Chemistry Methods Manual

1. Introduction

The analysis of a controlled substance will depend on the sample submitted for analysis. Samples come in the form of tablets and capsules, solids, liquids, powders, *vegetation*, and residue of any of the above. The choice of a method for the analysis of these materials will vary with each submission.

Analysts shall make every effort to maintain a clean, contamination-free workspace in areas where sampling and analysis occur. Consumables used for sampling or analysis shall follow manufacturer recommendations for storage. If no recommendations are provided, they should be stored away from direct sampling spaces, and if possible, in closed containers which can include, but is not limited to, drawers and cabinets. Consumables shall include, but are not limited to sampling utensils, test tubes, gloves, autosampler vials and caps, weigh boats and weigh paper. Additionally, consumables should be evaluated upon receipt to verify seals are intact, no damage or deterioration has occurred, and supplies have been received in accordance with vendor and manufacturer specifications, unless otherwise noted.

This manual is intended as a guideline for the analysis of controlled substances and other materials. It sets forth methods accepted by the forensic community and followed in the Chemistry sections at the Ohio BCI. Details of instrumental parameters, extraction preferences, note taking, suggested report wording, and other details cannot be exactly the same for every case and every examiner, therefore, variations are expected. Such variations will be documented in the case record and such that another experienced examiner is able to understand and evaluate the method used.

Generally, an analysis will consist of visual examination, mass determination, sampling, and a series of analytical tests. Attention should be given to visual examination, mass determination and sampling techniques before conducting analytical tests. Methods are included in this manual.

1.1 Evidence handling

All evidence submitted for drug analysis shall be handled in accordance with established laboratory policy. Submission, sealing, marking, custody and storage requirements are defined in the Laboratory Quality Assurance Manual and related practices. Safe handling and exposure information is contained in the Laboratory Safety Manual and specific sections of this document.

Evidence examination from multiple items is separated by time and/or physical space to prevent cross contamination.

The quantity of tablets or capsules should be documented prior to conducting mass determination or laboratory testing.

In order to determine what needs tested in order to reach the highest charge based on weight, scientists may elect one of the following approaches:

- Gross Weight-This value includes the packaging and its contents; this preliminary value is recorded in lab notes, but is not required to be on the laboratory report. Instead, a simple statement of "Not tested" is listed in the report, along with a clarification statement of "The gross weight was recorded in order to identify the items to be tested in accordance to the current submission policy, no further laboratory analysis was performed on items noted as "Not Tested"."

- Net Weight- This value includes only the contents of the package; this value is recorded in the notes and the laboratory report, along with the measurement uncertainty.
- Visual estimation only- No weight is taken; the reason for this approach is documented in the lab notes; the laboratory report states "Not tested".

Packaging is routinely removed prior to recording the mass of the substance. Packaging may include a portion of the item that is not typically consumed, such as a pipe, cotton balls, coffee filters, or capsules that appear to be illicitly filled.

The chemist must use discretion to ensure that the complete analysis does not consume more than half of the population amount.

If a multiple unit item is submitted and all units are opened, those items will be re-packaged separately (for example, 10 balloons of heroin will be packaged in 10 separate bags).

Syringe washings/rinsing - must be reported as 'trace amount' but a net weight may be recorded in the lab notes.

~~Saturated/soaked papers (excluding LSD papers) - Net weight must be included on the report. Each piece of paper will be treated as a separate sample.~~

1.2 Examination documentation

Examination documentation must be generated and retained in accordance with current accreditation standards and as specified in the Laboratory Quality Assurance Manual.

1.2.1 Traceability

All instruments and prepared reagents used to make analytical determinations must be traceable. Analysts will record in each case as part of the case examination documentation the lot number of the prepared reagent(s) used, *the lot number of stock reagent used*, the unique identifier for each instrument and balance used, and the lot number of the Quality Control mixture used.

1.2.2 Required elements of examination documentation

The following examples are provided to illustrate important aspects of note-taking in the matrix:

Open Date: the date that the evidence was opened must be documented; if an item is not opened, ~~a comment must be~~ it must be recorded ~~such~~ as "Not Opened" in the examination documentation, unless it was never taken into your custody.

Packaging: A description of all packaging must be included to document the condition of the evidence upon receipt by the scientist. If an item is not opened, packaging can be recorded as "Not Applicable" or "N/A" in the examination documentation.

Population (N), Sample Size (R), and Sampling plan (if applied): If the item is identified using only markings, N=R. If the item is not opened, both can be recorded as "Not Applicable", "N/A", "0" or left blank.

Contents: Upon opening, a description of the relevant characteristics of the contents in the packaging must be documented. Examples of characteristics include, but are not limited to, color, shape, texture, quantity, population and unique markings (as applicable).

Weight: If the item is less than 0.10 gram, the observed weight will be recorded in the case record. If the balance registers 0.00 gram, it is acceptable to add this or a statement such as "did not register on balance". If the gross weight is recorded for the purpose of estimating the sample amount required to test to charge, the weight must be recorded in the notes.

Analysis Methods: Documentation of the method(s) of testing must be recorded for each item. If an acid or base extraction is used, the scientist must indicate which type of acid or base was used for the extraction. The solvent used must be recorded. Information recorded on instrument data will be used for documentation run parameters (instrument name, method(s) run, injection volume, and standard manufacturer and lot# or unique identifier). Compliance with policies set out in this manual regarding blanks and correspondence of retention times will be directly ascertained from instrument data without further commentary in the examination documentation.

Sealing of Evidence: The date that the evidence *item* was sealed must be documented.

Test Results: Observational test results, such as color tests and microscopic examination, are documented to describe the original test results observed. Any rejected test data is documented in the case record in accordance to the policy in the Lab Quality Assurance Manual (section 7.5.1.5).

2. Administration Policies for Drug Analysis

2.1 Introduction

The Ohio Administrative Code lists all the substances controlled in the state of Ohio subject to section 4729:9 as well as sections 3719.41, 3719.43, and 3719.44 of the Ohio Revised Code.

While the protocols, methods, and procedures give direction for the analysis of controlled substances, there are some administrative considerations that may cause the specific method to be adjusted.

The workflows employed by the Drug Chemistry lab section are conducted in compliance to the ANSI ASTM E2329 standard (Identification of Seized Drugs).

2.2 Positive identification

A minimum of two testing procedures are required for the identification of a controlled substance. A single GC/MS injection serves as two testing procedures if both results are used to draw conclusions: GC retention time *with reference standard(s)* and mass spectrum. Approved instrument methods can be found in the appendix of this manual. Any instances of GC/MS testing *below* refers to *GC/MS* instruments using Helium unless noted for *GC/MS* instruments using Hydrogen.

General Testing Scheme:

(1) GC/MS (General Screen – QDS, GEN130, GEN170, GEN115-20M)
(2) N/A or FTIR or GC/MS *(Helium or Hydrogen)* or GC/FID or TLC

Determination of the total tetrahydrocannabinol (THC) *content* must be quantified (if sample amount permits) to report *cannabis or delta-9-tetrahydrocannabinol* This shall be determined by running an approved quantitative analytical scheme below.

Suspected Cannabis Vegetation Testing Scheme

(1) Modified Duquenois Levine OR GC/MS
(2) Microscopic Examination
(3) Moisture analysis (if sample permits)
(4) LC/UV/MS or LC/UV

Suspected Cannabis Products Testing Scheme

(1) GC/MS (*with reference standard(s)*)
(2) LC/UV/MS or LC/UV

The Forensic Laboratories in the State of Ohio have established a committee for newly

identified psychoactive substances (NPS). When a new substance is identified whose structure could be considered a potential NPS, the analyst provides a spectrum and proposed structure to the local FSC. The information will be distributed amongst the laboratory chemists. Reference NPS Statewide Review Committee process in appendix IV.

Opium- instrumental analysis identifying Morphine, Codeine, and at least two (2) of the following: Thebaine, Papaverine, Noscapine.

2.2.1 Alternative Positive Identification Schemes

There are times when other analytical schemes are appropriate and may be used in addition to or in lieu of the general testing scheme described above. They are listed below:

Use of a Hydrogen Instrument GC/MS (Genscreen-H2) – all samples require a second run using a general screen on a helium instrument ~~unless noted below~~ *with the exception of Methamphetamine and Cocaine*.

Mushrooms- if Weber's testing indicates psilocyn, run on GC/MS to confirm *(reference standard not required*) & report out results as "Psilocyn"; if Weber's testing is negative, run on GC/MS and if Psilocyn is found, perform derivatization or thin layer chromatography to confirm whether psilocyn is present. If psilocyn is present, report out results as "Psilocyn". If Psilocyn is not present, and the thin layer plate indicates Psilocybin, the scientist must derivatize the sample, run on GC/MS to confirm (*reference standard not required)*, and report out results as "Psilocybin".

Other Psilocyn/Psilocybin/4-AcO-DMT Items (e.g. gummies, chocolates, etc.)- (when not in vegetative form) – Perform a Weber's color test, if positive, run on GC/MS to confirm (*reference standard not required*) and report out Psilocyn. If a Weber's color test is not possible or it's negative, *run on GC/MS to confirm and* report out Psilocyn*. With the remark –*The Psilocyn in this case may be from the breakdown of Psilocybin/4-AcO-DMT.

Steroids/synthetic cannabinoids- instrumental analysis using an alternative high boiler method, such as HiB230, is acceptable.

Suspected carfentanil– A method that incorporates a lower split and higher injection volume, (for example: OPI212- 10S-2, OPI215-10S-2, OPI210-20m-10S-2, QDS-10S-2 or GEN115-10S-2 etc.) must be run after a general screen.

GHB – instrumental analysis using an alternative low boiler method, such as GHB510, is acceptable.

2C-X, Mescaline, 25X-NBOH- When a substance is identified as a 2C-X compound, 25X-

NBOH compound, or Mescaline, the analyst must derivatize using an approved derivatization method (such as BSTFA) *and run on GC/MS to confirm*, or alternatively run an FTIR.

THC Items *for qualitative analysis* only (includes substances, smoking devices, scales, etc.) – One GC/MS run on a general screen is acceptable (*reference standard not required*), no additional analysis is required if sample does not meet quantitative submission guidelines. Report 'Tetrahydrocannabinols (THC) indicated – Not confirmed'.

Cannabinoid~~s~~ *item for qualitative analysis only* – If positive Duquenois-Levine color test is observed, no additional analysis is required if sample does not meet quantitative submission guidelines. Report 'Cannabinoids indicated – Not confirmed'.

Marked Pharmaceuticals- Tablets with complete markings may use Logo ID and ~~run on~~ *confirm on either* GC/MS (helium or hydrogen) (*reference standard not required*) or FTIR ~~to confirm~~; Controlled pharmaceuticals with identifiable markings may be run using an alternative method, unless there are indications of tampering or of suspicion of counterfeit product.

Suspected Cocaine- Perform Cobalt Thiocyanate or Scott's color test. If positive, run on FTIR~~, or GC/MS (Genscreen-H2)~~ to confirm.

Suspected Methamphetamine- Perform Marquis and Sodium Nitroprusside color tests. If both are positive, run on FTIR~~, or GC/MS (Genscreen-H2)~~ to confirm.

2.3 ~~Insufficient~~ Inconclusive determination

~~Insufficient~~ Inconclusive for identification – Two GC/MS instruments (at least one being helium) are required to report ~~insufficient~~ inconclusive findings, with one of the runs incorporating a lower split. It is acceptable to use a shorter method on the second instrument, as long as it incorporates a lower split and higher injection volume, when applicable. *If the initial run was on a 10:1 split then it is also acceptable for the second instrument test to be a 10:1 split*.

2.4 No controlled substance determinations

A minimum of two independent instrumental tests are required for a no controlled substance determination, as indicated in the table below (listed in no particular order). If the sample has only a non-controlled substance(s) present, a second general screen method must be run.

If the sample has no substances indicated, then the second instrument test

must be an additional run using the initial vial run on a ~~stronger~~ *lower* split or alternatively, an additional sample of the evidence may be prepared for another instrument run. *If the initial run was on a 10:1 split then it is also acceptable for the second instrument test to be a 10:1 split.* The second instrument test must be run on a different instrument than the first instrument test.

	Testing Requirements
(1)	GC/MS (General Screen)
(2)	GC/MS (General Screen) or GC/MS (Genscreen-H2) or FTIR

Additional tests may be done at the discretion of the scientist. An alternative instrument screen method for the 2nd instrument test can be selected as a result of visual examination of the evidence. For example, for potential late-eluting compounds such as those in vegetation or liquids, HiB230 may be used for the second instrument method.

Non-controlled pharmaceuticals with identifiable markings that use logo identification and an instrumental run using a general screen may be reported as no controlled substance found, unless upon macroscopic observation, there is evidence of tampering or reasons to suspect a counterfeit.

Samples that are either acid or base extracted only and result in no controlled substances, must also be run neat with a solvent extraction or the alternate extraction method.

2.5 No Analysis Determinations

Not all items in a case need to be analyzed. Unless additional information is provided by the submitting customer, in a multiple item residue case only one type of each residue should be analyzed (e.g., five smoking devices, only one item needs to be tested per subject).

2.6 No Examination Determinations

Laboratory requests for the identification of non- drug manufacturing chemical precursors, poisons, or explosives are referred to the State Fire Marshal's Laboratory for testing. BCI

laboratory testing of clandestine laboratory evidence will include analysis for any controlled substances and non-controlled drugs (such as pseudoephedrine) in Manufacturing offenses.

2.7 Legal Limits Testing Approach

Cases containing multiple types of drugs should be worked to the highest criminal charge. This includes testing to bulk amounts. Evidence will be tested to the legal limit referenced in the Ohio Revised Code 2925 or applicable federal codes. A hypergeometric sampling plan may be utilized; however, the sampling plan may be abandoned if the legal limit requires fewer samples to be tested.

Inconsistent preparation/distribution of clandestine controlled substances and the lack of a legally defined mechanism to identify a "separately administered" quantity, (see unit dose definition, ORC Section 2925.01), restrict the laboratory's ability to ~~make an accurate unit dose determination~~ *report unit dose specifically. Separately identifiable quantities will be referred to as "units" and can be used to establish a legal limits testing approach*.

Forensic Scientists have discretion to *either* weigh the evidence or visually estimate whether the evidence meets the threshold for testing to the charge.

3. Macroscopic examination

3.1 Introduction

Initial examination will consist of a visual assessment of the form/condition of the evidence and determination of the number of unique sample populations present based on the similarity/dissimilarity of comparative visual characteristics. Relevant visual characteristics may include color, shape, size, thickness, texture and unique markings.

3.2 Population determination

3.2.1 The population determination shall take into account all typical forms and quantities in which exhibits may appear.

3.2.2 A population can consist of a single unit or multiple units.

3.2.3 A multiple unit population should consist of items which are similar in relevant visual characteristics.

3.3 Population Count

A physical count of units within a population must be obtained. Reporting a total number of units requires counting three times (triplicate) unless using grouping, grid counting, or a Fretwell Triangle. When counting in triplicate, if any of the three counts does not match, the analyst must recount the units using grouping, grid counting, or a Fretwell Triangle.

A grouping method may be used as follows:

- *Sort the units into groups of no more than 10 units each*
- *Count groups to mathematically determine the total number present*

Grid counting may be used as follows:

- *Align the units in distinguishable rows and columns to form a "grid" pattern*
- *Mathematically determine the total number present*

A Fretwell Triangle may be used to count round, uniform tablets as follows:

- *Form tablets into even rows in the triangle*
- *Count the number of rows*
- *Look up the total on the scale*

Note that the triangle must be cleaned using methanol before and after each use and stored in a designated enclosed location such as a drawer or cabinet.

4. Physical Identification of Tablets and Capsules

4.1 Introduction

The Physical Identification Procedure can be used on tablets*, capsules, and packaging*. ~~and capsules~~. These tablets and capsules can be from a commercial manufacturer or from a clandestine manufacturer. The markings on the tablet or capsule, together with its shape and color(s), can provide a tentative identification. The *unique* tentative identification of markings obtained on a commercial tablet ~~and/or~~ capsule*, or commercially produced package* using a listed reference can be used as a preliminary test. Markings on counterfeit "look-alikes" and/or clandestinely manufactured tablets and capsules may not be used as a preliminary test; however, the markings may be beneficial in the identification of the tablets and capsules.

4.2 Instrumentation

A thorough visual examination in most cases will provide enough information in identifying the markings on the tablets or capsules. However, because of the condition of some of the tablets and capsules submitted, an external light source and/or a stereomicroscope may aid in identifying the markings on the evidence.

Population counts of round, uniform tablets can be determined using a Fretwell Triangle per the following procedure.

Triangle Operation:

- *Form tablets into even rows*
- *Count the number of rows*
- *Look up the total on the scale*

Clean the triangle using methanol before and after each use. Store the triangle in a designated enclosed location such as a drawer or cabinet.

4.3 Minimum Standards and Controls

The reference used, year, page number where it was found (if applicable), and its corresponding results will be recorded in the examination documentation. An electronic copy will be included in the examination documentation if it is from an online or computer software source. The electronic copy will be marked with the corresponding item number(s) for which the reference is being used. If identification is made using a phone source, the agency called, date and time will be recorded in the examination documentation.

4.4 Procedure

The chemist will do a thorough visual exam in order to identify the markings on the capsules and tablets. If only partial markings are present, the logo assumption may not be used as a test that supports the identification of the substance. Notations of these observations will be recorded ~~in the examination documentation~~ with the following characteristics, if

applicable:

- Color
- Shape -The description of the item which may include a drawing.
- Markings -The description of the letters, numbers, and/or logo which may include a drawing.
- *National Drug Code (NDC) – on packaging only. May be used for logo identification on commercially produced pharmaceutical packaging. When confirmatory testing is performed on the contents of the package (sublingual films, tablets and/or capsules with markings and/or imprints), the physical markings will be used for the logo id, and not the NDC alone. If tampering is suspected, NDC will not be used for the logo identification.*

4.5 Identification References

The following is a list of generally recognized and commonly used reference sources. It is not to be considered the complete list, other legitimate references are acceptable. References used may be in text or electronic form. The reference used must be documented in the examination documentation as specified above.

1. Drug Identification Bible, Amera-Chem, Inc.
2. Ohio State Board of Pharmacy (1-614-466-4143)
3. Poison Control Center (1-800-872-5111)
4. The Logo Index for Tablets and Capsules
5. The Physician's Desk Reference (PDR), Thomson Healthcare, Inc.
6. The Physician's Desk Reference for Generics, Thomson Healthcare, Inc.
7. www.drugs.com
~~8. www.pharmer.org~~
8. www.rxlist.com
~~9. https://pillbox.nlm.nih.gov/~~
9. *National Drug Code Directory (fda.gov)- (for packaging only)*
10. *DailyMed (National Institutes of Health)*

5. Mass Determination

5.1 Cleaning

The balance must be cleaned of any debris and leveled, as needed.

5.2 Routine Inspection

There will be a routine inspection (i.e. tolerance check) on each balance weekly, when in use. If it is moved or overloaded a tolerance check must be performed prior to case use. The tolerance check will be performed using weights that approximate the normal weighing range. Tolerance check results will be recorded and maintained.

Balance tolerance check procedure (Shimadzu example):

1. Make sure that the balance is level and free of debris.
2. Use calibrated NIST weights. Be sure to record the serial number for the NIST weights.
3. Tare the balance.
4. Place a weight on the balance and record the actual value. (If equipped, the balance link feature must be utilized.)
5. Take the weight off of the balance and tare again if needed.
6. Repeat steps 4 and 5 for each subsequent NIST weight.
7. If the difference between the nominal and actual value of any weight deviates by more than ± 0.02 grams do not use the balance until it is rectified and passes the tolerance check.

NOTE: The above listed steps are also applicable to the unit's other balances (high-capacity top-loading and analytical); however, a failed tolerance check will be defined by the manufacturers' ~~repeatability~~ *linearity* specifications.

5.3 Maintenance

All pertinent information will be recorded in the equipment maintenance log. The documentation will include the following information, if available: nature of the defect, how and when the defect was discovered, action taken in response to the defect, comments on the type of maintenance performed, date, and scientist's initials.

5.4 Calibration

The balance must be calibrated annually by a vendor accredited to the current accreditation program standards and whose scope of accreditation includes the affected balances. Calibration certificates will be retained when a balance is calibrated.

5.5 Mass Determination

The net mass of all substances will be determined and recorded prior to instrumental analysis. Samples involving trace or residual amounts of material do not require weight determination.

The balance reading will be recorded and reported using the following conventions:

The mass shall be recorded in the examination documentation as displayed on the balance used. Any hardcopy record of the observed weight must be scanned and included with the case record.

For vegetation cases, the weight will be determined without roots, soil and foreign material.

The weight may be determined using one of the following methods:

5.5.1 Tare Method (Single weighing event)

A weighing vessel is placed on the balance and tared. The analyst immediately transfers the substance to the tared weighing vessel without removing it from the balance and records the net weight of the material. The entire operation is considered as a single weighing event.

5.5.2 Static Method (Two weighing events)

A weighing vessel is placed on the balance and tared. It is then removed from the balance and the substance is transferred to the weighing vessel, which is placed on the balance and a reading obtained.

5.5.3 Calculated Method (Two or more weighing events)

Application of acceptable sampling and mass determination methodology may result in an estimated total weight. Total weight determined as a result of actual measurement of only a subset of the group and subsequent calculation to determine the estimated total will be considered a “calculated weight”.

Total weight determined by addition or subtraction of actual recorded weights is not “calculated weight” under this definition.

Calculated weights will be indicated on the BCI Laboratory Report by adding “(calculated weight)” after the unit of measure and before the analytical finding.

The Calculated Weight spreadsheet must be used to determine any calculated weights and their associated uncertainties.
The calculated weight method relies on the assumption that the individually weighed units are uniform, so the analyst must determine which of the two calculated weight methods may be most appropriate depending on the case circumstances.
For determining a calculated weight using individual packaging weights:

1. *Weigh samples and packaging together to determine gross weight.*
2. *Determine the sample (R) amount.*

3. Empty (R) number of packaging units into separate containers and weigh each individual unit of packaging. This information is necessary to determine the measurement uncertainty.
4. Obtain the average weight of each empty packaging unit.
5. Using the average weight of the empty packaging units and the gross weight of the item, the net weight of the material can be extrapolated.
6. The resulting calculated weight must be truncated to the appropriate significant figure.

