharmaceutical reference materials include the Physician's Desk Reference, Drug Identification Bible, as well as websites from credible sources (e.g., pharmaceutical manufacturers or medical-related sites such as Ident-A-Drug or drugs.com). "Chatroom-type" websites open to contributions by the general public are not acceptable. The analyst's case notes will include a notation of or copy from the reference used. A list of approved references can be found in Appendix B.

10.3 Procedure

Note any logo markings on a tablet/capsule in the case file. A logo is any identifiable, legible impression, embossment, debossment, or marking on a commercially manufactured, legitimate (licit) pharmaceutical tablet or capsule that is complete, as intended by the manufacturer.

Compare the logo to recognized sources of pharmaceutical product information (Appendix B). Care will be taken to not compare any case data to blog website information.

Record the source and all active ingredients identified.

10.4 Documentation of pharmaceutical identifier

Examples of acceptable documentation are a conversation record (e.g. from Poison Control personnel), an email, or a notation in the case file.

Casefile notations for all logos in the case, whether analyzed or not, must include the following:

- Item type (e.g., tablet(s), capsule(s))
- Logo

- Source used for identification*
- Active ingredient(s) and dosage amount(s)*

*Note: If an image from a recognized source is present in the case file, it may serve as the ingredient/dosage information. This information does not need to be duplicated in the worksheet.

10.4.1 Examples

With no image in case file

- Pink tablets "5112 V" Propoxyphene/apap, 100/650mg; DEA Logo Index
- White tablets, "2121 V 4" codeine/acetaminophen, 60/325mg; DIB

With an image in the case file

- Blue tablets "GG 258"
- Red capsules "V 4206"

It will be recorded in the case file if any tampering is evident from the dosage unit appearance.

If tampering is suspected, then a complete analytical scheme including a structural elucidation (Category A) is required for identification.

If all dosage units are visually similar, and no tampering is suspected, take one representative sample for analysis.

A structural elucidation (Category A) must be used within the analytical scheme.

When the sample is not an identifiable pharmaceutical preparation, it is required that a definitive structural elucidation technique be used within the analytical scheme, if the substance is to be reported.

If the results of the analysis are consistent with the manufacturer's specifications with regard to content, the results shall be reported.

If the results of the analysis are inconsistent with the manufacturer's specifications with regard to content, further analysis may be required.

10.5 Reporting Results

10.5.1 Positive results

The logo of the tablet/capsule and physical characteristics where appropriate, matches the source.

If a package contains whole tablets and obvious parts of whole tablets that all appear to be

consistent in at least color, size, shape, and texture, then they will be considered to be one and the same kind of tablets. The parts of the logo must be consistent with the whole tablets' logo.

10.5.2 Inconclusive results

The logo of the tablet/capsule is partially obscured or missing and cannot be completely matched to the source.

The logo of the tablet/capsule corresponds to a different drug than determined by chemical and instrumental analysis.

11 Thin Layer Chromatography (TLC)

11.1 Scope

TLC is a separation technique used to separate and identify organic compounds. Chemical reference materials are compared to unknown compounds to make a preliminary determination as to the presence or absence of common drugs of abuse. TLC is recognized as a Category B analytical technique and therefore must be coupled with a Category A confirmatory test to positively identify a compound.

11.2 Preparation of Reagents, Standards, and Controls

The following are TLC systems that are used in drug identification. The solvent ratios are volume/volume and may be added to the thin layer tank in any order. In general, visualization will be achieved by exposure to gaseous iodine.

11.3 TLC Systems

Drug Category	Ratio	Solvents
General	85:13:2	Ethyl acetate: isopropanol: ammonium hydroxide
GHB	60:30:6	Chloroform:methanol:ammonium hydroxide
	100	Methanol

When preparing drug reference materials refer to Chapter23 for quality assurance requirements.

Method blanks and/or solvents will be used as negative controls for TLC analysis.

11.4 Procedure

Prepare a sample employing an approved procedure. Liquid samples may be analyzed directly or prepared according to any approved procedure and at the scientist's discretion.

Determine the samples, blanks (negative controls), and drug reference materials needed for

analysis. Label the TLC plate and/or template with appropriate blank, case numbers, exhibit number, and chemical reference material lot numbers.

One at a time, insert a clean capillary tube into the solution being tested and blot a small amount onto the TLC plate just above the solvent level (about 10 mm, denoted as the origin). Ensure the origin spot of each blank, sample, and drug reference material is equally spaced and as small in diameter as possible. Ensure that each solution is spotted and labeled correctly.

Place the spotted plate into the TLC tank using the appropriate system **(See 11.2 Preparation of Reagents, Standards, and Controls).**

Leave plate in the TLC tank until the solvent front is near the top of the plate.

Remove the plate from the TLC tank.

Allow solvents to evaporate or dry with air in the chemical fume hood.

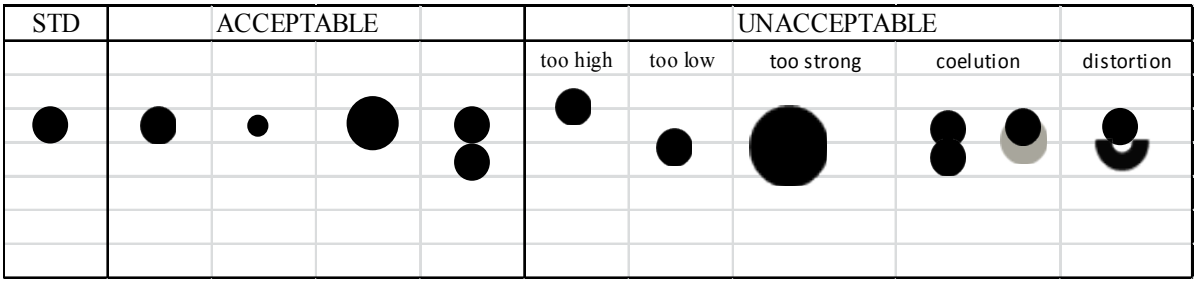
Visualize the plate using iodine vapor in the chemical fume hood.

11.5 Reporting Results

11.5.1 Positive Results

- The sample and the standard spots match at their greatest density in all positions and colors, record the result of the test as consistent with the reference material.
- If more than one set of the same reference material is used, then the sample must match each appropriate standard at their greatest density in positions and colors.
- The spot for the sample and all appropriate standards must be visible.

The following illustrations serve as a guideline in determining if the spots are acceptable matches. Note: The size of the spot does not have to correspond to the size in the illustrations.



11.5.2 Negative Results

- No spots visible in the channel.
- A channel with spots or bands may be used to support negative findings if consistent with other analysis.
- To comply with chapter 7.0 “Analytical Scheme for Non-Controlled Substances”, the

notes will indicate if a test yielded negative data.

11.5.3 Inconclusive Results

- Spots or band that are co-eluting or distorted when compared to reference materials.
- The standard used for reference is located with the solvent front (top of plate) or at the origin (bottom of plate), which indicates little or no partitioning occurred.
- The sample and all the appropriate standards do not align because of adsorptive competition, edge or matrix effects.

If data is inconclusive, the reason(s) must be recorded in the analytical case file.

11.5.4 Positional Isomers

If TLC is used as a test for samples which have ortho, meta, or para isomers, then all available isomeric reference standards will be spotted on the plate to demonstrate that the TLC is not able to distinguish the specific isomer.

11.6 References

Clarke's Isolation and Identification of Drugs

12 Infrared Spectroscopy

12.1 Scope

Infrared Spectroscopy (IR) is a structural elucidation technique, which is a Category A test. This method of spectral analysis is based on the molecular vibrational energies of an organic compound. Infrared light containing wavelengths from 4000 cm-1 to 400 cm-1 is generated and passed through the sample. When the frequency of light matches a frequency of vibration within the molecule, absorption occurs. The absorptions are translated electronically and recorded on a data system. The resulting spectrum will have characteristic bands corresponding to each different vibration among atoms in the molecule.

The IR spectrum of an unknown compound can be compared to the IR spectrum of a known or suitable reference spectrum for confirmation.

The Fourier Transform Infrared Spectrophotometer (FTIR) collects the composite spectrum in the time domain and mathematically transforms it to the frequency domain.

Non-chemical separations (spectral subtraction) may be performed to determine components of a mixture. The components would need to be separated and structural confirmation of the pure compounds done by this or other structural identification techniques, if needed.

Spectra may be collected using an Attenuated Total Reflectance (ATR) accessory and compared

to standards also collected utilizing the ATR. These standards may be stored in a user generated library. For unknown compounds, an ATR correction may be utilized in order to search a library of transmission spectra. The uncorrected unknown spectrum would then be compared to that of a known uncorrected standard spectrum.

Spectra may also be collected using the GC coupled with FTIR and compared to standards also collected on GC-FTIR. These standards may be stored in a user generated library.

12.2 Sampling

Samples should be relatively pure and can be cleaned up by extraction, recrystallization, or precipitation and filtration, depending upon the quantity and type of contaminants present.

Pure liquid organics can be run neat using the ATR accessory.

Solution Technique

- A small amount of the sample is dissolved in a non-polar solvent such as CCl_4 or CS_2. Polar solvents such as MeOH or EtOH should be avoided. Other slightly polar solvents, such as $CHCl_3$, can also be used but will have some interfering absorption bands due to C-H.

12.3 Gas Chromatography coupled with FTIR (GC/FTIR)

The MCT-A detector scans from 4000 – 650 cm^{-1} and requires cooling with liquid nitrogen prior to analysis.

The spectra produced must be compared to known spectra taken under similar conditions, such as those of vapor phase reference libraries.

GC-FTIR analysis is useful for diastereomer or positional isomer differentiation which may be required with synthetic cannabinoid and research chemical analysis.

Liquid nitrogen is added to the MCT-A detector daily approximately 30 minutes prior to use. The MCT-A detector holds 1 L of liquid nitrogen which allows for approximately 8 hours of instrument operation.

Samples should be dissolved in n-hexane, CH_2Cl_2, $CHCl_3$, ammonia saturated $CHCl_3$, or MeOH for GC/FTIR analysis. Depending on the nature of the samples, some samples must be cleaned up by extraction, but most may be directly dissolved in the solvent.

An amount of sample (1 – 4 µL) is injected utilizing an autosampler. Any amount other than 1uL will be documented on the data for the sample and the negative control.

Sample concentrations should be approximately the same concentration as the standard. Concentrations of approximately 2-5 mg/mL are recommended.

Basic extractions are recommended for suspected clandestine laboratory samples or other

phenethylamine type compounds.

If a standard is run for GC comparison purposes, the standard must be run using the same method conditions as the samples. Standards used in the comparison must be run on the same day as the sample. “Same day” is defined as an approximate 24 hour period.

After the data is processed, the sample spectrum will be compared to a known vapor phase spectrum. For identification purposes, the spectrum will be compared to the MNPD GC/FTIR vapor phase library. Another NIST traceable library may be used, but will require the approval of the Drug ID TL.

GC/FTIR will not be used in the salt form determination of cocaine samples. The GC/FTIR converts cocaine HCl into the base form during analysis; therefore, GC/FTIR cannot differentiate between cocaine HCl and cocaine base.

12.4 Reporting Results

At a minimum, the data shall include:

- MNPD Case number and Item (exhibit) number
- Date
- Instrument name
- Sampling information (e.g. ATR, GC/FTIR, etc.)
- Method used
- Analyst initials

12.4.1 Data Acceptance Criteria

These criteria must be evaluated prior to comparing the sample to a known reference material and/or reference library:

- The baseline is relatively flat from beginning to ending of the scale (4000-650 cm^{-1})
- The spectrum should exhibit strong peak resolution, especially in the fingerprint region (2000-650 cm^{-1})
- The spectra should be full, complete, and free from interference (solvents, moisture, and/or other compounds)

If GC/IR is being used, the following criteria must also be evaluated in addition to the ones listed above:

- The method blank and/or negative control immediately preceding the samples must be free of carryover from previous samples, target analyses, and analytes indicating possible contamination from either the extraction process or instrumental analysis.
- Chromatographic peaks to be used for confirmation should not demonstrate peak splitting or co-elution with other analytes.
- If drug reference materials are run for comparison purposes, the retention time must be within +/-2% of the corresponding reference material.

12.4.2 Positive Results

- When using FTIR as the primary structural elucidation technique, the sample spectrum will compare well with a spectrum of a known standard in both its overall appearance and in the presence and location of the peaks particularly in the fingerprint region. Due caution will be exercised when using the similarity index generated by the library search algorithm.

- When using FTIR to differentiate cocaine base from cocaine hydrochloride or another salt form where GC/MS has been previously performed, the areas of the spectrum which are different between cocaine base and cocaine hydrochloride will be clear. Other areas may have interfering peaks present that do not mask the "salt form" identity.

- In the presence of controlled substances, minor or inconsequential GC peaks may, at the scientist's discretion, be ignored (e.g., cis- and/or trans-cinnamoylcocaine in the presence of cocaine). However, a note must be made in the case file that all additional peaks have been examined.

12.4.3 Negative Results

The chromatogram/spectrum obtained exhibits no absorbance.

12.4.4 Inconclusive Results

A chromatogram/spectrum exhibiting absorbance that does not meet the minimum requirements of a positive result will be considered inconclusive.

A spectrum with no match to any available standard.

13 Gas Chromatography/Mass Spectrometry (GC/MS)

13.1 Scope

Gas Chromatography/Mass Spectrometry (GC/MS) is a specific method of identification for most drug substances. MS cannot differentiate between optical isomers. A sample is passed through a gas chromatographic column, effecting a separation of the components of the sample. The individual compounds then move into the mass spectrometer source where they are bombarded by electrons, producing charged ions. The ions of interest are positively charged fragments of the original compound. The ions are then separated, through a mass filtering process, according to their mass-to-charge ratios (m/z) and then collected by a detector. In the detector, the ions are converted to a proportional electrical current. The data system records the magnitude of these electrical signals as a function of m/z and converts this information into a mass spectrum. The mass spectrum is a record of the different ions (m/z) and the relative numbers of each ion (abundance). These spectra are characteristic for individual compounds, giving specificity for most types of drug substances.

Depending on the structure of the molecule, the amount and type of fragmentation will vary. Due

to this, some drugs do not exhibit a molecular ion using electron impact mass spectrometry. Examples include barbiturates, lorazepam, and methylphenidate.

For identification purposes, the spectrum will be compared to the MNPD GC/MS library. Another NIST traceable library may be used, but will require the approval of the Drug ID TL. Due caution will be exercised when using the PBM similarity index generated by the library search algorithm.

13.2 Procedure

Samples will be dissolved in a suitable solvent or extracted.

The general concentration should be determined (if applicable) by TLC or UV before being run on the GC/MS. Sufficient abundance of the total ion chromatogram peaks needs to be achieved in order to produce acceptable spectra, without overloading the chromatographic system.

The mass spectrum will be obtained in full scan mode using an appropriate scan range for the compounds to be analyzed.

13.2.1 Negative Controls

At a minimum, a blank consisting of the solvent(s) used to dissolve the samples or an instrument methanol blank must be run on the GC/MS systems, when any of the following conditions are met:

- After every chemical reference material. This includes performance check test mix and chemical reference materials used for comparison purposes.
- Between every case sample in an analytical sequence. This includes between samples from the same analyst.
- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded (except those that merely change the injection volume or split ratio) or run between blank and sample.
- Multiple blanks may be run after samples that are expected to be strong or when a completely unknown compound is encountered.
- Additional blanks may be run at the analyst's discretion.

Negative controls, which immediately precede case samples, QC data, and/or reference materials, must not produce unacceptable artifacts, excessive column bleed (methods utilizing a split ratio), carryover from previous sample(s), or peaks of target analytes nor will they contain any analytes indicating possible contamination from either the extraction process or instrumental analysis.

*Note: due to the nature of splitless injections, excessive column bleed will not be evaluated.

Any significant peaks in blank chromatograms must be properly investigated and documented in the referenced case file.

- If a controlled substance or related compound is present in any concentration, the

blanks and associated samples will be re-run.
- If an interfering substance is present, the blanks and associated samples will be re-run.
- Blanks and associated samples will be replaced and re-sampled, respectively, prior to further analysis if the same extraneous peaks are still present.

The solvent/method blank must be at least as large an injection volume of the same solvent as the sample to be injected. The upper limit injection volume is normally 4 µL.

The solvent blank must be run at the same split ratio as the sample.

For compounds identified and reported, anomalous mass peaks occurring above the molecular ion must be explained with data documentation in the case file. Easily recognizable column/septum bleed peaks, e.g., 207, 221, 267, 281, 327, 341, 355, 385, 415 and 429 *m/z*, occurring above the molecular ion may be labeled as such on the spectrum without further data documentation.

13.2.2 Drug Reference Materials

Drug reference materials in a sequence for comparison purposes must be run using the same instrument parameters as the samples and must be run within a 24 hour time period.

Drug reference materials used for comparison purposes may be run at any point in the sequence but must adhere to the requirements in this document.

Performance check drug reference materials (QC Mixes prepared from reference materials/drug standards) are suitable for retention time casework comparison when run under the same operating parameters as the case samples.

Performance check drug reference materials (QC Mixes) must bracket any sample data used for reporting purposes and must be run within a 24 hour time period.

13.2.3 Data

Sequences and/or samples (including standards and blanks) will be saved on the instrument.

- Data files will not be overwritten.
- Sequence files will not be overwritten unless additional data files have been added during the sequence run.
- Sequences and sequence log files shall be archived along with data files.

