Chemistry Methods Manual

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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 and gases, powders, plant material, and residue of any of the above. The choice of a method for the analysis of these materials will vary with each submission.

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 must 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 included in the laboratory report. Instead, a simple statement of “Not tested” is listed in the report, along with a clarification statement of “The gross weight may be estimated in order to identify the items to be testing in accordance to the current submission policy, in these instances no further laboratory analysis was performed on items.”

- 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.

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• Visual estimation only- No weight is taken; 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).
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. To this end, analysts will record in each case as part of the case examination documentation the lot number of the prepared reagent(s) 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, the open/sealed dates and packaging can be recorded as “Not Applicable” or “N/A”.

Packaging: A description of all packaging must be included to document the condition of the evidence upon receipt by the scientist.

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” or “N/A”.

Contents: A description of the relevant visual 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#). 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 was sealed must be documented.

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

2  Administration Policies for Drug Analysis

2.1  Introduction
The Ohio Revised Code lists all the substances controlled in the state of Ohio subject to amendment pursuant to section 3719.41, 3719.43, and 3719.44 of the Revised Code as well as OAC 4729.11-02.

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.

2.2  Positive identification
A minimum of two testing procedures are required for the identification of a controlled substance, as indicated in the table below (listed in no particular order). A single GC/MS injection serves as two testing procedures if both results are used to draw conclusions: GC retention time and mass spectrum.

Where applicable, instrument methods are selected as a result of preliminary testing indications, such as color tests or visual examination of the evidence. Approved instrument methods can be found in the appendix of this manual.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mushrooms</th>
<th>Tablets with complete markings</th>
<th>Suspected Cannabis products-post July 30, 2019</th>
<th>Suspected Cannabis vegetation post July 30, 2019</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Gen115-20m/Gen130/QDS</td>
<td>Logo ID</td>
<td>GC/MS screen</td>
<td>Modified Duquenois Levine or GC/MS</td>
<td>GC/MS (Gen115-20m / Gen130/Gen170/QDS)</td>
<td></td>
</tr>
<tr>
<td>(2) Weber or TLC or GC/MS</td>
<td>GC/MS or FTIR</td>
<td>LC/UV/MS</td>
<td>Microscopic Examination</td>
<td>N/A or FTIR or GC/MS or GC/FID or TLC</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td>LC/UV/MS</td>
<td></td>
</tr>
</tbody>
</table>

Specific analytical techniques are routinely performed for the identification of the following substances: additional testing may be conducted, at the discretion of the forensic scientist:

Mushrooms- if Weber’s testing indicates psilocyn, run on GC/MS to confirm & report out
results as “psilocyn”; if Weber’s testing is negative, run on GC/MS and if psilocyn is found, perform 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, derivatize the sample, run on GC/MS to confirm, and report out results as “Psilocybin”.
Opium- instrumental analysis identifying Morphine, Codeine, and at least two (2) of the following: Thebaine, Papaverine, Noscapine.

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

Analog and Pharmacophores-When a new substance is identified whose structure could be considered a potential analog or pharmacophore, the analyst provides a spectrum and proposed structure to the local FSC. The information will be distributed amongst the laboratory chemists. A unanimous vote amongst available scientists is required to make an inclusion of a substance in the list of identified analogs and pharmacophores routinely reported by BCI.

Suspected or indicated opiate, carfentanil or other fentanyl-related compound – An OPI method that incorporates a lower split and higher injection volume, (for example: OPI212-10S-2, OPI215-10S-2, OPI210-20m etc.) must be run after a general screen, when low level peaks and/or a critical ion to carfentanil or other fentanyl-related compounds are detected in areas of the GC/MS common to opioids, if no additional controlled substances were detected. The indicated presence of Carfentanil will be pursued using one of these OPI methods regardless of the presence of other controlled substances.

Insufficient for identification – It is acceptable to use a shorter method on the second instrument, as long as it incorporates a lower split and higher injection volume.

GHB – instrumental analysis using an alternative low boiling method, such as GHBS10, is acceptable.

2C-X or Mescaline- When a substance is identified as a 2C-X compound or Mescaline, the analyst must derivatize using an approved derivatization method (such as BSTFA).

Cannabis and Cannabis products after July 30, 2019 – Determination of the percent dry weight of tetrahydrocannabinol (THC) must be quantified (if sample amount permits) to report marihuana or hemp. Determination of the percent of THC must be quantified (if sample amounts permits) in cannabis products to determine if the cannabis product may qualify or is definitively not a hemp product. This shall be determined by running an approved quantitative method.

2.3 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

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must be an additional run using the initial vial run on a residue method/stronger split method or alternatively, an additional sample of the evidence may be prepared for another instrument run. The second instrument test must be run on a different instrument than the first instrument test.

<table>
<thead>
<tr>
<th>Testing Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) GC/MS</td>
</tr>
<tr>
<td>(Gen115-20m/Gen130/QDS)</td>
</tr>
<tr>
<td>(2) GC/MS or FTIR</td>
</tr>
<tr>
<td>(Gen115-20m/Gen130/QDS)</td>
</tr>
</tbody>
</table>

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 unknown vegetation or liquids, HiB230 may be used for the second instrument method.

Non-controlled pharmaceuticals with identifiable markings need not involve lengthy analysis unless there is evidence of tampering or reasons to suspect a counterfeit.

In multi-unit item cases where the items are observed to be similar, and results from testing the sampling plan number are negative, “No controlled substance” can be reported.

2.4 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 crack pipes, only one item needs to be tested).

2.5 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.

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.
2.6 Legal Limits Testing Approach
Cases containing multiple types of drugs should be worked to the highest criminal charge. This includes testing to bulk weight 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.

Forensic Scientists have discretion to 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 include color, shape, size, 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 shall consist of items which are similar in relevant visual characteristics.
4 Physical Identification of Tablets and Capsules

4.1 Introduction
The Physical Identification Procedure can be used on tablets 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 tentative identification of markings obtained on a commercial tablet and/or capsule 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.