For determining a calculated weight using individual sample weights:
1. Weigh samples and packaging together to determine gross weight.
2. Determine the sample (R) amount.
3. Remove (R) number of samples from their packaging and weigh each individual sample unit. This information is necessary to determine the measurement uncertainty.
4. Obtain the average weight of each sample unit.
5. Using the average weight of the sample units, the net weight of the material can be extrapolated.
6. The resulting calculated weight must be truncated to the appropriate significant figure.

~~1. Weigh samples and packaging together to determine gross weight.~~
~~2. Determine the sample (R) amount.~~
~~3. Empty (R) number of packaging in separate containers and weigh the empty packaging.~~
~~4. Collect weights of each individual packaging unit, as this information is necessary to determine measurement uncertainty.~~
~~5. Obtain the average weight of the empty packaging.~~
~~6. Using the average weight of the empty packaging and the gross weight of the items, the net weight of the material can be extrapolated.~~
~~7. Use of this method will result in a "calculated weight", as described above.~~
~~8. Weights (net/gross)- truncate to the appropriate significant figure~~

5.5.4 Subtraction method (Two weighing events)

1. Weigh sample(s) and packaging together to determine gross weight.
2. Remove sample(s) from packaging.
3. Weigh packaging only and subtract that amount from gross weight to obtain net weight of sample.

5.6 Measurement Uncertainty

Reported controlled substance weights and concentrations will include an estimation of the measurement uncertainty (MU):

- The reported estimated MU for weight will be that calculated for the balance or balance group on which the controlled substance weight was determined.
- The reported estimated MU for concentration will be that calculated for the pipettes or pipette groups, moisture analyzer, and standard for which samples were prepared.

- The reported estimated MU will include the coverage probability.
- The measurement result and the MU value will be reported to the same level of significance.
- The estimated MU value will not exceed two significant digits (rounded).
- Quantities reported as 'residue – trace amount', or 'less than' values do not require estimated MU inclusion.
- When multiple weighing events occur, the reported MU reflects an estimation of the uncertainty for all weighing events.
- When a calculated weight is reported, the reported MU reflects an estimation of the overall uncertainty based on the uniformity in weight of the *sample or* ~~empty~~ packaging units.

~~The LP- Measurement Uncertainty details the procedure for calculating and reporting estimated MU, including raw data and calculations. The laboratory Quality Assurance Manager, or designee, is responsible for initiating MU review /recalculation when contributing uncertainty elements change. Reporting examples are included in the procedure and in the Drug Reporting section of this manual.~~

6. Moisture Content Determination

The Ohio Revised Code excludes hemp from the definition of the controlled substance Marihuana on a dry weight basis. For this reason, the moisture content must be determined for a ~~submitted~~ cannabis vegetation sample, if sample amount permits.

6.1 Cleaning

The moisture analyzer must be free of any debris and leveled, as needed.

6.2 Routine Inspection

There will be a routine inspection (i.e. tolerance check) on each moisture analyzer weekly, when in use. If it is moved or overloaded, a tolerance check must be performed prior to case use. The tolerance check will be performed using weights that approximate the normal weighing range. Tolerance check results will be recorded and maintained. Additionally, a single measurement of a sample of known moisture content will be conducted each week of use to verify the instrument is working properly. Additionally, a monthly temperature and moisture check will be performed using a certified SmartCal sample.

Moisture analyzer tolerance check procedure:

1. Make sure that the moisture analyzer is level and free of debris.
2. Use calibrated NIST weights. Be sure to record the serial number for the NIST weights.
3. Tare the moisture analyzer.
4. Place a weight on the moisture analyzer and record the actual value. (If equipped, the balance link feature must be utilized.)
5. Take the weight off of the balance and tare again if needed.
6. Repeat steps 4 and 5 for each subsequent NIST weight.
7. If the difference between the nominal and actual value of any weight deviates by more than +/- 0.001 gram do not use the moisture analyzer until it is repaired and passes the tolerance check.
8. In conjunction with performing a tolerance check on the balance portion of the moisture analyzer, the heating element must also be checked as it has a greater impact on the accuracy of the moisture content reading. To check this, a certified temperature kit will be utilized monthly, when in use. If the difference between the nominal temperature (set point) and the actual value differ by more than 3 degrees do not use the moisture analyzer until it is repaired and pass the temperature tolerance check.
9. A sample of known moisture content (Sodium Sulfate Decahydrate) shall be analyzed weekly and the result recorded on the Moisture analyzer tolerance check worksheet. If the moisture analyzer is unable to measure the moisture content within the specifications of the manufacturer of the known sample, it will not be used until repaired and passes this tolerance check.

10. A sample of known moisture content shall be analyzed monthly (SmartCal) and the result recorded on the Moisture analyzer tolerance check worksheet. If the moisture analyzer is unable to measure the moisture content within the specifications of the manufacturer of the known sample, it will not be used until repaired and passes this tolerance check.

6.3 Maintenance

All pertinent information will be recorded in the equipment maintenance log, including the annual check and troubleshooting actions taken in response to a failed routine check. The documentation will include the following information, if available: nature of the defect, how and when the defect was discovered, action taken in response to the defect, comments on the type of maintenance performed, date, and scientist's initials.

6.4 Calibration

The moisture analyzer must be calibrated annually by a vendor accredited to ISO 17025 standards and whose scope of accreditation includes the affected moisture analyzer. Calibration certificates will be retained in accordance to established retention schedules.

7. Sampling and Analysis

7.1 Purpose

The purpose of this section is to clarify the difference between sampling and sample selection, when each is to be used, identify the recognized BCI Chemistry discipline sampling plan and provide general analysis guidelines.

7.2 Definitions

- Sampling – Taking a part of a substance, material or product for testing in order to reach a conclusion, make an inference about, and report on the whole. Sampling will only be used when there is reasonable assumption of homogeneity of the whole. If observations of evidence indicate non-homogenous sample, ensure that a representative sample is obtained for analysis.
- 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 (such as hypergeometric sampling) to determine the number of units 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 and therefore no inference about the whole population can be drawn.
- *Composite sampling – A method of sampling where an analyst may need to combine multiple individual units or portions into one "composite" sample or combined sample. This process is not commonly used and will require Supervisor approval prior to performing this sampling procedure.*

7.2.1 Hypergeometric Probability Distribution sampling plan

Unless otherwise identified, Hypergeometric Probability Distribution is the recognized sampling plan of the Ohio BCI Chemistry discipline. This sampling plan applies the probability theory of the hypergeometric distribution and provides a statistically valid approach to determining the number of units that must be tested in order to make an inference about the whole population.

Application

Hypergeometric sampling may be applied to a population under the following conditions:

- The units must appear to be homogeneous. If one or more of the units in the

population differ in appearance, they must be considered a separate population (a population may be considered 1 or more).

- Each unit comprising the sample must be fully tested to meet the requirements for the identification of that substance.
- When like evidence is submitted across multiple items, it is permissible for Hypergeometric sampling to be applied across multiple items. (i.e. 5 items, each consisting of 20 bags of crystalline substance can be treated as a single population of 100 instead of 5 individual populations of 20 for calculation of number of samples to be analyzed).
- Use of the sampling plan must be identified in the examination documentation and on the report for each group on which it was applied.
- A minimum confidence level of 95% will be used.
- A minimum proportion of positives of 90% will be used.

The ENFSI sampling calculator is used (available at www.Enfsi.eu) to determine sampling plans. When using the calculator, the Confidence Level used shall be 95% (0.95). For Population sizes of nine (9) or less, the value of (R) will be the same as (N).

7.2.2 Sample selection

Sample selection is used in lieu of Hypergeometric Probability Distribution Sampling when there is no intention to report a conclusion about the whole population of a multi-unit item. The selection of the unit to test is based on:

- The training and experience of the examiner
- Legal limits/charging guidelines
- Specific exceptions as may be provided in these methods

Sample selection analysis requirements

Minimum requirements for forensic drug identification shall be applied to at least one unit of the sample. The report must clearly reflect that conclusions do not apply to the whole population.

7.3 Sample and Analysis Procedures

7.3.1 Commercially produced tablets and capsules

Any commercially produced capsule or tablet that appears to have been tampered with and/or altered, shall be considered illicit and tested as such.

Tablets or capsules identifiable through a reference source as controlled substances: randomly select one and subject it to confirmatory testing.

When testing results identify a different substance than the markings indicate, the

population will be subjected to hypergeometric sampling, up to the legal threshold.

Tablets or capsules identifiable through a reference source as negative for controlled substances: no additional testing is required.

Commercially produced tablets or capsules that cannot be identified through a reference source (such as vitamins, supplements, aspirin, etc.): randomly select one and analyze it to meet the minimum requirements for no controlled substance determination.

7.3.2 Material of illicit origin

For illicit materials, quality control may be regarded as nonexistent. Though variation may occur, some of the active constituent should be expected in each unit of the group. Given these conditions, the sampling plan will be applied to visually homogenous groups of illicit materials unless the appropriate legal limit has been reached.

Careful attention should be taken while visually screening these substances in order to ensure that all are similar in appearance. The sampling plan will be aborted if results do not support the null hypothesis.

Clandestine tablets, powdery substances, paper (*excluding bound materials*), sugar cubes and gelatin forms

After uniformity and the population size (N) have been established:

- Randomly select the calculated sample size (R).
- The entire (R) amount shall be analyzed to meet the minimum requirements for forensic drug identification.
- A single piece of paper (with or without perforations, drawn lines, or definable units) and whole sheet gelatin forms will be considered a single population and one sample shall be analyzed.

Cocaine Base (Crack)

Evidence will occasionally come into the laboratory in an atypical wet or moist condition. Upon opening, the wet item will be weighed and this weight will be recorded in the examination documentation. The item is then to be dried out and this weight will also be recorded in the examination documentation. Both weights are to be reported.

~~There may be occasion where Federal prosecution could occur and therefore the analysts have the option of confirming base or salt form even if the case is not flagged as a Federal case. There will also be occasions when the agency may request the base or salt form be identified.~~ ~~The best option to confirm base or salt is through FTIR analysis.~~

There may be occasions when a department requests the salt/base form of Cocaine to be identified (e.g. federal prosecution). Salt/base form is only required to be reported when

requested; however, analysts may choose to report salt/base form even if no official request has been made. Salt/base determination must be performed using FTIR analysis.

Vegetation

It is recognized that conclusive determination of the exact number of individual plants in vegetation samples can be arbitrary and hypergeometric sampling requirements cannot be accurately established. Identifiable packaging is considered the basis for population size determination.

- Visually inspect contents of all packages for homogeneity. If the contents include multiple populations, such as both vegetation and hand-rolled cigarette/cigar remnants, the remnants will be separated. If the remnants are not tested, they will not be included in the weight measurement.
- Establish population size (N) of identifiable packaging units (bags, boxes, bricks, etc.)
- Calculate the sample size (R) and randomly select this number of packaging units
- Samples from the entire (R) number of packages will be analyzed to meet the minimum requirements for forensic drug identification
- Samples consisting of a bag of burned, hand-rolled cigarette/cigar remnants can be considered one population
- Additional samples may be tested at the analyst's discretion

Liquids

- A visual exam is conducted for color, viscosity, single or bi-level
- If suspected Clandestine laboratory, conduct pH and/or volume if sample size permits
- Weigh exhibit
- A representative sample is removed
- The sample is extracted using solvent (see extraction methods)
- The liquid will be analyzed to meet the minimum requirements for forensic drug identification
- If the liquid is determined to be bi-level, both layers will be analyzed to meet the minimum requirements for forensic drug identification

Residues

The sample can be removed from its container by using one of the following techniques; rinsing, swabbing or scraping. No more than half will be used for a complete analysis.

Synthetic cannabinoids or cathinones

The forensic community understands that the contents of synthetic cannabinoid or cathinones packets/containers may not be consistent. In these cases, confirmatory testing may be used as a determinant of a population. For instance, multiple, differently labeled packets have been tested and shown to contain the same controlled substance or potential controlled substance analog. If multiple packaging types are submitted, hypergeometric sampling may be used across the population of the positive packets in order to report a

weight for the substance in question. In this situation, it is acceptable to test one of each packet to determine if there is a common substance being detected.

Bound Materials (Soaked paper/soaked magazine paper)
There are instances where soaked papers may be submitted bound together as a magazine or book. With prior Supervisor approval, an analyst may proceed with composite sampling of all papers as one population. The analyst may take a portion (i.e. hole punch) from multiple pages of soaked papers and combine into one sample for instrumental analysis. The analyst must ensure that the report of analysis states that analysis was performed on a composite sample of all pages submitted.

7.4 References

1. Frank Richard S. et al., “Representative Sampling of Drug Seizures in Multiple Containers”, Journal of Forensic. Sciences., 1991, Vol. 36 No. 2, pp 350-357
2. Guidelines on Representative Drug Sampling, European Network of Forensic Science Institutes (ENFSI), 2004, www.enfsi.eu
3. Logan, Barry K. ET. al., "A Simple Laboratory Test for the Determination of the Chemical Form of Cocaine", Journal of Forensic Sciences, Vol. 24, No. 3, May 1898, pp 678-681.
4. Douglas M. Andrews, PhD, Professor of Statistics, Wittenberg University, Springfield, OH
5. Kiser, William O., “Analysis for Cocaine Base”, MICROGRAM, DEA Laboratory Notes, Vol. XXI, No. 2, February 1988, pp 28.

8. Cannabis Analysis

8.1 Introduction

Marihuana is defined in the state of Ohio under the Ohio Revised Code, 3719.01 (M) and 2925.01 (AA) and is controlled under OAC 4729:9-1-01, 2925.03 and 2925.11. Hashish is defined under 2925.01 (Z), for cases with offense dates prior to July 30, 2019. This is presented as a reference and should be referred to for weight limits, penalties, and other specific requirements of the law.

Cannabis can be visually characterized by observing its trichomes (cystolithic, covering and glandular) and their relative location ~~on the plant material~~. The cystolithic hairs and covering hairs must be observed on opposing surfaces of the same leaf. In cases after July 30, 2019, quantitative analysis must be performed to determine the percentage of THC in a sample on a dry weight basis, if sample amount permits.

The Duquenois-Levine test (modified Duquenois test) can be used for the identification of Cannabinoids found in the cannabis plant. Other tests may be substituted for the Duquenois, such as GC/MS analysis.

8.2 Marihuana/Hemp Definition

All parts of a plant of the genus cannabis, whether growing or not; the seeds of the plant of that type; the resin extracted from a part of a plant of that type; and every compound, manufacture, salt, derivative, mixture, or preparation of a plant of that type or of its seeds or resin. “Marihuana” does not include the mature stalks of the plant, fiber produced from the stalks, oils or cake made from the seeds of the plant, any other compound, manufacture, salt, derivative, mixture, or preparation of the mature stalks, “except the resin extracted there from”, fiber, oil, or cake, or the sterilized seed of the plant that is incapable of germination. "Marihuana" does not include "hemp" or a "hemp product" as those terms are defined in section 928.01 of the Revised Code.

The excluded parts (stalks, fiber, etc.) are excluded only when the material consists entirely of mature stalks or entirely of sterile seeds. Any mixture of excepted parts with other parts of marihuana such as leaves, flowers, stems, etc., is considered to be all illicit marihuana.

Several Ohio court decisions support this interpretation, including the Ohio Supreme Court decision in State v. Wolpe [(1984), 11 Ohio St.3rd 50.]; which ruled that excluded materials need not be separated from non-excluded materials in determining the weight of marihuana in a criminal prosecution.

Hemp is defined as the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than

three-tenths percent (0.3%) on a dry weight basis.

BCI will not differentiate between marihuana and hemp in reporting, but rather report "Cannabis" with its calculated delta-9-tetrahydrocannabinol concentration.

8.3 Safety Considerations

A fungus, Aspergillus fumigates, may be encountered on decaying *vegetation* ~~plant material~~. Spores are released when the plant is removed from its package. Breathing these spores may result in aspergillosis, which affects the pulmonary system in different ways. Wearing a dust mask and/or working with proper ventilation are advisable.

8.4 Procedure

It may not be necessary to test every item in a multiple item exhibit in order to make a determination as to the weight and presence of *cannabis* ~~Marihuana~~. Sampling and requirements for multiple item exhibits are addressed in the Sampling and Analysis section of this manual.

Refer to Mass Determination section for weighing procedures.

8.4.1 Macroscopic Examination

Visually examine the entire sample for homogeneity and note the description. When possible, separate all foreign material from the sample. Also, look for adulterants on the *vegetation* ~~plant material~~. If the plant material appears to be altered refer to the drug analysis method for the analysis of the adulterant. Common adulterants on ~~Marihuana~~ *cannabis* are Cocaine base (Crack) and Phencyclidine (PCP).

As a matter of policy, BCI does not separate stalks and seeds from ~~marihuana~~ *cannabis* mixtures for purposes of determining the weight of the ~~marihuana~~ *cannabis* sample.

8.5 Analysis Methods

8.5.1 Vegetation

A representative sample is observed under the microscope with a magnification strong enough to determine the necessary characteristics, if possible. A representative sample is removed and a Duquenois-Levine test is performed. A representative sample is then taken and subjected to quantitative analysis, as described later in this manual. Additional tests may be done at the discretion of the analyst.

Cystolithic hairs on the upper surface combined with covering hairs on the lower surface, using a stereomicroscope, are minimum criteria for a positive microscopic test. Identification of trichomes will be noted in the examination documentation.

8.6 Other Cannabis Related Products

~~Marihuana~~ *Cannabis* ~~plant material~~ can be processed into other forms including compressed resin, extracts/oils, and various edible food products. *Cannabis* ~~Plant material~~ THC/CBD levels can vary in strain, freshness and moisture content, all of which can affect the end product.

Visual examination is performed and recorded to document the physical state of the substance. A representative sample may be observed under the microscope or macroscopically. A substance that is essentially void of *vegetation* ~~plant material~~ with cystolithic hairs on the upper surface combined with covering hairs on the lower surface does not meet the criteria for ~~plant material~~ *cannabis* ~~Marihuana~~ and requires further instrumental testing.

Any prefilled cartridges contents are transferred into a vial for testing. A representative sample is prepared in an appropriate solvent for instrumental analysis. Follow quantitation procedures to determine the percent total THC.

Derivatization processes are needed to distinguish any of the acids from their corresponding neutral compound by GC/MS. ~~When reporting "Hashish" (or "Marihuana"), on cases prior to July 30, 2019, the acid/neutral state of any particular cannabinoid is not critical and derivatization is not required.~~

8.6.1 Resin

Hashish is a resinous preparation of cannabis. There are several manufacturing processes for hashish, it may be found in several different forms ranging from a free-flowing liquid to a hard/solid form.

~~No Hashish determination will be disclosed on the laboratory report for offense dates between July 30, 2019 and December 16, 2020.~~

House Bill 341, with an effective date of December 16, 2020 amended the definition of Hashish to mean a resin or a preparation of a resin to which both of the following apply:

1. It is contained in or derived from any part of the plant of the genus cannabis, whether in solid form or in a liquid concentrate, liquid extract, or liquid distillate form.
2. It has a delta-9 tetrahydrocannabinol concentration of more than three-tenths per cent.

~~As such, hashish determination will be disclosed on the laboratory report for offense dates on or after December 16, 2020.~~

~~The presence of a Tetrahydrocannabinol and one other cannabinoid or their acid derivative will constitute Hashish. For federal cases, the federal guidelines of the presence of Tetrahydrocannabinol and two other cannabinoids (Cannabinol, Cannabidiol, or Cannabichromene) will constitute Hashish.~~

BCI will not report an item as hashish, as that is a legal determination, but rather report the item description (along with solid/liquid determination) and its total delta-9-tetrahydrocannabinol concentration.

There may be occasions when a department requests the confirmation of Tetrahydrocannabinol and two other cannabinoids (Cannabinol, Cannabidiol, or Cannabichromene) necessary for federal hashish determination. Federal hashish guidelines are only required to be met when requested; however, analysts may choose to meet those guidelines even if no official request has been made.

~~**8.6.2 Marihuana Extract Definition (prior to July 30, 2019)**~~

~~Effective January 13, 2017, The Drug Enforcement Administration (DEA) created an Administration Controlled Substances Code Number for "Marihuana Extract", 21 CFR 1308.11(d)(58).~~

- ~~Marihuana extract: an extract containing one or more cannabinoids that has been derived from any plant of the genus Cannabis, other than the separated resin (whether crude or purified) obtained from the plant.~~

8.6.2 Edibles (i.e. products for oral consumption)

Products for oral consumption are currently not analyzed for quantitative purposes.

If analyst is unsure if the substance submitted is a food and/or health/beauty product, the sample can be run on the GC/MS and analyzed for common ingredients in those types of items. Examples of these substances are: cholesterol, sugars, sesame oil, olive oil, etc.

8.6.3 Residues

Residue, such as those left behind on smoking devices, may not demonstrate any physical characteristics of *cannabis* ~~plant material~~ products. Due to the limited amount of sample present, residues are unable to be analyzed for THC quantitation.

If the residue sample is not conducive to quantitation, and THC is identified, then report wording must state the presence of THC was indicated, but insufficient sample remains for quantitative analysis.

8.7 References

1. Thornton, J. And Nakamura, G., Journal of the Forensic Science Society, Vol. 12, No. 3, 1972, pp.461-519.
2. Ohio BCI Drug Chemistry Training Manual.
3. The Ohio Criminal Law Handbook.1996. Anderson Publishing Corporation, Cincinnati, OH.
4. Swisher, Thomas and Young, James, "Drug Abuse Control", Ohio State Bar Foundation, 1976.
5. Official Methods of the AOAC, 13th ed., AOAC, Washington, D.C., 1980, p686.

9. THC Quantitation by Liquid Chromatography/ Ultraviolet Detection (LC/UV)

9.1 Introduction

As of July 30, 2019, the Ohio Revised Code requires proof that a suspected Marihuana sample or a suspected Marihuana product contains more than 0.3% THC. The calculation used to quantify THC is specified in the ORC 928.01 (J) "Delta-9 tetrahydrocannabinol" means the sum of the percentage by weight of tetrahydrocannabinolic acid multiplied by 0.877 plus the percentage by weight of delta-9 tetrahydrocannabinol. Tetrahydrocannabinol (THC) as reported by BCI is equivalent to this definition. Total THC calculations will be conducted in the approved version of the controlled documents *found within* LF-Chem-QNT-Batch Worksheet.