All data shall be included in the case file.

At a minimum, the data will include:

- Data file name

- Date and time of analysis
- Instrument name/serial number
- Method name
- Sample name/case number /item number
- Lot number (for drug reference materials).

13.2.4 Data Acceptance Criteria

These criteria must be evaluated prior to comparing the sample to a known reference material and/or reference library.

- The method blank and/or the negative control immediately preceding the sample must be free of carryover from previous samples, target analytes, and analytes indicating possible contamination from either the extraction process and/or instrumental analysis.
- The abundance of the chromatogram (TIC) must be at least 200k but less than 30 million ($3x10^7$).
- Chromatographic peaks used for confirmation should not demonstrate any peak splitting or co-elution with other analytes.
- If drug reference materials are run for comparison purposes, the retention time must be within +/-2% of the corresponding reference material.

13.2.4.1 Splitless Samples

Individually prepared LSD and LAMPA standards will be used as a performance check in place of the GCMS QC Mix when using the Splitless method. GCMS data acceptance criteria will still apply with the following additions:

- LSD and LAMPA standards must contain less than 10% breakdown of the target peak
- Bracketing LSD and LAMPA standards must be run in the same order (ex: LSD Start, LAMPA Start, Samples, LSD End, LAMPA End)
- LSD and LAMPA standards must be stored in amber vials

13.3 Reporting Results

13.3.1 Positive Results

- The overall fragmentation pattern and relative ratios of the ions within the spectrum are compared for consistency. Matching spectra will also exhibit the same ion clusters.

- Data from in house drug reference materials used for comparison and to support positive conclusions will be maintained as part of the Drug ID Unit Reference Spectra collection. Library matches used as the comparative data to support positive

conclusions will be included in the case file.

- In the presence of controlled substances, minor or inconsequential GC peaks may, at the scientist's discretion, be ignored (e.g., cis- and/or trans-cinnamoylcocaine in the presence of cocaine). However, a note must be made in the case file that all additional peaks have been examined.

13.3.2 Negative Results

- No controlled substances or drugs of abuse are detected. The scientist will consider the method selected when using this information to support a negative conclusion. Reanalysis of the sample using a different method may be necessary if other testing indicates the possibility of a controlled substance. The notes will clearly indicate if a test yielded negative data.
- Samples must be run on the screen method to support a negative conclusion.

13.3.3 Inconclusive Results

The mass spectrum of a compound that does not meet the minimum requirements of a positive result will be deemed inconclusive. Examples include spectra that are too weak, spectra of co-eluting compounds, or spectra that have no apparent matches. Additionally, case sample(s) will be marked as inconclusive if the method blank does not meet the acceptance criteria stated under negative controls. Spectra with no match may be used to support a negative finding. If data is considered inconclusive, the reason(s) must be recorded in the analytical case file.

14 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is a Category B test. In liquid chromatography, the mobile phase is liquid, and the stationary phase is either solid or liquid.

Usually, a solid or a liquid is used as the mobile phase. (This includes the case where a substance regarded as a liquid is chemically bonded, or applied, to the surface of a solid.) The most common form of stationary phase consists of fine particles of, for example, silica gel or resin packed into a cylindrical tube. These packed particles are called "packing material" or "packing" and the separation tube into which they are packed is called the "separation column" or simply the "column". In day-to-day analysis work, "column" is sometimes used to refer to the stationary phase and "stationary phase" is sometimes used to refer to the column.

Various solvents are used as mobile phases. The mobile phase conveys the components of the dissolved sample through the separation field and facilitates the repeated three-way interactions that take place between the phases and the sample, thereby leading to separation. The solvent used for the mobile phase is called the "eluent" or "eluant". (In LC, the term "mobile phase" is also used to refer to this solvent.)

In general, it is possible to analyze any substance that can be stably dissolved in the eluent. This is one advantage that LC has over GC, which cannot be used to analyze substances that do not vaporize or that are thermally decomposed easily. The sample is generally converted to liquid form before being introduced to the system. It contains various solutes. The target substances (the analytes) are separated and detected.

14.1 Scope

The HPLC will be used to quantitate the total THC content (Delta 9-THC and THCA) (%w/w) in plant material submissions.

An aliquot of extracted plant material is placed in an autosampler vial for analysis. Qualitative analysis for the presence of one of the three main cannabinoids (THC, CBD, or CBN) will be performed on the GC/MS along with microscopic examination and Duquenois-Levine (where appropriate) prior to HPLC analysis. A portion of the sample is injected into a liquid chromatograph (LC) equipped with a diode array detector (DAD). Cannabidiol (CBD), Delta 9-Tetrahydrocannibinol (THC), Delta 8-THC, and Tetrahydrocannabinolic acid (THCA) are identified, if present, by retention time. Concentrations of all compounds are calculated automatically by quantitative software based on regression of a fortified calibration curve.

14.2 Procedure

See 17.1.6.1

14.2.1 Preparation of In-house Calibrators

CRMs from Cayman Chemical-4 Component Phytocannabinoid Mix

#	Compound	Conc. (mg/L)
1	CBD	1000
2	delta 9-THC	1000
3	delta 8-THC	1000
4	delta 9-THCA	1000

Calibration Range: 5-100 mg/L (ppm)

#	Sample Name	Sample ID
1	4 Component Mixture	5.0 ppm
2	4 Component Mixture	10.0 ppm
3	4 Component Mixture	50.0 ppm
4	4 Component Mixture	100.0 ppm

100 mg/L (100 ppm) Phytocannabinoid Standard Mixture (4 Components)

- Transfer 100uL of Cayman Chemical Phytocannabinoid Mixture -4 component to a sample vial and add 900 uL of MeOH.
- Vortex for 30 seconds

50 mg/L (50 ppm) Phytocannabinoid Standard Mixture (4Components)

- Transfer 500 uL of 100 mg/L standard mixture to a sample vial and add 500 uL of MeOH
- Vortex for 30 seconds

10 mg/L (10 ppm) Phytocannabinoid Standard Mixture (4 Components)

- Transfer 200 uL of 50mg/L standard mixture to a sample vial and add 800 uL of MeOH.
- Vortex for 30 seconds

5 mg/L (5 ppm) Phytocannabinoid Standard Mixture (4 Components)

- Transfer 500 uL of 10 mg/L standard mixture to a sample vial and add 500 uL of MeOH.
- Vortex for 30 seconds

14.2.2 Preparation of Control Samples

A 50 mg/L (50 ppm) of the 4 component phytocannabinoid mixture will be used as the control sample for batches. The control samples will be remade for each batch run.

50 mg/L (50 ppm) Phytocannabinoid Standard Mixture (10 Components)

- Transfer 50 uL of Cayman Chemical Phytocannabinoid Mixture -4 component to a sample vial and add 950 uL of MeOH.
- Vortex for 30 seconds

14.2.3 Negative Controls

At a minimum, a blank consisting of the solvent(s) used to dissolve the samples or an instrument (clean) blank must be run on the HPLC system when any of the following conditions is met:

- After every instrument control sample, this includes any performance check mix or CRM used for comparison purposes. This does not include calibrators run in a calibration curve.
- Between every case sample in an analytical sequence. This includes between samples from the same analyst.
- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded or run between sample and blank.
- Multiple blanks may be run after samples that are expected to be concentrated.
- Additional blanks may be run at the analyst's discretion.

Negative controls which immediately precede case samples and/or QC data must not produce carryover from previous samples, peaks of target analytes, and/or any anlaytes indicating

possible contamination from the extraction process and/or instrumental analysis.

Any significant peaks in blank chromatograms must be properly investigated, documented, and referenced in the case file.

- If there is evidence any of the four target cannabinoids (CBD, delta 9-THC, delta 8-THC, and/or THCA) is present in the negative controls, blanks immediately preceding samples, and/or blanks immediately preceding instrument controls, then the blanks and associated samples will be re-run.
- Blanks and associated samples will be replaced and re-sampled, respectively, prior to further analysis if there is still evidence of the four target cannabinoids after re-running.

The solvent/method blank must be the same injection volume as the sample.

14.2.4 Data

Sequences (batches) and/or samples (including CRMs and blanks) will be saved on the instrument.

- Data files will not be overwritten
- Sequence (batch) files will not be overwritten unless additional data files have been added during the run.
- Sequences (batches) will be archived along with data files.
- All data will be included in the casefile.

At a minimum, the data will include:

- Data file name
- Date and time of analysis
- Instrument name/serial number
- Method name
- Sample name/case number/item number
- Lot number (for CRMs)

14.2.5 Data Acceptance Criteria

These criteria must be evaluated prior to evaluating case sample data:

- The method blank and/or the negative control immediately preceding case samples must be free of carryover from previous samples.
- The instrument control samples must be within $\pm$20% of the target concentration of each of the four target cannabinoids (CBD, delta 9-THC, delta 8-THC, and THCA) in the mixture.
- The R^2 value of the calibration curves of the four target cannabinoids (CBD, delta 9-THC, delta 8-THC, and THCA) must be $\geq$ 0.995.

14.3 Reporting Results

Concentrations of delta 9-THC and THCA (where appropriate) will be reported as % w/w.

- Note: for Federal cases, the total THC (from the lab solutions generated report) will be reported. The uncertainty for the delta 9-THC *or* the THCA, whichever is greater, will be reported.

Concentrations are reported using one decimal place. The reported Measurement of Uncertainty shall be rounded to the same significance (decimal place) as the reported concentration.

If the delta 9-THC concentration is below the LLOQ and the sample was previously diluted (i.e. 10 or 20 dilution factor), then the sample will be re-extracted (without dilution) and re-run. If no dilution factor was previously used, the sample will be reported as "less than 0.3% delta 9-THC" and follow the guidelines listed in 17.4.2.

Concentrations above the ULOQ will be reported as "greater than (upper limit of calibration curve)" or diluted to get the concentration within the range of the calibration curve.

Since the retention times of delta 9-THC and delta 8-THC are so similar on the HPLC, data from the GC/MS will be used in conjunction with HPLC data to differentiate between the two compounds, if necessary.

14.4 References

Shimadzu, What is HPLC Basic Principles Power Point

15 General Analytical Methodology

TPM Sections 16-22 include the general methodology for specific drug groups and drug compounds. This section addresses the majority of drug classes and compounds encountered in Drug ID.

Extraction and solubility information are found in references such as *Clarke's Isolation and Identification of Drugs* and the *Merck Index*. For most compounds encountered, it is appropriate to use a single step extraction process (Appendix A) based on the compound's solubility. Extraction solvents used in case work will be recorded in the case notes along with their lot number.

For some compounds, (such as phenethylamines, mixtures, or where matrix effects are present), a multi-step extraction process may be required to improve chromatography on GC/MS and/or GC/IR. The scientist will use his/her training and experience to determine which extraction process from Appendix A is most appropriate for the sample being analyzed.

- A method extraction blank shall be run for each multi-step extraction and documented in the case notes.

- All lot numbers of each chemical/reagent used in the extraction process will be documented in the case notes.
- The type of extraction used will be documented in the case notes.

16 Lysergic Acid Diethylamide (LSD)

LAMPA (Lysergic acid methyl propyl amide) is included in Schedule 1 of the TCA (§39-17-406 subsection (d)) as a positional isomer of LSD.

16.1 Extraction

LSD may be dry extracted with Methanol from blotter paper and other matrices.

LSD can be extracted from basic aqueous solution with organic solvents. It may be necessary to allow the medium (blotter paper) to soak in 0.1N HCl for an extended period of time prior to basic extraction, especially in cases with limited sample available. See Appendix A for extractions.

It may be necessary to dissolve the samples in a MeOH/CHCl3 mixture to extract LSD out of samples like plastic "window panes."

If samples are in a matrix which is impervious to organic solvents, LSD may be extracted by creating the tartrate salt, followed by base extraction.

16.1.1 Procedure (Analysis of Drugs-DEA Publication)

- Soak sample in 1% aqueous tartaric acid solution
- Make basic with sodium bicarbonate powder
- Extract into chloroform for further analysis

16.2 References

The Botany and Chemistry of Hallucinogens, Shultes and Hofmann. 1980

17 THC and Cannabinoids

17.1 Plant Material

17.1.1 Macroscopic Identification

Morphological characteristics that may be observed include the palmate arrangement of the leaflets, the pinnate appearance of the leaflets, the serrated edges of the leaflet, the buds (with or without seeds) and, if present, fluted stems and stalks.

Due to the compressed or mutilated nature of many samples, many of these characteristics may not be discernible.

Positive macroscopic examination results may be recorded in the analytical notes.

17.1.2 Microscopic Identification

View the sample at varying magnifications (approximately 10 – 40x) using a stereomicroscope.

Cystolithic hairs are unicellular, “bear claw” shaped hairs with a cystolith of CaCO3 at the base. They are found in greatest abundance on the upper side of the leaf with longer covering hairs on the underside.

Seeds are coconut shaped, veined (with lacy markings) and have a ridge around the circumference.

The observation of the presence of appropriate cystolithic hairs with covering hairs is sufficient for a positive test. The observation of additional characteristics is considered supportive.

An image of the microscopic exam will be stored with the case file. The sample being tested will have the case number and item number clearly annotated on the image along with the date that the image was obtained.

17.1.3 Duquenois-Levine

1. Extract sample into a suitable solvent (e.g., hexane, petroleum ether, or methanol). If a large amount of solvent is used, most of it must be evaporated.

2. Add approximately equal amounts of Duquenois reagent and concentrated HCl to extract. A positive reaction to the Duquenois portion is a blue/purple color.

3. Add sufficient CHCl3 to form two discernable layers and mix. For a positive reaction to the Levine portion of the test, the bottom layer turns pink/purple in the presence of THC or other cannabinoids.

4. Run a solvent blank as a negative control with each batch of samples. An image of the negative control will be contained within the case file.

    - If a color develops in the blank, it will be repeated to determine the source of the contamination.

    - If the results of the second blank are acceptable, all samples will be re-run.

    - If the results of the second blank are unacceptable or if the blank and samples are not available to be re-tested, the analyst will take steps to resolve the issue (e.g., replacing the solvent in the bottle, checking the reagents) prior to re-sampling and any further analysis.

An image of the color test results, and negative control will be contained within the case file. The image will contain (at a minimum), the case number, item number, analyst initials, and date performed.

17.1.4 The Rapid Duquenois-Levine Procedure

1. Place a small amount of plant material in a culture tube, add Duquenois reagent and concentrated HCl in approximately equal proportions. Observe a blue/purple color. Add CHCl3 and observe extraction of pink/purple color into the CHCl3 layer.

2. A blank (negative control) will be run in a separate culture tube. An image of the negative control will be contained within the case file.

    - If color develops, reference 17.1.3 #4 above

If the Rapid Duquenois-Levine is utilized, it will be recorded in the case notes.

An image of the color test results, and negative control will be contained within the case file. The image will contain (at a minimum), the case number, item number, analyst initials, and date performed.

17.1.5 GC/MS

Plant material submissions will be analyzed by GC/MS in an appropriate solvent for the presence of tetrahydrocannabinol (THC), cannabinol (CBN), or cannabidiol (CBD).

17.1.6 HPLC

Plant material submissions which require determination of delta 9-THC shall be run on the HPLC. All plant material submissions requiring quantitation must be requested through the District Attorney's Office and have prior approval by the Crime Laboratory Director and/or the Drug ID Supervisor. Plant material submissions approved for quantitation will be analyzed for the delta 9-THC and THCA in the sample.

17.1.6.1 Procedure

- Weigh 200mg of material and homogenize using laboratory supplied grinder
- Add material to a 50 mL polypropylene centrifuge tube
- Add 20mL of methanol to the centrifuge tube and centrifuge for 1 minute at 1000 RPM
- Let sit for 15 minutes
- Vortex for 1 minute
- Transfer 1 mL to a test tube and centrifuge for 5 minutes at 3000 RPM
- Transfer 50uL of supernatant to a new micro-tube
- Add 950 uL of MeOH to micro-tube with supernatant
- Filter through a 0.45um syringe filter and transfer to a 1.5 mL sample vial

*Based on the concentration of the sample, it may be necessary to use a dilution factor. The following dilutions have been approved for casework:

- 100 uL of supernatant in 900 uL of MeOH (dilution factor of 10)
- 50 uL of supernatant in 950 uL of MeOH (dilution factor of 20)

If no dilution is required, then 1000 uL of supernatant will be used for testing (dilution factor of 1).

The use of traceable glassware and/or pipettes is required for quantitative procedures.

*Note: See Drug Identification Unit Instrument and Equipment Procedures for entering dilution factor into Lab Solutions software.

17.2 Food Products (candies, brownies, etc.)

If plant material is visible, remove sample of plant material and analyze appropriately. If an extraction is necessary, see below.

17.2.1 Extraction of THC from Food Products

A procedure blank shall be run with the extraction and documented in the case notes.

- Add hexane to suitable quantity of sample.
- Vortex and centrifuge, as necessary.
- Transfer hexane to a new test tube.
- Extract with 0.5N KOH (methanolic solution). The bottom layer retains THC if present.
- Discard top hexane layer.
- Wash with at least 3 aliquots of hexane.
- Acidify using 1N HCl to pH 1-2.
- Extract with hexane (top layer, retains THC).
- Remove and retain hexane.
- Concentrate hexane through evaporation.
- Resultant concentrated extract will yield THC.