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.

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.

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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.
7. [www.drugs.com](http://www.drugs.com)
8. [www.pharmer.org](http://www.pharmer.org)
9. [www.rxlist.com](http://www.rxlist.com)
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:
1. Make sure that the balance is level and there is no debris on it.
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 repaired 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 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 analysis with the following exceptions:
Samples involving trace or residual amounts of material do not require weight determination.

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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 contents 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 contents are 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.

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.

5.5.4 **Subtraction method (Two weighing events)**
1. Weigh samples and packaging together to determine gross weight.
2. Remove samples from packaging.
3. Weigh packaging only and subtract that amount from gross weight to get net weight of
sample.

5.7 Measurement Uncertainty
Reported controlled substance weights will include an estimation of the measurement
uncertainty (MU):
- The reported estimated MU will be that calculated for the balance or balance group
  on which the controlled substance weight was determined.
- 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.
- 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 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 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 on each day
of use to verify the instrument is working properly. Additionally, a monthly 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 there is no debris on it.
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 shall be analyzed daily 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 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 disciplines 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, consider taking two samples for testing purposes.
- 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.

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
Hybergeometric 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.
- This sampling plan is to be applied to each reported item, as necessary. Each additional item requires the sampling plan to be applied again.

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• 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). In regards to seeds, sample such that at least half of the population is preserved.

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.

The population will be subjected to hypergeometric sampling if:
• testing results identify a different substance than the markings indicate; or
• a legal threshold has not been met

Tablets or capsules identifiable through a reference source as negative for controlled substances: no additional testing is required.
Commerically 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 one or more of the sample units tests differently.

Clandestine tablets, powdery substances, paper, 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 in to 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.

On September 30, 2011, the Ohio Revised Code changed such that there is no longer a penalty difference between cocaine base and cocaine salt forms. With this in mind, there is no longer a need to distinguish between these forms at the state prosecution level. 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.

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

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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
- 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 (in its original form) 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.

### 7.4 References

4. Douglas M. Andrews, PhD, Professor of Statistics, Wittenberg University, Springfield, OH

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8 Cannabis Analysis

8.1 Introduction
Marihuana is defined in the state of Ohio under the Ohio Revised Code, 3719.01 (O) 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 to positively identify Marihuana, 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.

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8.3 Safety Considerations
A fungus, Aspergillus fumigates, may be encountered on decaying 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 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 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 are Cocaine base (Crack) and Phencyclidine (PCP).

As a matter of policy, BCI does not separate stalks and seeds from marihuana mixtures for purposes of determining the weight of the marihuana 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 Duquennois-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 plant material can be processed into other forms including compressed resin, extracts/oils, and various edible food products. 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 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 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 is needed to distinguish any of the acids in the table below 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.

<table>
<thead>
<tr>
<th>CBG</th>
<th>Cannabigerol</th>
<th>CBGA</th>
<th>Cannabigerolic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC</td>
<td>Cannabichromene</td>
<td>CBCA</td>
<td>Cannabichromenic acid</td>
</tr>
<tr>
<td>CBD</td>
<td>Cannabidiol</td>
<td>CBDA</td>
<td>Cannabidiolic acid</td>
</tr>
<tr>
<td>CBN</td>
<td>Cannabinol</td>
<td>CBNA</td>
<td>Cannabinolic acid</td>
</tr>
<tr>
<td>CBL</td>
<td>Cannabicyclol</td>
<td>CBLA</td>
<td>Cannabicyclolic acid</td>
</tr>
<tr>
<td>THC</td>
<td>Delta-9 tetrahydrocannabinol</td>
<td>THCA</td>
<td>Tetrahydrocannabinol acid</td>
</tr>
<tr>
<td>Δ8 THC</td>
<td>Delta-8 tetrahydrocannabinol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Figure 1 Relationship Between Cannabinoids](https://keytocannabis.com/blogs/cannabis/the-medical-benefits-of-cannabis-compounds)
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 prior to 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 percent.

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.

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

- 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.

8.6.3 Edibles (i.e. products for oral consumption)
Products for oral consumption may be processed with plant material or marihuana extracts. Due to the processing of these products, any visual examination may not indicate the presence of plant material products. A representative sample is prepared in accordance to the THC quantitative procedure.

8.6.4 Residues
Residue, such as those left behind on smoking devices, may not demonstrate any physical characteristics of 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
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.

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 involves 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.

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:

\[ \text{ug tincture} = \left( \frac{1250 \text{ ug THC}}{X \text{ ug THC}} \right) \times \left( \frac{100 \text{ ug tincture}}{100 \text{ ug THC}} \right) \]

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 and a 1:10 dilution will be performed.

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 will be within +/- 20 % of the calculated value.
- The quantitative value of the check standards will be within +/-15 % of the calculated value.
- The negative control will be considered acceptable if it contains less than 10% of the LOQ 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 weigh boat. Record weight
5. Using the same tip, repeat 5 times capturing the weight each time
6. Calculate the actual volume dispensed (avg wt. of vol * density @ 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. 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 upper limit of the calibration curve. If the sample
exceeds the upper limit of the calibration curve, the sample must be diluted and reran.

Quantitative values that fall below the lower limit of quantitation (LLOQ) will be reported as
less than 0.1%, if appropriate.

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:

\[
\% \text{ agreement} = \left( \frac{\text{Highest value} - \text{Lowest Value}}{\text{average of both}} \right) \times 100
\]

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

9.5 Cannabinoid Mix

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A quality control mix for the LC/UV/MS is designed to be run on a quantitative method. This QC mix includes Cannabidiol (CBD), Delta-8 THC, Delta-9 THC, Cannabinol (CBN), Cannabichromene (CBC), Cannabigerol (CBG), Cannabidiolic Acid (CBDA), Cannabinolic Acid (CBNA), Cannabigerolic Acid (CBGA), Cannabichromenic Acid (CBCA), 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. Proper lab attire must be worn.

