Department of Forensic Science

CONTROLLED SUBSTANCES PROCEDURES MANUAL

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1 INTRODUCTION

1.1 Introduction

The information in this Procedures Manual was collected from numerous sources and is presented here for easy reference for drug chemists. In all cases, it is acceptable to use the most current edition of listed literature references. This manual presents a basic outline of the types of drugs analyzed, a source book on reagent and standard preparation and a description of the analytical techniques used with a review of instrumentation. This manual is not all-inclusive, and will reference other sources where appropriate. It is always the chemist's responsibility to choose the best analytical scheme for each individual case. It is expected that supervisors will be consulted for extraordinary procedures.

1.2 Examination Documentation

1.2.1 Specific worksheets are provided as controlled forms and should be used in conjunction with other examination documentation to provide sufficient detail to allow another examiner to repeat the analysis under conditions as close as possible to the original, evaluate the data, interpret the results and come to the same conclusion.

1.2.2 The *General Drug Worksheet* is a generic worksheet for controlled substances casework. The comments section should be used for explanations of tests or lists of weights, etc.

1.2.3 The *Blank Worksheet* is available for cases requiring more notes than will easily fit on the *General Drug Worksheet*.

1.2.4 The *Pharmaceutical Identifiers* (PI) *Worksheet* is designed for tablet and capsule analysis. Tablet/capsule analysis may still be documented on the general worksheet; however, the PI worksheet is useful when cases contain multiple items of tablets and/or capsules.

1.2.5 The Controlled Substances *Weighing Event Uncertainty of Measurement (UoM) Calculation Worksheet* is used to calculate total measurement uncertainty for instances where a net weighing was performed on one or more specimens and shall be included in all case files accordingly.

1.2.6 Date(s) of examination shall be noted as “Date started” and “Date completed”. The completion date reflects the date when all data has been incorporated into a recorded conclusion.

1.2.7 If a test result, observation, or calculation is rejected, the reason for the rejection shall be documented. In addition, the identity of the individual taking the action and the date shall be documented. For example, if your sample is too weak or too strong based on poor chromatography (e.g., split peak, shifted retention time due to concentration differences) and you have to rerun it, print the total ion chromatogram or the peak of interest’s mass spectrum for the case file, write the reason for rejection on it, initial, and date. In the absence of a spectrum or TIC or when data collection was incomplete, include in your notes the descriptive reason for the rejection.

1.2.8 All examination documentation should include the applicable sample name(s), Item number(s), and/or sample/standard identifier(s). A “standard identifier” shall be used such that the specific standard could be determined (e.g., “coc”, lot #, lab assigned #).

1.3 The Department’s laboratory facilities provide sufficient environmental conditions to conduct all tests listed in the Procedures Manual with no further consideration required.

1.4 New procedures must be validated before use. Published procedures must be verified to work in each Regional Laboratory before use. Prior to beginning a validation process, consult the Chemistry Program Manager and the SWGDRUG guidelines for an appropriate validation plan.

2 ANALYTICAL SCHEMES

2.1 Introduction

There are three general analytical schemes to be used for controlled substances. At various times, a drug chemist will encounter drug substances for analysis that require specialized analyses. For these cases, the flowchart for general unknowns can be followed and any modifications must be approved by the Section Supervisor or Chemistry Program Manager as per ¶ 5.3.10 of the Quality Manual. It should be noted that sample size or other circumstances may require a rearrangement or modification of one or more steps.

2.2 Determination of Quantity

Documentation of the amount of sample present may be accomplished via weight, volume, and/or specimen count, depending on the nature of the evidence.