17.3 Hashish

Hashish is defined by the TCA §39-17-417 as a “non-leafy, resinous material containing tetrahydrocannabinol”

17.3.1 Analytical Scheme

- Weigh material
- Remove a representative sample for testing
- Dilute with appropriate solvent and perform the Duquenois-Levine test
- Take another sample and dilute with appropriate solvent and confirm the presence of THC using GC/MS or other confirmatory test.

17.4 Reporting

17.4.1 Plant Material without Request for Quantitation

Plant material submissions will be analyzed by GC/MS or GCIR for the presence of tetrahydrocannabinol (THC), cannabinol (CBN), or cannabidiol (CBD).

Microscopic analysis of the morphological characteristics of Cannabis following 17.1.1 and 17.1.2, Duquenois-Levine color test, and GC/MS will be performed on all plant material submissions. A positive Duquenois-Levine color test and a positive GC/MS for one of the three main cannabinoids (THC, CBD, and CBN) are required for reporting a positive result.

Examples of reporting include but are not limited to: “positive for THC, schedule VI in the sample tested” and/or “positive for Cannabidiol in the sample tested.”

17.4.2 Plant Material with Request for Quantitation

Plant material submissions will be analyzed by GC/MS or GCIR for the presence of tetrahydrocannabinol (THC), cannabinol (CBN), or cannabidiol (CBD).

Microscopic analysis of the morphological characteristics of Cannabis following17.1.1 and 17.1.2 and GC/MS and/or GCIR will be performed on all plant material submissions.

Plant material submissions which have a positive microscopic analysis and/or Duquenois-Levine color test, positive GC/MS and/or GC/IR confirmation of THC, and HPLC quantitative analysis will be reported as “positive for Cannabis: Total THCA content (% w/w) +/- UoM; Total Delta 9-THC content (% w/w) +/- UoM ”. Federal cases will be reported as “Positive for Cannabis: Total THC content (% w/w) +/- UoM.

17.4.3 Inconclusive for Cannabinoids

- If microscopic examination of the leafy material yields inconclusive results and subsequent analysis by GC/MS using an appropriate MS method reveals no cannabinoids, then the material must be tested further to preclude the potential of another controlled substance.

If a drug of higher severity is encountered, then the substances must be proven within the guidelines of 6.0 Analytical Scheme for Suspected Controlled Substances regarding that substance.

Due to the lack of acceptable protocols and quality control measures, no attempt will be made to germinate suspected marijuana seeds. Cases only containing seeds will be reported as “Service not performed per MNPD Crime Lab policy.”

17.4.4 Hashish

Hashish will be reported as positive for THC (Schedule VI) as defined by the TCA §39-17-417.

17.5 References

C.G Pitt, R.W. Hendron and R.S. Hsia, "The Specificity of the Duquenois Color Test for Marijuana and Hashish", Journal of Forensic Sciences, 1972, Volume 17, No. 4, Pages 693-700.

G.R. Nakamura, " Forensic Aspects of Cystolithic Hairs of Cannabis and Other Plants", Journal of the Association of Official Analytical Chemists, Volume 52, No. 1, 1969, Pages 5- 16.

Ely, Roger, CLIC List Communication, 2007.

Tennessee Annotated Code §39-17-417.

Shimadzu, Cannabis Analyzer for Potency Presentation

18 Psilocybin and Psilocyn

Mushrooms are commonly found growing wild in cow pastures in the wetter climates of the Pacific Northwest and are picked and dried. Mushrooms can also be grown and cultivated in a medium. The analysis of hallucinogenic mushroom material can be a challenge. Psilocyn, Psilocybin, or both may be present in hallucinogenic mushroom samples; however, Psilocybin is more stable and may be more predominate in dried mushroom material. Psilocybin will convert to Psilocyn when exposed to high heat, a strong acid, or a strong base.

18.1 Extraction

18.1.1 Dry Sample: Single Step Extraction

Take up to 1/2 of the sample (not to exceed 15 grams), ensure it is dry, break into small pieces, and soak in enough methanol to cover the sample for 12 - 96 hours in the dark. Filter off mushroom material prior to analysis. This extraction method will allow for the analysis of Psilocyn and/or Psilocybin. If this extraction scheme does not provide the desired results, continue with 18.1.2.

18.1.2 Dry Sample: Multi-Step Extraction

- Take up to 1/2 of the sample (not to exceed 15 grams), ensure it is dry, break into small pieces, and soak in enough methanol to cover the sample for 12 - 96 hours in the dark.
- Decant the methanol into a glass beaker, disposable test tube, or other appropriate container.
- Evaporate to a volume of approximately 1-3 mL.
- Filter the methanol solution into a separatory funnel or large disposable test tube.
- Add 10 - 15 mL of 0.1N hydrochloric acid to the methanol solution. Wash with an approximately equal volume of methylene chloride three times; discard the methylene chloride
- Adjust the pH to 8.5 with ammonium hydroxide.
- Extract with 5 - 10 mL of 9:1 methylene chloride: ethanol mixture.

- Filter the organic solution.
- Evaporate with gas (air or nitrogen) over heat to prevent condensation.
- If needed, reduce volume and transfer to an insert.
- Perform thin layer chromatography on the sample
- Adjust the sample concentration to closely resemble the strength of the psilocyn chemical reference standard

18.1.3 Simple Basic Extraction

- Add a representative sample of 2 to 5 grams of dried mushrooms to a test tube
- Add 0.1N HCl to cover material and soak for at a minimum of one hour
- Adjust pH to ~8 using NH4OH
- Extract using methylene chloride
- Filter organic layer, sample volume may need to be reduced for analysis

18.1.4 Mushrooms in Chocolate or other Matrices

- Dry up to approximately 3 grams material, as sample allows. (Easier to grind when dry.)
- Sample may be ground to increase extraction efficiency.
- Let soak in 6% acetic acid for 30 minutes - 1 hour.
- Filter off insoluble material.
- Extract acid portion with three aliquots of CHCl3.* (Discard CHCl3.)
- Basify acid portion with concentrated NH4OH to pH 8 - 10.
- Extract basic solution with three aliquots of CHCl3.*
- Combine aliquots of CHCl3.
- Evaporate CHCl3 with air (low heat).
- Resultant residue will yield Psilocyn.
- A procedure blank will be run and documented in the case notes

*To prevent an emulsion from forming, do not mix vigorously in the separatory Funnel.

18.2 Color Test Results

- Ehrlich's: purple (positive for Psilocin and Psilocybin)
- Marquis: greenish-brown: Psilocyn
- Marquis: dull orange: Psilocybin

18.3 TLC

A standard of Psilocyn and psilocybin will be run on the TLC plate.

Note: Psilocybin does not migrate on the silica plate using the General TLC Mobile Phase (85 Ethyl Acetate: 13 Isopropanol: 2 NH4OH). Therefore, by spotting both standards, movement and alignment of the sample with the psilocyn standard would indicate a positive result for

psilocyn for the TLC.

18.4 GC/MS

GC/MS will give only Psilocyn due to the Dephosphorylation of the Psilocybin caused by the GC injection port temperatures.

18.5 FTIR

ATR on extract from acetic acid extractions yields Psilocyn.

18.6 Reporting

Since Psilocybin undergoes thermal decomposition to Psilocyn in the injection port of the GC/MS, it cannot be used exclusively for the confirmation of Psilocyn/Psilocybin. TLC can be used in conjunction with GC/MS as a confirmatory test for Psilocyn.

A positive UV/Vis and/or color test for Psilocybin may be used in conjunction with a positive GC/MS for Psilocyn to report Psilocybin and/or Psilocyn as a result since no determination was made as to which compound or both is present. Alternately, a positive UV/Vis and/or color test for Psilocyn along with a positive Psilocyn GCMS may be reported as positive for Psilocyn.

18.7 References

www.swgdrug.org/Monographs/PSILOCIN.pdf

19 Cathinone and Cathine

Khat (Catha edulis; a.k.a. qat, quat) is a flowering evergreen shrub native to Eastern Africa and the Arabian Peninsula. Khat contains several compounds including two that are controlled psychostimulants similar to amphetamine. Cathinone and Cathine (norpseudoephedrine) produce stimulant effects with Cathinone being much more potent than Cathine. It is important to note that Cathinone converts to Cathine as the moist leaves age. Therefore, moist Khat will be refrigerated or frozen at the time of submission and extracted as soon as possible. In the case of dried Khat, the Cathinone does not appear to convert therefore no special storage precautions are required.

19.1 Extraction

- Cut up the leaves and stems to obtain the sample. The sample size will be based upon the amount submitted, and the need to retain material for reanalysis if requested.
- Homogenize sample
- Cover sample in 0.1N HCl (use of a separatory funnel is recommended). Let soak overnight
- Filter off insoluble material
- Wash material three times with CHCl3, retain aqueous layer
- Basify the solution with NaOH to pH 11-12

- Extract basic solution with aliquots of CH2Cl2
- Filter CH2Cl2 layer
- Concentrate CH2Cl2 for instrumental analysis
- Analyze residue as soon as possible or refrigerate to avoid degradation
- Resulting sample should contain Cathinone and Cathine

19.2 TLC

Cathinone and/or Cathine reference materials will be run on the plate for comparison based on whether one or both drugs are present in the sample being tested.

20 Anabolic Steroid

Drug Group Examples: Testosterone (associated esters), stanozolol, boldenone (Note: this list is not all inclusive).

20.1 Extraction

May be dry extracted into methanol or other organic solvents.

Injectables are often found in oils which may be extracted with methanol for further analysis.

- Care will be taken to note which layer the methanol occupies.
- If after vortexing or centrifuging, the sample does not separate into two distinct layers, add 2 drops of deionized water and repeat. Discard the layer opposite the methanol.
- Wash the methanol layer with petroleum ether, discard the petroleum ether.
- Filter the sample prior to analysis.

20.2 UV Analysis

Analysis is performed in Ethanol

20.3 Pharmaceutical Identifiers

Many substituted or negative preparations are encountered which make pharmaceutical identifiers less useful than with other types of preparations. They will not be ignored, but may need to be discounted.

Vials of liquid may contain labels and will be noted and examined.

20.4 GC/MS

Some of these materials will require elevated temperatures and have long retention times.

Molecular weights may exceed 500 and the usual mass spectral mass range would then need to be extended.

Note: Any changes made to the current GC/MS methods will require prior approval from the Drug ID TL. Approval will be documented in the case file of the affected cases. A deviation request may also be required.

21 Gamma Hydroxybutyric Acid (GHB)

21.1 Drug Group Examples

Gamma hydroxybutyric acid (GHB), gamma-butyrolactone (GBL), and butanediol (BD)

1. GHB: Pure GHB is a white powder. It is encountered dissolved into various liquids.
2. GBL: Pure GBL is a clear liquid. It is encountered dissolved into various liquids.
3. BD: Pure BD is a viscous clear liquid.
4. Other names for gamma hydroxybutyric acid include gamma hydroxybutyrate; 4-hydroxybutyrate; 4- hydroxybutanoic acid; sodium oxybate; and sodium oxybutyrate.

21.2 Extraction

If the sample is in a liquid form, take the pH of the solution prior to beginning analysis. GHB is generally found in basic solutions while GBL is generally found in acidic solutions. However, equilibrium occurs between the two in solution.

Refer to Appendix A for derivatization procedure if GC/MS will be employed.

21.3 GC/MS

GHB will form gamma-butyrolactone (GBL) in the heated injection port. The silyl derivative, prepared prior to injection, is required to differentiate GHB from GBL.

GHB - Derivatize dry sample (refer to Appendix A_GHB Derivatization)

GBL - A chloroform extract of a liquid containing GBL can be used to confirm GBL by GC/MS.

21.4 FTIR

- GHB: ATR on powder sample for GHB.
- BD: Light liquid smear on ATR.
- GBL: Light liquid smear on ATR. If results are unfavorable, perform a chloroform

extract of liquid. Use this extract to prepare a sample for the ATR by evaporating off the solvent.

21.5 References

Ciolino, L. A. et al. "The Chemical Interconversion of GHB and GBL" Forensic Issues and Implications" Journal of Forensic Sciences, 2001, Vol. 46, No. 6, pp. 1315-1323.

Bommrito, C. "Analytical Profile of Gamma-Hydroxybutyric Acid (GHB)" Journal of the Clandestine Laboratory Investigating Chemists Association, Vol. 3, No. 3, 1993.

Chappell, J. S. "The Non-equilibrium Aqueous Solution Chemistry of Gamma-Hydroxybutyric Acid" Journal of the Clandestine Laboratory Investigating Chemists Association, Vol. 12, No. 4, 2002.

22 Clandestine Laboratory Analysis

22.1 Introduction

Analysis of samples collected from clandestine labs may involve the use of both routine and non-routine analytical techniques.

Due to the nature of clandestine laboratories, it is not uncommon for a relatively large number of items to be submitted. In order to determine the best analytical approach, it may be necessary to confer with a supervisor, the investigating officer, and the prosecuting attorney to assess the probative value of each piece of submitted evidence.

The evidence submitted for a clandestine laboratory investigation 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. Due caution will be exercised when opening and examining evidence of this nature by utilizing appropriate personal protective equipment and sampling in appropriately ventilated areas (e.g., fume hood). Every effort will be made to prevent exposure of other employees to potentially hazardous materials. Special storage precautions may be necessary.

22.2 Selection of Items for Analysis

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

The evidence that has been determined to have the greatest probative value will be sampled and analyzed first. Section 3.0 Evidence Selection and Description should be followed once sufficient analysis has been performed to support charges of manufacture.

Target compounds from in-progress samples should be tested over other items not associated with the manufacturing process (e.g., smoking devices or packaged finished product) because of higher associated penalty. If no in-progress target compound is identified, then in-progress precursors and/or intermediates should be tested over other items.

Items seized from suspected clandestine laboratories are often residual in nature. Due to the non-routine nature of many items (e.g., coffee filters, biphasic liquids, swabs from contaminated equipment), special considerations must be taken when analyzing. These include ensuring appropriate sample size is obtained for analysis, back-extracting from water-immiscible liquids, and washing contaminants out of extractions.

The scientist may supplement the analysis with visual observation of materials (i.e. labeling on bottles) as well as any other details the scientist may note (examples: pH, recognizable odor detected while sampling).

If possible, the investigating officer will be encouraged to provide a copy of any notes or procedures found at the clandestine laboratory scene to aide in the identification of synthetic routes.

22.3 Safety

In addition to the normal precautions taken when working cases, the following conditions must be considered when analyzing clandestine lab submissions:

1. Anhydrous ammonia is corrosive. Contact with liquid ammonia may cause immediate, severe chemical burns as well as frostbite. Non-pressurized liquid ammonia will form ammonia gas. This gas is extremely corrosive and can burn and damage eyes, skin, mucous membranes, and other exposed tissue. If inhaled, irritation of the respiratory system may occur, along with coughing and breathing difficulty. Delayed pulmonary edema may occur following overexposure by inhalation. Overexposure to this gas may be fatal. See SDS for additional information.

2. Liquids may be strongly acidic or alkaline and may emit hazardous vapors.

3. Liquids may contain toxic solvents.

4. Extractions may release toxic gases (e.g., phosphine)

5. Sample containers may be under extreme pressure.

6. Solid materials may exhibit noxious odors. Weighing in fume hood may be required.

7. Appropriate personal protection equipment must be worn. Items required are, at a minimum, eye protection, hand protection, and lab coat. The fume hood will also be utilized when samples are taken.

22.4 Procedure

22.4.1 Solid Materials and Powders

Solid materials and powders will be sampled and analyzed following the schematic illustrated in 6.0 Analytical Scheme for Suspected Controlled Substances. Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

If a solid sample or powder is soluble in water, record the pH of an aqueous solution made from a portion of the sample.

Solid materials and powders that are most likely an inorganic essential chemical (e.g., iodine, red phosphorus) will not be analyzed.

22.4.2 Liquid Samples

Liquids will be sampled and analyzed following the procedure 6.0 Analytical Scheme for Suspected Controlled Substances. Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

The miscibility of liquid samples with both water and a water insoluble solvent (e.g., CHCl3, Hexane) will be determined.

For liquids with multiple layers, care will be taken to note the number of layers, the location of each layer relative to the others, and the color and clarity of each layer.

When a precipitate is discovered in a liquid sample, the precipitate will be sampled and analyzed in addition to or in conjunction with the liquid.

Samples that are most likely an essential chemical (e.g., a brown liquid in a labeled “Tincture of Iodine” bottle, or a bottle labeled “HCl”) will not be analyzed.

22.5 References

“Clandestine Lab Basic Guide” presented 12th Annual Training Seminar, Clandestine Laboratories Investigating Chemists, New Orleans, LA Sept. 4-7, 2002.

23 Quality Assurance

23.1 Introduction

The purpose of this section is to provide a uniform Quality Assurance Program for the Drug Identification Unit for the MNPD Crime Laboratory. It is to establish a baseline or reference point of reliability and system performance.

All Drug ID Unit personnel will report any unacceptable occurrences (Nonconforming Testing or Work), involving any Drug ID analytical system or work process, immediately to the Drug ID

Supervisor and/or Technical Leader or their designee. Appropriate steps to ensure resolution of the occurrence will follow as soon as possible by initiating the appropriate Quality Management System (QMS) Workflow. The QMS Workflow will be initiated by the person involved and/or the Drug ID Supervisor and/or Technical Leader or their designee.

All instruments and balances will be serviced by outside vendors that are accredited to ISO/IEC 17025 and whose scope of accreditation covers the service performed. If service requires shipment, the instrument and/or balance will be delivered to the vendor using trackable carrier services.