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 an 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.

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.

11.3.3 Acidic or Basic extraction
1. Add 2 or 3 mL of basic or acidic substance to a small amount of powder and mix.
2. Add a small amount 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. 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.

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• 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.
• Cathine and Cathinone break 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 appropriate method on SPEX Sample Prep 6875D freezer/mill Dual Chamber Cryogenic Grinder (See Appendix II). (Note – 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).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Amount of homogenized sample (g)</th>
<th>Volume of solvent (mL)</th>
<th>Sample volume for vial (mL)</th>
<th>IS Spike (640µg/mL) (mL)</th>
<th>Solvent volume (mL)</th>
<th>Final Volume (mL)</th>
<th>Final dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marihuana</td>
<td>0.2</td>
<td>5</td>
<td>0.020</td>
<td>0.100</td>
<td>1.880</td>
<td>2.000</td>
<td>100</td>
</tr>
<tr>
<td>Hash/Hashish</td>
<td>0.05</td>
<td>5</td>
<td>0.020</td>
<td>0.100</td>
<td>1.880</td>
<td>2.000</td>
<td>100</td>
</tr>
<tr>
<td>Food</td>
<td>1.0</td>
<td>5</td>
<td>0.200</td>
<td>0.100</td>
<td>1.700</td>
<td>2.000</td>
<td>10</td>
</tr>
<tr>
<td>General</td>
<td>0.2</td>
<td>5</td>
<td>0.020</td>
<td>0.100</td>
<td>1.880</td>
<td>2.000</td>
<td>100</td>
</tr>
</tbody>
</table>

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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 dH2O 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 distilled water 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.
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.

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

12.4 Preparations
- 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 °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.

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12.6 References
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
Screening 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 of cannabis and mushroom testing.

13.3 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
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. For these reasons, precautions should be taken when performing these tests.

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, 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

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

Stability: Up to six months, performance check monthly with reference material after three months. Performance checks will be documented on reagent preparation log sheets as well as the traceability log.

Problems: None

### 14.3.8 Mecke

**Formulation:**
0.25 g selenious acid
25 ml 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:**
5 g 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

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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 CHCl₃ 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 Silver Nitrate
Formulation: 5% w/v in water (1g in 20 mL, w/v)

Tests for:
- Ascorbic acid  Black precipitate with ascorbic acid
- Chloride ion  White precipitate with chloride ion
- Iodide ion  Yellow precipitate with iodide ion

Stability: Stable
Problems: None

14.3.12 Modified Sodium Nitroprusside (Simon’s Test)
Formulation:
Part A: 0.5g Sodium nitroprusside in 50 mL water, 1 mL acetaldeyde
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

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Problems: Few false positives

14.3.13 Sulfuric Acid
Formulation: Concentrated sulfuric acid

Result:
Tetracyclines Orange
Amitriptyline Purple

Stability: Stable
Problems: None

14.3.14 Wintergreen (Methyl Benzoate)
Formulation:
Methanol saturated with sodium hydroxide or potassium hydroxide.

Tests for:
• Cocaine Wintergreen odor with the addition of the solution upon drying

Stability: Must be tightly capped to avoid evaporation
Problems: Water will interfere with the reaction

14.3.15 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: Use within day of preparation

14.4 Standards and Controls
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.

14.5 Procedure
1. 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

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 # 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.

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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

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

### 15.6 References
1. BCI Drug Chemistry Training Manual
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. Gen115-20m, Gen 130, LondonScreen, BGScreen (7890) or or BGScreen) or a ClanLab method (ClanLab, ClanLab-20m, etc.). 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, alternative injection volume, instrument name and any reference material's manufacturer and lot number. 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 logbook 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 not exceed 2µl.

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• 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:
  o Use extraction reagents, chemicals, derivatizing agents, and/or solvent that corresponds to sample setup
  o Run using the same temperature range as the sample
  o Run using the same or lower split ratio as the sample
  o Prior to each instrumental test for residue
  o When obtaining retention time data, before both the reference material and the sample(s)
  o Before the first sample of each item
  o After the tenth item when running multiple samples (using the same method) in an item
  o After the OPI515-0S method is run

• 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 within +/- 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.

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.
17  Liquid Chromatography/Ultraviolet-Visible detection (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 that the instrument is used for quantitative analysis. The chromatogram is examined for relative retention time, peak shape, resolution, reproducibility, and concentration. This will be done on a daily basis, 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 on 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 Limit Of Quantitation (LOQ); 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.

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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 tube previously used to analyze THC containing samples that is soaked in 2mL of appropriate solvent. If this fails there must be sufficient measures taken to ensure the tubes 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 - 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. Blanks will be run at least every ten case samples. 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 LOQ≥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. Batch is acceptable with one passing positive control.

- **Check standards** – Every fifteen ten 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 approximately 20ug/mL: Cannabidiol (CBD), Delta-8 THC, Delta-9 THC, Cannabinol (CBN), Cannabichromene (CBC), Cannabigerol (CBG), Cannabidiolic Acid (CBDa), Cannabinolic Acid (CBNa), Cannabigerolic Acid (CBGa), Cannabichromenic Acid (CBCa), and Tetrahydrocannabinoic Acid (THCA).
  - The results are acceptable if the following criteria are met:

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• 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 conc exceeds high point (both THC and THCA above high point of curve) analyst will dilute. If concentration is less than LOQ analyst will concentrate (smaller dilution) unless calculated total THC with the analyte on the curve is greater than 5%. 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 carried through the same extraction / dilution scheme) 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%, 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

• **Batch**: The samples run continuously without change to the system (calibrators, mobile phase, etc.) and the check standards continue to meet criteria
### Table 1 Batch QC Sequence Example

<table>
<thead>
<tr>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>System blank</td>
</tr>
<tr>
<td>Calibration Curve</td>
</tr>
<tr>
<td>Check Standard</td>
</tr>
<tr>
<td>Positive control</td>
</tr>
<tr>
<td>Negative control</td>
</tr>
<tr>
<td>Method blank</td>
</tr>
<tr>
<td>Blank 1</td>
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<tr>
<td>Sample 1</td>
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<tr>
<td>Blank 2</td>
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<td>Sample 2</td>
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<tr>
<td>Blank 15</td>
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<tr>
<td>Sample 15</td>
</tr>
<tr>
<td>Check Standard</td>
</tr>
<tr>
<td>Calibration curve</td>
</tr>
</tbody>
</table>

Note – if additional samples will be run after this ending calibration curve, an additional check standard will be run.