Quantitative analysis using LC/UV is accomplished by preparing a calibration curve with a dynamic range that mimics the samples being analyzed. A series of calibrators are prepared at pre-defined concentrations and a known amount of an internal standard is added. The ratio of the response of the analyte being measured and the internal standard added is plotted on a curve. When an unknown sample is analyzed, a known amount of sample is extracted and has a pre-defined amount of certified reference material (CRM) internal standard added to it. The ratio of the response of the analyte to the internal standard is plotted on the same curve that was generated and a quantitative value is determined. Given the wide range of potential concentrations of unknown samples, dilution factors must be used to ensure that unknown sample concentrations will fall within the dynamic range of the calibration curve.

9.2 Safety Considerations

~~The sample preparation phase may involve the use of liquid nitrogen to freeze and homogenize samples. Cryogenic gloves must be utilized when handling items that have been in contact with liquid nitrogen.~~

Standard laboratory practices involving the use of solvents, acids, and bases will be used when preparing mobile phases for the LC/MS; these mobile phases and diluting solvents should be prepared in a fume hood.

9.3 Standards and Controls

Quantitative results for THC and THCA in each calibrator shall be within +/- 15% of their target value.

The calibration curves for THC and THCA shall have correlation coefficients ≥0.995
If the calibration curve fails to meet the ≥0.995 threshold, one point may be excluded to attempt to improve the linear fit of the curve.

9.3.1 Internal Standard preparation

Prepare an internal standard solution with a final concentration of approximately 640 ug/ml Androstenedione by weighing standard into vessel and diluting to volume with appropriate solvent using Class A volumetric glassware.

The same lot number of Internal Standard solution must be used in all calibration samples, check standards, and case samples.

9.3.2 Calibration Standard preparation

A five or six-point calibration curve shall be used. The six concentrations will be prepared using certified reference material. The concentrations of the calibrators are 8,16,32,64,96 and 128 µg/mL and will be prepared per reagent log sheet. References to the lowest limit of quantitation (LLOQ) in casework will refer to the 8 ug/mL calibrator and references to upper limit of quantitation (ULOQ) will refer to 128 ug/mL calibrator. These calibrators represent the values above and below which, respectively, quantitative results may be obtained.

Calibrators, at a minimum, will be run at the beginning and end of each batch, unless the batch consists of 15 samples or fewer. In these situations, it is acceptable for the batch to consist of a beginning set of calibrators and end with a check standard.

9.3.3 Positive and negative controls preparation

A positive control will be extracted alongside case samples each day that quantitative analysis is conducted and will consist of a preparation of a THC Tincture at approximately 25µg/mL. The amount of tincture needed will be calculated as follows:

$$\text{ug tincture} = (1250 \text{ ug THC}) \left((100 \text{ ug tincture})/(\text{X ug THC})\right)$$

Where X is the reported THC % of the tincture used.

The determined amount of tincture will be dissolved in 5 mL of appropriate solvent and carried through the extraction procedure using an appropriate dilution scheme.

A negative control will be extracted alongside case samples each day that quantitative analysis is conducted and will consist of hemp seed oil that is run through the sample preparation process. The negative control does not need to be matrix matched to the sample type being analyzed. The negative control will consist of approximately 0.0500g of matrix diluted 1:1 (i.e. 1900uL sample: 100uL internal standard).

Note: The positive control, negative control and method blank do not need to be reran when further dilutions are required.

The positive and negative controls shall be run following the calibrators and then the check standard shall bracket each fifteen case samples. (samples do not include blanks or QC samples). Acceptance criteria are as follows:

- Relative retention time of the Positive Controls and check standards must be

within +/- 2.5% of the average Relative Retention Time (RRT) of the calibrators
- The quantitative value of the Positive Control ~~and check standards~~ will be within +/- 20% of the calculated value.
- The negative control will be considered acceptable if it contains less than 10% of the LLOQ for both THC and THCA.

All calibrators, controls, and samples will be prepared using pipets which have been calibrated per the laboratories standard calibration protocol and which have had a monthly density check performed per the below listed procedure, when in use.

1. Place water in a beaker and allow to come to room temperature – record temperature of water
2. Set pipette for desired amount to expel (should test lowest volume used in casework and highest possible volume of pipette capability)
3. Pre-wet tip
4. Pull up liquid and expel into a tared weighing vessel. Record weight
5. Using the same tip, repeat 5 times capturing the weight each time
6. Calculate the actual volume dispensed (average weight of volume * density at measured temp)
7. Calculate accuracy (100*(answer from 6/ set volume). This should be 98-102% for all volumes greater than or equal to 100uL and 90-110% for volumes less than 100uL.
8. All results will be recorded on the appropriate pipette density check form.

9.4 LC/UV Interpretation Criteria

The peaks present should have good resolution (i.e. Not less than (NLT) 2) / symmetry, narrow peak width, and have minimal tailing or splitting.

The RRT of the sample must be within +/- 2.5% of the average Relative Retention Time (RRT).

The quantitative value must fall below the ULOQ and if the sample exceeds this value, the sample must be diluted and reran.

If duplicate preparations are made, the total THC concentration must be within +/-10% of each other and the lesser of the two runs will be reported. If a set of duplicate preparations does not fall within 10% of each other, the duplicate preparation will be repeated.
Percent agreement shall be calculated as follows:

$$\%\ \text{agreement} = (\ (\text{Highest value} - \text{Lowest Value})/\text{average of both}) \times 100$$

Area counts of the internal standard are evaluated to ensure consistency throughout the batch.

~~9.5 **Cannabinoid Mix**~~

~~A quality control mix for the LC/UV/MS is designed to be run on a quantitative method. This QC mix includes the following compounds, at a minimum: Cannabidiol (CBD), Delta-8 THC, Delta-9 THC, Cannabinol (CBN), Cannabichromene (CBC), Cannabigerol (CBG), Cannabidiolic Acid (CBDA), Cannabigerolic Acid (CBGA), and Tetrahydrocannabinolic Acid (THCA).~~

~~The results are acceptable if the following criteria are met:~~

- ~~Peak present for each reference material in quality control mixture; no unexplained shifts in Relative Retention Time (RRT) noted in comparison to previously run cannabinoid mixes.~~
- ~~Peaks exhibit good peak structure: they are symmetrical and lack significant fronting, tailing, or splitting.~~
- ~~Peaks exhibit baseline separation and Delta-8 THC and Delta-9 THC have a resolution of > 2.~~

10. Clandestine Laboratory Manufacturing Investigations

10.1 Introduction

This section is designed to guide the analyst in the analysis approach regarding evidence submitted relative to these investigations.

10.2 Safety Considerations

Special care should be taken when handling all items in these submissions. Many will contain strong acids and bases that can injure flesh and eyes. Other common safety concerns include; noxious fumes, spills and potential for explosion. Perform all analyses with adequate ventilation.

As per BCI policy, due to their hazardous nature anhydrous ammonia and lithium are not to be submitted or analyzed by any laboratory personnel.

10.3 Liquid Samples

1. Check the pH of the liquid to determine acidity or alkalinity and record volume.

When the sample is believed to be a strongly acidic solution, no further testing is required.

2. If the liquid is determined to be slightly acidic- basic pH, check for the presence of finished product-controlled substances and drug precursors (such as pseudoephedrine).

The customer may be referred to the State Fire Marshal's Office for any solvent identification. These submissions will be the responsibility of the customer.

10.4 Solid Samples

Solid samples will be examined visually and analyzed for the presence of finished product-controlled substances and drug precursors (such as pseudoephedrine) using standard chemical procedures.

11. Extractions and Separations

11.1 Introduction

Both extractions and separations are necessary for many of the methods and procedures found in the methods manual. Because of their variety and specificity, as well as the general approach to drug analysis, the general and more commonly used separations will be placed in this manual. The extractions listed are by no means exhaustive but can assist the examiner in some cases. The listed extraction, any deviation from the listed extraction, or information sufficient to reproduce any unlisted extraction used, must be recorded in the examination documentation. *Analyst should avoid injecting acidic or basic solutions onto the column. It can significantly alter the chemistry of the column, which may impact the retention time and detectability of some analytes*.

11.2 Safety Considerations

Standard laboratory practices involving the use of solvents, acids and bases.

11.3 Procedure

11.3.1 Solvent extractions

Solvent extractions (dry extractions) are based on the differences in solubility between substances. Dry extractions involve washing a powder with a solvent in which the desired component is soluble and the other mixture components are not soluble.

- The solvent can be separated from the insoluble material either by filtering or using a centrifuge.
- The solvent is then dried down to yield the desired component (if necessary).

11.3.2 Acid/Base extractions (liquid/liquid)

The liquid/liquid extractions use two immiscible solvents. Water or aqueous acid/base, and immiscible organic solvents are normally used. The desired component is partitioned into one solvent while the other components are partitioned into another solvent. Separating the two phases will yield the desired substance. *The acid/base remains in the aqueous layer, and the immiscible organic layer is separated and injected into the instrument*.

11.3.3 Acidic or Basic extraction

1. Add approximately 2 or 3 mL of basic or acidic solution to a small amount of powder and mix.
2. Add a small amount of organic solvent and mix.
3. Let stand or centrifuge, then remove and keep the organic layer.
4. Evaporate the organic layer for testing, if desired.

Note: Chloroform is best for general screens (with the exception of instruments using hydrogen as a carrier gas, in these instances ethyl acetate is a suitable alternative). Fewer drugs extract into hexane, however it usually yields a cleaner sample.

If the substance being extracted is volatile, e.g. methamphetamine, concentrated HCl gas should be bubbled through the solvent layer before evaporation to yield the insoluble hydrochloride salt.

11.3.4 Pseudoephedrine/Ephedrine extraction

1. Add 1N NaOH to tablet material
2. Add appropriate solvent (e.g. hexane or chloroform)
3. Vortex and Centrifuge
4. Decant
5. Bubble HCl fumes through liquid (precipitate will form)
6. Dry down
7. Run IR

11.3.5 MDMA/BZP tablet extraction

1. Add Saturated Sodium Carbonate
2. Add an organic solvent
3. Remove the organic layer
4. Run on GCMS

11.3.6 Mushrooms

1. Place 0.5-1 g of mushrooms in a beaker cover with 5mL of MeOH, tap down and soak (2-5 min).
2. Pour off half the methanol into a separate beaker, filter and air evaporate.
3. Perform TLC with samples and standards of Psilocybin and Psilocyn.
4. Spray with p-DMB
   - Psilocybin turns pink
   - Psilocyn turns blue
5. Add 10% acetic acid (4mL) to beaker with mushroom pieces. Tap down, soak (2-5 min).
6. Pour in test tube, extract with Chloroform (x2, 4mL) – keep acid layer (top).
7. Make basic with ammonia (4-5 drops), check with pH paper.
8. Extract with Chloroform (2mL), filter – keep chloroform layer (bottom).
9. Air Evaporate, then run sample on GC/MS.

11.3.7 Mushroom extraction (for GC/MS only- dephosphorylates psilocybin)

1. Grind mushrooms with water and solid sodium bicarbonate into a paste with a mortar and pestle.
2. Extract 3x with diethyl ether.
3. Dry down and bring up in chloroform.

11.3.8 Khat extraction

1. Place 5-6 g of shoots, leaves, or stalks in a blender and add 30 mL of 0.1N HCl.

2. Liquefy the sample.
3. Using a large syringe packed with glass wool, separate the liquid from the vegetation.
4. Place the liquid in a separatory funnel and wash 5x with 50 mL of CHCl3. The CHCl3 layer and the "fatty" layers can be discarded into waste.
5. Make the acidic solution in the funnel basic by adding conc. Na2CO3. (pH around 10).
6. Rinse with 10 mL of CHCl3.
7. Collect the CHCl3 layer and dry it down until about 1 mL for GC/MS and/or GC-FID analysis.

- If emulsions are generated anytime in the process, centrifuge. Keep all waste, etc. until the end of the analysis in the event restart is required. Dried Khat leaves may be blended before adding the acid.
- Cathinone breaks down at room temperature or in the basic stage of the extraction process. It is stable in the acidic stage. After collection of the CHCl3 layer (step 6), re-acidify the solution by adding concentrated HCl to pH 1 (approximate).

11.3.9 Salvia

1. Take a sample of the vegetation
2. Chop, if necessary
3. Extract in CHCl3
4. Run on GC/MS and/or GC/FID

11.3.10 THC Quant extraction

~~1. Transfer a portion of the sample to cryogenic freezer/grinder tube/equivalent.~~
~~2.~~ Homogenize sample utilizing a ~~appropriate method on SPEX Sample Prep 6875D freezer/mill Dual Chamber Cryogenic Grinder or~~ coffee grinder, or via vortexing (See Appendix II). (Note – *not applicable to* samples that are considered sufficiently homogenous ~~and those that will cause irreparable damage to the grinding tubes (i.e. liquids and hash) will not be processed through the freezer/mill.~~
3. Refer to the table below and transfer the appropriate amount of homogenized sample to a disposable centrifuge tube (record weight taken in examination documentation) followed by the addition of 5000µL of appropriate solvent.
4. Cap and sonicate the sample for 15 minutes and centrifuge if necessary.
5. Filter the sample through a 0.45µm membrane filter, if necessary.
6. Using the table below as guidance, carry out the appropriate dilution based on sample type and run on the appropriate quantitative method (see Appendix II).

NOTE: Pipettes and balances used for sample preparation must be approved and considered in the measurement uncertainty calculations

Sample type	Amount of homogenized sample (g)	Volume of solvent (uL)	Sample volume for vial (uL)	IS Spike (640µg/mL) (uL)	Solvent volume (uL)	Final Volume (uL)	Final dilution
Vegetation	0.2	5000	20	100	1880	2000	100
Hashish type solid or oils	0.05	5000	20	100	1880	2000	100
General	0.2	5000	20	100	1880	2000	100

Note – other dilutions schemes may be used at the analyst's discretion.

11.3.11 Steroid extraction (for oils)

1. Mix 20 mL of acetonitrile with 2 mL of hexane in a bottle and shake (Reagent A).
2. Mix 20 mL of hexane with 2 mL of acetonitrile in a separate bottle and shake (Reagent B).
3. In a clean test tube mix about 2 mL of the bottom layer from Reagent A with about 2 mL of the top layer from Reagent B and vortex. When the layers separate, remove the bottom acetonitrile layer as blank.
4. In another test tube mix about 2 mL of the bottom layer of Reagent A with about 2 mL of the top layer from Reagent B and vortex. Then add 1-5 drops of the oil (depending on the concentration of your sample) to the test tube. The oil remains in the hexane layer and the steroid to the lower acetonitrile layer.
5. Collect the acetonitrile layer and run on the GC/MS or dry down to run an IR.

11.3.12 Modafinil extraction

1. Take a sample of the tablet and place in a separatory funnel.
2. Add 50 mL of dH20 and 50 mL of methylene chloride.
3. Shake the funnel for approximately 1 min. while venting.
4. Take a portion of the lower layer and filter it.
5. Evaporate the liquid in a vial.
6. Add ~0.5 mL of BSTFA to the dried vial.
7. Cap the vial and incubate at approximately 70 degrees for about 30 min.
8. Run on the GC/MS and/or GC/FID.

11.3.13 LSD sugar cube/gel pane - Tartaric Acid extraction

1. Make up a 1% Tartaric Acid Solution (1g tartaric acid to 100 mL water).
2. Dissolve sample in Tartaric Acid solution (~1 hr).
3. Wash with CHCl3 and discard Chloroform.
4. Make basic with NaHCO3 Use pH paper to verify alkalinity.
5. Extract with CHCl3 - evaporate down.
6. May be used for GCMS and TLC.

11.3.14 Chocolate-covered mushroom extraction

1. Begin with sufficient amount of starting material (suggested 1-2 g)
2. Grind in mortar, add covering layer of 10% acetic acid and grind further.
3. Add 5-7 mL of water (i.e. lab grade, at a minimum) and grind an additional 2 minutes to create a thin slurry.
4. Place slurry in centrifuge tube(s) and add equal volume of chloroform, then centrifuge for 3 min.
5. Collect aqueous layer and place 2-3 drops in a spot plate for testing with Ehrlich's reagent. A deep purple color indicates indolic compounds.
6. Slowly add sodium bicarbonate to the remaining aqueous solution until effervescence stops.
7. Check the pH to ensure it is between 8-9, more sodium bicarbonate may be required.
8. Place the aqueous solution into centrifuge tube(s) and add equal volumes of chloroform.
9. Centrifuge for about 5 min,
10. Retain the chloroform layer for further testing.

11.3.15 Pseudoephedrine/Methamphetamine extraction

Part 1 - Methamphetamine

1. Add 1.0N sodium hydroxide and vortex.
2. Add hexane and vortex. Centrifuge.
3. Pipette out the hexane layer (upper) into another test tube and save the lower layer.
4. Add water to hexane and vortex. Centrifuge.
5. Pipette out the hexane layer into another tube.
6. To hexane from step 5 add water and vortex. Repeat this step one more time. Save hexane.
7. Pass HCl vapors through the hexane and methamphetamine will precipitate out or can run hexane on GC/MS.

Part 2 – Pseudoephedrine

1. To lower layer from step 3 add chloroform and vortex. Centrifuge.
2. Pipette chloroform (lower layer) into a test tube.
3. Dry the chloroform down.
4. To the powder add hexane and shake the tube to dissolve powder.
5. Pass HCl vapors through the hexane solution and pseudoephedrine will precipitate out.

12.Sample Derivatization

12.1 Introduction

Some samples do not lend themselves to gas chromatographic analysis, such as enantiomers (d,l-methamphetamine), thermally labile compounds (psilocybin), and samples whose solubilities prevent their introduction into a GC (sugars). Chemical derivatization of these types of compounds will overcome these problems and aid in their separation and identification.

12.2 Safety Considerations

Good laboratory practices are essential when dealing with the hazardous materials associated with these procedures.

MSDS or other references should be consulted unless the analyst is familiar with the hazards associated with a certain chemical.

12.3 Instrumentation

Gas chromatograph (GC)
Gas chromatograph/Mass Spectrometer (GC/MS)

12.4 Preparations

- TPC Reagent - [N-Trifluoroacetyl-L-Prolyl Chloride] available from REGIS® Technologies, Inc.
- BSTFA Reagent - [N,O-bis(Trimethylsilyl)trifluoroacetamide], available from PIERCE Chemical Co.
- Tri-Sil® Z Reagent - [Trimethylsilylimidazole in pyridine] from PIERCE.
- STOX™ Reagent - (a pyridine solution containing hydroxylamine HCl and phenyl-3-D-glucopyranoside. For forming oximes of sugars prior to derivatization).
- Methanol
- Pyridine

12.5 Procedures

12.5.1 Methamphetamine or Amphetamine enantiomers

1. For an extracted powdered sample, add several mg to an autosampler vial. (Work with purified extracts since cutting agents and contaminants may also be derivatized.)
2. Add 1/2 mL of the derivatizing agent to the tube.
3. Place the tube in a heating block (setting of 5 or 6 on high or about 65 ^{o}C) for 10 to 15 min.

4. Remove from heat and if necessary, dilute with dichloromethane, and analyze by GC or GC/MS. Do not use methanol or ethanol because they can also be derivatized.

12.5.2 Psilocybin/Psilocyn or Naproxen

1. Extract dried mushroom material with methanol, filter, and evaporate 5-10 drops in an autosampler vial under vacuum or a nitrogen stream.
2. Add 5-10 drops of BSTFA, cap, and place in a heating block at 140°C for 15 min and analyze by GC/MS. Use hexane as the wash solvent for the syringe.

12.5.3 Sugars

1. Mix 5-10 mg of sample (may be dry or wet) and ~0.5 mL of Tri-Sil Z in an auto vial. Swirl to dissolve (additional pyridine may be added to aid dissolution).
2. Heat at 70°C for 10-15 min. Analyze by GC or GC/MS.

An alternative method is to treat the sample with STOX® Reagent to form oximes of the sugars. The oximes are then derivatized with Tri-Sil Z.

12.6 References

1. Mckibben, T., Separation and Identification of Drug Enantiomers Via N-TFA-(S)- Prolyl Chloride Derivatization. J. CLICA, Vol.1, No. 2, January 1992.
2. REGIS® Technologies, Inc., Chromatography Catalog 1998-1999
3. PIERCE Chromatography Catalog & Handbook 1992-1993 and 1994-1995
4. Repke, David B., et.al., Journal of Pharmaceutical Sciences, Vol. 66, No. 5, May 1977, pp743-744.
5. Fuelster, R.G., “Quantitation of Sugars in Street Drug Samples”, Journal of Forensic Sciences, JFSCA, Vol. 37, No. 1, Jan. 1992, pp. 77-81.
6. Nowicki, J. and Pauling, S., “Identification of Sugars in Explosive Residues by Gas Chromatography-Mass Spectrometry,” Journal of Forensic Sciences, JFSCA, Vol. 33, No. 5, Sept. 1988, pp. 1254-1261.
7. Poole, P., DEA, Private communications.

13. Analytical Tests

13.1 Introduction

Analytical tests for the analysis of drug samples may be classified into three categories based on their discriminating power.

13.2 Presumptive tests

Presumptive tests provide the lowest discriminating power. These tests establish either a sample probably does belong to a generic group of controlled substances or is definitely not part of a generic group of controlled substances. Color (spot) tests described in the following methods are presumptive tests and do not count as one of the required tests for reporting a controlled substance, with the exception mushroom testing, *cannabis*, cocaine, and methamphetamine.

13.3 Preliminary tests

Preliminary tests provide greater discrimination than presumptive tests. Preliminary tests offer a strong indication of the identity of the unknown substance, but are not considered definitive. Preliminary tests available to the analyst range from a simple visual inspection to instrumental methods. Some common choices are:

- Physical identification from a reference source
- Thin-layer Chromatography
- Gas Chromatography/FID

13.4 Confirmatory tests

Confirmatory tests provide the highest level of discrimination. A confirmatory test will be conducted providing structural information for the confirmation/identification of the compound.