Any maintenance performed on instrumentation will be recorded in the electronic maintenance log along with the date, issue, initials of person performing maintenance, and if instrument is in service. The maintenance logs are maintained in Excel spreadsheets on the L Drive.

Guidance on instrument maintenance as well as general troubleshooting and routine/preventative maintenance can be found in the respective instrument user manuals which are located in both hard copy and electronic form with each instrument. Additionally, the instrument manufacturer may be contacted if the required maintenance is beyond the expertise and/or scope of the analyst.

Any changes to instrument search libraries and/or method parameters will be updated on all affected instruments, as appropriate. These changes will be communicated via email to the Drug ID Unit staff so that the appropriate revisions can be made.

23.2 Reagents and Chemicals

All chemicals and solvents used in the Drug ID Unit will be of the highest possible quality (e.g., HPLC grade, Omnisolv, Optima).

All chemicals, reagents, and solvents used for analytical purposes will be labeled with the chemical name, preparer's initials, lot number, and expiration date. All chemicals, reagents, and solvents will be stored per the manufacturer's recommendations.

The reliability testing of all chemicals, reagents, and solvents used for analytical testing purposes will occur prior to use, or where appropriate, concurrent with testing.

Chemicals and/or solvents received without an expiration date will be assigned an expiration date of 5 years from the date the chemical and/or solvent is received.

All chemicals, commercial reagents, and solvents shall be replaced when their stated expiration date or shelf life has expired and/or when they fail the quality check. Expiration dates can be extended if the validity of the chemicals/reagents can be proven through passing quality control checks.

For chemicals/reagents whose expiration dates provided by the manufacturer have expired, a quality control test/check must be performed prior to each use (prior to use is defined as a 24-hour window). The corresponding data must be uploaded into the casefile of each case where the expired chemical/reagent was used.

Expiration dates for reference material solutions prepared in-house will be set for 2 years from date of preparation or for the earliest expiration of a reference material or solvent component used in the final reference material solution. Any solvent and/or chemical used only in the extraction, but not present in the final solution does not need to be considered in the determination of the expiration date. Solutions may be retested to extend the stated expiration date, if necessary.

Water used in reagent preparation will be either deionized (DI) or ultra-pure from the Millipore water filtration system.

Stock solutions of general color test reagents will be prepared as needed. The QMS Drug Reagent and Chemical Prep Workflow will be used to record reagent preparation. After preparation, they will be verified with the corresponding check compound listed in the table below and results, date, and initials will be recorded.

Common Reagents and Check Compounds

Reagent	Check Compound
Duquenois	delta-9 THC
Cobalt Thiocyanate	Cocaine
Marquis	Heroin and/or Methamphetamine

Color test reagents will expire every six months except for the Cobalt Thiocyanante preparation in H2O, which will expire three months after the date of preparation. The Drug Reagent and Chemical Prep Workflow in the QMS will be used for documentation.

23.3 Drug Reference Materials

Purchased Reference Materials (Drug Standards) will, where possible, be traceable to certified reference materials (CRMs) or SI units of measurement. Internal reference materials shall be checked as far as is technically and economically practicable.

Receipt, storage, transportation, and use of reference materials will be recorded.

Purchased reference materials are delivered to the Drug ID Unit by the manufacturer using trackable carrier services. Once in the unit all purchased reference material information will be recorded electronically and/or manually.

Purchased reference materials will be stored in secure labeled containers and will be stored in a lockable cabinet, lockable refrigerator, or lockable freezer in the Drug ID Unit to prevent deterioration.

Purchased reference materials will be transported, handled, and opened with care, using appropriate laboratory safety measures and equipment (Fume hoods, personal protective equipment, etc.), in order to protect the integrity of the material and the safety of the analyst.

Purchased reference materials will be opened, sampled, and resealed one at a time ensuring that cross contamination does not occur.

Purchased reference materials may be used as received or to prepare liquid stock solutions (prepared reference materials) for use in case analysis.

Purchased reference materials will have an expiration date of five years from the date received, unless otherwise noted. Purchased reference materials may have their expiration date extended by the manufacturer, in which case a new CoA will be obtained from the manufacturer and the expiration date will be updated. Purchased reference materials may also be re-verified in-house by a structural elucidation technique if a reverification is not available from the manufacturer. In-house reverifications will be used to extend the expiration date for one year from the date of reverification. All external reverifications from the manufacturer will be valid to extend the expiration date of the expired reference materials for up to five years. In-house reverifications can be used in conjunction with external reverifications, if applicable. Reverifications of expired reference materials, whether external or internal, may only be used to extend the expiration date of the expired reference materials for a maximum of five years after the original date of expiration, after which they will be discarded. Expired reference materials awaiting reverification will be stored in a separate location. Expiration dates will be listed on the reference material label, packaging container, or reference material documents. Reverifications will be documented in the QMS.

Purchased and/or prepared Reference materials used in casework, are considered critical supplies and shall be purchased from manufacturers approved by the Drug ID Unit Supervisor.

Pharmaceutical preparations may, when necessary, be purchased from a licensed pharmacy or the patented drug manufacturer for use as reference materials. In such instances traceability to ISO 17034 or ISO 34 standards will, where possible, be documented.

Drug reference materials will be verified prior to use in case work with the exception of Certified Reference Materials (CRMs). Reference materials that are not CRMs will at a minimum be verified by GC/MS, FTIR, **or** GC/FTIR. If the reference material has isomers of other controlled and/or non-controlled substances, or exhibits polymorphism, then GC/FTIR is the most suitable instrument for verification. The spectra obtained from the verification will be compared to published reference data for those compounds, where available. Data generated by the manufacturer of the reference material is excluded as a source of comparison, unless prior approval is granted from the Drug ID TL.

The spectra for all newly verified reference materials will be added to the appropriate MNPD instrument library and noted that it was completed in the corresponding QMS Verification Workflow. Each reference material should only be added once to each instrument's respective MNPD reference library.

The MNPD reference libraries will be inventoried at least annually to ensure that all library entries are consistent on each instrument.

After the reference material has been verified, it is deemed suitable for casework. All data will be stored electronically in the LIMS case file for standard verification.

All stored electronic data will include the following:

- Lot#
- Expiration date
- Standard name
- Concentration, as appropriate
- Analyst's initials and date

All prepared reference materials in the Drug ID Unit, used for analytical purposes, will be labeled with the drug name, preparer's initials, lot number, and expiration date. All prepared drug reference materials that are deemed suitable for casework will have a shelf life of two years, unless otherwise noted.

When positive results are achieved in casework, the corresponding standard(s) must be properly documented in the case file with the name of the compound and the MNPD lot number or manufacturer's lot number (if applicable).

23.4 Balances and Reference Standard Weights

All analytical, precision, and high-capacity balances shall be performance checked for accuracy using OIML F1 weights or better the day sampling occurs and before any sample weights are collected. Analytical and precision balances in the analyst's work area shall both be performance checked before sampling occurs. Weights will be recorded using the QMS Drug Balance Performance Check Workflow. If the QMS experiences a system failure, weights will be recorded manually on Drug Balance Log Sheets and then scanned into the QMS Records-Logs Workflow when the system is restored. All balances will be calibrated annually by an ISO/IEC 17025 accredited vendor whose scope of accreditation covers the calibration performed. The specifications of the calibration service provider will be used to determine the effectiveness of the calibration

Working reference standard weights (working weights) used to check balance accuracy shall be checked against the in house set of primary reference standards weights (primary weights) annually. The in-house primary weights will be calibrated every five years by an ISO/IEC 17025: accredited vendor whose scope of accreditation covers the calibration performed. The specifications of the calibration service provider will be used to determine the effectiveness of the calibration.

The balance will be checked with a minimum of 3 working weights (Class OIML F1 or better) prior to use. The weights must bracket the low, mid, and upper range of samples to be weighed. Acceptable values are ± 3 times the readability of the balance being used. The exception will be the large Capacity (Pound) balance which will have ± the actual readability value as its acceptable range. The readability of each balance is copied below.

Balances and Readability

Balance Type	Balance Examples	Readability
Analytical	Mettler Toledo XP205DR	0.0001g
Precision	Mettler Toledo XP4002S	0.01g
Large Capacity (pound)	Mettler Toledo IND236	0.05lb

Tare balance and add weight.

If a result from the performance check is outside of the acceptable range, first ensure that the balance is level and clean prior to rechecking.

Check the balance with the primary weight set to determine if the working weight set or the balance is out of tolerance.

- If the primary weight set is outside the acceptable range, then the balance will be performance adjusted by the analyst by following the manufacturer's guidelines found in the balance written operation manual, located near the balance. The primary weight set will be used for this external performance adjustment. The adjustment will be documented.

- If the primary weight set is within the acceptable range, then the working weight set will be taken out of service until they have been brought back into tolerance and re-verified.

- If a result is outside of the acceptable range after performing the actions found in the bullets above, the balance will be immediately taken out of service until maintenance and/or calibration are performed by an approved vendor.

Accuracy and precision must be established after a balance has been put into service after purchase or repair, or if it has been moved to a new location. The following procedure will be performed and recorded.

- The working weights listed in Table 3 are weighed and recorded five times.
- The mean and % relative standard deviation (%RSD) are calculated for each weight. %RSD= 100*(standard deviation/mean)

23.4.1 Acceptance Criteria

- The accuracy of each weight will meet the criteria in Table 3
- %RSD must be less than or equal to 5 %.
- The balance will be immediately taken out of service if these criteria are not met

Table 3: Working Weights Used for Placing a Balance into Service

Balance Type	Balance Examples	Working Weights

Analytical	Mettler Toledo XP205DR	0.1000 (±0.0003) gram 1.0000 (±0.0003) gram 50.0000 (±0.0003) grams
Precision	Mettler Toledo XP4002S	1.00 (±0.03) gram 100.00 (±0.03) grams 400.00 (± 0.03) grams
Large Capacity (pound)	Mettler Toledo IND236	2.00 (± 0.05) lb. 25.00 (± 0.05) lbs. 100.00 (± 0.05) lbs.

Receipt, transportation, and use of reference standard weights will be recorded.

Purchased reference standard weights used in the Drug ID Unit will be transported to and from an accredited recertification vendor using trackable carrier services when recertification is necessary. Once in the unit all recertification documents will be recorded electronically and/or manually.

Purchased reference standard weights will be stored in secure labeled containers in the Drug ID Unit to prevent deterioration or contamination.

Purchased reference standard weights will be transported, handled, and opened with care, using appropriate laboratory safety measures and equipment (disposable gloves, forceps, tweezers, etc.), in order to protect the integrity of, prevent contamination of, and prevent deterioration of the weight.

23.5 Thin Layer Chromatography

TLC reagents will be made up as needed. QMS workflows will be used to record reagent preparation. After they are made, they will be checked with the compound(s) listed below in Table 4 and results, date, and initials will be recorded on the TLC Template in the case record. Limited use baths not listed in the table below will be checked by running appropriate standards along with the sample(s).

Previously prepared TLC baths may be refreshed daily before use as an alternative to preparing a new TLC bath solution.

Day-to-day performance is checked by running the standard along with the sample(s).

Bath	Check Compounds
All “General”	BCPH (benzocaine: cocaine:

systems listed in TPM Section 11 TLC	procaine: heroin)

23.6 Gas Chromatography/ Mass Spectrometry

23.6.1 Daily (Prior to Running a Sequence)

1. The column performance is checked with the injection of a mixture of reference materials in a suitable solvent injected prior to case sample(s).
2. Rinse the syringe with an appropriate solvent(s) (Example: Methanol)
3. Run a mixture containing reference materials of Phentermine, Methamphetamine, Cocaine, and Hydromorphone. (Formula can be found in Appendix H) The mix standard will be run on the Screen method.
4. The chromatogram will demonstrate base line separation between analytes and retention time agreement within +/- 2 % for each test mix component ran before and after the examiner's instrument data. (See Instrument Tune and QC Log Stored on the "L" Drive)
5. The mass spectrum of each standard will match the overall fragmentation pattern and ion clusters of published reference material (IDDA) or searchable instrument libraries.
6. The mass spectrum of each standard will be free of excessive column contribution which would require a baseline subtraction and/or average of the spectrum.
7. GC/MS data will not be used for reporting purposes until a subsequent daily check has been performed.
8. All QC reports will be stored electronically and backed up.

Note: The daily check is not required unless samples are being analyzed.

23.6.2 Weekly

1. Change septum (unless a Merlin Microseal is installed), as needed
2. Autotune - use for GC/MS systems for routine "seized" drug analysis. If the autotune meets the requirements in Table 5, then the instrument is considered suitable for use.
3. Check Helium, replace as needed
4. Monitor air and water in tune report (H2O, N2, O2, CO2 percentages)

Tune Acceptance Parameters

Tune Parameter	Specific Parameter	Acceptance Range
Peak Widths (PW50)	0.6 Da	±0.1 Da
Mass Assignment	69.00, 219.00, 502.00 Da	±0.2 Da
Base Ion		69 or 219
Relative Abundance	69	100%

Relative Abundance	219/69	≥70% but ≤250%
Relative Abundance	502/69	≥3%
Isotope Ratios	70/69	≥0.5% but ≤1.6%
Isotope Ratios	220/219	≥3.2% but ≤5.4%
Isotope Ratios	503/502	≥7.9% but ≤12.3%

23.6.3 Monthly

1. Change injection port liners, as needed
2. Check oil level in foreline-mechanical vacuum pump, as needed.

23.6.4 Semi-Annually

1. Replace GC column, if needed
2. Clean source, if needed
3. Clean injection port, if needed
4. Check and change foreline-mechanical vacuum pump oil, if needed

23.6.5 Placement of Instrument into Service

After maintenance has been performed, (excluding septum changes)

1. Tune and run air and water check, as necessary, as outlined in 23.6.2
2. Run standard mixture as outlined in 23.6.1.
3. If tune values meet acceptance range and a mixture of reference materials in a suitable solvent shows good chromatographic performance, then place the instrument back into service.

23.6.6 New Instrument Installation

1. Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.
2. After methods are created, run a standard on a representative sample of the methods (e.g., splitless, screen, etc.) to demonstrate efficacy.
3. Run either the QA mixture or a cocaine standard ten times to demonstrate chromatographic reproducibility.
4. Load applicable user libraries.
5. A summary of the verification will be sent to the Drug ID Supervisor and/or Technical Leader for approval prior to placing a new instrument into service.
6. Retain instrument verification documentation in the instrument case file.

23.6.7 Compounds with Similar Mass Spectra

Since some compounds (i.e. positional) exhibit very similar mass spectra, a retention time is needed to distinguish similar compounds. These compounds are listed in Table 6 along with the required drug reference materials needed if GCMS is used as a confirmatory test. These

drug reference materials must be run as controls at the same time the evidence sample is being analyzed.

- Note: this table is not all inclusive. Some isomers such as ortho-, meta-, para- *may* be able to be distinguished with retention times on the GC/MS, but could require a more discriminating technique such as FTIR and/or GC/IR.

As an alternative to retention time matching, these compounds may also be differentiated by use of another, different structural elucidation technique (Ex: GCIR) providing discrimination between the two isomers.

Target Analyte	Required Drug Reference material(s)
Amobarbital	Amobarbital and pentobarbital
Butabarbital	Butabarbital and butethal
Butalbital	Butalbital and secobarbital
Butethal	Butabarbital and butethal
N-ethylamphetamine	N-ethylamphetamine, N-N-dimethylamphetamine, mephentermine
N,N-dimethylamphetamine	N-ethylamphetamine, N-N-dimethylamphetamine, mephentermine
GHB (BSTFA derivatized)	GHB (BSTFA derivitized)
LAMPA (lysergic acid methylpropylamide)	LAMPA and LSD
LSD	LSD and LAMPA
Mephentermine	Mephentermine, N-N-dimethylamphetamine, N-ethylamphetamine
Methamphetamine	Methamphetamine and phentermine
α-methyltryptamine	α-methyltryptamine and N-methyltryptamine
N-methyltryptamine	α-methyltryptamine and N-methyltryptamine
Pentobarbital	Pentobarbital and amobarbital
Phentermine	Methamphetamine and phentermine
Secobarbital	Secobarbital and butalbital

23.7 FTIR

23.7.1 Daily (Prior to Running a Sample)

1. A background will be run prior to each sample collected.
2. A negative control will be run after the background and prior to each sample collected.
3. A NIST traceable polystyrene standard will be run on the ATR accessory using Val-Pro test (Smart iTR accessory-EP). All the parameters will pass the acceptance

criteria. The traceable standard has an expiration date for the purpose of traceability to a NIST standard and for the initial qualification of the instrument at the time of installation before it was placed into service. This standard is also used for monthly and daily Valpro Qualification Checks to ensure that the instrument is performing to specifications. The NIST Traceable Polystyrene Standard will be used regardless of expiration date as long as the instrument and standard pass monthly and daily Valpro Qualification Checks as indicated by a passing result on this report. If the Valpro Check fails, the standard will be further checked with another FTIR. The standard will be replaced when it fails repeated Valpro Checks on multiple instruments.

4. If the Valpro qualification Report indicates a problem with the instrument the instrument service provider will be contacted, and the instrument will be taken out of service.

- Note: Daily check is not required unless samples are being analyzed.

All QC reports will be stored electronically and backed up.