2.2.1 Refer to ¶ 4 for Weighing Practices.

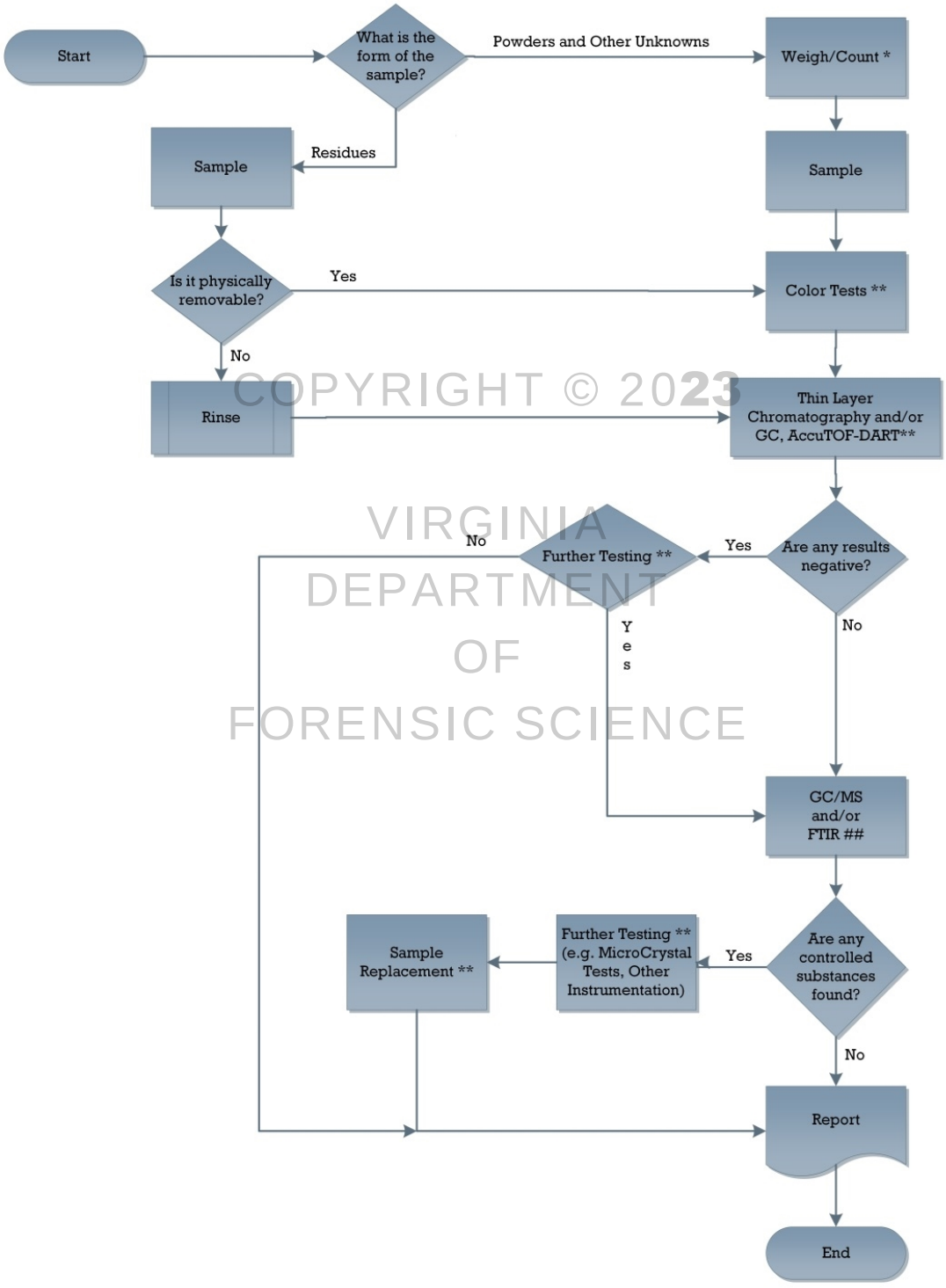
2.2.2 Dosage units (e.g., cigarettes, cigarette butts, blotter papers, tablets, marked pharmaceutical capsules, or electronic-smoking device cartridges) will not routinely be weighed. Unmarked capsules and their contents shall be weighed, with the exception of those that appear to be supplements or vitamins.

2.2.3 Handling miscounts

2.2.3.1 All undercounts shall be verified by another Controlled Substances examiner or a FLS (who is authorized to handle evidence) before consulting with the customer. The verification will be documented in the case notes or on the RFLE and shall include the initials of the verifier and the date of verification. This may be accomplished by the verifier writing and initialing the word “verified” near the correction. Customer consultation with further instructions is required before proceeding with analysis.