- Gas Chromatography/Mass Spectrometry
- Liquid Chromatography/Mass Spectrometry
- Infrared Spectrophotometry
- Other forms of confirmatory testing as they arise (i.e., GC/IRD)

14. Color and Functional Group Tests

14.1 Introduction

Many substances give distinct colors when brought into contact with various chemical reagents. Color tests, also known as spot tests, are non-specific screening tests that react to a particular functional group. These tests are not a positive identification. These tests are only presumptive in nature, and constitute an effective screening test because they indicate the type of compound that may be present.

Although most of these tests have been empirically derived, their accuracy being dependent of many years of observation, both color development and lack of color, can furnish the chemist with valuable information as to what may be contained in the substance. There will always be a certain amount of subjectivity that must be taken into account when a color is reported. The color reaction may be described differently by chemists.

The concentration of the sample, adulterants within the sample, and the time the sample remains in the reagent, may affect the color change. Allowances should be made for these differences, especially with street samples, where the concentrations of the drug or the adulterants of the substance, is unknown.

14.2 Safety Considerations

Precautions should be taken when handling these color test reagents. Many of these reagents contain concentrated acids that can injure flesh and eyes, so proper lab apparel must be worn. These reagents, under certain conditions, can splatter, effervesce, or emit noxious or harmful vapors.

14.3 Formulations

Common reagent formulations are given below. The formulations include the name of the test, how to prepare the reagent, and the type of drug that reacts with each test. The amounts of reagent used in testing the samples are suggested guidelines and can be varied from sample to sample. ACS grade chemicals will be used to make up reagents when possible. All reagents will be quality control tested with the appropriate standard following preparation. Preparation information will be recorded in the reagent preparation log., and will include:

- Substance used, weight or volume used, manufacturer, lot number, expiration date, and storage conditions
- Reference standard used for quality control check, lot number of standard (or unique identifier), the observed result, and the initials of the individual performing the check.

14.3.1 Cobalt Thiocyanate $Co(SCN)_2$

Formulation:

2g cobalt thiocyanate in 100 mL water

Tests for:

- Cocaine HCl Blue
- Cocaine Base Blue, only after the addition of HCl
- PCP Blue
- Lidocaine HCl Blue
- Procaine Blue
- Amitriptyline/Doxepin Blue

Stability: Very stable
Problems: There is a large group of false positives.

14.3.2 Dille-Koppani

Formulation:
Part A: 0.1g cobalt acetate in 100 mL of methanol, acidified with 0.2 mL of glacial acetic acid.
Part B: 5- mL isopropylamine in 95- mL methanol.
Use two drops of A and one drop of B.

Tests for:

- Barbituric acid derivatives Purple
- Ampicillin Brown

Stability: Very stable when stored as two solutions
Problems: Few false positives

14.3.3 Duquenois-Levine

Formulation:
1. One gram of vanillin is added to 50 mL of ethanol.
2. To this solution, 0.6 mL or 12 drops of acetaldehyde is added.

Tests for:

- Cannabinoids Purple chloroform wash

Stability: Refrigerate stock
Problems: Few false positives

The Rapid Modified Duquenois-Levine test is conducted in two steps:

1. The Duquenois reagent is added to the sample followed by a few drops of concentrated Hydrochloric Acid. Alternatively, after the Duquenois-Levine reagent has been allowed to mix with sample, the liquid may be poured off into a separate container before adding the

acid. A blue-green to purple color is indicative of the presence of Cannabinoids.
2. Add chloroform. Note the color. The transfer of a purple to violet color is indicative of the presence of Cannabinoids.

14.3.4 Ferric Chloride

Formulation:
5% ferric chloride in water. (w/v, 5 g in 100 mL water)

Tests for:

• Salicylates	Violet
• Acetaminophen	Blue
• GHB	Red/brown

Stability: Stable
Problems: None

14.3.5 Froehde's

Formulation:
50 mg molybdic acid or sodium molybdate
10 mL hot concentrated sulfuric acid.
The solution should be colorless.

Tests for:

• Heroin	Purple to green
• Codeine	Green to red/brown
• Morphine	Deep purple to slate
• Aspirin	Blue to purple
• Pentazocine	Blue
• Acetaminophen	Pale blue

Stability: Stable
Problems: None

14.3.6 Mandelin's

Formulation:
0.5 g Ammonium Vanadate to 50 mL Concentrated Sulfuric Acid

Tests for:

• Narcotics	Violet or Green
• Amphetamines	Violet or Green
• Hallucinogens	Violet or Green

Stability: Stable
Problems: None

14.3.7 Marquis

Formulation: One mL of formaldehyde to 10 mL of concentrated sulfuric acid

Tests for:

- Opiates — Purple
- Amphetamines — Orange/brown
- Phentermine — Orange
- MDMA/MDA — Blue/black
- Aspirin — Pink to red
- Diphenhydramine — Yellow
- Tryptamines — Green
- Methylenedioxy cathinones — Yellow
- *Fentanyl* — *Orange*

Stability: Six months

Problems: None

14.3.8 Mecke

Formulation:
0.25 g selenious acid
25ml concentrated sulfuric acid

Tests for:

- Alkaloids — Green/Blue
- Heroin — Green/blue
- Codeine — Bright green to blue/green
- PCP and Quinine — Light yellow

Stability: Stable
Problems: None

14.3.9 PDMB – Ehrlich's – Look – Van Urk Formulation

Formulation:
5g paradimethylaminobenzaldehyde
50 mL concentrated HCl
50 mL ethanol

Tests for:

- LSD, Psilocyn Purple
- Benzocaine, Procaine Yellow

Stability: Stable - Refrigerate stock solution
Problems: None

14.3.10 Scott's test for Cocaine

Formulation: 2 g cobalt in 100 mL of water, add 100 mL of glycerin
Solution 1 2% aqueous cobaltous thiocyanate, diluted 1:1 with 96% Glycerin (w/v)
Solution 2 Concentrated hydrochloric acid (HCl)
Solution 3 Chloroform

Procedure:

1. Place a small amount of suspected cocaine in a test tube and add 5 drops of solution 1 and shake. Note color change. If blue color does not develop, the sample does not contain cocaine hydrochloride, although it could still contain cocaine base. Continue with step 2.
2. Add a drop of solution 2. Any blue color from Step 1 may disappear resulting in a clear pink solution. Cocaine base will result in blue color with addition of Solution 2. Proceed to step 3.
3. Add several drops of Solution 3 and shake. The $CHCl_3$ layer will develop an intense blue color if cocaine or cocaine base is present.

Test for:

- Cocaine hydrochloride Blue, Pink over Blue
- Cocaine base Pink, Pink over Blue

Stability: Stable
Problems: Some false positives

14.3.11 Modified Sodium Nitroprusside (Simon's Test)

Formulation:
Part A: 0.5g Sodium nitroprusside in 50 mL water, 1 mL acetaldehyde
Part B: 5g Sodium carbonate in 100 mL water.
Use one drop of A, then two drops of B

Tests for:
Methamphetamine, secondary amines Blue
BZP Blue

Stability: Keep refrigerated
Problems: Few false positives

14.3.12 Weber's

Formulation:
0.01 g Fast Blue B in 10 mL DI water/concentrated HCl

Tests for:

- Psilocyn — Red, blue after the addition of acid
- Psilocybin — No reaction

Stability: One week, Stored between -20-10°C

14.4 Standards and Control

Stock bottles of the reagents are to be tested when prepared, or when needed, using primary reference material. A record will be kept on the preparation of these reagents, including the initials and date, along with the standard used in testing the reagent.
Tests will be performed in individual spot plate well(s) cleaned with appropriate solvent prior to use or using disposable vessel(s).

14.5 Procedure

Add the recommended amount of reagent to the spot plate before adding the unknown. Alternatively, transfer a small amount of the unknown to a disposable container (such as a weigh boat or test tube) and add the recommended amount of the reagent.

Any reaction with the substance, such as color, odor, or effervescence will be recorded in the case examination documentation.

14.6 References

1. Ohio BCI Drug Chemistry Training Manual
2. Clark, E.G.C. Isolation and Identification of Drugs, 2nd Edition; Pharmaceutical Press: London, England, 1986.
3. Feigl, F. Spot tests in Organic Analysis, 7th ed. Elsevier Publishing: New York, NY. 1966.
4. Johns, S.H., Wist A.A., Najam A.R., Journal of Forensic Sciences, Spot Tests: A Color Chart Reference for Forensic Chemists
5. Garrett, S.A., Clemens, S. R., Gaskill, J. H., SWAFS Journal, Vol. 15, No. 1, April 1993.

15. Thin Layer Chromatography

15.1 Introduction

Thin layer chromatography (TLC) is an analytical technique that offers a rather quick and easy separation of chemical compounds. TLC can be used as a preliminary test. The distance traveled and visualized colors are compared to that of a standard run at the same time.

15.2 Safety Considerations

The mobile phase and visualizers will be prepared in the hood. Also, any spraying of the visualizers will be performed in the hood and the spray will be directed in a spray booth.

15.3 Standards and Controls

The following are requirements to meet the standards and controls for thin layer chromatography:

- Reference material and a negative control consisting of the extraction solvent will be run on each plate. A spot in the blank near the area of interest requires the plate to be re-run. Observed results and a copy or photograph of the plate will be included in the examination documentation.
- Manufacturer and lot # (or unique identifier) of the reference material will be recorded in the examination documentation.
- The solvent system and the method of visualization will be indicated in the examination documentation.
- The TLC chambers will be properly labeled with date prepared, solvent system, and initialed by the preparer.

15.4 Procedure

Commercially prepared TLC plates will be used with or without a pre-adsorbent layer. A standard will be run on each plate with the unknown. The developing solvent is placed in a closed developing chamber that has been allowed to equilibrate.

The samples are then spotted at the bottom of the plate, above the solvent line on a non-pre-adsorbent plate, or on the pre-adsorbent layer. The plate is then placed in the developing chamber and developed to the desired height. The plate is then placed in a hood where it will be air dried before using the visualization spray. Positive identification of a compound is achieved by matching the vertical distance traveled on the plate by the reference material and evidence sample. The visual appearance of the spots must be consistent (color or fluorescence). These results will be recorded in the case file in a visual format such as a photographed or scanned image.

15.5 Common Solvent Systems and Visualizers

Drug	System	Visualizers
Marihuana (Cannabis)	Hexane: Ether (4:1) Hexane: Acetone (4:1)	Fast blue B Fast blue BB
General screen	Methanol: Ammonia (95:5) Cyclohexane: diethylamine (90:10) Methylethylketone: diethylamine (95:5) Ethyl acetate: methanol:ammonia (70:25:5)	Iodoplatinate
LSD	Acetone Acetone: chloroform (50:50) Acetone: ammonia saturated chloroform (9:1) Cyclohexane:Ether:Acetone: Diethylamine (35:30:30:5)	PDMB
Psilocyn/Psilocybin	n-butanol: acetic acid: water (2:1:1) Methanol: ammonia (100:1.5)	PDMB
Steroids	Chloroform: Ethyl Acetate (4:1) Ethanol:Sulfuric acid (4:1)	

15.6 References

1. BCI Drug Chemistry Training Manual
2. Clarke, E.G.C., (1986), Isolation and Identification of Drugs, The Pharmaceutical Press, London.
3. Stahl, Egon, (1973), Drug Analysis by Chromatography and Microscopy, Ann Arbor Science, Ann Arbor, MI.
4. Skoog, Douglas A., (1985), Principles of Instrumental Analysis, Saunders College Publishing

16. Gas Chromatography

16.1 Introduction

Gas chromatography (GC) is a method of separating the components of a volatile mixture by partitioning them between a stationary liquid phase and a moving gaseous phase. Gas chromatography when combined with an acceptable detector can be used as a preliminary test.

16.2 Safety Considerations

Standard laboratory practices

16.3 Preparations

The samples will be dissolved in an appropriate solvent, e.g., chloroform, ethanol, methanol, hexane etc.

16.4 Standards and Controls

To ensure the instrument is working properly, a quality control mixture of known reference material is run using a general drug screen program (e.g. QDS, Gen115-20m, Gen 130, GENscreen-H2, LondonScreen, BGScreen (7890), or BGScreen). The chromatogram is examined for retention time (RT), peak height, shape, baseline separation and reproducibility. This will be done on a monthly basis, after maintenance and as the chemist deems necessary. If an irregularity is noted, each reference material used to create the quality control mixture could be run to verify the mixture components are reproducible.

The stock quality control mixture could also be run on a GC/MS to determine the nature of the irregularity. A passing quality control mixture is required for the instrument to be used for casework.

The following are the requirements to meet the standards and controls for gas chromatography:

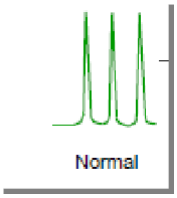
- The chromatogram will indicate case number, item number, method used, injection volume, instrument name and any reference material's manufacturer/lot number or identifier. An electronic copy of the chromatogram will be kept in the case file as data.
- Examination documentation will list the results of the GC/FID.
- A log will be kept for each instrument recording maintenance, monthly results of known reference materials (QC mix), the type of column used and installation date.
- A single reference material chromatogram may be used multiple times within a month to the day of the injection time stamp on the printed file (e.g. a cocaine standard run on August 2nd may be used for a case sample run on September 2nd).

- The injection volume ~~should~~ *shall* not exceed 2µl.
- The main peak in the reference material should be 75% of the total area or greater. If it is not, that reference material should be run on a GC/MS for structural verification. If the reference material passes GC/MS verification, the reference material can be used for GC/FID RT comparison. A fresh reference material should then be created. If the reference material fails both the 75% total area and GC/MS verification steps, the reference material cannot be used as a GC/FID reference material.
- A blank shall be run under the following circumstances; however, additional blanks may be run at the analyst's discretion:
    - Use extraction reagents, chemicals, derivatizing agents, and/or solvent that corresponds to sample setup
    - Run using the same temperature range as the sample
    - Run using the same or lower split ratio as the sample
    - Run using the same or higher injection volume as the sample
    - Prior to each instrumental test for residue
    - Before the first sample of each item
- A blank result should be void of unacceptable artifacts, peak(s) in the area of interest, integrated peaks or carryover from previous sample(s). Documentation of the testing of blanks will be maintained in the case record, each with a unique file identifier.

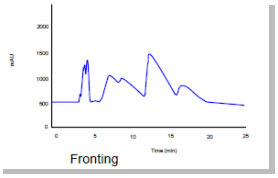
16.5 Interpretation Criteria

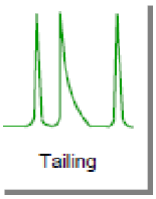
Prior to comparison to a known reference material, the chromatogram will be evaluated to ensure suitability for comparison.

The peaks present should have good peak resolution/symmetry, narrow peak width, and with minimal peak tailing or split peaks.



Poor peak shape can be attributed to small peaks eluting on the tail of the larger peak, contamination, column overload or a bad column.





Qualitative comparisons will be made with known reference material.

The RT of the sample must be less than or equal to +/- 0.050 min of the RT of the reference material. If the reference material has more than one integrated peak, the reference material will be evaluated for consistency.

The unknown sample and reference material will be compared using the same method parameters. Methods are considered interchangeable when altered instrument parameters do not affect the RT of a compound.

16.5.1 Inconclusive Results

The chromatograph does not meet the minimum requirements of a positive result or the blank does not meet the acceptable criteria. Examples include: retention time falls outside the accepted range, poor baseline separation of peaks, carry-over in blanks from previous cases, and instances when the standard did not meet the 75% rule.

Samples can be extracted, diluted and/or re-run to obtain acceptable results.

16.6 References

1. BCI Drug Chemistry Training Manual.
2. Saferstein Ph.D., Richard. Forensic Science Handbook, Volume II; Prentice Hall: Englewood Cliffs, NJ, 1988, p.p. 39-67.
3. Clarke, E.G.C., (1986), Isolation and Identification of Drugs**,** The Pharmaceutical Press, London.
4. Skoog, Douglas A., (1985), Principles of Instrumental Analysis**,** Saunders College Publishing.

17. Liquid Chromatography/Ultraviolet-Visible detection (LC/UV)

17.1 Introduction

Liquid Chromatography/Ultraviolet-Visible detection (LC/UV-Vis) is an instrumental technique used to separate a sample into its individual components based on their interaction between a stationary solid phase and a liquid mobile phase. When paired with an appropriate detector (i.e. PDA, DAD, etc.), LC can be used as a preliminary or quantitative test. BCI's detector system is UV-VIS and refers to a UV system that scans a range of wavelengths in the ultraviolet / visible region.

17.2 Safety Considerations

Standard Laboratory Practices

17.3 Preparations

Samples will be dissolved in an appropriate solvent.

17.4 Standards and Controls

To ensure the instrument is working properly, quality control samples will be run each ~~day~~ time a batch is run for quantitative analysis. The chromatogram is examined for relative retention time, peak shape, resolution, reproducibility, and concentration. This will be done when in use, after maintenance, and as the chemist deems necessary. If an irregularity is noted, the controls will be remade and run again. If the irregularity persists, maintenance will be done on the instrument to correct the problem. The following are the requirements to meet the standards and controls for Liquid Chromatography:

- An electronic copy will be kept in each case file.
- The method used, case number, item number, alternative injection volume, instrument name, reference material manufacturer, and lot number and date will be recorded on the electronic copy.
- The examination documentation will indicate the results of the analysis and all chromatographs and spectra will be included in the case file.
- A logbook will be kept for each instrument to record any maintenance done on the instrument.
- Chromatographic peaks appear symmetrical (i.e. no co-elution, split peaks, shoulders, etc.)
- Relative Retention Times (RRT) for target compounds and internal standards shall be +/- 2.5% of the average Relative Retention Time (RRT)
- Blanks (all) – Continuous and smooth, target analytes <10% of peak area of LLOQ this includes:
    - **System blank** proves instrument is free of contamination prior to curve. If established acceptance criteria are not achieved, correct and rerun with acceptable results before curve is considered acceptable for

use.

- **Method blank** proves grinder process is clean and free of THC/THCA – If failure occurs repeat sample prep on a new sample from evidence. This is a dry swab of a grinding device previously used to homogenize THC containing samples that is soaked in 2mL of appropriate solvent. If this fails there must be sufficient measures taken to ensure the ~~tubes~~ *cups* that will be used for the prep aren't the cause for the contamination.
- **Matrix Blank (Negative Control)** proves the entire matrix including reagents are free of THC/THCA - *if failure occurs, re-run within the batch. If re-run fails, repeat batch prep and re-run. If re-prep fails, re-prep batch with new control lot.* ~~repeat prep if failure occurs~~. This is a Hempseed oil sample.
- **Sample Blanks** prove no carryover was present from sample to sample – repeat analysis of sample and blank if failure occurs. A blank will be run at minimum prior to each item. In the situation where duplicate analysis is performed a blank will be run for each sample.

- **Calibration Curve** Correlation coefficient on a 5-6point calibration curve ≥ 0.995, Accuracy +/- 15% (of known values or to another curve), Signal to Noise of LLOQ≥10, repeat prep if failure occurs. Calibration curve will be run at the beginning and end of each batch, unless the batch consists of no more than 15 samples. In this situation it is acceptable for the analyst to run only a beginning curve. The ending calibration curve concentrations must be within +/- 15% of the expected concentration and the Relative Retention Time (RRT) must be within 2.5% of the average RRT of the calibrators.
- **Positive control** Grow Ohio Tincture (approximately 25 ug/mL). Acceptance criteria 80-120% recovery, relative retention time within 2.5% of average relative retention time of target analyte on curve. If failure occurs rerun within the batch. *If re-run fails, repeat batch prep and re-run. If re-prep fails, re-prep batch with new control lot.* Batch is acceptable with one passing positive control. *Positive control is usable for up to one year past the manufacturer's date of expiration,* as long as the pass criteria noted above are achieved.
- **Check standards** – Every fifteen evidence samples must be bracketed by a check standard with the exception of the ending samples in a batch, which may be bracketed by one check standard or the ending calibration curve. Check standards are made with certified reference materials of a different manufacturer than the calibration curve and are diluted to 16 µg/mL of THC and THCA. If a different manufacturer is not available, then the check standard will be prepared by an analyst who did not prepare the calibrators. Acceptance criteria 85-115% recovery, relative retention time within 2.5% of average relative retention time of target analyte on curve. If a failure occurs data will be

reprocessed using a curve within the same batch. A check standard must be run if the sequence stops.

- **Cannabinoid Mix** – this is a quality control mix designed for the LC/UV/MS and consists of the following (at a minimum) ~~at approximately 20ug/mL~~: Cannabidiol (CBD), Delta-8 THC, Delta-9 THC, Cannabinol (CBN), Cannabichromene (CBC), Cannabigerol (CBG), Cannabidiolic Acid (CBDA), Cannabigerolic Acid (CBGA), and Tetrahydrocannabinolic Acid (THCA).
    - The results are acceptable if the following criteria are met:
        - Peak present for each reference material in quality control mixture; no unexplained shifts in relative retention times noted in comparison to previously run cannabinoid mix samples.
        - Peaks exhibit good peak structure: they are symmetrical and lack significant fronting, tailing, or splitting.
        - Peaks exhibit baseline separation and Delta-8 THC and Delta-9 THC have a resolution of > 2.
- **Samples** – Relative retention time within 2.5% of the average relative retention time of target analyte on curve. If concentration exceeds the ULOQ (both THC and THCA) analyst will dilute. If concentration is less than LLOQ analyst will concentrate (smaller dilution) unless calculated total THC with the analyte on the curve is greater than 3%. If a subsequent dilution scheme yields a result where one analyte is on the curve and one is still below LOQ, a further dilution is only required if it would yield both analytes on the curve. (i.e. 1st run THC and THCA are below LOQ, 2nd run (1:2 dilution) yields THC at 10ug/mL and THCA at 1.2ug/mL. In this case doing a 1:1 dilution should not yield a THCA result that is on the curve, thus performing it is not required.)
- **Duplicate analysis-** Perform a duplicate analysis ~~(independent sample~~ *2 samples* carried through the same extraction / dilution scheme *simultaneously*) if total THC concentration falls between 0.1 and 1%. Report lower of the 2 duplicate results, if samples are within 10% of each other. If outside 10%, *perform duplicate analysis again* ~~prepare original sample and duplicate again.~~
- **To verify a prepared calibration stocks** – ~~Run old calibrators to establish a curve, run new calibrators as unknowns, and check standards. Correlation coefficient ≥ 0.995, Accuracy of new calibrators must be +/- 15% of old calibrators.~~ *Run old calibrators, new calibrators, and check standards in a single batch. Then, process the new calibrators and check standards using the old calibrators as the established curve. Finally, process the old calibrators and check standards using the new calibrators as the established curve. Correlation coefficient ≥ 0.995 and accuracy of +/- 15% must be met in both instances.*
- **Batch**: The samples run continuously without change to the system (calibrators, mobile phase, etc.) and the check standards continue to meet criteria

17.5 Interpretation Criteria

The peaks present should have good resolution / symmetry, narrow peak width, and have minimal tailing or splitting.