### 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 5%, 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 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) 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 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 insure the instrument is operating properly, the IR will be calibrated monthly using polystyrene or an internal validation method. The validation will be recorded in the IR logbook 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 logbook will be kept on 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 (if a published source is used, it should be recorded in the examination documentation).

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 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
Mass Spectrometry

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 auto-tuned the first working day of the workweek. A known drug mix using the general drug screen method to insure the instrument is operating properly will be run 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 spectrometer 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, injector insert changes, and oil changes. The date and chemist performing the tasks will be logged.

Safety Considerations
Standard laboratory practices

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

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, alternative injection volume, instrument name, reference material manufacturer and lot number and date will be recorded on the electronic copy.

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• 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 within 0.050 min of one another.
• A logbook will be kept on 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:
  o Use extraction reagents, chemicals, derivatizing agents, and/or solvent that corresponds to sample setup
  o Run using the same temperature range as the sample
  o Run using the same or lower split ratio as the sample
  o Prior to each instrumental test for residue
  o When obtaining retention time data, a blank shall be run before both the reference material(s) and the sample(s).
  o Before the first sample of each item
  o After the tenth case sample when running multiple samples (using the same method) in an item.
  o After the OPI515-05 method is run
• 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 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

Unknown substance spectra must be compared to a primary reference material, a published literature source or library source (if a published source is used, it must be recorded in the examination documentation). 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 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
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 ORC. 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.

20.2.2 Quantitative results
Quantitative values will be reported in accordance with any measurement uncertainty requirements as determined by accreditation program requirements.

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 statute “not more than three tenths percent”, rounding considerations should be reported as follows:

- THC level below Limit of Quantitation (LOQ) – Less than 0.1%
- THC level between 0.1% and 0.3% - Hemp
- THC level between 0.301 and 0.500 – Cannabis, with a Tetrahydrocannabinol content of “X”
- THC level above 0.501% - Marihuana

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.

- Gross weight estimates are documented in the lab notes to determine the amount of sample needed to test to charge limits. The gross weight is not included in laboratory reports.
- 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%.”

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 ORC 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 drive 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 it is present in a greater amount than heroin as measured by peak area on the GC/MS TIC. 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
This document is uncontrolled if viewed outside the BCI document management system.
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 report:

**Marihuana**
Vegetation- 0.67 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. Determined using chemical testing, microscopic examination, instrumental analysis and quantitative analysis.

**Edible product**
Brownie- 7.95 g +/- [Current Estimated MU (g)] - found to contain a Tetrahydrocannabinol (THC) content of 15.4% +/- [Calculated MU (%)] Determined using chemical testing, instrumental analysis and quantitative analysis.

**Hemp / Product below Limit of Quantitation (LOQ)**
Vegetation – 225.75g +/- [Current Estimated MU (g)] found to contain Hemp (a non-controlled substance) with a Tetrahydrocannabinol (THC) content of less than 0.1% calculated on a dry weight basis. Determined using chemical testing, microscopic examination, instrumental analysis and quantitative analysis.

**Cocaine powder**
Off-white substance - 0.23 g +/- [Current Estimated MU (g)] - found to contain Cocaine. Determined using chemical testing and instrumental analysis.

**Cocaine base** - As of September 30, 2011, “cocaine base” was removed from the Ohio Revised Code as a named substance. All variations of cocaine are now reported as Cocaine. Off-white substance - 1.89 g +/- [Current Estimated MU (g)] - found to contain Cocaine. Determined using chemical testing and instrumental analysis.

If asked to report out as cocaine base, use the following wording:
Off-white substance - 1.89 g +/- [Current Estimated MU (g)] - found to contain Cocaine base (Crack Cocaine)- Determined using chemical testing and instrumental analysis.

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

“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.”

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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- Determined using chemical testing and instrumental analysis.

Clandestine tablets
Five (5) orange tablets – 1.52 g +/- [Current Estimated MU (g)] - found to contain 3,4-Methylenedioxymethamphetamine (MDMA)- Determined using chemical testing and instrumental analysis.

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- Determined using logo identification and instrumental analysis.

Controlled Tablets or Capsules identifiable via reference source (not tested) Twelve (12) orange tablets marked “N8”<word imprint – Markings indicate Buprenorphine. Not confirmed. Determined using 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 - Determined using chemical testing and instrumental analysis.

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 -determined using logo identification and instrumental analysis.

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- Determined using 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 - Determined using chemical testing and instrumental analysis.

Heroin
Twenty-eight (28) packets of powder - 0.68 g (Calculated weight) +/- [Calculated MU (g)] - found to contain Heroin- Determined using hypergeometric sampling, chemical testing and instrumental analysis.

One (1) balloon with brown solid substance - 0.27 +/- [Current Estimated MU (g)] - found to contain Heroin.

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contain Heroin- Determined using chemical testing and instrumental analysis.

**Mushrooms (Weber’s positive and GC/MS found psilocin)**
Brown vegetable matter - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocyn. Determined using chemical testing and instrumental analysis.