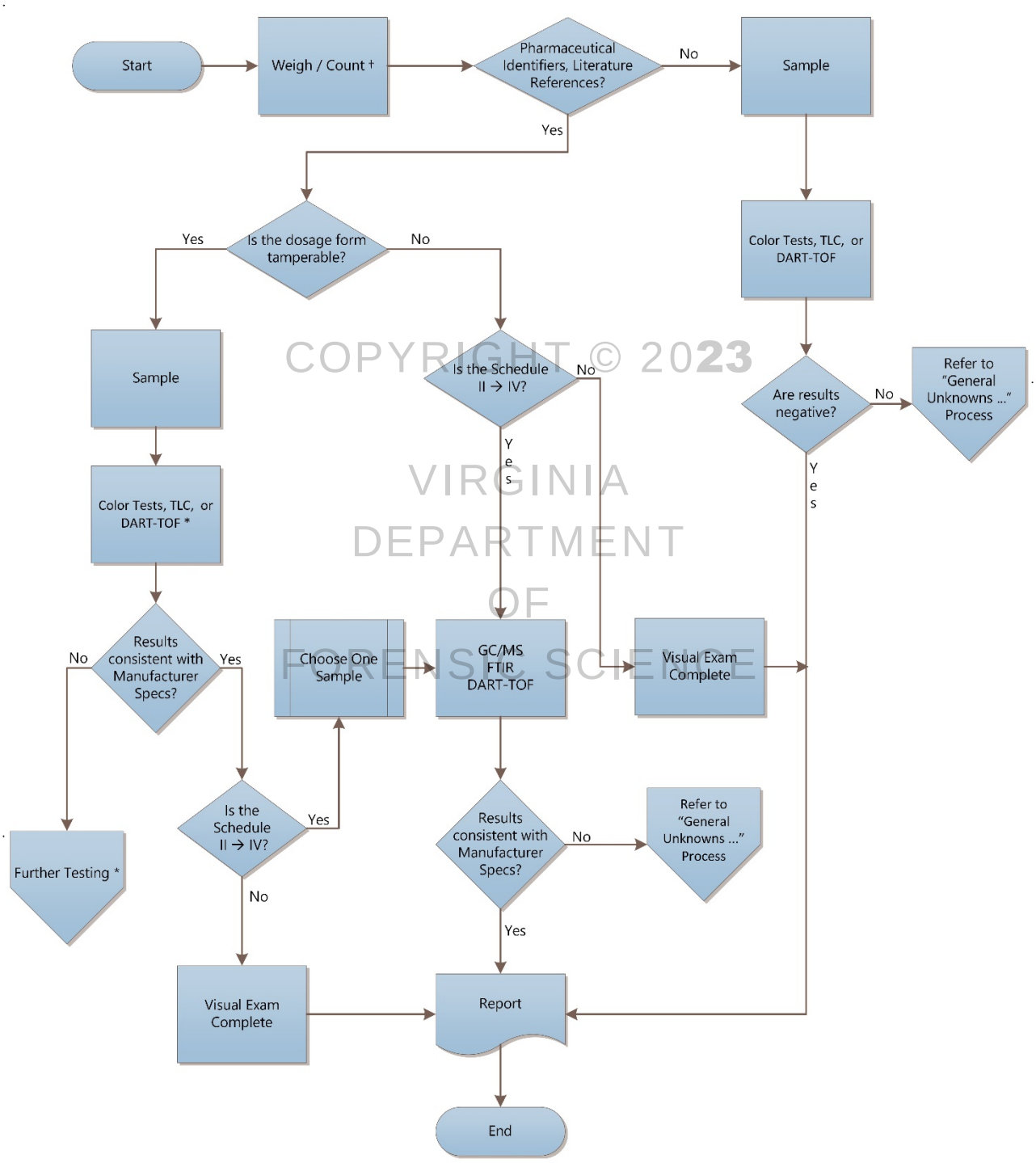
2.2.3.2 As a courtesy, all overcounts shall be communicated with the customer and the communication documented on a MFR or email stored within the case file, in the object repository, or in the communication log in the LIMS. Overcounts do not require verification or necessitate further instructions from the customer before proceeding with analysis.