The relative retention time of the sample must be within +/- 2.5% of the RRT of the reference material (calibration curve).

The quantitative value falls within the linear range of the calibration curve.
Note: It is understood that there could be situations where one analyte falls within the calibration curve and another does not. If a particular dilution scheme produces a total THC content of greater than 3%, no additional analysis is required.

When an analyst chooses to prepare a dilution to achieve a result in the middle range of the calibration curve, the result with the lower MU will be reported.

17.6 ~~Inconclusive~~ Other results

The chromatograph does not meet the minimum requirements of a positive result or the blank does not meet the acceptable criteria. Examples include: relative retention time falls outside the accepted range, poor baseline separation of peaks, and carry-over in blanks from previous cases.

No Controlled Substance Found: To report No Controlled Substance Found, two runs on a GC/MS under general screen parameters (GEN115-20M, QDS, ~~or~~ GEN130, or GEN170) are required, in accordance with *general qualitative chemistry policy* ~~(i.e. 2nd run must be on a lower split or higher injection).~~

Insufficient Sample for Quantitative Identification: To report Insufficient Sample for Identification, one GC/MS run with indications of Tetrahydrocannabinol (THC) followed by quantitative analysis on the lowest dilution feasible for the sample with no reportable THC, is required.

18. Infrared Spectroscopy

18.1 Introduction

Infrared Spectroscopy (IR) is most commonly used as a tool for structural identification of a substance. The substance should be in a near pure state to obtain a positive identification of the substance, which may require an extraction or other separation means prior to analysis. Comparing the position and relative intensity of each peak to that of a known standard spectrum a chemist can make a positive identification of an unknown. The IR computer is used to acquire data and make data searches and create an electronic copy for comparison.

18.2 Safety Considerations

The FTIR uses a laser beam to calibrate the proper mirror motion in the interferometer. Do not look directly into the beam as this could cause damage to the eye.

18.3 Preparations

Common sampling accessories, such as Attenuated Total Reflectance (ATR) or diffuse reflectance, require little or no sample preparation.

18.4 Standards and Controls

To ensure the instrument is operating properly, the IR will be calibrated monthly *when in use* in accordance to the FTIR performance check and maintenance procedure. The performance check will be recorded in the IR log along with any maintenance necessary for the proper operation of the instrument. If the instrument is used less than once a month the validation will be conducted prior to the scan.

The following are the requirements to meet the standards and controls for Infrared Spectroscopy:

- An electronic copy of the spectrum will be kept in each case file.
- The spectrum will indicate the case number, item number, method used and instrument name.
- The examination documentation will indicate the result of the IR analysis.
- Typically, the IR analysis technique utilized will be ATR. Other techniques, such as pellet or diffuse reflectance, are permissible. Any technique used, exclusive of ATR, must be reflected in the case file.
- A log will be kept for each instrument to record any maintenance done on the instrument and the calibration or reference checks.
- A background is run each day of instrument use
- A Contamination Check control is run prior to each sample analysis:
    - Clean the diamond crystal with a solvent, such as methanol
    - Acquire a spectrum under sample analysis instrument parameters, which includes lowering the compression arm if it is used with the sample.

- Contamination Check results should be void of unacceptable artifacts or carryover from previous samples. Documentation of the contamination checks will be maintained in the case record, each with a unique file identifier.

18.5 Procedure

1. The sample is placed on the IR and a spectrum of the substance is obtained.
2. The spectrum is then compared to that of reference material spectra, which is included (be it from the instrumental library or published literature source).

18.6 Interpretation

Prior to comparison to a primary reference source, the spectrum will be evaluated to ensure suitability for comparison using the following criteria, at a minimum:

- Minimal baseline noise
- Sufficient details present in the spectrum for comparison purposes

The unknown substance spectrum must be compared to a primary reference material, a published literature source or library source and the source must be included.

Visual comparison of the spectra from the unknown substance to the primary reference material shall be conducted taking into account the wavenumbers and intensity of each stretch. The overall appearance and location of the major peaks in the sample should correspond with the reference spectrum.

18.6.1 Inconclusive *Instrument* Results

The spectrum does not meet the minimum requirements of an identification or the blank does not meet the acceptable criteria. Examples include: the unknown substance is a mixture, additional stretches or the absence of stretches noted, spectrum is weak/poor quality.

18.7 References

1. Ohio BCI Drug Chemistry Training Manual
2. Saferstein Ph.D., Richard. Forensic Science Handbook, Volume III; Prentice Hall: Englewood Cliffs, NJ, 1993.
3. Clark, E.G.C. Isolation and Identification of Drugs; 2nd ed.; Pharmaceutical Press: London, England, 1986.
4. Skoog, Douglas A., (1985), Principles of Instrumental Analysis, Saunders College Publishing.

19. Mass Spectrometry

19.1 Introduction

Mass Spectrometry (MS) is most commonly used for structural information for the positive identification of a compound, but can also be used for screening purposes. The instrument can be used with or without an auto sampler.

Identification by mass spectrometry can be accomplished by comparing the unknown spectrum with that of a standard spectrum run on the same instrument, or with a reference source.

The computer is used to generate data from the unknown and to make library searches. It should not be used to alter the fundamental data (the analyst has the discretion to enhance the data through background subtraction or manual integration).

The mass spectrometer will be tuned weekly. A known drug mix using a general drug screen method (e.g. QDS, Gen115-20m, Gen 130, Genscreen-H2) will be run to ensure the instrument is operating properly after a mass spectrometer cleaning, mass spectrometer/GC repair, GC column change and at the chemist's discretion. A record of the auto-tune and quality control mix will be kept in the mass spectrum logbook. The quality control mixture will include low and high boilers and two closely eluting reference materials that baseline separate. The quality control mix should be run monthly. The logbook will also indicate any maintenance done on the instrument including source cleanings, column changes, liner/septum changes, and oil changes. The date and chemist performing the tasks will be logged.

19.2 Safety Considerations

Standard laboratory practices

Hydrogen carrier gas is highly combustible. Antistatic mats will be used to prevent sources of static electricity and potential ignition. Maintenance practices developed by the instrument manufacturer for the safe use of hydrogen will be utilized.

19.3 Preparations

Prior to injection into the gas chromatograph, the sample will be dissolved in an appropriate solvent such as:
Methanol
Ethanol
Chloroform
Hexane

19.4 Standards and Controls

The following is a list of requirements to meet the standards and controls for mass spectrometry:

- An electronic copy will be kept in each case file.
- The method used, case number, item number, injection volume, instrument name, reference material manufacturer and lot number (or unique identifier) and date will be recorded on the electronic copy.
- Ultra-high purity Hydrogen is required. Gas supplied from high pressure cylinders or gas generators are acceptable.
- GC/MS models 7890/5977B or newer with turbo-molecular pumps can be converted for Hydrogen carrier gas method(s). Hardware modifications are necessary to retain mass spectral fidelity which allows users to continue using existing helium-based mass spectral libraries. Modifications include but aren't limited to the use of a multimode inlet, Hydroinert source or EI extractor source with 9.0 mm draw-out plate lens, high temperature filaments and narrower capillary columns.
- The examination documentation will indicate the result of the MS analysis and all spectra will be included in the case file.
- If a GC/MS is being used for retention time purposes, the evidence sample and reference material must be equal to or less than +/- 0.050 min of one another.
- A log will be kept for each instrument to record any maintenance done on the instrument and the calibration or reference checks.
- A blank must be run under the following circumstances; however, additional blanks may be run at the analyst's discretion:
    - Use extraction reagents, chemicals, derivatizing agents, and/or solvent that corresponds to sample setup
    - Run using the same temperature range as the sample
    - Run using the same or lower split ratio as the sample
    - Run using the same or higher injection volume as the sample
    - Prior to each instrumental test for residue
    - Before the first sample of each item
- A blank result should be void of unacceptable artifacts, excessive column bleed, or carryover from previous sample(s). Documentation of the testing of blanks will be maintained in the case record, each with a unique file identifier.

19.5 Procedure

Various solvents can be used to introduce the sample into the GC and solubility plays an important role in identifying a substance. For introduction into the mass spectrometer the gas chromatography procedure should be followed. Programs (methods) can be created for specific drugs as needed and will be performance checked. depending on the results of preliminary tests. Procedures used in obtaining mass spectra may vary depending on the substance being analyzed. (Note: For specific methods and conditions see Appendix.)

19.6 Interpretation

Prior to drawing conclusions regarding comparison to a primary reference source, the spectrum will be evaluated to ensure suitability for comparison using the following criteria, at a minimum:

- Minimal background noise observed
- Presence of major ions

Hydrogen Carrier Gas Methods:

- Higher background and reduction in signal to noise (S/N) ratios are typical.
- Use of chlorinated solvents in a Hydrogen environment can lead to an increased presence of HCl which can lead to column degradation and the observation of siloxane peaks.

Unknown substance spectra must be compared to a primary reference material, a published literature source or library source, and the source must be included. Examination of the fragmentation pattern and relative ratios of the ions within the spectrum should be compared.

The spectra from the unknown substance to the primary reference material/ reference standard will have consistent fragmentation patterns and be void of any major contributions from unexplained artifacts.

At times, the unknown substance spectrum's fragmentation pattern is not consistent with the primary reference material; however the substance may be structurally similar to a controlled substance. See the procedure for analog report in this document.

19.6.1 Inconclusive *Instrument* Results

The spectrum does not meet the minimum requirements of a positive result or the blank does not meet the acceptable criteria. The analyst should consider other testing methods if the spectrum quality is considerably weak.

19.7 References

1. Ohio BCI Drug Chemistry Training Manual
2. Saferstein Ph.D., Richard. Forensic Science Handbook, Volume II; Prentice Hall: Englewood Cliffs, NJ, 1982, pp. 92-137.
3. McLafferty, Fred W., and Turecek, Frantisek. Interpretation of Mass Spectra; University Science Books: Sausalito, California.
4. Skoog, Douglas A., (1985), Principles of Instrumental Analysis, Saunders College

Publishing.
5. Agilent 5994-2312EN: Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion
6. Agilent Inert Plus GC/MS System with Hydroinert Source: Applying H2 carrier gas to real world GC/MS analyses
7. Lokits, K. (2022). The Science Behind He to H2 Carrier gas Method Conversion [PowerPoint Slides]. Agilent technologies
8. Agilent G7003-90053: Agilent GC/MS Hydrogen Safety

20. Drug Reporting

20.1 Introduction

Format and content will meet requirements of the current accreditation standard and as specified in the Laboratory Quality Assurance Manual and related practices.

20.2 General Reporting Guidelines

20.2.1 Controlled substance names

Controlled substances will be reported by the name or the abbreviation with which they are referenced in the OAC. In the cases where analogs are reported and compared to a controlled substance, the naming convention may be altered to better illustrate the substantially similar core structures.

In instances where the Federal government has temporarily, emergency, or permanently scheduled a compound, the compound will follow that guidance and report with the name only, even when the compound has been voted on as an analog, pharmacophore, or substituted cathinone.

20.2.2 Quantitative results

Quantitative values will be reported with a coverage probability of 95.45% (k=2) and in accordance with any measurement uncertainty requirements as determined by accreditation program requirements.

Total THC results (% THC) will be truncated to the appropriate significant figure.

~~The laboratory routinely reports values two or three decimal places (0.32), therefore, any result up to and including 0.349 should technically be reported as Hemp instead of Marihuana. To ensure clear reporting of the legal statue "not more than three tenths per cent", rounding considerations should be reported as follows (factoring in the associated measurement uncertainty):~~

~~Vegetative Samples~~

- ~~Total % THC level below 0.1% – Less than 0.1%~~
- ~~Total % THC level between 0.1% and 0.3% – Hemp~~
- ~~Total % THC level between 0.301 and 0.500 – Cannabis, with a Tetrahydrocannabinol content of "X"~~
- ~~THC level above 0.501% - Marihuana~~

~~Approved Non-Vegetative samples (oils, vape cartridges, waxes, etc)~~

- ~~Total % THC level below 0.1% – Less than 0.1%~~

- ~~Total % THC level between 0.1% and 0.3% - Delta-9-Tetrahydrocannabinol with a Tetrahydrocannabinol content of "X"~~
- ~~Total % THC level above 0.3% - Delta-9-Tetrahydrocannabinol or Hashish (solid/liquid) based on sample type, with a Tetrahydrocannabinol content of "X"~~

20.2.3 Weights

Weights will be reported in accordance with the accuracy of the balance(s) used and any measurement uncertainty requirements as determined by accreditation program requirements.

- Weights (net/gross) will be truncated to the appropriate significant figure
- Gross weight records are documented in the lab notes to determine the amount of sample needed to test to charge limits.
- Samples involving trace or residual amounts of material do not require weight determination. These quantities may be reported as "trace amounts".
- Samples weighing less than 0.10 g and not reported as residue will be reported as less than 0.10 g.
- Estimated measurement uncertainty values offered with reported weights must include coverage probability information. The following is an example of standardized language that may be included in the report:

  "When a measurement uncertainty value is offered above, the coverage probability (i.e. probability the true weight is contained within the specified coverage interval) is XX.XX%."
- Estimated measurement uncertainty values offered with reported weights will be rounded to the appropriate significant figure, which is no more than two

20.2.4 Bulk amounts

Bulk amounts will not be referenced in reports.

20.2.5 Drug combinations

Drug combinations which are listed as exempted or excepted, as listed in the Controlled Substance Reference Table in the OAC will contain wording to such effect in the report.

20.2.6 Schedule

Any pharmaceutical whose schedule can change as a result of its drug makeup will have the schedule reported; otherwise, schedules will not be reported.

20.2.7 Analog and Pharmacophores

The approved list is located in the Laboratory shared location and the contents are available in the LIMS matrix.

20.2.8 Known breakdown products

In samples where heroin and 6-MAM are found, only report 6-MAM if heroin cannot be confirmed in the sample. If only 6-MAM and morphine are found, report both.

20.2.9 Sampling plan applied

For items on which the sampling plan is applied, findings for the whole item may be reported provided the customer is notified in the report that the sampling plan was used.

20.2.10 Sample selection applied

For items in which sample selection is applied, the laboratory report may state what was received, what was tested, and report findings on only that which was tested.

20.2.11 Method of Testing

The method(s) of testing performed on each evidence item must be included in the laboratory report.

20.2.12 Items not tested

Any items that were received at the laboratory but not tested will be included in the report.

20.2.13 Evidence Disposition

The disposition of any items that were received at the laboratory will be included in the report. All items will be returned to the department once testing is completed.

20.2.14 Reporting Examples

Drug chemistry reports will be formatted in accordance with laboratory protocol and will include all applicable information as specified by ANAB accreditation program requirements and the BCI laboratory quality management system.

Examples offered below reflect typical laboratory report wording for analytical conclusion and sampling elements of the quantitative lab report:

~~**Marihuana**~~

~~Vegetation- 7.67 g +/- [Current Estimated MU (g)] - found to contain Marihuana with a Tetrahydrocannabinol (THC) content of 12.2% +/- [Calculated MU (%)] calculated on a dry weight basis. Method(s) of testing used: chemical testing, microscopic examination, and LC-UV.~~

Cannabis (THC content greater than or equal to 0.1%)

Vegetation - 333.74g+/- [Current Estimated MU (g)] - found to contain Cannabis. Method(s) of testing used: chemical testing, microscopic examination and LC-UV. The Tetrahydrocannabinol (THC) content of each sample, calculated on a dry weight basis, is shown in the table below:

Sample	*Total Tetrahydrocannabinol (THC) Content (%)*	*Measurement Uncertainty (%)*
1	*15.0*	*[Calculated MU (%)]*

~~**Hemp / Product below 0.1% total THC**~~ ***Cannabis (THC content less than 0.1%)***
Vegetation – 225.75g +/- [Current Estimated MU (g)] found to contain ~~Hemp (a non-controlled substance)~~ *Cannabis* with a Tetrahydrocannabinol (THC) content of less than 0.1% calculated on a dry weight basis. Method(s) of testing used: chemical testing, microscopic examination, GC-MS, ~~FTIR, GC-FID,~~ and LC-UV.

~~Vegetation- 225.75 g +/- [Current Estimated MU (g)] - found to contain Hemp (a non-controlled substance with a Tetrahydrocannabinol (THC) content of 0.215% +/- [Calculated MU (%)] calculated on a dry weight basis. Method(s) of testing used: chemical testing, microscopic examination, and LC-UV.~~

THC Items (Instrumental analysis – no further confirmation)
Yellow-brown substance - 4.02 g +/- [Current Estimated MU (g)] – Tetrahydrocannabinols (THC) indicated. Not confirmed. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Cannabinoids (Positive Duquenois-Levine color test only)
Brown substance - 0.56 g +/- [Current Estimated MU (g)] - Cannabinoids indicated. Not confirmed. Method(s) of testing used: chemical testing.

~~**Hashish**~~
~~Brown substance- 0.5 g +/- [Current Estimated MU (g)]- found to contain Hashish in solid/liquid form with a Tetrahydrocannabinol (THC) content of 12.4% +/- [Calculated MU (%)]. Method(s) of testing used: chemical testing, GC-MS, FTIR, GC-FID, and LC-UV.~~

Approved non-vegetative samples (oils, vape cartridges, waxes, etc.) (THC content greater than or equal to 0.1%)

In order to address statutory differences in solid and liquid hashish, sample type will be included in the item description

Solid amber substance - 15.84g+/- [Current Estimated MU (g)] - found to contain Delta-9-Tetrahydrocannabinol/Delta-9-Tetrahydrocannabinolic Acid (THCA). Method(s) of testing used: chemical testing, GC-MS and LC-UV. The Tetrahydrocannabinol (THC) content of each sample is shown in the table below:

Sample	*Total Tetrahydrocannabinol (THC) Content (%)*	*Measurement Uncertainty (%)*

1	*78*	*[Calculated MU (%)]*

Approved non-vegetative samples (oils, vape cartridges, waxes, etc.) (THC content less than 0.1%)
Liquid amber substance – 4.82g +/- [Current Estimated MU (g)] found to contain Delta-9-Tetrahydrocannabinol with a Tetrahydrocannabinol (THC) content of less than 0.1%. Method(s) of testing used: chemical testing, GC-MS, and LC-UV.

Quantitative analysis requested for item- unable to Quantitate

Green substance – 10.85g +/- [Current Estimated MU (g)] - Quantitation of THC could not be determined at this time due to the capabilities of the laboratory.

Examples offered below reflect typical laboratory report wording for analytical conclusion and sampling elements of the qualitative lab report:

Cocaine powder (*All variations of Cocaine are now reported as Cocaine*)
Off-white substance - 0.23 g+/- [Current Estimated MU (g)] - found to contain Cocaine- Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC.

~~**Cocaine base** - All variations of cocaine are now reported as Cocaine.~~
~~Off-white substance - 1.89 g+/- [Current Estimated MU (g)] - found to contain Cocaine- Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.~~

If ~~asked to report out~~ ~~as cocaine base~~ *reporting out with salt or base determination*, use the following wording:
Off-white substance - 1.89 g+/- [Current Estimated MU (g)] - found to contain Cocaine base (Crack Cocaine) *OR Cocaine Hydrochloride*- Method(s) of testing used: chemical testing, *and FTIR* ~~GC-MS, FTIR,~~ ~~and GC-FID~~.

If this request is received after the original report was issued- prepare an Amended Report and include the following wording, for example:

"This report supplements the report previously issued in this case dated (original report date). Further testing was performed on the item(s) previously found to contain cocaine to make a cocaine / cocaine base determination. Reported weights are transcribed from the previous report."

Wet/Dry reporting
Off white substance - weight including excessive moisture, 9.83g +/- [Current Estimated MU (g)] -analytical weight after drying, 7.12 g+/- [Current Estimated MU (g)] - found to contain Cocaine. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC.

Clandestine tablets
Five (5) orange tablets – 1.52 g+/- [Current Estimated MU (g)] - found to contain 3,4-Methylenedioxymethamphetamine (MDMA). Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Tablets or Capsules identifiable via reference source (sample selection applied)
Seven (7) white tablets marked “MYLAN457” (Referenced strength: 1mg) – tested one (1) - 0.12 g+/- [Current Estimated MU (g)] - found to contain Lorazepam. Method(s) of testing used: logo identification, GC-MS, FTIR, and GC-FID.

Controlled Tablets or Capsules identifiable via reference source (not tested) Twelve (12) orange tablets marked “N8”<>sword imprint – Markings indicate Buprenorphine. Not confirmed. Method(s) of testing used: logo identification.

Unmarked Tablets or Capsules (sample selection applied)- appears legitimately manufactured
Seven (7) white tablets – tested one (1) - 0.12 g+/- [Current Estimated MU (g)] – No controlled substance found - Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Blister Packs (sample selection applied)
One (1) blister pack containing four (4) red round tablets marked "44 112" - tested one (1)- 0.13 g+/- [Current Estimated MU (g)]- found to contain pseudoephedrine. Method(s) of testing used: logo identification, GC-MS, FTIR, and GC-FID.

Non-controlled substance reporting for marked pharmaceuticals
As needed or when requested,
Seven (7) white tablets marked “MYLAN457”– Markings indicate [insert drug names], which is a non-controlled substance- Method(s) of testing used: logo identification.

Logo Identification on pharmaceutical packaging
Twenty-one (21) commercially sealed foil pouches marked "Buprenorphine and Naloxone 8 mg / 2 mg NDC #####-####-###". Package markings indicate Buprenorphine. Not confirmed. Method(s) of testing used: logo identification.