23.7.2 Monthly Checks

1. The bench will be aligned monthly using the FTIR Operations Guide. If the instrument does not pass any of the acceptance criteria, perform maintenance as needed
2. Val-Pro test (Nicolet iS10 System KBr-EP) will be run monthly and all the parameters need to pass the acceptance criteria.

All QC reports will be stored electronically and backed up.

23.7.3 Placement of Instrument into Service

After maintenance has been performed

- Run the daily and monthly QC as outlined above.

23.7.4 New Instrument Installation

1. Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.
2. After experiments are created, run a cocaine base and a cocaine hydrochloride standard on each to demonstrate efficacy. An additional standard of procaine hydrochloride may be run.
3. Run a cocaine standard ten times to demonstrate reproducibility.
4. A summary of the verification will be sent to the Drug ID Supervisor and/or Technical Leader for approval prior to placing a new instrument into service.
5. Instrument verification documentation will remain with the instrument.

23.8 GC/FTIR

23.8.1 Daily (Prior to running a sequence)

1. Fill the MCT/A detector with liquid nitrogen, if necessary.
2. Rinse the syringe with an appropriate solvent(s) (Example: Methanol)
3. Run a performance check with the QA standard mixture (Butalbital, Cocaine, and Testosterone). The formula can be found in Appendix H. The concentration of each standard will be above 2mg/mL.
4. The chromatogram will demonstrate base line separation between analytes and retention time agreement of less than +/- 2 % for each test mix component ran before and after the examiner's instrument data. (See Instrument Tune and QC Log Stored on the instrument)
5. The infra-red spectrum of each standard must match the overall pattern of published reference material (Ex. IDDA) or searchable instrument library reference materials.
6. GC-FTIR data will not be used for reporting purposes until a subsequent daily check has been performed.
7. All QC reports will be stored electronically and backed up

Note: The daily check is not required unless samples are being analyzed.

23.8.2 Monthly Checks

1. Change the septum of GC, as needed
2. Change the injection port liners of GC, as needed
3. For is50 FTIR, Val-Pro test (Nicolet iS50 system with DTGS in built-in position KBr-EP) will be run monthly and all the parameters need to pass the acceptance criteria.
4. All QC reports will be stored electronically and backed up.
5. *Note: The GC-FTIR contains a validation/attenuation wheel which is used by the ValPro software to validate the performance of the spectrometer. The wheel contains standards which are traceable to NIST. The NIST traceable standards within the wheel will be used regardless of expiration date as long as the instrument passes the monthly ValPro Qualification checks as indicated by a passing result on the report. If the ValPro checks fail, and the wheel is determined to be the cause of the failure, then the instrument will be taken out of service and the validation/attenuation wheel will be replaced.

23.8.3 Annual Checks

- Replace GC column, if needed

23.8.4 Placement of Instrument into Service

After maintenance has been performed (excluding septum changes)

- Run the daily and monthly QC as outlined above.

23.8.5 New Instrument Installation

1. Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.
2. After experiments are created, run QC standard mixture to demonstrate efficacy.
3. Run QA standard mixture ten times to demonstrate GC/FTIR reproducibility.
4. A summary of the verification will be sent to the Drug ID Supervisor and/or Technical Leader for approval prior to placing a new instrument into service.
5. Retain instrument verification documentation in the instrument case file.

23.9 HPLC

23.9.1 Daily (Prior to Running a Batch)

1. Purge the instrument lines
2. Check solvent levels
3. Run a new calibration curve, as appropriate
    - Run LC system check prior to running new calibration curve.
4. Run a 50 ppm sample of the phytocannabinoid mix CRM and adjust retention times in method, if necessary
5. At the end of the day, if sequence has finished, ensure that the system is "soft" off

23.9.2 Weekly

1. Check solvent levels
2. Solvent bottles will be changed bimonthly to prevent microbial growth
3. Ensure the instrument has been shut down prior to the end of the work week.

23.9.3 Monthly

1. Empty solvent waste containers into containers in trash room
2. Replace pump seal wash

23.10 UV

23.10.1 Daily (Prior to Running Samples)

1. The UV spectrophotometer will be checked daily before samples are analyzed with a 0.55 mg/mL Ephedrine HCl standard. Peak values must be 251, 256, and 262 nm ± 4 nm. The %T values for these three peaks must be between 10% T and 80% T.
2. 0.1N HCl negative control must be between 96% T and 104% T
3. EtOH negative control must be between 96% T and 104% T

Note: Daily check is not required unless samples are being analyzed.

23.10.2 Monthly

Verification testing will be performed using the procedure outlined in UV Operations on a monthly basis or when daily check values are not met. The instrument must pass the wavelength accuracy and wavelength reproducibility tests, both located on the Agilent verification certificate. The verification certificate will be acceptable if both tests pass in the working range of the predetermined Agilent instrument specifications.

23.10.3 Yearly

Annual testing with a holmium oxide standard will be performed using the procedure outline in UV Operations. The instrument must pass wavelength accuracy and noise tests.

23.11 Refrigerators/Freezers

The temperature of refrigerators and freezers which store reagents, standards, or evidentiary material should be checked and recorded on a weekly basis. These values will be recorded on the Drug ID Freezer and Temperature Log.

For refrigerators, the temperature shall be between 0ºC to10ºC.

For freezers, the temperature shall be between -15ºC to -25ºC.

If temperatures fall outside the range, the thermostat will be adjusted. If necessary, the contents of the refrigerator or freezer will be moved to another refrigerator or freezer.

Critical reagents and standards will be re-verified if the temperature in the refrigerator exceeds 15ºC or the freezer exceeds 0ºC prior to use in case work.

23.12 Pipettes

All pipettes will be visually monitored with each use to ensure adequate volume of solution is being delivered.

Analysts will clean pipettes with isopropanol or a 10% bleach solution when needed.

Pipettes will be calibrated by an outside vendor annually and will be serviced by outside vendors that are accredited to ISO/IEC 17025 and whose scope of accreditation covers the calibration performed. The specifications of the calibration service provider will be used to determine the effectiveness of the calibration.

23.13 Stereoscopes

A general lens cleaning should be performed as needed.

- Using a soft lens cloth, lens cleaner paper, or a Kim wipe gently wipe the eye piece lens in a back to front motion until the lens is free of debris.
- DO NOT wipe the lens in a circular motion. This could possibly damage the lens.
- When the stereomicroscope is not in use, it will be covered with the furnished cover.

23.14 Instrument Data Backup (QC data, maintenance logs, case data, and reference material databases/libraries)

Instrument data (including QC data, maintenance logs, case data, and reference material databases/libraries) will be stored on the JusticeTrax software system and/or individual instrument computers in an Excel spreadsheet, which are networked to Metro Police IT Department servers and will be backed up per their network backup schedule.

Archived QC, Tune, and Maintenance logs can be found in the QMS under the Records-Logs Workflow.

In the event, that the network is down, or backup is unavailable, all instrument data (as described above) will be manually backed up by transferring the data to each examiners flash drive daily if data is being generated. The data will then be transferred from the examiner's flash drive to the secure crime laboratory network drive.

23.15 Auto Samplers

For any instrumentation which utilizes an auto sampler for analysis (-GC/MS, GC/FTIR, HPLC), two different people will load and unload the sample vials on the instrument to ensure that the vials are in the correct order before and after the analysis is performed.

The instrument operator manually checks the sample vial position in the instrument auto sampler against the sequence log before acquisition begins.

Any discrepancies between the sequence log and the position of the sample vials in the auto sampler are corrected prior to starting the run.

After completion of the analytical sequence, the sample vials are checked against the sequence log as the samples are removed from the instrument auto sampler to ensure that all samples are still in the correct order. This step will be performed by a staff member other than the one who loaded the sequence.

If during the process of unloading the instrument, sample vials are found to be in a different order than reflected on the sequence log, those samples must be reanalyzed, and a notation made in the case file.

The initials of the person loading and unloading the automated sequence will be recorded on the sequence log along with the procedure performed (i.e., load/unload).

The sequence logs will be stored in an electronic instrument case file in LIMS.

24 Case (Peer) Review (Also see MNPD Quality Manual)

All case materials must be reviewed using the parameters described below. The scientist performing the review will set the appropriate milestone in LIMS.

All Technical and Administrative reviews must be completed and documented by the individual performing the review. The "Drug ID Technical and Administrative Review Checklist" form will be used to define the criteria for, and documentation of, these reviews and will be stored in the case record. Written, or electronic, date and signature of this form is acceptable.

Technical and/or Administrative discrepancies found during the review process will be handled following MNPD Quality Manual policies.

24.1 Technical Review

Technical review is intended to review the casefile analytical data to ensure that the data complies with technical procedures and supports the conclusions stated in the report.

24.2 Administrative Reviews

Administrative review is intended to review the casefile documents to ensure that proper administrative procedures are followed (Examples: Spelling, grammar, documentation of technical review, chain of custody, etc.)

24.3 Dissemination of Report

If a deficiency is detected during the review process, it will be corrected and, if necessary, a new report generated. After both Technical and Administrative review have been successfully completed and the appropriate milestones in the LIMS set, the report is ready for dissemination.

25 Estimation of the Uncertainty of Measurement (UoM)

Sample Weights

An estimation of the Uncertainty of Measurement of weight measurements shall be calculated for sample weights that are reported on the official MNPD Crime Lab report.

The expanded uncertainty shall be reported to a 99.73% level of confidence. In addition, the expanded uncertainty shall be reported to the same number of decimal places as the readability of the balance used.

Surrogate weights shall be weighed weekly on all balances as an ongoing component of measurement assurance. The policies and procedures in the Drug Identification unit account for balance drift and quality measures are taken to ensure the balance is operating properly before and after samples are weighed. Therefore, any balance identified with having a combined uncertainty greater than the readability can still possess the same measurement of uncertainty as the other balances due to our operations protocol.

Concentration of THC in Plant Material

An estimation of the Uncertainty of Measurement shall be calculated for the concentration of delta 9-THC.

- Note: For Federal cases, the samples will be analyzed for total THC. The UoM for delta 9-THC *or* THCA will be used, whichever is greater.

The expanded uncertainty shall be reported to a 99.73% level of confidence. The expanded uncertainty shall be reported to the same number of decimal places as the concentration of delta 9-THC.

- Any adjustments, modifications, or changes to the plan outlined below are submitted to the Drug Identification Supervisor and/or Technical leader.
- These measurements, along with the uncertainty of measurement program, will be evaluated annually by the Drug ID TL.

25.1 Uncertainty of Measurement Determination for Balances in Drug Identification

25.1.1 Scope

Weight determination of a solid (non-liquid) material using a balance. All balances in the Drug ID Unit will be delivered, calibrated, and serviced by outside vendors whose scope of accreditation covers the calibration or service performed. Calibrations will be performed annually. Measurements are traceable through reference standards provided by the calibration vendor, which are traced back to SI units.

25.1.2 Equipment

Balances: Mettler Toledo XP205DR Analytical Balance (Serial# B241413430)
Mettler Toledo XPE205DR Analytical Balances (Serial Numbers: B518887210, B518887217, B518887212)
Mettler Toledo XP4002S Precision Balances (Serial Numbers B240379118, B240379116, B240379119, B24346032)
Mettler Toledo IND236 (Serial Number 032050)

Weight Sets: B246537942 (OIML F1)

0.2lb (Serial#5TCJ); 2lb. (Serial# 5TCY); 5 lb. (Serial#5TCW); 10 lb. (Serial#5TCL); 25 lb. (Serial#5TDC)

25.1.3 Possible Uncertainty Components

25.1.3.1 Measuring equipment-Balance

Display Resolution: impact of rounding at load and zero

Balance Calibration Uncertainty

Balance Linearity

Balance Bias (using calibrated mass reference standards)

Temperature Coefficient of Sensitivity

25.1.3.2 Staff

Multiple Analysts
Training
Experience

25.1.3.3 Test Method

Differences in centering of measurand on balance
Buoyancy effect of weighing measurand in air

25.1.3.4 Environment

Temperature variation
Drafts
Location of balance
Vibration
Humidity
Static Electricity
Temperature Coefficient of Sensitivity

25.1.3.5 Sampling

Another possible source of uncertainty can arise from sampling in which residual material is left behind in the packaging and not transferred to a weighing vessel. Since this would always result in a reported weight less than the true weight of the test material, it will not be included in the measured uncertainty.

25.1.4 Evaluation of Uncertainty Components

Uncertainty Component	Method of Evaluation[1]	Included	Notes
Measuring Equipment			
Display Resolution	Type B	Yes	
Balance Calibration Uncertainty	Type B	Yes	
Balance Linearity	Type B	Yes	
Balance Bias	Type B	Yes	Calibrated mass reference standards are used to confirm the continued calibration status of the balances. This provides the laboratory with an ongoing evaluation of bias. The evaluation of bias will be done after calculation of combined

Uncertainty Component	Method of Evaluation[1]	Included	Notes
			standard uncertainty.
Temperature Coefficient of Sensitivity	Type B	Yes	
Staff			
Multiple Analysts	Type A	Yes	Staff contribution to UoM will be included
Training	Type A	Yes	in the evaluation of the process
Experience	Type A	Yes	reproducibility data
Test Method			
Differences in centering measurand on balance	Type A	Yes	Contribution to UoM will be included in the evaluation of the process reproducibility data
Buoyant effect of weighing measurand in air	Type B	No	SWGDRUG Supplemental document SD-3[2]
Environment			A surrogate compound will be used to evaluate environmental effects on weighing, which includes all of the environmental factors below. Contribution to UoM of all environmental factors will be included in the evaluation of the process reproducibility data.
Temperature Variation	Type A	Yes	
Drafts/Air Flow	Type A	Yes	
Location of Balance	Type A	No	Equipment is in same location since external calibration and is included in the balance calibration uncertainty.
Vibration	Type A	Yes	
Humidity	Type A	Yes	
Static Electricity	Type A	Yes	

[1]**Type A evaluation** (of uncertainty): method of evaluation of uncertainty by the statistical analysis of series of observations (GUM 2.3.2)

Type B Evaluation (of uncertainty): method of evaluation of uncertainty by means other than the statistical analysis of observations (GUM 2.3.3)

[2] "Buoyance is difficult to account for in seized drug cases because the density of the material being weighed must be known. However, for material that has a lower density than the steel calibration weights (8.0 g/cm^3) the bias imparted is always negative and the weight displayed by the balance will be less than the true weight of the material. Ignoring buoyancy contributes a small systematic error that represents no more than 0.1% bias to the weight. Therefore, buoyancy corrections are not made in any uncertainty computations shown in this document (SD-3)."

25.1.5 Calculation of Uncertainty of Measurement

25.1.5.1 Type A Evaluation

To calculate the components of uncertainty using Type A evaluation, a minimum of 100 measurements of a NIST traceable mass reference standard were taken for each balance type. Every balance in the Drug ID section was used in the collection of this data. (see Table below)

Balance Type	Weight
Analytical	1 gram
Top Loading (2 decimal places)	100 gram
High Capacity (g)	200 gram
High Capacity (500 lb.)	25.00 pound

Every analyst in the Drug ID section participated in the data collection. The uncertainty components relating to staff (multiple analysts, training, experience); test method (differences in centering measurand on balance); and environment (temperature variation, airflow, vibration, humidity, static electricity) will be represented by the standard deviation of the recorded weights.

The standard uncertainty of the reproducibility is considered a normal distribution, thus the standard deviation; "s" is defined as:

$$s = [\sum (x_i - x_{barr})^2 / n]^{1/2}$$

Where n= the number of individual measurements (x_i)

x_{barr}= the arithmetic means of the individual measurements (x_i)

To directly monitor the effects of the environment on sample weights as well as uncertainty associated with staff, a minimum of 100 weights were recorded for a surrogate compound over the course of one month. Every analyst in the Drug ID section

participated in the data collection. The uncertainty from this evaluation will also be calculated using the standard deviation formula referenced above.

25.1.5.2 Type B Evaluation

The standard uncertainty of the display resolution and linearity of the balance will be calculated using a rectangular distribution. The rectangular distribution is summarized below:

$$\text{Upper limit} = +a$$

$$\text{Lower limit} = -a$$

$$\text{Possible range of values} = (+a)-(-a) = 2a$$

$$\text{Standard uncertainty} = a/\sqrt{3}$$

Display Resolution

There is an outside limit associated with the readability of the balance at zero and at load. Both will be included in the standard uncertainty calculation. Each outside limit will be determined by:

$$\text{Outside Limit} = 1/2 \text{ readability of balance}$$

Balance Linearity

The linearity of the balance is measured during the external calibration of the balance and is obtained from the manufacturer.

The standard uncertainty of the balance calibration and the balance bias will be calculated by dividing values that were obtained from the manufacturer by the coverage factor k.

Balance Calibration

The balance calibration uncertainty was reported at a confidence level of 95.45% (k=2). The standard uncertainty will be divided by 2.

Balance Bias

The uncertainty of the calibration of the reference standards was reported at 95.45% (k=2). The standard uncertainty will be divided by 2. The mass reference standard with the largest uncertainty will be used to calculate bias. The balance bias will be calculated and compared to the combined uncertainty. If it is not significant compared to the combined uncertainty, then it will be included in the estimation of uncertainty. If the bias is deemed significant, then it will be eliminated or a correction for the bias will be made in the estimation of uncertainty.