**Mushrooms (Weber’s negative, GC/MS finds psilocin, and TLC finds psilocin and psilocybin)**
Brown vegetable matter - 1.36 g +/- [Current Estimated MU (g)] - found to contain Psilocyn. Determined using chemical testing, thin layer chromatography, and instrumental analysis.

**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. Determined using chemical testing, thin layer chromatography, and instrumental analysis.

**Exempted or excepted preparations**
Three (3) tablets marked “0111” - 0.37g(s) +/- [Current Estimated MU (g)] - Markings indicate Butalbital in an exempted form- Determined using logo identification.

**No controlled substance identified**
Off-white powder - 1.63 g +/- [Current Estimated MU (g)] - no controlled substance found - Determined using chemical testing and instrumental analysis.

**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- Determined using logo identification.

**Residue (not weighed)**
Residue - trace amount - found to contain Cocaine- Determined using chemical testing and instrumental analysis.

**Residue (THC identified)**
Residue- trace amount- found to contain Cocaine and delta 9-Tetrahydrocannabinol (THC)- Determined using chemical testing and instrumental analysis. The Tetrahydrocannabinol in this item may be from the thermal breakdown of Tetrahydrocannabinolic Acid (THCA). Due to limited sample size, quantitative analysis could not be performed.

**No identification due to insufficient sample**
Residue - trace amount – Insufficient sample for identification- Determined using instrumental analysis.

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

**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] - determined using chemical testing and instrumental analysis.

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 and instrumental analysis.

**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] - determined using chemical testing and instrumental analysis. Confirmation of the isomer could not be determined due to the instrumental capabilities of this laboratory.

**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 - determined using
chemical testing and instrumental analysis.

**Transdermal patches**
Three (3) transdermal patches (Referenced strength: 125 µg/hr) – found to contain Fentanyl- determined using logo identification and instrumental analysis.

**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 - determined using 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.

**Hashish**
Brown substance- 0.5 g +/- [Current Estimated MU (g)]- found to contain a Tetrahydrocannabinol (THC) content of 12.4% +/- [Calculated MU (%)]. Determined using chemical testing, instrumental analysis and quantitative analysis.

**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 - Determined using microscopic examination and instrumental analysis.

**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- Determined using chemical testing and instrumental analysis.

Vegetation – 2.56 g +/- [Current Estimated MU (g)] – found to contain [Identified substance], which is a naphthoylindole- Determined using microscopic examination, chemical testing, and instrumental analysis.

Vegetation - 2.28g +/- [Current Estimated MU (g)]- No controlled substance found. Determined using instrumental analysis based on dry weight.

Vegetation – 3.00g +/- 0.04g – found to contain ADB-FUBINACA. Determined using microscopic analysis and instrumental analysis.

**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.
21 Report Examples

21.1 Example 1

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- Determined using hypergeometric sampling, chemical testing and instrumental analysis.

Remarks

All items will be returned to your department. The packaging from Item #1 was preserved for future testing.

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

Analytical findings offered above were determined using macroscopic examination and other accepted forensic drug chemistry methods. 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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212  Example 2

To:  Ohio Police Department  BCI Lab Number:  19-00002
     Detective Joe Jones
     123 Columbus Road  Analysis Date:  Issue Date:
     State, OH 12345  April 18, 2019  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 - Determined using microscopic examination and instrumental analysis.

Remarks
All items will be returned to your department.

Robert Jones
Forensic Scientist
(330) 609-4600
Robert.Jones@ohioattorneygeneral.gov

Analytical findings offered above were determined using macroscopic examination and other accepted forensic drug chemistry methods. 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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Example 3

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

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 +/- 0.04 g - found to contain Fentanyl. Determined using chemical testing and instrumental analysis.
1.2 Two (2) capsules with residue - Not tested.
1.3 United States one-dollar ($1) bill - Not tested.

Remarks

All items will be returned to your department.

Robert Jones
Forensic Scientist
(330) 609-4600
Robert.Jones@ohioattorneygeneral.gov

Analytical findings offered above were determined using macroscopic examination and other accepted forensic drug chemistry methods. 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 may be estimated in order to identify the items to be testing in accordance to the current submission policy, in these instances no further laboratory analysis was performed on those items.

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21.4 Example 4

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

BCI Lab Number:  19-00004
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. 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. Determined using microscopic examination, chemical testing, and quantitative analysis.

Remarks
All items will be returned to your department.

Robert Jones
Forensic Scientist
(513) 659-4600
Robert.Jones@ohioattorneygeneral.gov

Analytical findings offered above were determined using macroscopic examination and other accepted forensic drug chemistry methods. 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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Example 5

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 +/- 0.04g- found to contain Marihuana. Determined using microscopic examination, chemical testing and quantitative analysis. The Tetrahydrocannabinol (THC) content for each sample tested, determined on a dry weight basis, is listed in the table below:

<table>
<thead>
<tr>
<th>Item</th>
<th>Tetrahydrocannabinol (THC) content</th>
<th>Uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-000051- Sample 1</td>
<td>10.20%</td>
<td>1.5</td>
</tr>
<tr>
<td>19-000051- Sample 2</td>
<td>9.62%</td>
<td>1.3</td>
</tr>
<tr>
<td>19-000051-1-Sample 3</td>
<td>8.64%</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Remarks

All items will be returned to your department.