LSD
One piece of paper divided into one hundred (100) squares - 0.15 g +/- [Current Estimated MU (g)] - found to contain Lysergic Acid Diethylamide (LSD) in solid form. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Heroin
Seven hundred twenty-eight (*728)* packets of powder - *20.68* g (Calculated weight) +/-

[Calculated MU (g)] - found to contain Heroin. Method(s) of testing used: hypergeometric sampling, chemical testing, GC-MS, FTIR, and GC-FID.

One (1) balloon with brown solid substance - 0.27 +/- [Current Estimated MU (g)] - found to contain Heroin. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Mushrooms (Weber's positive and GC/MS found psilocyn)
Brown vegetable matter - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocyn- Method(s) of testing used: chemical testing GC-MS, FTIR, and GC-FID.

Other Psilocyn Items (edibles, gummies, tinctures, etc)
Edible substance - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocyn- Method(s) of testing used: GC-MS, FTIR, and GC-FID.

Include the following wording in the Remarks section of the report (only include 4-AcO-DMT if it is present in the item):

"The Psilocyn in this case may be from the breakdown of Psilocybin/4-AcO-DMT.'

Mushrooms (Weber's negative, GC/MS finds psilocin, and TLC finds psilocyn and psilocybin)
Brown vegetable matter - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocyn. Method(s) of testing used: chemical testing, thin layer chromatography, GC-MS, FTIR, and GC-FID.

Mushrooms (Weber's negative, TLC finds only psilocybin, GC/MS finds derivatized psilocybin)
Brown vegetable matter - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocybin. Method(s) of testing used: chemical testing, thin layer chromatography, GC-MS, FTIR, and GC-FID.

Exempted or excepted preparations
Three (3) tablets marked "0111" - 0.37g(s) +/- [Current Estimated MU (g)] - Markings indicate Butalbital in an exempted form- Method(s) of testing used: logo identification.

No controlled substance identified
Off-white powder - 1.63 g +/- [Current Estimated MU (g)] - no controlled substance found - Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Visually identified as no controlled substance without chemical analysis
Twenty-three (23) white tablets marked "IP 132<>600" - 22.78 g +/- [Current Estimated MU (g)] – Markings indicate a non-controlled substance- Method(s) of testing used: logo identification.

Residue (not weighed)
Residue - trace amount - found to contain Cocaine- Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Diazepam (outside of a pharmaceutical preparation)
Residue – trace amount – found to contain Diazepam*. Method(s) of testing used: GC-MS, FTIR, and GC-FID.

Include the following wording in the Remarks section of the report:

"The Diazepam in this case may be from the thermal breakdown of Ketazolam.'

No identification due to ~~insufficient~~ *inconclusive result(s)*
Residue - trace amount – ~~Insufficient sample~~ *- inconclusive* for *positive* identification- Method(s) of testing used: GC-MS, FTIR, and GC-FID.
-or-
White powder-2.63 g +/- [Current Estimated MU (g)]- inconclusive for positive identification- Method(s) of testing used: GC-MS, FTIR, and GC-FID.

No analysis due to insufficient sample
One (1) piece of plastic– insufficient sample for analysis.

-or-

One (1) piece of plastic- insufficient sample for analysis. Testing can be performed upon receipt of written consent to consume the evidence.

Submitted items that are not tested
Ten (10) plastic zip bags with residue – Not tested.

One (1) plastic bag containing crystalline substance - 2.30 g (gross weight) +/- ~~0.05 g~~ *[Current Estimated MU (g)]* - Not tested

Standard on Order

A.) Off-white powder - 0.80 g +/- [Current Estimated MU (g)] - A substance has been detected for which the laboratory does not have a standard. The standard has been ordered and a supplemental report will be issued after further testing is performed.

B.) Off-white powder - 0.80 g +/- [Current Estimated MU (g)] - A substance has been

detected for which the laboratory does not have a standard. The standard has been ordered and a supplemental report can be issued upon request.

A is for single Item case and B is for a larger case where potential analogs and/or controlled substances already confirmed.

Multiple suspected analogs in a single container/controlled substance and multiple suspected analogs in a single container
White powder – 0.80 g +/- [Current Estimated MU (g)] – found to contain [Identified substance], the chemical structure of which is substantially similar to [Schedule I or II controlled substance]. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Additional substances have been detected for which the laboratory does not have standards. The standards have been ordered and a supplemental report can be issued upon request.

No standard available
Off-white powder - 0.80 g +/- [Current Estimated MU (g)] - No definitive identification can be made at this time because there is currently no available reference standard- -examined using chemical testing, GC-MS, FTIR, and GC-FID.

Beyond the capability of our instrument (2, 3 and 4 isomers)
Off-white powder - 0.80 g +/- [Current Estimated MU (g)] - Analysis indicated the presence of [Identified substance], which has a chemical structure substantially similar to [Controlled substance]. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID. Confirmation of the isomer could not be determined due to the instrumental capabilities of this laboratory.

Not Listed Positional Isomers
Brown crystalline substance - 25.76 g +/- ~~0.05 g~~ *[Current Estimated MU (g)]* - found to contain 4-(2-aminopropyl)benzofuran (4-APB), which is a positional isomer of 6-(2-aminopropyl)benzofuran (6-APB). Method(s) of testing used: chemical testing and GC-MS, FTIR, and GC-FID.

True unknown
Off-white powder - 0.80 g +/- [Current Estimated MU (g)] - No definitive identification can be determined at this time due to current instrumental capabilities-). Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Transdermal patches
Three (3) transdermal patches (Referenced strength: 125 µg/hr) – found to contain Fentanyl. Method(s) of testing used: logo identification, GC-MS, FTIR, and GC-FID.

Strong acids
If only an acidic pH is determined, the report should state:
Colorless liquid- 15.01g +/- [Current Estimated MU (g)]- found to be an acidic liquid.
Method(s) of testing used: chemical testing.

Clandestine Laboratory Manufacturing Cases
If additional general chemical testing may be performed on evidence submitted, the following remark should be included in the report:

Additional general chemical testing may be performed at the State Fire Marshall Laboratory.

Pharmacophores
Vegetation - 1.25 g +/- [Current Estimated MU (g)] – found to contain [Identified substance], which meets the structural requirements outlined for a synthetic cannabinoid pharmacophore. Method(s) of testing used: microscopic examination, GC-MS, FTIR, and GC-FID.

Substituted Cathinones or other substances listed by class
Off-white substance- 0.10g +/- [Current Estimated MU (g)] – found to contain [Identified substance], which is a substituted cathinone. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

Vegetation – 2.56 g +/- [Current Estimated MU (g)] – found to contain [Identified substance], which is a naphthoylindole. Method(s) of testing used: microscopic examination, chemical testing, GC-MS, FTIR, and GC-FID.

Submitted for Quantitative Analysis – no THC indicated
Vegetation - 2.28g +/- [Current Estimated MU (g)]- No controlled substance found.
Method(s) of testing used: GC-MS, FTIR, GC-FID, and LC-UV based on dry weight.

Remarks:
Item(s) X were originally submitted for quantitative analysis, however no controlled substances were indicated in the sample.

Vegetation – 3.00g +/- [Current Estimated MU (g)] – found to contain ADB-FUBINACA.
Method(s) of testing used: microscopic analysis, GC-MS, FTIR, and GC-FID.

Remarks:
Item(s) X were originally submitted for quantitative analysis, however no

Tetrahydrocannabinol (THC) was indicated in the sample. Other substances were identified and those results are reported.

Bound materials
One (1) "AutaBuy" magazine - 195.65 g +/- [Current Estimated MU (g)] – found to contain Methyl-2-(1-(5-fluoropentyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (5F-ADB). Method(s) of testing used: composite sampling and GC-MS.

Instances in which hypergeometric sampling is performed across items
Remarks:
Items were mathematically combined to meet highest penalty threshold. Then hypergeometric sampling was performed across these items.
-or-
A hypergeometric sampling plan was used across items X and Y.

Additional Remarks for Latent Print Cross-assignment:
Remarks:
All items will be returned to your department. The packaging from Item X was preserved for testing.

~~**Additional Remarks for Forensic Biology Cross-assignment:**~~
~~Remarks:~~
~~The swab(s) from item X were preserved for testing~~
Note: Item packaging must also be documented in report item description.

Optical Isomers (with pharmaceutical markings)
One (1) oval peach tablet marked "b 974 / 3 0" (Referenced: Amphetamine and Dextroamphetamine, 30 mg) – 0.25 g +/- [Current Estimated MU (g)] – found to contain Amphetamine (see remarks). Method(s) of testing used: Logo identification and GC/MS*

Remarks:
**Based on the logo identification, this tablet is referenced as a mixture of Amphetamine and Dextroamphetamine. Due to instrumentation limitations, the laboratory cannot differentiate between optical isomers.*

One (1) round peach tablet marked "b 953 / 1 0" (Referenced: Dextroamphetamine 30 mg) – 0.25 g +/- Current Estimated MU (g)] – found to contain Amphetamine (see remarks). Method(s) of testing used: Logo identification and GC/MS*

Remarks:
**Based on the logo identification, this tablet is referenced as Dextroamphetamine. Due to instrumentation limitations, the optical isomer Dextroamphetamine cannot be differentiated from*

Amphetamine.

One (1) round yellow tablet marked "5276 / 9 03" (Referenced:Dexmethylphenidate 10 mg) – 0.25 g +/- Current Estimated MU (g)] – found to contain Methylphenidate (see remarks). Method(s) of testing used: Logo identification and GC/MS*

Remarks:
**Based on the logo identification, this tablet is referenced as Dexmethylphenidate. Due to instrumentation limitations, the optical isomer Dexmethylphenidate cannot be differentiated from Methylphenidate.*

21 Report Examples

21.1 Example 1 (Cross Assignment)

To: Ohio Police Department
Detective Joe Jones
123 Columbus Road
State, OH 12345

BCI Lab Number: 19-00001
Analysis Date: April 18, 2019
Issue Date: April 19, 2019

Re: Possession
Agency Case Number: A100

Submitted on December 30, 2018 by Abby Schwaderer:

1. Plastic bag containing unknown substance (PR# 12345)

Findings

1. Twenty-eight (28) packets of powder - 0.68 g (Calculated weight) +/- [Calculated MU (g)] - found to contain Heroin. Method(s) of testing used: hypergeometric sampling, chemical testing, GC-MS, FTIR, and GC-FID.

Remarks

All items will be returned to your department. The packaging from Item #1 was preserved for testing. (added for LP cross assignments). The swabs from the packaging of item #1 were preserved for testing (added for FB cross assignments).

_____

Robert Jones
Forensic Scientist
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Robert.Jones@ohioattorneygeneral.gov

The reported results correspond only to the items tested unless it is otherwise denoted. BCI reserves the right to select the most appropriate methods and apply threshold testing, when applicable. A visual examination of tested evidence was conducted. Methods of testing performed may include microscopic examination, moisture analysis, hypergeometric sampling, logo identification, chemical testing, thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), gas chromatography-flame ionization detection (GC-FID), and liquid chromatography-ultraviolet spectroscopy (LC-UV).

Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2). Hypergeometric sampling may be specified in the findings above. The application of hypergeometric sampling establishes a 95 % confidence level that at least ninety percent 90% of the units in the sample are as reported.

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

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21.2 Example 2 (Pharmacophore example)

To: Ohio Police Department
Detective Joe Jones
123 Columbus Road
State, OH 12345

BCI Lab Number: 19-00002
Analysis Date: April 18, 2019
Issue Date: April 19, 2019

Re: Possession
Agency Case Number: A100

Submitted on December 30, 2018 by Abby Schwaderer:

1. Plastic bag containing unknown substance (PR# 12345)

Findings

1. Vegetation - 1.25 g +/- [Current Estimated MU (g)] – found to contain N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-3-carboxamide (ADB-PICA), which meets the structural requirements outlined for the synthetic cannabinoid pharmacophore. Method(s) of testing used: microscopic examination and GC-MS, FTIR, and GC-FID.

Remarks

All items will be returned to your department.

Robert Jones
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Robert.Jones@ohioattorneygeneral.gov

The reported results correspond only to the items tested unless it is otherwise denoted. BCI reserves the right to select the most appropriate methods and apply threshold testing, when applicable. A visual examination of tested evidence was conducted. Methods of testing performed may include microscopic examination, moisture analysis, hypergeometric sampling, logo identification, chemical testing, thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), gas chromatography-flame ionization detection (GC-FID), and liquid chromatography-ultraviolet spectroscopy (LC-UV).

Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2). Hypergeometric sampling may be specified in the findings above. The application of hypergeometric sampling establishes a 95% confidence level that at least ninety percent 90% of the units in the sample are as reported.

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

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21.3 Example 3 (gross weight without reporting weight)

To: Ohio Police Department
Detective Joe Jones
123 Columbus Road
State, OH 12345

BCI Lab Number: 19-00003
Analysis Date: April 18, 2019
Issue Date: April 19, 2019

Re: Possession
Agency Case Number: A100

Submitted on December 30, 2018 by Abby Schwaderer:

1. Plastic bag containing unknown substance (PR# 12345)

Findings

1.1 Five (5) capsules containing off-white powder - 0.10 g +/- [Current Estimated MU (g)] - found to contain Fentanyl. Method(s) of testing used: chemical testing, GC-MS, FTIR, and GC-FID.

1.2 Two (2) capsules - Not tested.

1.3 United States one-dollar ($1) bill - Not tested.

Remarks

All items will be returned to your department.

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(330) 659-4600
Robert.Jones@ohioattorneygeneral.gov

The reported results correspond only to the items tested unless it is otherwise denoted. BCI reserves the right to select the most appropriate methods and apply threshold testing, when applicable. A visual examination of tested evidence was conducted. Methods of testing performed may include microscopic examination, moisture analysis, hypergeometric sampling, logo identification, chemical testing, thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), gas chromatography-flame ionization detection (GC-FID), and liquid chromatography-ultraviolet spectroscopy (LC-UV).

Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2). Hypergeometric sampling may be specified in the findings above. The application of hypergeometric sampling establishes a 95% confidence level that at least ninety percent 90% of the units in the sample are as reported.

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

The gross weight was estimated in order to identify the items to be testing in accordance to the current submission policy, no further laboratory analysis was performed on items noted as "Not Tested"

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21.4 Example 4 (QNT vegetation)

To:	Ohio Police Department Detective Joe Jones 123 Columbus Road State, OH 12345	BCI Lab Number: Analysis Date: March 20, 2020	19-00004 Issue Date: March 21, 2020
Re:	Possession	Agency Case Number:	A100

Submitted on December 30, 2019 by Abby Schwaderer:

1. Plastic bag containing unknown substance (PR# 12345)

Findings

1. Vegetation- 2.28g +/- [Current MU (g)]- found to contain Marihuana (Cannabis) with a total THC content of 10.20% +/- [Calculated MU (%)]) calculated on a dry weight basis. Method(s) of testing used: microscopic examination, chemical testing, and LC-UV.

Remarks

All items will be returned to your department.

Robert Jones
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(330) 659-4600
Robert.Jones@ohioattorneygeneral.gov

The reported results correspond only to the items tested unless it is otherwise denoted. BCI reserves the right to select the most appropriate methods and apply threshold testing, when applicable. A visual examination of tested evidence was conducted. Methods of testing performed may include microscopic examination, moisture analysis, hypergeometric sampling, logo identification, chemical testing, thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), gas chromatography-flame ionization detection (GC-FID), and liquid chromatography-ultraviolet spectroscopy (LC-UV).

Where applicable, an estimate to the measurement uncertainty associated with the weight of an item and the overall quantitative process has been provided. The coverage probability in such instances is 95.45% (k=2). Hypergeometric sampling may be specified in the findings above. The application of hypergeometric sampling establishes a 95% confidence level that at least ninety percent (90%) of the units in the sample are as reported.

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

ORC 928.01 (J) "Delta-9 tetrahydrocannabinol" means the sum of the percentage by weight of tetrahydrocannabinolic acid multiplied by 0.877 plus the percentage by weight of delta-9 tetrahydrocannabinol. Tetrahydrocannabinol (THC) as reported by BCI is equivalent to this definition.

ORC 928.01 (F) "Hemp product" means any product, containing a delta-9 tetrahydrocannabinol concentration of not more than three-tenths per cent, that is made with hemp. Note that testing done at BCI can statutorily exclude certain products from being hemp products by virtue of having a tetrahydrocannabinol (THC) concentration of more than three-tenths per cent (>0.3%). Testing done at BCI cannot speak to the "made with hemp" requirement. A tetrahydrocannabinol (THC) content that is less than three-tenths per cent neither confirms nor denies that the product in question was made using hemp. Similarly, the definition of "hashish" involves the preparation of the resin of "marihuana". Testing done at BCI, regardless of THC content, will be unable to definitively state the source of a particular preparation of cannabis resin as from "marihuana" in lieu of the purification or synthetic modification of hempresin.

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21.5 Example 5 (QNT multiple samples tested per item)

To: Ohio Police Department
Detective Joe Jones
123 Columbus Road
State, OH 12345

BCI Lab Number: 19-00005

Analysis Date: March 20, 2020

Issue Date: March 21, 2020

Re: Possession Agency Case Number: A100

Submitted on December 30, 2019 by Abby Schwaderer:

1. Plastic bag containing unknown substance (PR# 12345)

Findings

1. Three (3) plastic bags containing vegetation 255.28 g +/- [Current Estimated MU (g)] found to contain Marihuana. Method(s) of testing used: microscopic examination, chemical testing and LC-UV. The Tetrahydrocannabinol (THC)content for each sample tested, determined on a dry weight basis, is listed in the table below:

Item	Tetrahydrocannabinol (THC)content	Uncertainty (%)
19-000051- Sample 1	10.20%	1.5
19-000051- Sample 2	9.62%	1.3
19-00005-1 Sample 3	8.64%	1.1

Remarks

All items will be returned to your department.

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The reported results correspond only to the items tested unless it is otherwise denoted. BCI reserves the right to select the most appropriate methods and apply threshold testing, when applicable. A visual examination of tested evidence was conducted. Methods of testing performed may include microscopic examination, moisture analysis, hypergeometric sampling, logo identification, chemical testing, thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), gas chromatography-flame ionization detection (GC-FID), and liquid chromatography-ultraviolet spectroscopy (LC-UV).

Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2). Hypergeometric sampling may be specified in the findings above. The application of hypergeometric sampling establishes a 95% confidence level that at least ninety percent 90% of the units in the sample are as reported.

Based on scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

ORC 928.01 (J) "Delta-9 tetrahydrocannabinol" means the sum of the percentage by weight of tetrahydrocannabinolic acid multiplied by 0.877 plus the percentage by weight of delta-9 tetrahydrocannabinol. Tetrahydrocannabinol (THC) as reported by BCI is equivalent to this definition.

ORC 928.01 (F) "Hemp product" means any product, containing a delta-9 tetrahydrocannabinol concentration of not more than three-tenths per cent, that is made with hemp. Note that testing done at BCI can statutorily exclude certain products from being hemp products by virtue of having a tetrahydrocannabinol (THC) concentration of more than three-tenths per cent (>0.3%). Testing done at BCI cannot speak to the "made with hemp" requirement. A tetrahydrocannabinol (THC) content that is less than three-tenths per cent neither confirms nor denies that the product in question was made using hemp. Similarly, the definition of "hashish" involves the preparation of the resin of "marihuana". Testing done at BCI, regardless of THC content, will be unable to definitively state the source of a particular preparation of cannabis resin as from "marihuana" in lieu of the purification or synthetic modification of hemp resin.

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Appendix I - Chemistry Notes Abbreviations

PACKAGING

Adh- = Adhesive
Bet = Blue evidence tape
Bln = Balloon
Brn = Brown
Bl = blue
Bld = suspected blood
Blk = black
BPB = brown paper bag
Cello = Cellophane
Cl = Clear
Ct = Clear tape
Cts = Clear tape sealed
Cont = Containing
Contr = Container
Dk = dark
Env = Envelope
Et = Evidence tape
Gp = glassine paper
HS = Heat Sealed
Kn = Knotted
Lg = Large
Lt = light
Man = Manila
Me = Manila envelope
Med = Medium
Mkd = Marked
Pap = Paper
PB = Paper Bag
Pkg = Package
Pkt = Packet
Pl = Plastic
Plb = Plastic bag
Ret = Red evidence tape
Rets = Red evidence tape sealed
Rec'd = Received
Rx = Prescription
Sand = Sandwich
Sld = Sealed
SM = Small
Sme = Small manila envelope
Sub = Submitted
Un-sld = Unsealed
Wht = White
Wpb = White paper bag
Yet = Yellow evidence tape
Yets = Yellow evidence tape sealed
Zlb = zip loc bag

EXAMINATION:

Aq = Aqueous
ABE or ABx = Acid/Base Extract with
Alq = aliquot
AX = Acid Extraction
AXW = Acid Extract With
Bx = base extraction
BXW = Basic Extract With
BSB = Background Subtraction
Bkg = Background
Brn = Brown
CI,CII = Schedule 1, Schedule 2 etc..
Cap = Capsule
Cig = Cigarette
Cmpd = compound
Conc = Concentrated
Cont'd = continued
DDR I= Dried down reconstituted in
DF = Dilution Factor
DNU = Data Not Used
Eff = Effervescence
EV = Evidence
Ext = Extraction
Frag = Fragment
GW = Gross Weight
Hr = Hand rolled
Hyper-G, HGS = Hypergeometric Sampling
ID = Identification
Ind = Indicated
Indiv = Individually
Inj = injection
Insuff = Insufficient
IS= Internal Standard
Liq = Liquid
LP = Latent Prints
Lt = Light
LOD = limit of detection
LOQ = limit of quantitation
LLOQ =Lower limit of quantitation
ULOQ =Upper limit of quantitation
Mat = Material
MC = Moisture Content
Micro = Microscopy
MT = empty
N = Population Size
NA = No Analysis
Neg-, (-) = Negative
NT = Not tested
NW = Net Weight
Pc = Piece
Pl = Plastic
Pos, (+) = Positive
Pow = Powder
Ppt = Precipitate
Psw = post sampling weight
PW = Population weight
R = Sample Size
rX# = Run, where * # = number
RRT = Relative Retention Time
Res = Residue
RT = Retention Time
Rxn = reaction
Sat = Saturated
Sd = Smoking Device
Sq = Square
Subt = Substance
Sw = Swab
Tb or tab = Tablet
UD = unit dose
Veg = Vegetable, Vegetation
Vol = volume
# WE = number of weighing events
W/O = Without
Wt = Weight
X, 2X = Times one, Times two, etc.