25.1.5.3 Combined Uncertainty

The combined uncertainty is defined by the square root of the sum of the squares of the standard uncertainties.

$$\text{Combined Uncertainty} = [(U_1)^2 + (U_2)^2 + (U_3)^2 + (U_4)^2 + \ldots]^{1/2}$$

25.1.5.4 Expanded Uncertainty

The expanded uncertainty is then calculated by multiplying the combined uncertainty by the confidence interval constant (k). For the MNPD Crime Lab Drug Identification section, a 99.73% (k=3) will be employed, therefore:

$$\text{Expanded Uncertainty} = 3(\text{combined uncertainty})$$

The expanded uncertainty will be reported to the readability of the balance being used.

25.2 Weights

25.2.1 Items with Single Specimens

The calculated expanded uncertainty is the uncertainty for that measurement.

25.2.2 Items with Multiple Specimens

When weights are added to calculate a total net weight, the uncertainties associated with each individual value must be taken into account in the total uncertainty. The estimated expanded uncertainty is multiplied by the number of specimens weighed to determine the total expanded uncertainty.

25.2.3 Case File Records and Reporting for Weights

Weights shall be recorded and reported to reflect full balance readability.

Expanded uncertainty values shall be derived from the appropriate budget worksheet specific to the class of balance used. (See QMS)

25.3 Uncertainty of Measurement Determination for Concentration of Delta 9-THC/THCA in Plant Material

25.3.1 Uncertainty Components

- Measurement Process Reproducibility (%CV): This component is based off of the control values run within each batch of cases. The control values will take into account many sources of uncertainty: environmental effects, materials used, sample preparation, different analysts, instrumentation, etc. The %CV of the control values is considered in this calculation.
- Sample Pipette (100-1000 uL): A variable volume pipette is used in the aliquoting of calibrators, controls, and case samples for this type of analysis. The largest % error

rate of this pipette is used for this uncertainty component.

- The CRMs purchased for analysis are altered by dilution to create mixes used in spiking different levels for calibration curves and controls. The sources of uncertainty associated with calibration preparation are accounted for in the process reproducibility. Each CRM has an associated Certificate of Analysis used in traceability. The uncertainty (k=2, approximately 95% confidence interval) for delta 9-THC and THCA is used for this uncertainty component.

25.3.2 Quantifying the Uncertainty Components

Once the sources of uncertainty have been identified and reconciled, the significance of each component must be estimated. The first step in this process is to determine what information is available for each listed source. Then the source of uncertainty is categorized as either *Type A* or *Type B*.

Random (*Type A*) Uncertainty Data

This method of evaluation of uncertainty is determined by repeating a measurement a number of times and performing statistical analysis on the results. This data can be obtained through quality control data, validation studies, collaborative studies and/or results from proficiency tests. This data is expressed as the Measurement Process Reproducibility (%CV) of controls in the Measurement Uncertainty Estimation.

- *Quality Control (QC) Data*: The QC samples are CRMs. In the Drug ID Unit the following are available to be used as QC materials.

  - Cayman Chemical Phytocannabinoid Mixture 10 (CRM)
  - Cayman Chemical Phytocannabinoid Mixture 4 (CRM)

- The data produced by the historical quantification of QC samples provide information about the reproducibility of the analytical method. This captures the day-to-day capability of the laboratory and takes into account many variables such as: working calibration standards, various instruments, several analysts, and environmental conditions.

- Control charts are utilized to monitor trends in an analytical process. The relative standard deviation (%CV) used for uncertainty calculations shall be updated at least annually or as necessary.

- *In-House Validation Study Data*: New methods or infrequently used methods might not have an extensive amount of QC sample data generated. In this situation, validation studies that were conducted to bring the method online can be used to assist in uncertainty determinations. For example, precision and bias studies are conducted on QC samples in which the samples are analyzed several times over a period of several days, resulting in data that can be statistically analyzed.

Statistical Analysis for *Type A* Uncertainty Data

There should be enough data points acquired for uncertainty estimation to achieve a normal distribution. The data sources allow for the statistical calculation of the mean measured value of the spiked sample using the following equation:

Mean = Sum of all measured values / Number of measurements

Since the data used is part of a larger population, the standard deviation of the population is estimated using the following equation:

$$\text{Standard Deviation (s)} = [\sum (x_i - x_{barr})^2 / n]^{1/2}$$

With a normal distribution of results, it can be assumed that 1 standard deviation includes 68.26% of the measurements, 2 standard deviations include 95.45% of the measurements, and 3 standard deviations include 99.73% of the measurements.

The Relative Standard Deviation (RSD) or % CV is estimated using the following equation:

$$\% \text{ RSD} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

The RSD or % CV is the value used to express the measurement process reproducibility in the Measurement Uncertainty Estimation Spreadsheet.

Systematic (*Type B*) Uncertainty Data

This uncertainty is derived from techniques other than repeated analysis and statistical calculations. This systematic uncertainty may be reduced by optimizing the method of measuring equipment, but can never be completely eliminated. The following are examples of *Type B* uncertainties sources that are considered in the uncertainty budget in the Drug ID Unit:

- The use of an analytical pipette to dispense sample.
- Uncertainty associated with Certificates of Analysis of CRMs

Systematic (*Type B*) uncertainties in the Drug ID Unit have an equal chance of being any value within the outside limits (± a), therefore a rectangular (or uniform) distribution is applied. The standard deviation for this type of distribution is calculated using $\sigma = a/\sqrt{3}$. This distribution is applied to the pipettes used in the preparation of samples.

Type B evaluation of calibration certificates from all CRMs assume a normal distribution, a coverage factor of k = 2 and a coverage probability of 95%. Therefore, the uncertainty on the calibration certificate will be divided by 2 to arrive at the standard uncertainty.

25.3.3 Conversion to Standard Uncertainties

In this step, all calculated standard deviations are simply expressed as standard uncertainties. It is

useful to ensure that common units are used throughout the budget. If the units are not the same, then all units are converted into percentages.

Ex. If measuring 10mL has a standard deviation of 0.2mL, the percentage standard deviation is calculated as 0.2mL/10mL x 100 = 2.0 %.

25.3.4 Combining Standard Uncertainties

The mechanism of combining uncertainties is the Root Sum Squares technique. The combined uncertainty is equal to the square root of the sum of all uncertainty components squared.

25.3.5 Expressing Expanded Uncertainties

The laboratory's accrediting body requires that a coverage factor (k) be applied in the final step of the uncertainty measurement. The coverage factor is a number that, when multiplied by the combined standard uncertainty, produces an interval around the measurement result that is expected to include a specified percentage.

For routine measurements with a large amount of historical data:

- A (k) value of 2 represents a 95.45% confidence interval.
- A (k) value of 3 represents a 99.73% confidence interval.

- For analysis with insufficient historical data, a corrected coverage factor (kcorr) can be used based on the Student's t table.

25.3.6 Reporting Results with Uncertainties

The uncertainty of measurement for delta 9-THC concentration will be stated on the final report.

Since the uncertainty of measurement is only an estimate, it shall be rounded to the same level of significance (decimal places) as the reported concentration.

- Example 1: delta 9-THC 15.0% $\pm$ 3.1%
- Example 2: delta 9-THC 0.7% +/- 0.1%

Measurement Uncertainty is reported at a 99.73% level of confidence for all delta 9-THC concentration determinations in plant material.

25.4 References

ASCLD/LAB Policy on Measurement Uncertainty. (AL-PD-3060 Ver. 1.1)

ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX B, Drug Chemistry Discipline. (AL-PD-3063).

Joint Committee for Guides in Metrology (JCGM), *Evaluation of measurement data-Guide to the expression of uncertainty in measurement* (GUM) (GUM 1995 with minor corrections). (Sèvres, France: International Bureau of Weights and Measures [BIPM]-JCGM 100], September 2008).

SWGDRUG***, SUPPLEMENTAL DOCUMENT SD-3 FOR PART IVC-Quality Assurance/Uncertainty Measurement Uncertainty for Weigh Determinations in Seized Drug Analysis***. (SWGDRUG 2011-07-07).

MNPD Crime Laboratory Toxicology TPM

26 Reports

Upon completion of case analysis, the analyst will prepare a case report. The analyst will utilize the standard report format in LIMS to report findings. Reports will be free formed when results do not coincide with standard formats.

All items that are submitted, whether tested or not, will be included on the final report.

All reports must be clear, accurate, unambiguous, and objective. Therefore, it may be necessary to clarify which results pertain to specific items that were tested, within the evidence description submission. This can be done by sub-itemizing evidence in the evidence description, or stating the population tested in the results.

Tablets and/or capsules encountered in an item will be sub-itemized. Each population within each bag or container must be uniquely referenced in the evidence description. It is not necessary to sub-itemize tablets and/or capsules when multiple bags contain the same population.

26.1 Reporting Guidelines

The report must state what was received, what was tested, and must be clear that the result/conclusion pertains to what was actually tested. When applicable, the weight and number of the sub items actually tested must be reported.

Analysts will ensure the number of packages (bags, bundles, etc.) received is detailed in the evidence description. For all cases which have an excess of 100 tablets, only a weight will be required for reporting.

For all samples where a weight is reported (including solid material, plant material, tablets/capsules, etc.), the measurement of uncertainty and confidence interval associated with that sample must be included on the report along with the quantity actually tested as in the following example: “analysis confirms the presence of cocaine in the sample tested, Schedule II. The net weight of sample tested: 30.05 grams +/- 0.30 grams. The net weight of total sample: 45.02 grams +/- 0.42 gram, tested five of seven bags.”

- Exceptions:
    - If only one bag of material is received, the report does not have to state how many bags were tested.
    - If all bags are tested within the item, and the report clearly states that all the bags were tested, it is not necessary to report the net weight of the sample tested in addition to the net weight of the total sample (since these values will be equal). Reporting the net weight of total sample in both these exceptions will suffice.

In addition to the weight, the number of tablets/capsules for all tablet/capsule pharmaceutical, illicit, counterfeit cases must be reported. For counterfeit tablets/capsules, an additional statement of "Analysis is not consistent with logo markings from a pharmaceutical preparation" will be placed on the report.

When residue samples are positive for controlled substances, "residue" must be recorded on the report.

The net weight of the total sample will be reported for all solid material, plant material, tablet/capsule submissions, substances saturated into secondary mediums, and mushroom cases that yield negative results. The number of units will also be recorded for tablets and capsules.

Due to safety concerns associated with fentanyl and fentanyl analogues, evidence which contains small or "trace" amounts of these compounds will contain the following statement on the final report:

> "Analysis indicates that the sample tested could potentially contain (fentanyl or name of analogue), but was not confirmed"
>
> Small or "trace" amount applies to cases in which concentrating and/or resampling the evidence would not result in a confirmation of fentanyl or one of its analogues. Additionally, the sample must match to an MNPD reference library.

26.2 Positive Results

A positive result is obtained when a scientist has successfully completed at least the minimum tests (see Chapters 6.0 and 7.0) and can state that the analysis confirms the presence of (drug) in the sample tested. With some exceptions, the scientist will assign the applicable code or schedule. Information on the schedule or code of a substance will be obtained from the most current version of the TCA 39-17, Part 4.

If positive findings are made on a compound that falls into a controlled drug class or structural category, and that compound is not specifically listed in the Tennessee Code Annotated (TCA) then a statement of how the specific compound relates to the drug class or structural category will be made along with the positive finding.

Positional isomers (i.e., meta, para, ortho) produce data that is nearly identical. GC/FTIR and FTIR *may* be able to determine the specific isomer if reference data is available and/or the sample produces data that meets the minimum data acceptance criteria. If the specific isomer is not able to be confirmed, then the sample will be reported as "positive for xxx, isomer not identified."

26.3 Negative Results

26.3.1 No Controlled Substances Detected

A "negative" or "no controlled substances detected" result is obtained when the analytical tests performed did not yield data that would support the conclusion that a

controlled substance (as defined by the TCA) is present in the sample tested. This category applies to those exhibits in which the following condition is met:

•All tests performed to satisfy minimum requirements yield no controlled substances.

26.3.2 No Controlled Substances Confirmed

If any of the following criteria are met, then half of the sample up to a gram will be retested. If this sample does not meet the minimum requirements for data acceptance, then the sample will be reported as "no controlled substances confirmed".

•One test yields no controlled substances, but the other test indicates the presence of a controlled substance.

•One test yields positive results, and the other test(s) indicate the presence of a controlled substance, but the data does not satisfy the requirements for positive results (i.e. weak spectrum, etc.).

•Two or more tests indicate a controlled substance, but do not yield positive results.

When reporting cases with no controlled substances, there are instances where it is more appropriate to report "analysis confirms the presence of (drug) in the sample tested." An analyst should use his/her training and experience to make this determination. All attempts should be made to do this if reference materials are available. The final report must reflect that the substance is a non-controlled substance.

Marked pharmaceutical preparations (e.g. tablets or untampered capsules) indicated as non-controlled or over the counter preparations from recognized reference sources (i.e. Drug ID Bible, DEA logo Index, Ident-a-Drug, etc.) may be reported as "Not Analyzed" or "No chemical examinations performed as item markings/logo identification are consistent with the presence of a pharmaceutically prepared [enter drug classification], a non-controlled substance."

For cases that contain mushrooms and no psilocyn/psilocybin is detected, the case will be reported as "No psilocyn/psilocybin detected in the sample tested."

26.4 Partially Tested Items

There may be occasions when items in a case are only partially tested due to sample condition, screening of items in a larger population, and/or other items analyzed in the case reveal higher schedules and/or associated penalties. In these instances, the items that have been partially tested will be reflected on the final report.

Wording on reports must clearly indicate what was partially tested and will make no assumptions about the identity of the compound:

"A full and complete analysis was performed on [number of item(s) fully tested] of [total number of items] submitted items. The remaining [number of remaining items] items were each partially

tested and were consistent with the fully tested items(s)."

Items will not routinely be partially tested.

The Drug ID TL should be consulted prior to reporting a partially tested item.

26.5 Items Tested Using the Hypergeometric Sampling Plan

For items that are tested using the hypergeometric sampling plan, one of the following statements will be placed on the report:

"Utilizing a hypergeometric sampling plan, the [contents of] (number of items tested) were analyzed fully and separately. Analysis confirms the presence of (drug, Schedule) in the samples tested. Based on these results, there is a 95% level of confidence that at least 90% of the (total sample population) contain (drug)."

Or

"Utilizing a hypergeometric sampling plan, the [contents of] (number of items tested) were analyzed fully and separately. No controlled substances were detected in the samples tested. Based on these results, there is a 95% level of confidence that at least 90% of the (total sample population) contain no controlled substances."

Only the total weight of the entire population will be included on the report. The net weight of the sample tested, however, will be included in the case notes.

27 Laboratory Safety

27.1 MNPD Crime Lab Laboratory Safety Plan

The laboratory Safety Plan can be found in the QMS, the laboratory document control system. That plan will be followed in the Drug ID Unit. The following additional procedures are specific to the Drug ID Unit.

27.2 Personal Protective Equipment (PPE) in the Drug ID Unit

The only area designated as a clean room in the Drug ID Unit is the office area. PPE will not be worn in this area.

The Drug ID Extraction Room and Drug ID Prep Room are designated as hazardous chemical areas. PPE will be worn anytime evidence is being processed or chemicals are being handled in these areas.

The Drug ID Instrument Room and Evidence Storage Room are areas where analysts may be working with small amounts of chemicals, drugs, and other substances many of which are contained in sealed vials, bottles, or plastic bags. In these areas analysts can use their judgement as to whether PPE is necessary.

Any reagent or solution preparation for use where the chemicals, reagents, or their vapors are known to be hazardous will be performed in a chemical fume hood and then safely transported to where they will be used.

Instrument maintenance and service repair procedures requiring the use of organic solvents or liquids will be performed in a chemical fume hood. Some exceptions to this policy can be made when, in the judgment of the analyst, he/she is working with small amounts of solvents that would not be hazardous (Ex: diamond cell cleaning, UV sampling, etc.)

Any exceptions to the above listed procedures will be handled following the MNPD Crime Laboratory Safety Plan.

27.3 Work Area Cleaning

All floors in the Drug ID laboratory area are maintained by contract custodial services. All work surfaces are not maintained by contract custodial services due to the nature of their use and the desire to protect custodial personnel from possible exposure to hazardous substances and evidence. These surfaces will be maintained by the Drug ID Staff.

Work surfaces in the Drug ID Extraction Room and the Drug ID Prep Room will be decontaminated with an appropriate disinfectant after completion of procedures as required by the Laboratory Safety Plan.

Work stations in the instrument areas of the lab will be routinely checked and when necessary cleaned with appropriate cleaning solvents.

Laboratory trash bags will be tied and removed from the laboratory area by lab staff, in order to safely transfer them to Housekeeping staff. Used glassware receptacles will be sealed and safely disposed of by laboratory staff. Empty glass bottles will be disposed of by laboratory staff.

Due caution will be used when bringing cell phones and/or personal belongings into the lab area to ensure that they are not contaminated.

27.4 Submissions containing mold

Moldy submissions will not be tested. Mold is a respiratory hazard.

27.5 Syringe Evidence Safety

If a syringe must be analyzed, unprotected hands will not be used to remove or replace the needle cover on the syringe. The syringe will be considered as biohazardous. Needle Stick Resistant gloves and a laboratory vise will be used to carefully remove the syringe from its puncture resistant container, then remove the contents of the syringe for analysis, and finally to repackage the syringe in its puncture resistant container.