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

Analytical findings offered above were determined using macroscopic examination and other accepted forensic drug chemistry methods. 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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https://www.surveymonkey.com/Q7V2N6H

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## Appendix I - Chemistry Notes Abbreviations

<table>
<thead>
<tr>
<th>PACKAGING</th>
<th>EXAMINATION</th>
<th>INSTRUMENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adh = Adhesive</td>
<td>Aq = Aqueous</td>
<td>ATR = Attenuated Total Reflectance</td>
</tr>
<tr>
<td>Bet = Blue evidence tape</td>
<td>ABE = Acid/Base Extract with</td>
<td>FID = Flame Ionization Detector</td>
</tr>
<tr>
<td>Bln = Balloon</td>
<td>AXW = Acid Extract With</td>
<td>FTIR = Fourier Transform Infrared Spectrometer</td>
</tr>
<tr>
<td>Brn = Brown</td>
<td>BAX = Basic Extract With</td>
<td>GC = Gas Chromatography</td>
</tr>
<tr>
<td>Bld = suspected blood</td>
<td>BSB = Background Subtraction</td>
<td>LC = Liquid Chromatography</td>
</tr>
<tr>
<td>Blk = black</td>
<td>Bkg = Background</td>
<td>MS = Mass Spectrometer</td>
</tr>
<tr>
<td>BPP = brown paper bag</td>
<td>CIL, CL II = Schedule 1, Schedule 2 etc.</td>
<td>TLC = Thin Layer Chromatography</td>
</tr>
<tr>
<td>Bx = Box</td>
<td>Cap = Capsule</td>
<td>DAD = Diode Array Detector</td>
</tr>
<tr>
<td>Cello = Cellophane</td>
<td>Cig = Cigarette</td>
<td>PAD = Photo Diode Array</td>
</tr>
<tr>
<td>Cl = Clear</td>
<td>Compd = compound</td>
<td>MA = Moisture Analyzer</td>
</tr>
<tr>
<td>Ct = Clear tape</td>
<td>Conc = Concentrated</td>
<td>QNT = Quant</td>
</tr>
<tr>
<td>Cts = Clear tape sealed</td>
<td>Cont'd = continued</td>
<td>&amp;</td>
</tr>
<tr>
<td>Cont = Containing</td>
<td>DNU = Data Not Used</td>
<td>&amp;</td>
</tr>
<tr>
<td>Contr = Container</td>
<td>Eff = Effervescence</td>
<td>&amp;</td>
</tr>
<tr>
<td>DK = dark</td>
<td>EV = Evidence</td>
<td>&amp;</td>
</tr>
<tr>
<td>Env = Envelope</td>
<td>Ext = Extraction</td>
<td>&amp;</td>
</tr>
<tr>
<td>Et = Evidence tape</td>
<td>Frag = Fragment</td>
<td>&amp;</td>
</tr>
<tr>
<td>Gp = glassine paper</td>
<td>Hr = Hand rolled</td>
<td>&amp;</td>
</tr>
<tr>
<td>HS = Heat Sealed</td>
<td>ID = Identification</td>
<td>&amp;</td>
</tr>
<tr>
<td>Kn = Knotted</td>
<td>Ind = Individually</td>
<td>&amp;</td>
</tr>
<tr>
<td>Lg = Large</td>
<td>Insuff = Insufficient</td>
<td>&amp;</td>
</tr>
<tr>
<td>Lt = light</td>
<td>Liq = Liquid</td>
<td>&amp;</td>
</tr>
<tr>
<td>Man = Manila</td>
<td>LP = Latent Prints</td>
<td>&amp;</td>
</tr>
<tr>
<td>Me = Manila envelope</td>
<td>Lt = Light</td>
<td>&amp;</td>
</tr>
<tr>
<td>Med = Medium</td>
<td>Mat = Material</td>
<td>&amp;</td>
</tr>
<tr>
<td>Mkd = Marked</td>
<td>MC = Moisture Content</td>
<td>&amp;</td>
</tr>
<tr>
<td>Pap = Paper</td>
<td>MCLR = McLaafferty Rearrangement</td>
<td>&amp;</td>
</tr>
<tr>
<td>PB = Paper Bag</td>
<td>Micro = Microscopy</td>
<td>&amp;</td>
</tr>
<tr>
<td>Pkg = Package</td>
<td>MT = empty</td>
<td>&amp;</td>
</tr>
<tr>
<td>Pkt = Packet</td>
<td>N = Population Size</td>
<td>&amp;</td>
</tr>
<tr>
<td>Pl = Plastic</td>
<td>NA = No Analysis</td>
<td>&amp;</td>
</tr>
<tr>
<td>Plb = Plastic bag</td>
<td>Neg, (-) = Negative</td>
<td>&amp;</td>
</tr>
<tr>
<td>Ret = Red evidence tape</td>
<td>NT = Not tested</td>
<td>&amp;</td>
</tr>
<tr>
<td>Ret's = Red evidence tape sealed</td>
<td>Pc = Piece</td>
<td>&amp;</td>
</tr>
<tr>
<td>Rec'd = Received</td>
<td>PI = Plastic</td>
<td>&amp;</td>
</tr>
<tr>
<td>Sand = Sandwich</td>
<td>Pos, (+) = Positive</td>
<td>&amp;</td>
</tr>
<tr>
<td>Sld = Sealed</td>
<td>Pow = Powder</td>
<td>&amp;</td>
</tr>
<tr>
<td>SM = Small</td>
<td>Ppt = Precipitate</td>
<td>&amp;</td>
</tr>
<tr>
<td>Sm = Small manila envelope</td>
<td>R = Sample Size</td>
<td>&amp;</td>
</tr>
<tr>
<td>Sub = Submitted</td>
<td>RRT = Relative</td>
<td>&amp;</td>
</tr>
<tr>
<td>Un-sld = Unsealed</td>
<td>Retention Time</td>
<td>&amp;</td>
</tr>
<tr>
<td>Wht = White</td>
<td>Res = Residue</td>
<td>&amp;</td>
</tr>
<tr>
<td>Wpb = White paper bag</td>
<td>RT = Retention Time</td>
<td>&amp;</td>
</tr>
<tr>
<td>Yet = Yellow evidence tape</td>
<td>Rxn = reaction</td>
<td>&amp;</td>
</tr>
<tr>
<td>Yets = Yellow evidence tape sealed</td>
<td>Sat = Saturation</td>
<td>&amp;</td>
</tr>
<tr>
<td>Zlb = zip loc bag</td>
<td>Sd = Smoking Device</td>
<td>&amp;</td>
</tr>
</tbody>
</table>