INSTRUMENTATION:

~~ATR = Attenuated Total Reflectance~~
FID = Flame Ionization Detector
FTIR = Fourier Transform Infrared Spectrometer
~~GC = Gas Chromatography~~
~~LC = Liquid Chromatography~~
~~MS = Mass Spectrometer~~
~~TLC = Thin Layer Chromatography~~
DAD = Diode Array Detector
PDA = Photo Diode Array
MA = Moisture Analyzer
~~QNT = Quant~~
S/Z = Stereo zoom microscope

REAGENTS:

AIP = Acidified Iodoplatinate
Co Thio = Cobalt Thiocyanate
H+ = Acid
Hex = Hexane
p-DMB or p-DMAB = para-Dimethylaminobenzaldehyde
Sod carb = sodium carbonate
EtAC = Ethyl Acetate
MP = Mobile Phase

DRUGS/RESULTS:

APAP = Acetaminophen
Coc, Coke = Cocaine
CBC = Cannabichromene
CBCA = Cannabichromenic acid
CBD = Cannabidiol
CBDA = Cannabidiolic acid
CBG = Cannabigerol
CBGA = Cannabigerolic acid
CBL= Cannabicyclol
CBLA = Cannabicyclolic acid
CBN = Cannabinol
CBNA = Cannabinolic acid
CBDV = Cannabidivarin
D8 = Delta-8-THC
D9 = Delta-9-THC
IBU = Ibuprofen
ISFA = Insufficient Sample for Analysis
ISFI = ~~Insufficient~~ *Inconclusive* Sample for Identification
INSFQA = Insufficient for Quantitative analysis
NCC = No color change
NCSF = No Controlled Substance Found
P/E = Pseudoephedrine/ephedrine
THC = Tetrahydrocannibinol
THCA = Tetrahydrocannibinolic acid

REFERENCES:

DIB = Drug Identification Bible
Drugs = Drugs.com Reference
IDDA = Instrumental Data for Drug Analysis
NIST = National Institute of Science & Technology
PDR = Physicians Desk Reference
PTOX = Pfleger Toxicology Library

Appendix II – Instrument Analysis Methods

The following methods are approved for use in the analysis of evidentiary samples commonly encountered by the Chemistry Unit. Each method specifies recommended target(s). As situations warrant, an alternative method, or deviation from the described method is permissible. Method used and any deviation from the method, as described below, must be approved by management and recorded in the case examination documentation.

Although not considered a critical parameter, injection port split value is currently set as a 50:1 ratio for most methods unless otherwise noted in the method charts. Modifiers to the method name represent a change in the split ratio of the method. "-30S" represents a 30:1 split "-10S" represents a 10:1 split. All methods will be run with a 1 µl injection volume unless noted otherwise. Modifiers will be indicated in the examination documentation. (i.e. COC212-10S, GEN130-10S, LoB110-10S or OPI212-30S for splits or -2 for alternative injection volume).

Although the Gas Chromatograph methods have been separated based on instrument type, these validated methods can be used on any Gas Chromatograph of the appropriate length and non-polar stationary phase column, unless indicated otherwise below. The suggested solvent delay is listed for some methods, however other delays are permitted at the analyst's discretion.

GC/MS Methods Using Helium (30 Meter Column)

Method	Typical Use	Mass Range	Run Time (min)	Temperature / hold (min)	Ramp	Hold (min)	Solvent Delay (min)	Gain Factor	Scan Speed	Threshold
ClanLab	Separate Ephedrine from PSE; medium or high polarity columns only	40-400	11	100°C/ 2 min	20°C/min - 120°C 4°C/min - 140°C 30°C/min - 200 °C	- - 1	3	1	N=2	100
ClHy420	Chloral hydrate, dichloralphenazone	20-400	20	40°C / 4 min	10°C/min - 95°C 50°C/min – 270°C	3 4	3	1	N=2	100
Coc209	Cocaine	40-400 or 500	9	200°C /-	20°C/min - 280 °C	5	2.5	1	N=2	100
Coc212	Cocaine, Opiates	40-400 or 500	12	200°C / -	20°C/min - 280°C	8	2.5	1	N=2	100
COC215	Opiate mixtures that contain tramadol, cocaine and/or fentanyl	40-400 or 500	15	200°C /-	20°C/min - 280°C	11	2.5	1	N=2	100
Gen130	General Screen	29-500	30	100°C / 2 min	20°C/min - 300°C	18	3	1	N=2	100
Gen170	General Screen	29-500	70	100°C / 2 min	4°C/min - 300°C	18	3	1	N=2	100
Gen220	General Screen for late eluting compounds	40-500	20	200°C /-	20°C/min -280 °C	16	2.5	1	N=2	100
GHB510	GHB	20-500	10.33	50°C / 1 min	30°C/min - 180°C	5	2.5	1	N=2	100
GLY440	Ethylene glycol	10-100	9	40°C / 1 min	10°C/min - 70°C	5	2	1	N=2	100
HiB230	Steroids	40-500	30	200°C /-	20°C/min - 300°C	25	2.5	1	N=2	100

Method	**Typical Use**	**Mass Range**	**Run Time (min)**	**Temperature / hold (min)**	**Ramp**	**Hold (min)**	**Solvent Delay (min)**	**Gain Factor**	**Scan Speed**	**Threshold**
HT216	Separate Hydrocodone and Delta-9-THC, Separate CBC and CBD	40-400 or 500	16	200°C / 2 min	5°C/min - 240°C	6	2.5	1	N=2	100
IoD405	Iodine	40-400 or 500	5.2	40°C / 1 min	50°C/min - 200°C	1	2.5	1	N=2	100
ISO125	Separate Cathine and PPA	40-400	9	125°C /-	-	-	2.5	1	N=2	100
LoB110	Methamphetamine, MDMA	40-400 or 500	10	100°C /-	25°C/min - 250°C	4	2	1	N=2	100
LSD218	LSD 5:1 Split (default)	28-400	18	200°C /-	20°C/min - 280°C	14	10	1	N=2	100
OPI212	Opiates (avoids APAP)	40-400 or 500	12	200°C /-	20°C/min - 280°C	8	3.75	1	N=2	100
OPI215	Opiates (avoids APAP and detects noscapine)	40-400 or 500	15	200°C /-	20°C/min - 280°C	11	3.75	1	N=2	100
OPI515	Opiates	40-400	15	50°C / 0.5 min	50°C/min -200°C 20°C/min - 280°C 20°C/min – 300°C	0 3 3.50	7.25	1	N=2	100
OPI515- 0S	Split-less Opiate Method (suspected fentanyl-related compound in small amounts)	40-400	15	50°C / 0.5 min	50°C/min -200°C 20°C/min - 280°C 20°C/min – 300°C	0 3 3.50	7.25	1	N=2	100
Pho708	Phosphorus	40-400	8	70°C / 2 min	20°C/min - 190°C	-	2	1	N=2	100
QDS	General Screen	29-500	19.8	100°C / 2 min	30°C/min - 305°C	11	3	1	N=2	100

GC/MS Methods Using Helium (20 Meter Column)

Method	**Typical Use**	**Mass Range**	**Run Time (min)**	**Temperature / hold (min)**	**Ramp**	**Hold (min)**	**Solvent Delay (min)**	**Flow Ramp**	**Gain Factor**	**Scan Speed**	**Threshold**
ClanLab- 20m	Separate Ephedrine from PSE; medium or high polarity columns only	29-500	6.25	100°C/ 1 min	40°C/min - 120°C 8°C/min - 140°C 30°C/min - 200 °C	- - 0.25	1.6		1	N=2	100
Gen115-20m	General Screen	29-600	15	100°C / 1 min	40 ° /min – 300°C (40° C/min – 315°C)	1 (7.625)	1.5	1.2 mL/min for 8 min; ramp 10 mL/min to a flow of 2mL/min	1	N=2	100
OPI210-20m	Opiates	40 - 400 or 500	10	200°C /-	32°C/min - 280°C	7.5	1.6		1	N=2	100

GC/MS Methods Using Hydrogen (20 Meter Column)

Method	**Typical Use**	**Mass Range**	**Run Time (min)**	**Temperature / hold (min)**	**Ramp**	**Hold (min)**	**Solvent Delay (min)**	**Flow Ramp**	**Gain Factor**	**Scan Speed**	**Threshold**
Genscreen-H2	General Screen	29 - 500	21	70°C/ 1.0 min	40°C/min - 170°C 12°C/min - 315°C	0.2 5.217	~~2.3~~ *2.0*		2	N=1	100
SHT210	Opiates, Cannabinoids	29 - 500	10	200° C /-	25°C/min – 275°C/1.575 40°C/min – 310°C	1.575 4.55	0.7	0.9 ml/min	2	N=1	100

GC/FID Methods Using Helium (15 Meter Column)

Method	Typical Use	Run Time (min)	Temperature / hold	Ramp	Hold (min)	Solvent Delay (min)	Split
BGScreen	General Screen; 15 m column	10	150°C / 1 min	25°C/min - 250°C 50°C/min - 300°C	- -	1	30:1
BGScreenHB	High boilers and Sterioids; 15 m column	12	220°C /-	10°C/min - 320°C	2	1	30:1
BGScreen(7890)	General Screen; 15 m column	10	150°C / 1 min	50°C/min - 300°C		1	30:1
ClanLab	Separate Ephedrine from PSE; medium or high polarity columns only	11	100°C/ 2 min	20°C/min to 120°C 4°C/min to 140°C 30°C/min to 200 °C	- - 1	3	30:1
Gen130	General Screen	30	100°C / 2 min	20°C/min - 300°C	18	3	30:1
FID175	Low boilers	6	175°C/ 2.5 min	30°C/min - 280°C	-		
FID250	Mid-range boilers	5	250°C / 5 min	-	-		
FID300	High boilers	6	280°C / -	20°C/min - 310°C	4.5		
FID_Screen	General Screen	12	175°C / 2.5 min	30°C/min - 280°C 20°C/min - 310°C	- 4.5		
Steriod_Screen	Steroids and High boilers	20	175°C / 2.5 min	30°C/min - 280°C 20°C/min - 310°C	- 12.5		
LondonScreen	General Screen; 15 m column	12.33	150°C / 2.5 min	30°C/min - 280°C 20°C/min - 310°C	- 4	0.85	30:1
LondonScreenHB	Steroids and High boilers; 15 m column	13.83	225°C / 2.5 min	30°C/min - 280°C 20°C/min - 310°C	- 8	0.85	30:1
Screen	General Screen	10	175°C / 2.5 min	30°C/min - 280°C 20°C/min - 310°C	- 0.5		
ISO175	Amphetamine, Phentermine, Methamphetamine	4	175°C /-	-	-	2.5	
ISO250	Opiates, Cocaine	4	250°C /-	-	-		
ISO300	Alprazolam, LSD	4	300°C /-	-	-		

FTIR Methods

Method	Minimum Scans	Minimum Background Scans	Resolution	Wave Number
Thermo Nicolet iS5	32	32	4	4000-400
Thermo Nicolet 4700	32	32	4	4000-500
JChem	16	16	4	4000-400
Nicolet iS5	16	16	4	4000-450
PE Spectrum 100	4	4	4	4000-450

Moisture Analyzer Methods

Drying Program	Standard
Drying Temperature	90°C
Switch-off criterion	5 (1mg / 140s)
Display Mode	% MC
Start Weight	1.5g
Start Weight Tolerance	25%

Note: During validation of the moisture analyzer method, it was noted that there was statistically relevant moisture loss after 48 hours. This statistical relevance however, did not and does not appear to transfer relevance to the quantitation of % THC in a sample.

Shimadzu LC/MS/UV

General Method Parameters:

Parameter	Value
Method	SHQNT-Hemp
Run Time	27.5min
Oven Temp	40°C
Autosampler Temperature	10°C
Flow	0.35 ml/min
Injection Volume	1ul
Calibration Levels (6)	8, 16, 32, 64, 96, 128 ug/ml
Quantitative method	Internal Standard
UV Wavelength	228nm
MS mode*	SIM

**SHQNT-Hemp_NoMS method has the same method parameters but does not utilize the MS*

Mobile Phase Gradient:

Mobile Phase: A (Aqueous): 0.1% (V/V) Formic Acid in High Purity HPLC Water B (Organic): 0.1% (V/V) Formic Acid in High Purity HPLC 50/50

Acetonitrile/Methanol

Time (min)	**%A**	**%B**
1.10	35	65
8.82	25	75
13.23	25	75
14.33	20	80
19.00	20	80
21.00	10	90
24.00	~~35~~ *5*	~~65~~ *95*
25.00	35	65
27.50	controller	stop

UV Conditions:

Parameter	**Value**
Lamp	Deuterium
Range (nm)	190-800
Cell Temperature	30°C
Slit width (nm)	8
Resolution	512
Bandwidth	4
Wavelengths evaluated	228
Acquisition Start	1.5 min
Acquisition Stop	25.00 min

~~MSD Conditions:~~

~~**Parameter**~~	~~**Value**~~
~~Ionization Mode~~	~~ESI Positive (Neutrals) ESI Negative (Acids)~~
~~Nebulizer Gas~~	~~1.5 L/min~~
~~Drying gas~~	~~Nitrogen at 15 L/min~~
~~Scan Range~~	~~SIM~~
~~Desolvation Line Temperature~~	~~300 °C~~
~~Heat Block Temperature~~	~~500 °C~~
~~Acquisition Start~~	~~2.50 min~~
~~Acquisition Stop~~	~~24.00 min~~

~~**Compound**~~	~~**SIM m/z ion**~~	~~**ESI Mode**~~
~~Androstenedione~~	~~287.2~~	~~Positive~~
~~Cannabidiol (CBD)~~	~~315.2~~	~~Positive~~
~~Cannabigerol (CBG)~~	~~317.2~~	~~Positive~~
~~Cannabidiolic Acid (CBDA)~~	~~357.2~~	~~Negative~~
~~Cannabigerolic Acid (CBGA)~~	~~359.2~~	~~Negative~~
~~Cannabinol (CBN)~~	~~311.2~~	~~Positive~~

~~Delta 9 Tetrahydrocannabinol (D9-THC)~~	~~315.2~~	~~Positive~~
~~Delta 8 Tetrahydrocannabinol (D8-THC)~~	~~315.2~~	~~Positive~~
~~Cannabichromene (CBC)~~	~~315.2~~	~~Positive~~
~~Cannabinolic Acid (CBNA)~~	~~353.2~~	~~Negative~~
~~Tetrahydrocannabinolic Acid (THCA)~~	~~357.2~~	~~Negative~~
~~Cannabichromenic Acid (CBCA)~~	~~357.2~~	~~Negative~~

~~SPEX Sample Prep 6875D freezer/mill Dual Chamber Cryogenic Grinder methods: Gummy method~~

~~Parameter~~	~~Value~~
~~Precool~~	~~10 minutes~~
~~Run time~~	~~2 minutes~~
~~Cool~~	~~1 minute~~
~~Cycles~~	~~4~~
~~Rate~~	~~10~~

~~Plant material method~~

~~Parameter~~	~~Value~~
~~Precool~~	~~5 minutes~~
~~Run Time~~	~~1 minute~~
~~Cool~~	~~1 minute~~
~~Cycles~~	~~3~~
~~Rate~~	~~10~~

Coffee Grinder method:

1. Add ~1.5 g raw sample to Coffee grinder cup and grind
2. Weigh ~0.2 g ground sample into ~~two~~ disposable test tube(s)
3. Add 5 mL isopropanol (IPA)

Vortex method:

1. Sample ~0.2 g raw sample into ~~two~~ disposable test tube(s)
2. Add 5 mL isopropanol (IPA)
3. Vortex on at least a speed setting of 7 for 15 minutes

Appendix III: Analog Letter

All drug chemistry reports which report an analog shall include a copy of the "Analog Letter for BCI Reports". This will make it available for download from OHLEG.

Appendix IV: NPS Committee Process

BCI Initiated Review

1. When a new psychoactive substance (NPS) is identified, the analyst will provide a spectrum and proposed structure/substance name to their local FSC via email. The BCI representatives to the NPS Statewide Review Committee will be cc'd on the email.
2. FSCs will distribute the information amongst the laboratory chemists to vote. BCI's designated representatives will provide the same information to the Statewide Committee for consideration.
3. Vote counts and any additional pertinent information provided by the scientists will be collected by the FSC (or designee) and will be forwarded to BCI's designated representatives within 7 days of the vote being sent out. The collected information will be evaluated and brought forward for discussion during the Statewide Committee meeting.
4. Each laboratory system will receive one vote during the Statewide Committee meeting. A unanimous decision amongst the participating laboratories is required to determine if the substance will be reported as a controlled substance.
5. A notification will be distributed to staff by BCI's designated representatives as to the outcome of the Committee's vote. BCI's internal list of new substances will be updated to reflect the outcome of the Statewide Committee vote.

Statewide Committee Initiated Review

1. When a new psychoactive substance (NPS) is identified by a non-BCI Ohio laboratory, the laboratory will provide the spectrum and proposed structure/substance name to the Statewide Committee for consideration.
2. BCI's designated representatives will receive the information and provide it to FSCs for distribution amongst the laboratory chemists to vote.
3. Vote counts collected by the FSCs and any additional pertinent information scientists would like included in the Statewide Committee evaluation, will be forwarded to BCI's designated representatives within 7 days of the vote being sent out.
4. During the Statewide Committee meeting, each laboratory system will receive one vote. A unanimous decision amongst the participating laboratories is required to determine if the substance will be reported as a controlled substance.
5. A notification will be distributed to staff by BCI's designated representatives as to the outcome of the Committee's vote. BCI's internal list of new substances will be updated to reflect the outcome of the Statewide Committee vote.

Appendix V: Laboratory Practices for Measurement Uncertainty

The laboratory reports measurement uncertainty (1) when values are reported for the weight of controlled substance evidence and (2) when values are reported for the % THC in controlled substance evidence.

The estimations for measurement uncertainty may change when any significant parameters that affect the measurement result are varied. Estimations are recalculated upon changes in the affected equipment, personnel, and/or measuring process; following significant changes in laboratory facility; and following recalibration/certification of the measuring equipment.

The purpose of this laboratory practice is to provide a detailed procedure for the on-going collection of data, performance of subsequent calculations, evaluation of the results and reporting.

Applicable Statutes:
Ohio Revised Code Chapters 2925 (Drug Offenses) and 928 (Hemp and Hemp Products) include all applicable legislation; included Section 2925.51 (Evidence in Drug Offense Cases) specifies laboratory analysis and reporting requirements.

Scope
The BCI Drug Chemistry laboratory section previously applied the NIST 8-Step Process for estimating and reporting Measurement Uncertainty. The results of this initial study identified parameters that require on-going evaluation in order to assess the measurement uncertainty for the weight of controlled substance evidence. The results of the initial reproducibility study are retained by the QA Manager.

A re-calculation of the measurement uncertainty is initiated whenever one of the following laboratory changes occurs: relocation to a new laboratory facility, newly qualified staff begins casework or measuring equipment is newly installed, re-calibrated/re-certified.

Estimating Measurement Uncertainty (MU) for Weight of a Controlled Substance
Initial Measurement Process Reproducibility studies included approximately 200 measurements collected from the analysts working Drug Chemistry casework.

On-going measurement data should be collected weekly by each of the qualified analysts in the Drug Chemistry section. When an analyst re-locates to a different laboratory bench or balance, additional data should be collected. *The data collected is*

combined and continuously used for each subsequent re-calculation and is removed when an analyst no longer conducts casework, separates from the workplace, or when a balance is placed out of service or discarded. All measurement data collected should be used if possible, however a minimum of 2 years' worth of data should be combined to complete the calculation, if applicable.

If a measurand is repackaged or a new measurand is created, all analysts working Drug Chemistry casework need to collect additional data.

Existing analysts receiving a new balance or relocating to a different laboratory bench (with either a new or previous balance) should collect measurement data over 5-10 business days. At least two measurements should be collected per day. *This should also be done if a new or repackaged measurand is created.*

Trainees should collect measurement data during the final month of their training period. At least two measurements should be collected per day *over 5-10 business days*.

i. Measurement Process Specifications

The weighing vessel is placed on a balance, the balance is tared, and the measurement assurance check standard is immediately added to the weighing vessel without removing it from the balance. A single measurement is made where the weight is determined through a functional relationship based on the amount of force on the balance. The functional relationship can be expressed by the mathematical equation:

$$y=mx + b +/- U$$

The weight is determined using balances with readabilities of 0.01 gram, 0.1 gram or 2 grams.

Range of Measurement: Minimum balance load to maximum balance load.
[y= the measurement result; m= slope or sensitivity of the measurement instrument linearity; x = the indication; b= bias; U = expanded uncertainty]

Each qualified analyst documents measurement data using the previously established measurement assurance check standard set on each brand and model of measuring equipment. The measurement assurance check standards were designed to mimic case evidence commonly encountered in the Drug Chemistry section. The measurement assurance check standards are secured in heat-sealed plastic containers to prevent loss. The contents include:

- Paper
- Vegetation
- Brown powdery substance

- Capsules (non-controlled)
- *Metal block*

Uncertainty Component	Factors Considered
Measuring Equipment	Multiple equipment of the same model
Staff	Multiple analysts, Training, Experience, Time of Day, day of week, Interruptions, Workload
Test Method	Differences in centering of measurement assurance check standard on the balance
Facility	Temperature Variation, Air flow, Vibration, Humidity, Static Electricity, Location of balances in the laboratory buildings

Type B Evaluations
A Type B Evaluation is a method of evaluation of uncertainty by means other than the statistical analysis of a series of observations.

Uncertainty components assessed as Type B evaluations:

- Display resolution – impact of rounding at zero and at load value displayed
- Balance calibration uncertainty
- Balance linearity
- Balance bias
- Bias

Display resolution – rounding at zero and at load:
All measuring equipment in use are "single range" balances.
The display resolution of the Shimadzu balances is ~0.01 gram at both zero and at load.
The display resolution of the Mettler balances is ~0. 1 gram at both zero and at load.
The display resolution of the Ohaus balances is ~2 grams at both zero and at load.
The purpose of this uncertainty component is to account for the rounding that is automatically performed by the balance. Because rounding automatically occurs at both zero (taring) and at load, two components will be included.