27.6 New Psychoactive Substances (NPS) Safety Precautions

27.6.1 PPE

When cases containing solid material are analyzed, the analyst will wear at a minimum: safety glasses/goggles, disposable lab coat (with elastic cuffs), particle masks (N95 or higher), and gloves.

If prior information or testing indicates that Fentanyl, Fentanyl analogs, or other NPS is present in pure form, an additional level of PPE is required.

Gloves will have a minimum thickness of at least 5 mil, or double gloving will be mandatory.

Clean PPE for all unit staff personnel will be stored and available in the lab changing vestibule when not in use. Reusable mask will be routinely decontaminated according to the manufacturer's instructions after use for casework and before storing in the changing vestibule.

When staff members are in the chemical extraction area of the lab during sampling, the PPE outlined above will be worn by all staff present, even if they are not actively sampling cases.

Staff who are in the instrument area of the lab will not be required to wear full PPE as outlined above when cases are being sampled but may use clean PPE at their own discretion.

Every attempt will be made to use clean PPE when an analyst is transferring samples from the lab extraction area to the instrument area.

The air pressure interlock system for the lab changing vestibule can also be activated as a third level of safety anytime unit staff feels it is necessary.

27.6.2 MX 908

The MX 908 is a handheld high-pressure mass spectrometer. It can be utilized when bulk cases suspected to contain New Psychoactive Substances (NPS) are submitted. When the MX908 is used it shall be listed in the case notes along with the serial number. The MX908 will not be used on residue/trace amounts.

Required levels of PPE for solid material must be implemented when using the MX908. PPE must be maintained even when no target is detected. When an NPS and/or other controlled substance(s) are detected, two tests are still required for confirmation.

27.6.3 Sampling

During sampling of cases, a "buddy" system will be employed.

Sampling will occur only in the Drug ID Extraction area or the Drug ID Prep Room area of the lab. The analyst will notify another member of the unit so that he/she may be monitored in the event of exposure. The buddy will remain in the Drug ID Unit or Lab for the duration

of sampling until the evidence has been repackaged and sealed. The analyst will make the final decision on the location of the buddy during sampling and when he/she can be released. Sampling cases alone is prohibited.

- Acceptance of being a buddy means the willingness to administer Narcan should the analyst become incapacitated.
- If the buddy is in the lab area, he/she will be required to wear the same PPE as the analyst.
- The designated buddy will have access to Narcan should the analyst become incapacitated during testing.

In the event of a confirmed exposure, any available lab employee will immediately call EMS. This person will preferably not be the assigned buddy.

Access to the lab area will be limited while sampling/testing is conducted to prevent exposure. Signage will be placed on the door entering the lab area warning personnel about hazardous sampling conditions.

The analyst will use his/her best judgement to determine if and when sampling under the fume hood is necessary. If necessary, the analyst will take a small amount and use the GC/MS to screen for the presence of fentanyl, fentanyl analogs, or other new psychoactive substances (NPS). If the result of this test is positive, then only a gross weight will be required, no reserve weight will be required, and all further sampling will be conducted in the fume hood and documented in the case file. If the result is negative, then the analyst will proceed as normal. Due caution will be used when sampling and weighing to prevent agitation of the sample.

- If additional information is available about the case (i.e. 282 information, correspondence with customer, etc.), indicating possible Fentanyl or NPS then sampling under the fume hood will be required. Documentation of why the case was sampled in the hood will be included in the case record as it relates to common indicators that a substance may create a heightened safety concern.

Warning stickers will be placed on all evidence found to contain illicit fentanyl, fentanyl analogs, and new psychoactive substances (NPS) even if they are not confirmed. This does not include licit sources such as lollipops and patches.

Unnecessary movement between lab areas when sampling is taking place is prohibited.

The transfer of evidence/samples containing (or expected to contain) fentanyl, fentanyl analogs, and/or new psychoactive substances (NPS) will be minimized to prevent exposure.

NPS Evidence will be stored away from regular evidence, when possible.

27.6.4 Post Exposure

If the analyst is incapacitated, the buddy will administer Narcan.

The buddy will wear appropriate PPE prior to administration of Narcan.

Any available lab employee will immediately call 911.

During an evacuation and/or emergency event, protective covers will be placed over the sampling area to prevent environmental exposure.

HAZMAT will be called for decontamination and clean up after an exposure.

Appendix A Extractions

Basic Extraction

Purpose: This procedure is used to extract basic organic compounds for analysis.

Materials

Petroleum ether

Methylene Chloride

Chloroform

50% w/w Sodium Hydroxide (NaOH)

Concentrated Ammonium Hydroxide (NH_4OH)

0.1N Hydrochloric Acid (HCl)

Disposable test tubes

pH paper

Disposable pipets

Procedure

- Take a small representative sample of material and place into test tube
- Add 0.1N HCl to dissolve sample. The amount of 0.1N HCl needed may vary from sample to sample. Agitate if necessary.
- Add 50% w/w NaOH to solution. (Note: If heroin is suspected, use NH_4OH to avoid converting the sample to morphine). Agitate sample.
- Confirm that sample is strongly basic (pH $\geq$ 12) with pH paper. Do not add paper directly to sample to avoid contamination.
- Add appropriate solvent to sample. The amount of solvent needed may vary depending on sample concentration. Agitate sample to mix both layers.
- Remove organic layer for analysis.
- Extractant may be filtered if desired.

Results

Perform required analytical tests for identification of substance.

References

"Separation of Cocaine from Impure Crack." Microgram Vol. XXII, #2, Feb 1989.

Georgia Bureau of Investigation-Division of Forensic Sciences, Drug Identification Procedures Manual, AN DI 1, November 1, 2004.

Bradford-Brackett Extraction Scheme

Purpose: The Bradford Brackett (BB) procedure is employed to prepare a wide variety of organic substances for analysis using various analytical techniques. These substances are isolated through acid/base solubilities into five fractions. This procedure has been modified to isolate target analytes into three fractions.

Fraction Three (BBIII): Acid compounds that are weaker acids than a 4% solution of sodium bicarbonate (e.g., barbiturates)

Fraction Four (BBIV): Compounds soluble in methylene chloride as free bases (e.g., phenethylamines, some opiates, etc.)

Fraction Five (BBV): Amphoteric compounds (e.g. morphine)

Materials:

Deionized/Ultrapure Water

Methylene chloride (CH_2Cl_2)→Chloroform ($CHCl_3$) may be substituted

Ethanol (EtOH)

Ammonium hydroxide (NH_4OH), concentrated

Sodium hydroxide (NaOH), concentrated

Hydrochloric acid (HCl), concentrated

Hydrochloric acid (HCl), 0.1N

Filter paper

Disposable test tubes

Separatory funnels

pH paper

Pipettes

Mortar and pestle

Funnels

Procedure:

1. Add sample to a mortar/test tube/ beaker/separatory funnel. Add deionized/ultrapure water and mix.

2. Acidify the solution with 0.1N HCl and extract with 1-20 mLof CH_2Cl_2. Depending on the analyte(s) requiring isolation, it may be necessary to perform multiple extractions with CH_2Cl_2. Separate the CH_2Cl_2 from the aqueous solution. The CH_2Cl_2 layer is **Fraction 3.**

3. Using the aqueous layer from step 2, perform the following:

a. Adjust the pH of the aqueous solution to at least pH 10 by adding concentrated NaOH (check using pH paper). Extract with 1-20 mL of CH_2Cl_2 . Separate the CH_2Cl_2 from the aqueous solution. The CH_2Cl_2 is **Fraction 4.**
Note: Adjusting the pH of the aqueous solution to between 8 and 9 using NH_4OH for the Fraction 4 extraction may be necessary for suspected analytes known to decompose in strongly alkaline matrices.
b. Take the aqueous layer from step 3 and acidify with concentrated HCl. Adjust the pH of the solution to ~8.5 with concentrated NH_4OH. Extract this with a 9:1 v/v mixture of methylene chloride: ethanol. Separate the methylene chloride: ethanol from the aqueous layer. The methylene chloride: ethanol layer is **Fraction 5.**

4. Filtration will be performed as necessary.

5. If the target analyte is known, it is unnecessary to perform the entire series of fractions. All steps unrelated to the specific fraction containing the target analyte may be eliminated. Modifications to the fraction procedure are permitted if certain isolation steps are not needed. Sufficient justification must be contained in the case file for any such modification. For instance, it is not required to extract with CH_2Cl_2 in step 2 when performing a BBV on morphine tablets where logo identification does not indicate any additional compounds. Any modifications made to the extraction procedure must be fully documented in the case file.

6. If the target analyte is unknown, the analyst shall perform, at a minimum, BBIII, BBIV, and BBV fractions without any modifications. Fraction extractions may be performed in any order to prevent decomposition of potential analytes. If a controlled substance is indicated at any point during the analysis of the unknown item, it is unnecessary to complete the full BBIII, BBIV, BBV fractions. For example, if a Schedule I substance is indicated in a BBIV fraction during the extraction process, the completion of BBIII and BBV fractions is not required.

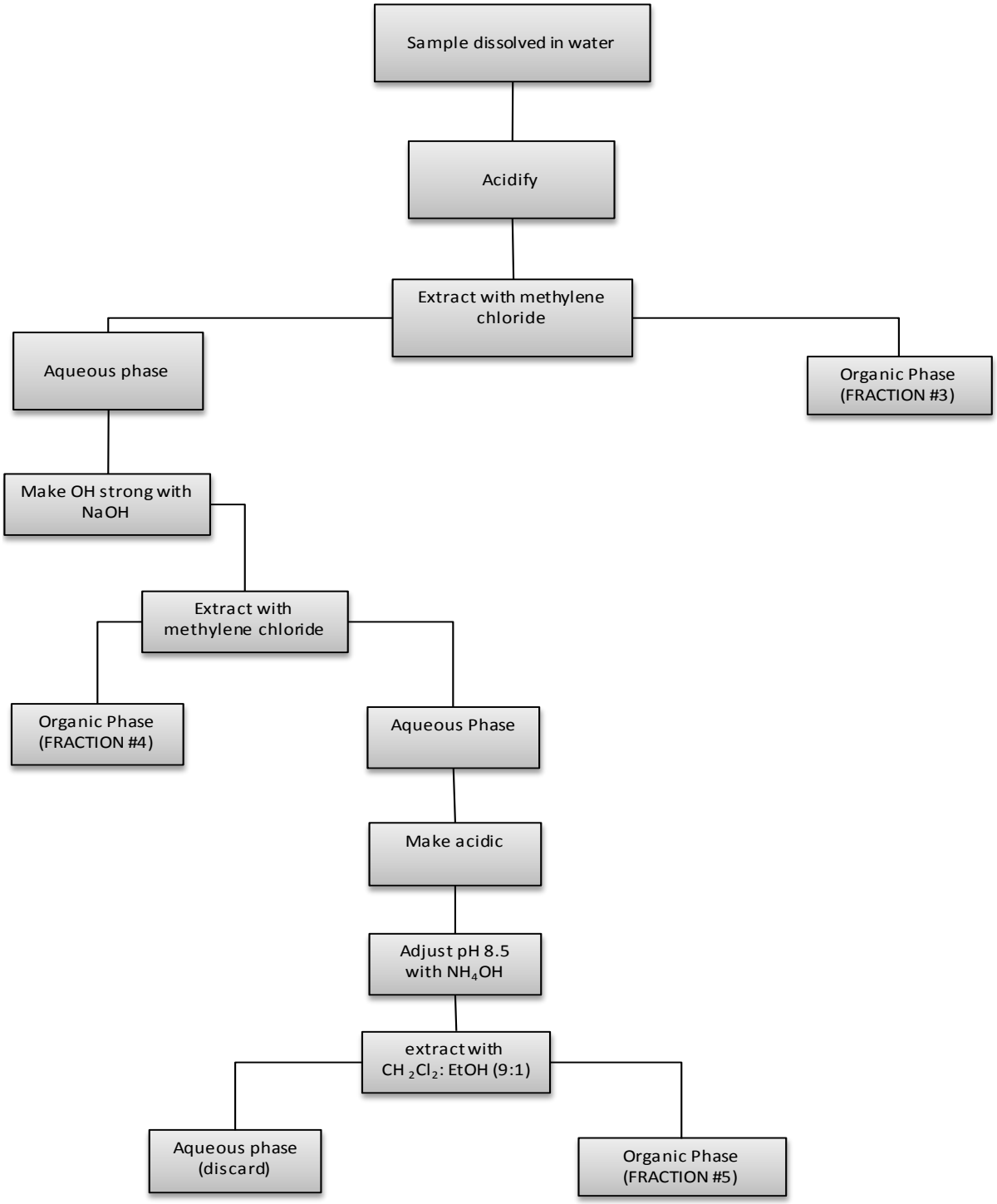
Results

The extracted substance may then be used for further analysis.

References

Clarke's Isolation and Identification of Drugs, 2nd Ed., (1986)

Georgia Bureau of Investigation-Division of Forensic Sciences, Drug Identification Procedures Manual, AN DI 04, September 6, 2013.



GHB and Pregabalin Derivatization

Purpose: GHB will form gamma-butyrolactone (GBL) in the heated injection port. The silyl derivative will differentiate GHB from GBL. Derivatization procedure can also be used for GC/MS analysis of pregabalin by starting at the fifth bullet-point.

Materials, Reagents, and Equipment

Acetone

Bis(trimethylsilyl) trifluoroacetamide (BSTFA)

Ethanol

Methylene Chloride

Suitable aprotic solvent

Appropriate neat standard (if in solution, solvent must be fully evaporated)

Auto sampler vials

Auto sampler vial inserts

Beakers

Glass pipettes

Spatula

Steam bath or hot air blower

Procedure

1. Place sample in glass beaker
2. Dry sample with heat until crystals begin to form
3. Wash sample two times with acetone and discard acetone.
4. Dry sample with heat. Crystals should form.
5. Dissolve about 1 mg of crystals or powder in BSTFA in an insert.
6. Stir the solution with a needle or glass pipette to ensure target analyte dissolves in the BSTFA.
7. *Note: if the target analyte does not dissolve in BSTFA, repeat step 5, adding a minimal amount of solvent (preferably a suitable aprotic solvent) before derivatizing.
8. Allow any particulate matter to settle and transfer the supernatant to a new insert.
9. Place the insert into an auto sampler vial, cap, and place it into the refrigerator until ready to inject.
10. Inject one microliter of the appropriate standard that has been derivatized in the same manner as the case sample.
11. Inject a BSTFA blank
12. Inject one microliter of the derivative solution.
13. Inject a solvent blank if used to dissolve before adding BSTFA

References

"*The Identification of GHB*" Microgram Vol. XXIV, #7, July 1991.

"Silylation and Acylation Derivatives for GLC and GLCMS Drug Analysis," Microgram Vol. XII #4, April 1979.

Georgia Bureau of Investigation-Division of Forensic Sciences, Drug Identification Procedures Manual, AN DI 39, June 14, 2010.

Single Step Extractions

Purpose: Drugs are directly extracted from impurities, excipients, and adulterants using a single step extraction process using aqueous or organic solvents.

Materials:

- Ethanol
- Methanol
- Isopropanol
- Acetone
- Ethyl Acetate
- Chloroform
- Methylene Chloride
- Hexanes
- Petroleum Ether
- Acetonitrile
- 0.1N HCl
- Deionized Water
- Disposable Pipettes
- Filter Paper
- Beakers, test tubes, or other glassware
- Funnel

Note: This list of solvents is not all inclusive

Procedure

- Choose an appropriate solvent based upon the chemical and physical properties of the target analyte (i.e. solubility, polarity) to ensure compatibility with subsequent testing.
- Add the appropriate solvent to the sample and agitate.
- Filter the solution or decant (if solution is clear) into the appropriate container.

Results: The solution may be used for further analysis.

References:

Merck Index

Georgia Bureau of Investigation-Division of Forensic Sciences, Drug Identification Procedures Manual, AN DI 12, October 21, 2013.

Appendix B References

Reference List

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Smith, Fundamentals of Fourier Transform Infrared Spectroscopy

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RX-ID computer subscription

All commercial pharmaceutical manufacturer websites

CLIC Journals

Code of Federal Regulations

Tennessee Code Annotated

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Portions of this manual were written based off of information gathered from the following accredited Crime Laboratories:

Arkansas State Crime Laboratory

Georgia Bureau of Investigation

Tennessee Bureau of Investigation

Virginia Department of Forensic Science

Note: This list is not all inclusive

Appendix C Abbreviations

Note: These abbreviations may be upper or lower case or a combination of both.