### 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
- Ret = Red evidence tape
- Rets = Red evidence tape sealed
- Rec'd = Received
- Sand = Sandwich
- Sld = Sealed
- SM = Small
- Sm = 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

### 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
- IBU = Ibuprofen
- SUF = Insufficient Sample for Analysis
- ISFI = Insufficient Sample for Identification
- NCSF = No Controlled Substance Found
- P/E = Pseudoephedrine/ephedrine
- THC = Tetrahydrocannabinol
- THCA = Tetrahydrocannabinolic 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. 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

<table>
<thead>
<tr>
<th>Method</th>
<th>Typical Use</th>
<th>Mass Range</th>
<th>Run Time (min)</th>
<th>Temperature / hold (min)</th>
<th>Ramp</th>
<th>Hold (min)</th>
<th>Solvent Delay</th>
<th>Split</th>
<th>Flow Ramp</th>
</tr>
</thead>
<tbody>
<tr>
<td>ClanLab</td>
<td>Separate Ephedrine from PSE; medium or high polarity columns only</td>
<td>40-400</td>
<td>11</td>
<td>100°C / 2 min</td>
<td>20°C/min to 120°C 4°C/min to 140°C 30°C/min to 200°C</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ClanLab-20m</td>
<td>Separate Ephedrine from PSE (20mx0.180mmx0.18um 35MS stationary phase columns only)</td>
<td>29-500</td>
<td>6.25</td>
<td>100°C / 1 min</td>
<td>40°C/min to 120°C 16°C/min to 140°C 30°C/min to 200°C</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>ClHy420</td>
<td>Chloral hydrate, dichloralphenazone</td>
<td>20-400</td>
<td>20</td>
<td>40/ 4 min</td>
<td>10°C/min to 95°C 50°C/min to 270°C</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Coc209</td>
<td>Cocaine</td>
<td>40-400 or 500</td>
<td>9</td>
<td>200/-</td>
<td>20°C/min to 280°C</td>
<td>5</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Coc212</td>
<td>Cocaine</td>
<td>40-400 or 500</td>
<td>12</td>
<td>200/-</td>
<td>20°C/min to 280°C</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gen115-20m</td>
<td>General Scan (20mx0.180mmx0.18um columns only)</td>
<td>29-600</td>
<td>15</td>
<td>100 / 1 min</td>
<td>40°C /min – 300°C 40°C /min – 315°C</td>
<td>1</td>
<td>7.625</td>
<td>1.5</td>
<td>1.2 mL/min for 8 min; ramp 10 mL/min to a flow of 2mL/min</td>
</tr>
<tr>
<td>Gen130</td>
<td>General Scan</td>
<td>29-500</td>
<td>30</td>
<td>100 / 2 min</td>
<td>20°C/min - 300°C</td>
<td>18</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gen170</td>
<td>General Scan</td>
<td>29-500</td>
<td>70</td>
<td>100 / 2 min</td>
<td>4°C/min - 300°C</td>
<td>18</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gen220</td>
<td>General Scan</td>
<td>40-500</td>
<td>20</td>
<td>200/-</td>
<td>20°C/min - 280°C</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHBS10</td>
<td>GHB</td>
<td>20-500</td>
<td>10.33</td>
<td>50 / 1 min</td>
<td>30°C/min - 180°C</td>
<td>5</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>GLY440</td>
<td>Ethylene glycol</td>
<td>10-100</td>
<td>9</td>
<td>40 / 1 min</td>
<td>10°C/min - 70°C</td>
<td>5</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HiB230</td>
<td>Steroids</td>
<td>40-500</td>
<td>30</td>
<td>200/-</td>
<td>20°C/min - 300°C</td>
<td>25</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>HT216</td>
<td>Separate Hydrocodone and Delta-9-THC, Separate CBC and CBD</td>
<td>40-400 or 500</td>
<td>16</td>
<td>200 / 2 min</td>
<td>5°C/min - 240°C</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IoD405</td>
<td>Iodine</td>
<td>40-400 or 500</td>
<td>5.2</td>
<td>40 / 1 min</td>
<td>50°C/min - 200°C</td>
<td>1</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>ISO125</td>
<td>Separate Cathine and PPA</td>
<td>40-400</td>
<td>9</td>
<td>125/-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoB110</td>
<td>Methamphetamine, MDMA</td>
<td>40-400 or 500</td>
<td>10</td>
<td>100/-</td>
<td>25°C/min - 250°C</td>
<td>4</td>
<td></td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

This document is uncontrolled if viewed outside the BCI document management system.
<table>
<thead>
<tr>
<th>Method</th>
<th>Typical Use</th>
<th>Run Time (min)</th>
<th>Temperature / Ramp</th>
<th>Hold (min)</th>
<th>Solvent Delay</th>
<th>Split</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD115</td>
<td>LSD</td>
<td>15</td>
<td>100 / 1 min</td>
<td>9</td>
<td>9 min</td>
<td>50: 0.5</td>
</tr>
<tr>
<td>LSD218</td>
<td>LSD</td>
<td>18</td>
<td>200 / -</td>
<td>14</td>
<td>10 min</td>
<td>5:1</td>
</tr>
<tr>
<td>OPI210-20m</td>
<td>Opiates (20mx0.180mmx0.18um columns)</td>
<td>10</td>
<td>200 / -</td>
<td>7.5</td>
<td>1.6 min</td>
<td>2.1min</td>
</tr>
<tr>
<td>OPI212</td>
<td>Opiates (avoids APAP)</td>
<td>12</td>
<td>200 / -</td>
<td>8</td>
<td>3.75 min</td>
<td></td>
</tr>
<tr>
<td>OPI215</td>
<td>Opiates (avoids APAP and detects noscapine)</td>
<td>15</td>
<td>200 / -</td>
<td>11</td>
<td>3.75 min</td>
<td></td>
</tr>
<tr>
<td>OPI515-05</td>
<td>Split-less Opiate Method (suspected fentanyl-related compound in small amounts)</td>
<td>15</td>
<td>50 / 0.5 min</td>
<td>0</td>
<td>3.50 min</td>
<td>7.25 min</td>
</tr>
<tr>
<td>Pho708</td>
<td>Phosphorus</td>
<td>8</td>
<td>70 / 2 min</td>
<td>-</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>QDS</td>
<td>General Scan</td>
<td>19.8</td>
<td>100 / 2 min</td>
<td>11</td>
<td>3 min</td>
<td></td>
</tr>
</tbody>
</table>