2.3 General Unknowns/Powders/Illicit Tablets



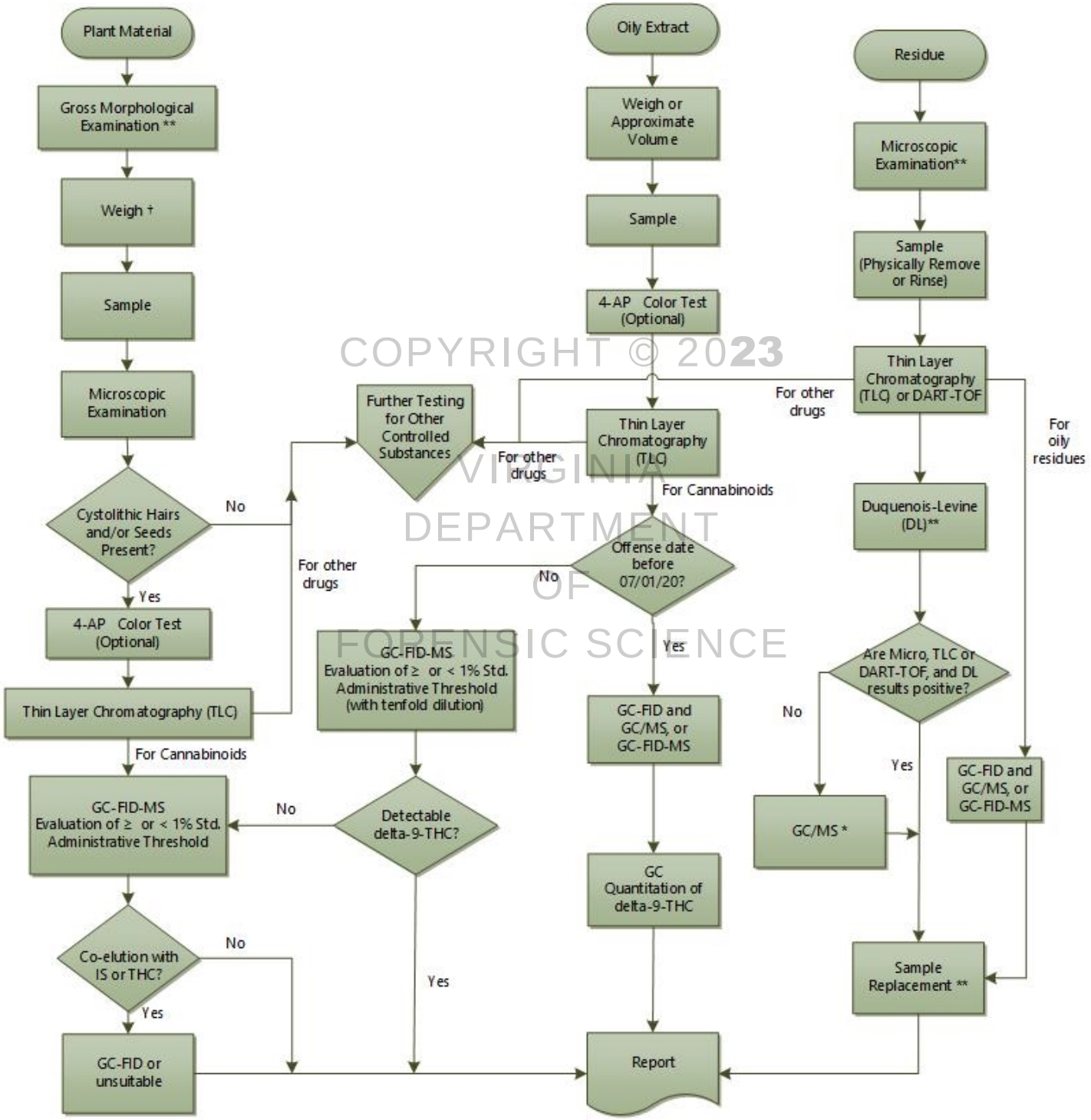
* Gross weight suitable if less than statutory threshold
** As appropriate
\#\# Or other appropriate definitive structural elucidation method

2.4 Tablets and Capsules



† As appropriate (dosage forms not generally weighed)
* As needed

2.5 Cannabis



† Gross weight suitable if < 1 ounce w/ innermost packaging or simple possession
* Required if microscopic characteristics are absent or if another test is inconclusive, negative, or omitted (see ¶ 6.9.4)
** As needed

2.6 Pharmaceutical Identifiers

2.6.1 Check the Physician's Desk Reference (PDR), Poison Control, DEA Logo Index, Identadrug, Drug ID Bible/Amera-Chem, Inc CD, Epocrates, webpoisoncontrol.org, NIH-DailyMed, Drugs.com, or manufacturer's resources for information relating to inscriptions on tablets and capsules. Two unrelated references are recommended for unfamiliar tablets. Additional references may be used following approval by Chemistry Program Manager.