(+)	Positive
(-)	Negative
→, ->, ~>, => >	Containing
AC#	Agency case number
ACETA	Acetaminophen
ACN	Acetonitrile
ALP	Alprazolam
AMPH	Amphetamine
APAP	Acetaminophen
APEX	Alkaline petroleum ether extraction
ATR	Attenuated total reflectance
BCPH	Mixed standard (benzocaine, cocaine, procaine, heroin)
BLK	Blank
°C	Degrees centigrade

CAP/CAPS	Capsules/capsules
CB	Corner bag
CBD	Cannabidiol
CBN	Cannabinol
CDA	Controlled drugs of abuse
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane (Methylene Chloride)
CIG	Cigarette
CLIC	Clandestine Lab Investigating Chemists
COC	Chain of custody
COMB	Combined
CONC	Concentration
C/	Containing
COT	Containing
CPB	Corner plastic bag
CRM	Certified reference material
CRYST	Crystalline
DI	Deionized
DIB	Drug ID Bible
DL	Duquenois-levine
DNU	Do not use
ED	Edition
ENV	Envelope
EPH	Ephedrine
ETOH	Ethanol
EXP	Expired
EXT	Extraction
EVID	Evidence
FTIR	Fourier Transform Infrared Spectrometry
G(s)	Gram(s)
GC	Gas Chromatography
GCIR	Gas chromatography Fourier Transform Infrared Spectrometry
GCMS	Gas chromatography mass spectrometry
GTS	Gross total sample
GW	Gross weight
H_2O	Water
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
H+	a solution having an acidic pH
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HYPERG	Hypergeometric sampling
ID	Identification
INC	Inconclusive
IDDA	Instrumental Data for Drug Analysis
INDIC	Indicative

IPA	Isopropyl alcohol
IR	Infrared spectroscopy
KG	Kilogram
L	Liter
LB(s)	Pounds
LCMS	Liquid Chromatography Mass Spectrometry
LIMS	Laboratory Information Management System
LIQ	Liquid
M	Mole or molarity
MEOH	Methanol
METH	Methamphetamine
MFG	Manufacturer
MG(s)	Milligram(s)
MICRO	Microscopic
MJ	Marijuana
ML	Milliliter
MOD	Modified
MU	Measurement uncertainty
N	Normal or normality
NA	Not analyzed
NAOH	Sodium hydroxide
NCDA	Negative for controlled drugs of abuse
NEG	Negative
NH_4OH	Ammonium hydroxide
NM	Nanometers
NPS	New Psychoactive Substances
NU	Not used
NW	Net weight
OH-	Solution having a basic pH
OXY	Oxycodone
PDR	Physician's Desk Reference
PET ETHER	Petroleum ether
PFTBA	Perfluorotributylamine
PHENT	Phentermine
PKG	Package
POS	Positive
PPA	Phenylpropanolamine
PPT	Precipitate
PSC	Personal Storage Cabinet
PSL	Personal Storage Locker
QMS	Quality Management System
REF	Reference
RM	Rock-like material
RGW	Reserve Gross Weight
RNW	Reserve Net Weight
RT	Retention time

RTP	Room Temperature
RX	Prescription
SAT	Saturated
SD	Smoking device
SM	Solid material
SN, S/N	Serial Number
SOLN	Solution
STD	Standard
SUB	Submission
SYNCAN(s)	Synthetic cannabinoid(s)
TAB	Tablets
TFMPP	Trifluoromethylphenylpiperazine
TGW	Total gross weight
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid
TIC	Total ion chromatogram
TLC	Thin Layer Chromatography
TNW	Total net weight
TOT	Total
UBSC	Unit Bulk Storage Cage
UL	Microliter
UV	Ultraviolet Spectrophotometry
V/V%	Volume/volume percent
VOL	Volume
W/O	With out
W/W%	Weight per weight
W/V	Weight per volume
WGT	Weight
WKSH	Worksheet
WP	White powder
WT	Weight
XTC	Ecstasy
ZIP	Ziploc

Drug ID Staff

NMD	Nicole Dowell
RDH	Reika Haskell
SRT	Sarah Turner
MWB	Michael Brown

Appendix D Definitions

Carryover: Peaks (signal) which contain four or more major ions (as defined by the IDDA) of a target analyte.

Confirmatory Test - Second test by an alternate chemical method for unambiguous identification of a drug or metabolite.

Certified Reference Material: reference materials characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Critical solvent(s), reagent(s), and solution(s): Any solvent(s), reagent(s), and solution(s) used in the extraction process and/or in the analysis of a sample(s) with the exception of GC carrier gas and DI water which can directly affect the quality of the final result.

Excessive Column Bleed: Peaks (signal) which have a relative abundance that is greater than 10% of the lowest acceptance criteria for data comparison.

Forensic Technician – An employee of the MNPD-CL Laboratory who works under the supervision of the unit supervisor and performs tasks in support of the unit including, but not limited to, Quality Assurance checks, reagent preparation, supply inventory control, and assisting forensic scientists in the examination and analysis of forensic evidence within a particular discipline or sub-discipline.

Long-term Personal Storage Locker- a locker(s) assigned to each analyst that cannot be accessed by other unit staff. These lockers have a location barcode and are used for long term storage (over 120 days) of drug evidence. These are located in the Drug ID Unit Evidence Storage Room.

Measurement – The process of experimentally obtaining one or more values that can reasonably be attributed to a quantity.

Measurand – Quantity to be measured

Negative - A negative result is obtained when the analytical tests performed did not yield data that would support the conclusion that a controlled drug of abuse (as defined by the Tennessee Annotated Code §39-17-417) is present in the sample.

Negative Control - A blank solution used to monitor instrument conditions and/or prevent carryover. Negative controls, also referred to as solvent blanks, are used to confirm that an instrument or procedure is capable of producing a negative result

Personal Storage Cabinet (PSC)- a lockable storage cabinet assigned to each analyst that cannot be accessed by unit staff. These are located at each examiner's assigned lab work area.

Personal Storage Locker (PSL)- a locker(s) assigned to each analyst that cannot accessed by

other unit staff. These are located in the Drug ID Unit Evidence Storage Room.

Positive - A positive result is obtained when the analyst has successfully completed at least the minimum tests

Positive Control - A known chemical reference standard used to confirm that an instrument or procedure is capable of producing a positive result.

Random Sample(s) - A sample(s) selected for analysis without consideration of any characteristics of the item(s) being analyzed.

Representative Sample(s) - A sample(s) selected for analysis from a collection of uniform or similar items as determined by visual or scientific means.

Sampling Plan - For an item that consists of a multi-unit population (e.g., tablets, baggies, bindles), a sampling plan is a statistically valid approach to determine the number of sub-items that must be tested in order to make an inference about the whole population.

Sampling Procedure - A defined procedure used to collect a sample or samples from the larger whole, to ensure that the value obtained in the analysis is representative of the whole. The sampling procedure may include details about size and number of sample(s) to be collected, locations from which to collect the sample(s), and a method to ensure the homogeneity of the larger whole (or to make it so.)

Sample Selection - A practice of selecting items to test, or portions of items to test, based on training, experience and competence. In sample selection, there is no assumption about homogeneity.

Unit Bulk Storage Cage (UBSC)- There are two lockable UBSCs available in the unit's evidence storage room that can be used by any Drug Unit analyst to store evidence too large for their PSL or PSC. When bulky evidence is locked in a UBSC, only the analyst has access to the cage at that time. Evidence stored in a UBSC is also tracked in the LIMS COC since it is not a PSL or PSC. Each cage has its own barcode ID.

Appendix E Weight Thresholds

Drug	Class E	Class D	Class C	Class B	Class A
Schedule I				Any amount (see exclusions, fine changes at 200g)	2000g and above
Heroin				15g	150g and above
LSD				5g	50g and above
Peyote				1000g	10,000g and above
*Acetylfentanyl, Acryl fentanyl & schedule 1 analogues				15g	150g and above
Schedule II			< 200g	200g - 2000g	2000g and above
Amphetamine				26g	300g and above
*Cocaine and Methamphetamine			<0.5g	0.5g - 26g - 300g (Fine changes at 26g)	300g and above
*Fentanyl, Carfentanil & schedule 2 analogues			<0.5g	0.5g - 15g – 150g (Fine changes at 15g)	150g and above
Morphine				15g	150g and above
Hydromorphone				5g	50g and above
Phencyclidine (PCP)				30g	300g and above
Phenmetrazine				50g	500g and above
Schedule III		Any amount			
Barbituric Acid				100g	1000g and above
Schedule IV		Any amount			
Flunitrazepam (IV)			Any amount		

Drug	Class E	Class D	Class C	Class B	Class A
Pentazocine w/ Tripelennamine				5g	50g and above
Schedule V	Any amount				
Schedule VI					
Marijuana	14.175g - 4535g (10lbs)	4536g (10lbs, 1g) - 31,696g (70lbs)		31,697g (70lbs, 1g) - 136,050g (300lbs)	136,050 (300lbs) and above
Non-Leafy Resinous Material (THC)	<905g (2.0lbs)	906g (2lbs, 1g) - 1810g (4lbs)	1811g (4lbs, 1g) - 3620g (8lbs)	3621g (8lbs, 1g) - 6,792g (15lbs)	6,793g (15lbs, 1g) and above
Plants		10 - 19 plants	20 - 99 plants	100 - 499 plants	500 plants and above
Schedule VII	Any Amount				

*Must meet weight thresholds independently

References

Tennessee Code Annotated §39-17-417

Tennessee Code Annotated §39-17-423

State of Tennessee Public Chapter NO. 386

Appendix F Hypergeometric Sampling Table (UN 1998)

Total number of items in exhibit	Number of units to be tested
1-9	All units
10 – 12	9
13	10
14	11
15 – 16	12
17	13
18	14
19 – 24	15
25 – 26	16
27	17
28 – 35	18
36 – 37	19
38 – 46	20
47 – 48	21
49 – 58	22
59 – 77	23
78 – 88	24
89 – 118	25
119 – 178	26
179 – 298	27
299 – 1600	28
1600+	29

The Hypergeometric Sampling Table was constructed using the hypergeometric calculation based on a 95% probability that 90% of the population contains the identified compound.

SWGDRUG, *Recommendations,* Version 7.0: August 14, 2014.

ENFSI (European Network of Forensic Science Institutes). *Guidelines on Representative Drug Sampling*. Version 1-1, 2003.

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ASTM. Standard Practice for Probability Sampling of Materials. Designation E 105 – 58 (Reapproved 1996).

Moffat, Anthony C., Osselton, M. David, and Widdop, Brian and Watts,Jo(Eds.). Clarke's Analysis of Drugs and Poisons. . Fourth Edition, London/Chicago 2004. Vol. 1 p. 192-193.

Additional Sampling for Inconsistent Units

Total # Units in Exhibit	Sampling required for one negative (#positives/additional sample size)	Sampling required for two negatives (#positives/additional sample size)
13-37	****	****
38-59	****	****
60-68	30/32	****
69-73	31/33	****
74-84	34/36	****
85-106	38/40	****
107-126	41/43	****
127-156	44/46	****
157-198	47/49	****
199-222	48/50	12/133
223-246	49/51	131/142
247-259	50/52	137/148
260-287	51/53	147/159
288-324	52/54	150/162
325-372	53/55	162/175
373-392	53/55	172/186
393-465	65/68	175/189
466-590	67/70	185/200
591-770	68/71	195/201
771-940	69/72	208/224
941-1,150	69/72	221/238
1,151-1,500	70/73	234/252
1,501-10,000	72/75	245/264
10,001-30,000	72/75	255/274
30,000+	73/76	256/275

Tennessee Bureau of Investigation, Forensic Chemistry Standard Operating Procedure Manual, Appendix C: Hypergeometric Sampling for Negative Results. Revision 11/1/2016.

Appendix G Instrument Methods

Approved Methods for GC/MS Analysis of Controlled Substances

Method Name	Initial Temp	Initial Time	Rate#1 (°/min)	Temp #1	Time #1	Rate #2 (°/min)	Final Temp	Final Time	Inlet Temp	AMU Range	Notes
Screen	70	2	30	--	--	--	275	10	275	40-500	Screening and non-controlled substances
Splitless	60	2	35	--	--	--	275	12	270	40-500	LSD/LAMPA and other low dose drugs
Mid	200	1	25	--	--	--	275	7	275	40-500	Late eluting drugs (Ex: Opiates, THC, etc.)
Opioid	200	1	25	250	0	5	275	1	275	40-550	Opioids and Fentalogues

Universal Parameters

GC/MS Parameters

GC Interface Temperature: 280°C (split methods), 275° C (splitless methods)

MS Source Temperature: 230°C

MS Quad Temperature: 150°C

Injection Parameters (minimum requirements)

Solvent A Washes (Pre-Injection): 1

Solvent A Washes (Post-Injection): 3

Sample Pumps: 6

All injections are split injections with the exception of the **Splitless** method.

The methods listed are automated.

Sample volume will be 1µL with the exception of samples consistent with pharmaceutical preparations with dosages of 2 mg or less where a 2 µL injection may be used as needed.

Final times listed above are the minimum time requirements. Final times can be extended to include late eluting compounds, as necessary. AMU ranges listed above are minimum requirements and may be expanded, as needed.

- Full instrument parameters and validation information for the methods can be found in the validation notebooks.
- Changes to the solvent delay setting, which result in starting the mass spectrometer scan earlier (Ex: changing from 2 minutes to 1 minute), are considered minor adjustments to the method and will not require completion of a Deviation Request Form.

FTIR Instrument Method

Instrument: Nicolet iS10 FT-IR Spectrometer

Smart iTR ATR accessory

Software: OMNIC 9.2.98

Experiment: MNPD Crime Lab FTIR/ATR

Bench Parameters: Sample Compartment: Main
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
Accessory: Smart iTR
Window: Diamond
Max Range Limit: 4000 cm^{-1}
Min Range Limit: 650 cm^{-1}
Gain: Autogain
Optical velocity: 0.4747
Aperture: Medium resolution

Collection Parameters: Number of scans: 32

Resolution: 4

Data Spacing: 0.482 cm^{-1}

Final format: %Reflectance

Sample Introduction: Neat sample will be applied to Smart ATR accessory

GC-FTIR Instrument Method (MNPD Crime Lab GC-IR or MNPD CL GCIR Low Temp)

Instrument: Thermo Fisher Trace 1310 GC with autosampler
iS50 GC-IR and iS50 FT-IR

Software: Chromeleon 7.2.8 for GC
OMNIC 9.8.372 for FTIR

GC/IR Interface: Transfer Line: 280°C
Flow Cell: 280°C

GC Conditions:

GC Method: "**MNPD Crime Lab GC-IR**" or "**MNPD CL GCIR**

	Low Temp"
Column:	TR-1MS capillary column
	5m (L) x 0.32 mm (i.d.), d_f 1 µm
	Thermo Scientific
Carrier gas:	Helium
Sample Introduction:	1µL, split ratio: 5:1,
	Constant carrier flow: 1 mL/min
	Split flow 5.0 mL/min
	Makeup gas: 40.0 mL/min
Temperatures:	Injector: 250°C
	"**MNPD Crime Lab GC-IR**" Oven Program: 120° C (1 min), 70 °C/min, 270°C (12 min), 70 °C/min, 120°C (1 min)
	"**MNPD CL GCIR Low Temp**" Oven Program:
	80 C (1 min), 25C/min to 220 C (4 min)

FTIR Conditions:

FTIR Experiment:	MNPD Crime Lab iS50 GC-IR
Bench Parameters:	Sample Compartment: GC-IR
	Detector: MCT/A
	Beamsplitter: KBr
	Source: IR
	Accessory: None
	Window: None
	Max Range Limit: 4000 cm^{-1}
	Min Range Limit: 650 cm^{-1}
	Gain: 1
	Optical velocity: 2.5317
	Aperture: 100
Collection Parameters:	Number of scans: 4
	Resolution: 8
	Data Spacing: 3.857 cm^{-1}
	Final format: Absorbance
	Collect 64 scans for the background
Series Parameters:	Data collection type: GC/IR
	Profiles: Gram-Schmidt
	Auto Scale profiles
	Synchronize data collection

HPLC Instrument Method for Quantitation of Cannabinoids

- Column: Shimadzu NexLeaf CBX for Potency, 2.7 umx150 mmx4.6 mm ID
- Column Thermostat: 50°C
- Solvent A: Mobile Phase A (0.1% Formic Acid in Water)
- Solvent B: Mobile Phase B (0.1% Formic Acid in Acetonitrile)
- Flow rate: 1.5 mL/min
- Injection volume: 5 uL
- Stop time: 8 min
- Detector wavelength: 220 nm
- Gradient:

Time	%Mobile Phase A	%Mobile Phase B
5	20	80
6	15	85
6.01	30	70
8	STOP	

Appendix H QC Mix Formulas

Preparation of GCMS QC Mix

1. Add approximately 9.0 mg of Methamphetamine and Phentermine to a test tube
2. Add approximately 19.0 mg of Hydromorphone to the same test tube
3. Add approximately 6.0 mg of Cocaine Base to a separate test tube
4. Dissolve Methamphetamine, Phentermine, and Hydromorphone in approximately 5 mL of 0.1N Hydrochloric Acid
5. Adjust the pH to approximately 11 using Sodium Hydroxide
6. Extract solution three times (~2 mL for each pull) using Petroleum Ether and add to a 10 mL volumetric flask
7. Add approximately 0.5 mL of concentrated Hydrochloric acid to the remaining aqueous layer
8. Adjust the pH to approximately 8.5 using Ammonium Hydroxide
9. Extract solution three times (~1 mL for each pull) using 9:1 Methylene Chloride: Ethanol and add to the volumetric flask
10. Dissolve Cocaine Base in approximately 1 mL Petroleum Ether and add to the volumetric flask
11. Fill to the line with Petroleum Ether
12. Cap and invert the volumetric flask

For verification purposes, the GC/MS QC mix will be run on all GC/MS instruments which are in service. The QC mix must pass all data acceptance requirements on each instrument to be suitable for casework.

Preparation of GCIR QC Mix

1. Add approximately 19 mg of Butalbital and Cocaine to a test tube
2. Add approximately 20 mg Testosterone to the same test tube
3. Dissolve Butalbital, Cocaine, and Testosterone in approximately 3 mL of Methylene Chloride
4. Add solution to a 5 mL volumetric flask
5. Fill to the mark with Methylene Chloride
6. Cap and invert the volumetric flask