**GC/FID Methods**

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

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FTIR Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Minimum Scans</th>
<th>Minimum Background Scans</th>
<th>Resolution</th>
<th>Wave Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Nicolet iS5</td>
<td>32</td>
<td>32</td>
<td>4</td>
<td>4000-400</td>
</tr>
<tr>
<td>Thermo Nicolet 4700</td>
<td>32</td>
<td>32</td>
<td>4</td>
<td>4000-500</td>
</tr>
<tr>
<td>JChem</td>
<td>16</td>
<td>16</td>
<td>4</td>
<td>4000-400</td>
</tr>
<tr>
<td>Nicolet iS5</td>
<td>16</td>
<td>16</td>
<td>4</td>
<td>4000-450</td>
</tr>
<tr>
<td>PE Spectrum 100</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4000-450</td>
</tr>
</tbody>
</table>

21.5 Moisture Analyzer Methods

<table>
<thead>
<tr>
<th>Drying Program</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying Temperature</td>
<td>90°C</td>
</tr>
<tr>
<td>Switch-off criterion</td>
<td>5 (1mg / 140s)</td>
</tr>
<tr>
<td>Display Mode</td>
<td>% MC</td>
</tr>
<tr>
<td>Start Weight</td>
<td>1.5g</td>
</tr>
<tr>
<td>Start Weight Tolerance</td>
<td>25%</td>
</tr>
</tbody>
</table>

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.
21.6 Shimadzu LC/MS/UV

General Method Parameters:

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

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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>8.82</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>13.23</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>14.33</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>19.00</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>21.00</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>24.00</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>25.00</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>27.50</td>
<td>controller</td>
<td>stop</td>
</tr>
</tbody>
</table>

UV Conditions:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp</td>
<td>Deuterium</td>
</tr>
<tr>
<td>Range (nm)</td>
<td>190-800</td>
</tr>
<tr>
<td>Cell Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>8</td>
</tr>
<tr>
<td>Resolution</td>
<td>512</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>4</td>
</tr>
<tr>
<td>Wavelengths evaluated</td>
<td>228</td>
</tr>
<tr>
<td>Acquisition Start</td>
<td>1.5 min</td>
</tr>
<tr>
<td>Acquisition Stop</td>
<td>25.00 min</td>
</tr>
</tbody>
</table>

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MSD Conditions:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization Mode</td>
<td>ESI Positive (Neutrals) ESI Negative (Acids)</td>
</tr>
<tr>
<td>Nebulizer Gas</td>
<td>1.5 L/min</td>
</tr>
<tr>
<td>Drying gas</td>
<td>Nitrogen at 15 L/min</td>
</tr>
<tr>
<td>Scan Range</td>
<td>SIM</td>
</tr>
<tr>
<td>Desolvation Line Temperature</td>
<td>300 °C</td>
</tr>
<tr>
<td>Heat Block Temperature</td>
<td>500 °C</td>
</tr>
<tr>
<td>Acquisition Start</td>
<td>2.50 min</td>
</tr>
<tr>
<td>Acquisition Stop</td>
<td>24.00 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>SIM m/z ion</th>
<th>ESI Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>287.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Cannabidiol (CBD)</td>
<td>315.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Cannabigerol (CBG)</td>
<td>317.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Cannabidiolic Acid (CBD-A)</td>
<td>357.2</td>
<td>Negative</td>
</tr>
<tr>
<td>Cannabigerolic Acid (CBGA)</td>
<td>359.2</td>
<td>Negative</td>
</tr>
<tr>
<td>Cannabinol (CBN)</td>
<td>311.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Delta 9 Tetrahydrocannabinol (D9-THC)</td>
<td>315.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Delta 8 Tetrahydrocannabinol (D8-THC)</td>
<td>315.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Cannabichromene (CBC)</td>
<td>315.2</td>
<td>Positive</td>
</tr>
<tr>
<td>Cannabichromenic Acid (CBCA)</td>
<td>353.2</td>
<td>Negative</td>
</tr>
<tr>
<td>Cannabichromenic Acid (CBCA)</td>
<td>357.2</td>
<td>Negative</td>
</tr>
<tr>
<td>Tetrahydrocannabinolic Acid (THCA)</td>
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21.7 SPEX Sample Prep 6875D freezer/mill Dual Chamber Cryogenic Grinder methods:

Gummy method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precool</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Run time</td>
<td>2 minutes</td>
</tr>
<tr>
<td>Cool</td>
<td>1 minute</td>
</tr>
<tr>
<td>Cycles</td>
<td>4</td>
</tr>
<tr>
<td>Rate</td>
<td>10</td>
</tr>
</tbody>
</table>

Plant material method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precool</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Run Time</td>
<td>1 minute</td>
</tr>
<tr>
<td>Cool</td>
<td>1 minute</td>
</tr>
</tbody>
</table>
22 Appendix III: Analog Letter
All drug chemistry reports which report an analog shall include a copy of the “Analog Letter for BCI Reports” letter, dated January 14, 2019. The letter, formatted as a portable network graphics (.png) document, should be copied and pasted into the Word document version of the draft report and saved in LIMS. This will make it available for download from OHLEG.