2.6.2 Additional analyses may be required as described in the general analytical scheme in ¶ 8.2.

2.7 Color Tests

2.7.1 If the size of the sample is sufficient, perform the appropriate color tests required to provide an indication of any compounds present.

2.7.2 For the interpretation of color test results, check relevant sections of this Manual, available literature (e.g., Clarke), and/or ask other chemists, as necessary.

2.8 Chromatography

2.8.1 Dissolve the sample directly into a suitable solvent (e.g., methanol). If appropriate, extract the sample from an acidic or basic medium (or both if the contents of the sample are still unknown at this time).

2.8.2 Unknown samples can be screened via TLC or GC. If the technique chosen does not indicate the possible presence of a controlled substance in combination with the results obtained from any color test(s) performed, the examiner may halt the analysis and report "No controlled substances found". The examiner should exercise caution and continue the analytical scheme when circumstances suggest that doing so may result in the identification of a controlled substance (e.g., information provided on the RFLE, visible residue on the evidence).

2.8.3 If sample identity was indicated previously, choose the appropriate two system TLC and/or two system GC systems, as needed, with a standard (also known as the two-system chromatography requirement). The choice of TLC or GC should be based on which is more appropriate under the circumstances. Note: GC is more effective at distinguishing closely related compounds, such as fentanyl and fentanyl analogs, while TLC is better suited to analyze thermally labile compounds.

2.8.4 The chromatography requirement is waived for pharmaceutical preparations that are confirmed with a structural elucidation technique following visual examination under ¶ 8 Pharmaceutical Identifiers.

2.8.5 In addition to two system TLC, sample retention times obtained from GC/MS systems should be compared to standard retention times. The exception to this being those compounds which inherently demonstrate poor chromatography under normal method conditions (e.g., acetaminophen, ibuprofen, sildenafil).

2.9 Infrared Spectroscopy/Mass Spectrometry

2.9.1 If the identity of the sample is still unclear at this point, the IR or GC/MS will provide further information.

2.9.2 The DART-TOF screening method provides accurate mass information, which may assist in identifying components present in the sample. This screening method can be useful at any point in the analytical scheme.

2.9.3 A definitive structural identification technique such as GC/MS or IR is required to be used on all substances where the identity will be reported. Confirmation of pharmaceutical identifiers may be accomplished using DART-TOF as described in ¶ 16.4.

2.10 Further Testing

2.10.1 If the sample is still an unknown or other confirmation is needed, the chemist should use any instrumental techniques available (or combinations thereof) to arrive at a sound analytical conclusion about the identity of the substance(s) in the sample. This may involve using Department Instrument Support as approved by the Chemistry Program Manager.

2.10.2 Microcrystal tests are used for isomer determination only. They are to be used only in combination with a structural elucidation technique.

2.11 Liquids

2.11.1 Liquid samples may be submitted for analysis as part of a clandestine lab prosecution or for general drug analysis. These types of samples may require additional steps in the general analytical scheme in ¶ 2.2. Occasionally, it will be necessary to consult with a supervisor, the investigating officer, or both to determine the best analytical course.

2.11.2 Information provided on the RFLE or by the investigating officer should be used to determine the purpose of the examination. In some cases, evidence may need to be transferred to Trace Evidence for analysis or, depending on the overall case, may not require analysis.

2.11.3 Information such as package labeling, visible precipitates, number of layers present, viscosity and color of liquid should be considered when deciding on an analytical scheme.

2.11.4 The approximate volume of the liquid will be determined as sample size allows. The pH of aqueous liquids, density, and miscibility may also be determined.

2.11.5 Additional considerations regarding liquids submitted in association with a clandestine laboratory or the intent to manufacture methamphetamine, methcathinone or amphetamine as per § 18.2-248(J) are addressed in the Clandestine Laboratory section.