Equal to one half the display resolution = ½ of 0.01 g = 0.005g (Shimadzu balances)
Equal to one half the display resolution = ½ of 0.1 g = 0.05g (Mettler balances)
Equal to one half the display resolution = ½ of 2 g = 1g (Ohaus balances)

The measurement process reproducibility data may double-count variation separately

quantified for the display resolution at zero and at load. Any double counting will result in an overestimation of the measurement uncertainty and as such is considered acceptable by the laboratory.

Balance calibration uncertainty: A review of balance calibration certificates from the accredited external calibration laboratory identifies the greatest expanded uncertainty.

This uncertainty may be provided as a static value or as a function (line, parabola, etc.) of the load on the balance. In cases where expanded uncertainty is provided as a function, suitable weight brackets may be used, using the maximum value of the uncertainty function in the given weight bracket as the value used in calculating measurement uncertainty for any measured weight within the bracket.

Balance linearity: The laboratory procedures to confirm the continued calibration status and ensure proper functioning of the balances have pre-defined performance criteria across the useable range of the balances used for these measurements.

Balance bias: Calibrated mass reference standards are used to confirm the continue calibration status of the balances. This provides the laboratory with an ongoing evaluation of bias.

ii. Converting Quantities to Standard Uncertainties

The measurement unit is the gram expressed in decimal format, where the extended decimal value is later rounded to the appropriate number of significant figures.

Type A evaluation components:
Reproducibility data is recorded in the correct unit (gram) and format (decimal).

When a set of several repeated measurements have been recorded, the mean and the estimated standard deviation should be calculated for the data set.

Type B evaluation components:
Display resolution – rounding at zero and at load

This component is evaluated as a rectangular distribution- see appendix for calculation specifics.

Balance calibration uncertainty:
A review of the calibration certificates from the accredited external laboratory for all the balances used throughout the laboratory system identifies the greatest calibration expanded uncertainty.

Each certificate indicates this expanded uncertainty assumes a normal distribution, a coverage factor of k, where k correlates to the coverage probability of approximately 95% or 99%.

The uncertainty of the calibration certificate will be divided by the coverage factor, k, to arrive at a standard uncertainty- see appendix for calculation specifics.

Balance Linearity:
This component is evaluated as a rectangular distribution- see appendix for calculation specifics.

iii. Calculating the Combined Standard Uncertainty

This estimation assumes that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components. The combined standard uncertainty (uc) is the positive square root of the variance of all components combined.

$$u_c(y) = \sqrt{\sum (c_i u_i)^2}$$

The laboratory recognizes the Type A measurement process reproducibility component may double-count variation quantified individually by the Type B evaluation components. This double counting cannot be quantified. The laboratory recognizes any double counting will result in an acceptable over estimation of the measurement uncertainty.

iv. Expanding the Combined Standard Uncertainty by Coverage Factor (k)

To expand the calculated uncertainty to 95.45% coverage probability the coverage factor k = 2 is used. To increase coverage probability to 99.73%, the coverage factor k = 3 is used.

In cases where a limited number of measurements have been taken (where the number of degrees of freedom less is than 200), the appropriate value of k will be selected by ~~from a Student's T table~~ *using the related Microsoft Excel function (T.INV.2T).*

The Drug Chemistry Measurement Uncertainty Estimation Form uses a budget table to display the expanded uncertainty calculations. *The form for each balance type can be found in PowerDMS*. ~~This form is attached to this laboratory practice~~.

$$U = k * (u_c)$$

v. Measurement Uncertainty with a Calculated Weight

Under certain casework circumstances, it may be appropriate to estimate the overall weight of an item by extrapolating the average weight of a statistically significant number (R) of either individual samples or their packaging. This practice is referred to as a calculated weight and is generally reserved for very large submissions of visually consistent, individually packaged units. When a calculated weight is used, the uniformity of the collected weights becomes a critical contributing factor in the overall uncertainty. If there is a large amount of variability in the collected weights, it will result in a greater uncertainty associated with the total calculated weight. The relative standard deviation (RSD) is used to express the spread of values within a data set and can indicate whether a data set is expected to be normally distributed. To ensure that the assumption of uniformity is met, the RSD must be less than 10%. If the RSD is 10% or greater, then the calculated weight scheme must be abandoned for another mass determination method (ex. Subtraction method). Case circumstances may be considered by the analyst when deciding whether to use the calculated weight method, even if acceptance criteria are met.

Calculated weights and their associated uncertainties must be determined using the appropriate Calculated Weight and MU Worksheet for the respective method (either based on packaging weights or sample weights). Explanation sheets for each method are provided in the same workbook for more in-depth information on each step of calculations.

When calculating the weight based on packaging weights, the expanded uncertainty is determined as follows:

$$U = kN\sqrt{{u_c}^2 + \left(\frac{s}{R+1}\right)^2}$$

The expanded uncertainty for a calculated weight based on sample weights is determined using the equation:

$$U = kN\sqrt{{u_c}^2 + \left(\frac{s}{R}\right)^2}$$

Where k, u_c, and R have the same meanings as elsewhere in this document, N is the population size, and s is the standard deviation of individual weights of the "R" number of packaging or sample units.

~~An overall weight may be estimated via an extrapolation of the average weight of a statistically significant number of packaging units (the "R" number). This practice is generally reserved for very large submissions. A new, critical component to the overall uncertainty is introduced by this technique – the uniformity in weight of the empty~~

~~packaging units. Logically, if there is wide variability in the weight of the individual packaging units, there must be a greater uncertainty in a total weight calculated from extrapolating the average weight of a packaging unit.~~

~~In circumstances where a calculated weight is employed, the expanded uncertainty shall be determined as follows:~~

$$U = k\sqrt{n^2 u_c{}^2 + \sigma^2}$$

~~Where k and u_c have the same meanings as elsewhere in this document, n is the total number of measurement events needed to determine the total gross weight and the weight of the "R" number of packaging units, and "s" is the standard deviation of individual weights of the "R" number of packaging units.~~

vi. Evaluation of the Expanded Uncertainty

~~The laboratory evaluates the estimation of uncertainty to ensure the following:~~

- ~~The estimation is void of calculation errors~~
- ~~Ensure the estimation is within the limits of acceptable expanded uncertainty~~

~~The expanded uncertainty for a single measurement event should be less than half of the lowest critical weight (1 gram), per applicable ORC.~~

The laboratory evaluates the expanded uncertainty through a review performed by an independent scientist using the guide provided in the LF-Chem-MU Estimation form. This ensures that all applicable data has been entered and verified, the estimation is void of calculation errors and is suitable for its intended use.

vii. Reporting the Uncertainty

Report structure/content is controlled by the LIMS Drug Chemistry Matrix, ensuring consistent Drug Chemistry reporting throughout the BCI laboratory system. Upon approval of the new estimate, the updated measurement uncertainty estimates are configured into the LIMS Drug Chemistry Matrix. The LIMS Drug Chemistry Matrix is equipped with a pick list to insert the current Measurement Uncertainty estimations into a report draft for each balance type given either one or two measuring events.

The expanded measurement uncertainty value will be expressed as the quantity value, y, along with the expanded uncertainty, U, in the form y +/- U. The units of the measurement result and the expanded uncertainty will be the same and the values reported to the same significance. The reported uncertainty should not exceed 2 significant figures.

Reporting examples:

Off-white substance - 0.23g +/- [Current Estimated MU (gram)] - found to contain

Cocaine.
Vegetable matter - 1027.6g +/- [Current Estimated MU (gram)] - found to contain ~~Marihuana (~~Cannabis~~)~~.
Seven hundred twenty-eight (728) packets of powder - 20.68 gram (Calculated weight) +/- [Current Estimated MU (gram)] - found to contain *Fentanyl.*

The report should also include remarks regarding the coverage probability. The following coverage probability statement may be used:

"Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2)."

Rounding
If an overall weight is reported for an item containing two or more measuring events, then the uncertainty in each measurement must be accounted for in the reported overall uncertainty. In order to accurately calculate the combined standard uncertainty, a correlation coefficient that describes the relationship between the weighings must be determined. A conservative approach is to assume that the consecutive weighing events are completely positively correlated. Thus, the sum of each respective uncertainty should be reported, which will likely result in an overestimation of the uncertainty.

The analyst must refer to the current Drug Chemistry Measurement *Estimation* spreadsheet *for the balance type needed*, (located in the Labshare location) to ensure that the expanded numerical value is considered and appropriate rounding mechanisms are applied.

Estimating Measurement Uncertainty (MU) for THC Quantitation in cannabis related items

viii. Measurement Process Specifications

The batch containing the sample run will have two linear, 5-point (or greater) calibration curves, with internal standard. One calibration curve will equate THC peak response to THC concentration; the other calibration curve will equate THCA peak response to THCA concentration. Consider the example below for THC.

$$\frac{I_{Sample,THC}}{I_{IS}} = m_{cal,THC}\ xC_{Sample,THC} + b_{cal,THC}$$

Where:

$I_{Sample,THC}$ is the THC peak response of the sample

I_{IS} is the peak response of the internal standard

$m_{cal,THC}$ is the slope of the THC calibration curve

$C_{Sample,THC}$ is the THC concentration of the sample

$b_{cal,THC}$ is the "y-intercept" (peak response ratio - intercept) of the THC calibration curve

Solving for the Concentration of the sample:

$$C_{Sample,THC} = \frac{\frac{I_{Sample,THC}}{I_{IS}} - b_{cal,THC}}{m_{cal,THC}}$$

Using this, the %THC for the sample is:

$$\%THC = \frac{C_{Sample,THC}\left(\frac{\mu g}{mL}\right) x\ Dilution\ (mL)}{mass_{sample}\ (mg)\ x\ 1000\ \left(\frac{\mu g}{mg}\right)}\ x\ 100\%$$

Using the same technique with the pertinent THCA data will also generate a %THCA value. The total THC (the reported value) is given by:

Total THC = %THC + (0.877 x %THCA)(by definition, see ORC 928.01 (J)).

ix. Traceability

The traceability for this measurement process is established through the calibration of the balances, pipettes, temperature kits and moisture analyzers used to perform the measurement, the mass reference standard weight sets used to confirm the continued calibration status of the balance, and the use of known drug standards for calibration curve development.

- The calibration of the balances is performed annually by an external calibration laboratory that is accredited to ISO/IEC 17025, with a scope of accreditation that includes the specifics of the calibration performed.
- Continued balance calibration is confirmed weekly using certified weight sets. Weight sets are regularly recertified by an external laboratory that is accredited to ISO/IEC 17025, with a scope of accreditation that includes the specifications of the certification performed.
- Balance calibration and weight set certification information is maintained by the affected unit and/or the QA Manager.
- The calibration of the pipettes is performed annually by an external calibration

laboratory that is accredited to ISO/IEC 17025, with a scope of accreditation that includes the specifics of the calibration performed.

- Continued pipette calibration is confirmed monthly at the low and high end of the mechanical range.
- The calibration of the moisture analyzer is performed annually by an external calibration laboratory that is accredited to ISO/IEC 17025, with a scope of accreditation that includes both the weighing accuracy and temperature. The heating element will be checked monthly with a certified SmartCal Test Substance. The sodium sulfate decahydrate reference will be checked weekly. The temperature kits are used to perform a monthly check of the moisture analyzers. The temperature kits are calibrated every two years by an external vendor to ISO/IEC 17025, with a scope of accreditation that includes the temperature.
- Continued moisture analyzer calibration is confirmed monthly.
- NIST Traceable certified glassware is used in quantitative casework procedures.
- THC/THCA known drug standards records are retained by the affected unit.

x. Identification of Uncertainty Components

- U(prep) – uncertainty associated with sample and standard preparation
  - Uncertainty in standard concentration (x 2, THC and THCA)
  - Uncertainty in pipette volume in standard dilution
  - Uncertainty with volumetric flask in standard dilution
  - Uncertainty in sample mass
  - Uncertainty in pipette volume in sample extraction
  - Uncertainty in moisture content
- U(calib)- uncertainty associated with the calibration curve- generated from the linear regression data with each batch
- U(rep)- uncertainty associated with method repeatability- generated from pertinent section of the method validation data
- U(bias)-uncertainty associated with bias- generated from the spike recovery section of the method validation data

Staff:

- Analysts from each laboratory
- Training
- Experience
- Time of day, day of week, interruptions, workload

Test Method:

- Differences in centering of measurement assurance check standard on the balance

Facility:

- Temperature variation of laboratory and difference from the temperature during calibration
- Drafts – air flow in the laboratory area of the balance or moisture analyzer
- Location of measurement equipment in the laboratory buildings
- Vibration
- Humidity
- Static electricity

xi. Quantification of Uncertainty Components

Type A Evaluations

A Type A Evaluation is a method of statistical analysis regarding a series of observations.

The data results of the uncertainty components specified in the table below are evaluated to ensure the following criteria demonstrates fitness for purpose:

- The data collected is a normal, non-skewed distribution

- The data falls within 1, 2 and 3 standard deviations of the mean. The statistic that will be calculated is the standard deviation for each measurement assurance check standard on the balance.

Uncertainty Component	Factors Considered
Staff	Multiple analysts, Training, Experience, Time of Day, day of week, Interruptions, Workload
Test Method	Differences in centering of measurement assurance check standard on the balance
Facility	Temperature Variation, Air flow, Vibration, Humidity, Static Electricity, Location of balances in the laboratory buildings
U(Mass of Sample)	Multiple equipment of the same model

U(Moisture content)	Multiple equipment of the same model
U(Calib)	See below
U(Rep)	See below
U(Bias)	See below

U(calib): The standard uncertainty for the concentration of THC as a result of the calibration correlation being applied is given by the following relationship:

$$u(C_{Sample,THC}) = \frac{s_{residual}}{m_{cal,THC}}\sqrt{\frac{1}{p}+\frac{1}{n}+\frac{([C_{Sample,THC}]-\bar{x})^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$

Where:

$$s_{residual}=\sqrt{\frac{\sum_{i=1}^{n}[\frac{I_{i,THC}}{I_{IS}}-(m_{cal,THC}\times x_i + b_{cal,THC})]^2}{n-2}}$$

$I_{i,THC}$ is the THC peak response of the i-th calibration standard
I_{IS} is the peak response of the internal standard
$m_{cal,THC}$ is the slope of the THC calibration curve
$b_{cal,THC}$ is the "y-intercept" (peak response ratio - intercept) of the THC calibration curve
p is the number of repeated measurements for the given sample
n is the total number of standards used for plotting the calibration curve
$\bar{x}$ mean value of the concentrations of all the calibration standards
x_i concentration of the i-th calibration standard

Relative standard uncertainty u(calib)= $u(C_{Sample,THC})/C_{Sample,THC}$ This value is be calculated on a per-batch basis.

U(rep): A significant component of uncertainty is with the repeatability of measurements. This will be assessed based on repeated measurements of the same material collected in conjunction with the method validation.

This value will be reassessed as new analysts begin cannabis casework using data from their training mirroring the repeated measurements performed in the method

validation.

U(bias): Was modeled after the similar section in the Eurachem guide, and taken from spike recovery measurements in the method validation.

This value will be reassessed as new analysts begin cannabis casework using data from their training mirroring the spike recovery measurements performed in the method validation.

U(sample mass): Will be calculated using both Type A and Type B evaluations and will revert to the procedures outlined in section 11.2.

Type B Evaluations
A Type B Evaluation is a method of evaluation of uncertainty by means other than the statistical analysis of a series of observations.

U(Standard concentration): standard preparation
A review of the THC and THCA standard certificates identifies the concentration expressed with an expanded uncertainty at the 95.45% confidence interval. A coverage factor of k = 2 is used and incorporates uncertainties from the corrected purity*, solution preparation, homogeneity, and long- and short- term stability.
*Note: Corrected purity is a measure that corrects for residue on ignition, chromatographic purity, and either loss on drying or Karl Fisher water titration and residual solvents]

These values will be reassessed whenever a new reference material is utilized. If the standard's certified concentration uncertainty has not changed, then no recalculation of overall uncertainty is needed.

U(dilution):
Appropriate factors will be included from the certificates of analysis of the various glassware and pipettes used in the preparation of the calibration standards and the samples.

At present, the uncertainty from volumetric flasks has been determined insignificant (more than an order of magnitude less impact than other components).

These values will be reassessed whenever equipment is switched (different pipettes) or new calibration standards are prepared (using different volumetric flasks).

xii. Converting Quantities to Standard Uncertainties

Each individual quantity (for example, an uncertainty in pipette volume, measure in mL) will be rendered unitless by dividing the uncertainty by the volume utilized in the measurement.

xiii. Calculating the Combined Standard Uncertainty

This estimation assumes that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components. The combined standard uncertainty (u_c) is the positive square root of the variance of all components combined.

$$u_c(y) = \sqrt{\sum (c_i u_i)^2}$$

The laboratory recognizes the Type A measurement process reproducibility component may double-count variation quantified individually by the Type B evaluation components. This double counting cannot be quantified. The laboratory recognizes any double counting will result in an acceptable over estimation of the measurement uncertainty.

xiv. Expanding the Combined Standard Uncertainty by Coverage Factor (k)

To expand the calculated uncertainty to 95.45% coverage probability the coverage factor k = 2 is used. To increase coverage probability to 99.73%, the coverage factor k = 3 is used.

In cases where a limited number of measurements have been taken (where the number of degrees of freedom is less than 200), the appropriate value of k will be selected from a Student's T-table or using the related Microsoft Excel function (T.INV.2T).

The Drug Chemistry Measurement Uncertainty Estimation Form used a budget table to display the expanded uncertainty calculations. This form is attached to this laboratory practice.

$$U = k * (u_c)$$

xv. Evaluation of the Expanded Uncertainty

The laboratory evaluates the estimation of uncertainty to ensure the following:

- The estimation is void of calculation errors

- Ensure the estimation is within the limits of acceptable expanded uncertainty

The expanded uncertainty for a single measurement event should be less than 25% relative of the measured value.

xvi. Incorporation of MU into Quality Management System

The expanded measurement uncertainty will be updated in the following records to ensure the appropriate MU is reported:

- The Equipment Records associated with any updated calibrations performed
- Active Lab Forms associated with MU Calculations
- LIMS Matrix Panel options for MU

xvii. Reporting the Uncertainty

The expanded measurement uncertainty value will be expressed as the quantity value, y, along with the expanded relative uncertainty, U, in the form y +/- y*U. The units of the measurement result and the expanded uncertainty will be percent and the values reported to the same significance, using traditional rounding rules. The reported uncertainty should not exceed 2 significant figures.

The report should also include remarks regarding the coverage probability. The following coverage probability statement may be used:

"Where applicable, an estimate to the measurement uncertainty associated with the weight of an item has been provided. The coverage probability in such instances is 95.45% (k=2)."

xviii. References

a) Tomić, T., Uzorinac Nasipak, N. & Babić, S. Accred Qual Assur (2012) 17: 291. https://doi.org/10.1007/s00769-011-0872-0.
b) EURACHEM/CITAC Guide CG 4. "Quantifying Uncertainty in Analytical Measurement". Third edition. https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf

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ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty- ANNEX A. AL-PD-3062Ver 1.0 May 22, 2013.

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ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty- ANNEX C. AL-PD-3064 Ver 1.0 May 22, 2013.

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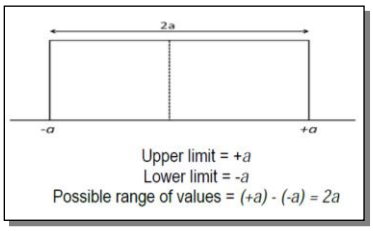
ENFSI-DWG Guidelines on Sampling of Illicit Drugs for Qualitative Analysis (2016). http://enfsi.eu/wp-content/uploads/2017/05/guidelines_on_sampling_of_illicit_drugs_for_qualitative_analysis_enfsi_dwg_2nd_edition.pdf

Drug Chemistry Calculations

Display resolution – rounding at zero and at load

This component is evaluated as a rectangular distribution:



Standard uncertainty for rectangular distribution is calculated by:

Standard uncertainty = a/√3

Outside limit = ½ the readability of the balance at zero
Examples:
Shimadzu (*Model UW4200H or UP4202X*) = ½ (0.01 g) = 0.005 g
Shimadzu standard uncertainty = 0.005 g/ √3 = 0.0028867513 g

Mettler (Model MS32001L or XS32001L) = ½ (0.1 g) = 0.05 g
Mettler standard uncertainty = 0.05 g/ √3 = 0.028867513 g

Ohaus (Model D50RQV) = ½ (2 g) = 1 g
Ohaus standard uncertainty = 1 g/ √3 = 0.577350269 g

Outside limit = ½ the readability of the balance at load
Examples:
Shimadzu (*Model UW4200H or UP4202X*) = ½ (0.01 g) = 0.005 g
Shimadzu standard uncertainty = 0.005 g/ √3 = 0.0028867513 g

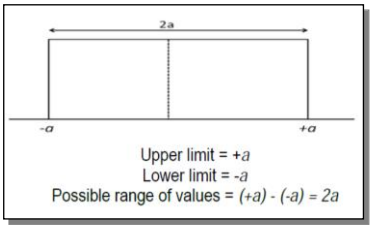
Mettler (Model MS32001L or XS32001L) = ½ (0.1 g) = 0.05 g
Mettler standard uncertainty = 0.05 g/ √3 = 0.028867513 g

Ohaus (Model D50RQV) = ½ (2 g) = 1 g
Ohaus standard uncertainty = 1 g/ √3 = 0.577350269 g

Balance calibration uncertainty:
The uncertainty of the calibration certificate will be divided by the coverage factor, k, to arrive at a standard uncertainty. For example:

Calibration Uncertainty = 0.23103490 g
Standard Uncertainty= 0.23103490 g / 2.00 = 0.11551745 g

Balance Linearity:
This component is evaluated as a rectangular distribution:



Outside limit
Examples:
Shimadzu (*Model UW4200H or UP4202X*) = +/- 0.02 g
Shimadzu standard uncertainty = 0.02 g/ √3 = 0.011547005 g

Mettler (Model MS32001L or XS32001L) = +/- 0.3 g
Mettler standard uncertainty = 0.3 g / √3 = 0.1732050807 g

Ohaus (Model D50RQV) = +/- 4 g

Ohaus standard uncertainty = 4 g / √3 = 2.30940107 g