2.11.6 It may not be sufficient to simply screen a neat liquid with TLC or FTIR. Liquids submitted for general drug analysis may require extraction prior to TLC or GC/MS to concentrate the analyte during the screening process or to remove the analyte from the matrix. Screening for suspected amyl nitrite will require GC/MS headspace analysis. Examples of samples which come in liquid forms are:

- Solutions containing GHB, GBL or 1,4-butanediol
- Cough syrups
- Suspected amyl nitrite or other inhalants
- Eye drops or other liquids in dropper bottles
- Injectables such as tubexes

2.12 Plant Material

2.12.1 Initially, all plant material shall be screened via stereomicroscopy for cystolithic hairs.

2.12.1.1 In the presence of cystolithic hairs or Cannabis seeds, the analytical scheme for Cannabis shall be completed.

2.12.1.2 Visual observation via macro/microscopic examination in conjunction with contextual clues from the evidence might indicate, for example, an herbal blend (see Cannabimimetic Agent Methodology section) or khat (see Cathinone Methodology section). Contextual clues, such as package labels or banana leaf wrappers, shall be recorded in the case notes.

2.12.1.3 In the absence of cystolithic hairs and in conjunction with contextual clues from the evidence, samples shall be screened for controlled substances including, but not limited to, Salvinorin A, cathinone/cathine and cannabimimetic agents.

- Apparent plant material residues, if analyzed, should be handled in the same manner.

2.12.2 If other drugs are indicated at any point during screening methods (e.g., TLC), further testing for controlled substances is necessary.

2.13 Fungal Material

2.13.1 Fungal material shall be screened as per the Psilocybin and Psilocyn Methodology section.

2.14 References

2.14.1 ASTM Standard E2329, 2017, "Standard Practice for Identification of Seized Drugs," ASTM International, West Conshohocken, PA, 2017, DOI: 10.1520/E2329-17, www.astm.org.

3 DRUG ITEM REDUCTION PROGRAM

3.1 Introduction

3.1.1 The Drug Item Reduction Program (DIRP) allows for the analysis of key items within a case to maximize the resources of the laboratory.

3.1.2 In every case, the most significant items in terms of quantity and schedule are analyzed. This "rule of thumb" cannot address every drug case. Consideration must be given to the information contained on the Request for Laboratory Examination (RFLE). This includes things such as the specific charges or types of offense, items unique to a single suspect, the statement of fact and examinations requested and the descriptions of evidence submitted as well as the chemist's visual inspection of the items. Currency lacking visible residue should not be analyzed.

3.1.2.1 In the presence of an identifiable controlled substance, subsequent identification and reporting of minor constituents (that do not meet the identification criteria for the technique) or non-controlled cutting agents is not required.

3.1.2.2 If a controlled adulterant or cutting agent is present, identification and subsequent reporting of its presence and schedule should be pursued when it is of value to our customers or for Department data sharing purposes (i.e., trend tracking). For example, xylazine, often found in fentanyl mixtures, should be identified when possible.

3.1.2.3 Controlled substances of a higher schedule should be confirmed within reason, however, this is not required if the instrument must be overloaded with the major constituent.

3.1.3 If, during the pretrial process, it becomes apparent that items not analyzed will require analysis for prosecution, then upon re-submission, those items will receive top priority at the laboratory.

3.2 Procedures

3.2.1 Steps should be taken to discourage "no suspect, information only" requests.

3.2.2 Syringes should only be analyzed if they are the only item in the case.

3.2.3 The Department does not routinely perform drug quantitations unless the quantitation is specifically requested or required by the Code of Virginia. If the customer does not require a quantitation required by the Code of Virginia, an MFR or email shall be placed in the case file documenting the communication.

3.2.3.1 The purity of evidence found to contain methamphetamine will not be routinely determined unless it is specifically requested by a Commonwealth's Attorney.

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

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

3.2.4.2 Example 2: Submitted evidence includes five tablets containing oxycodone and a plastic straw section with residue. The tablets would be analyzed and the straw section would not.

3.2.4.3 Example 3: Submitted evidence includes five tablets containing alprazolam and a plastic straw section with residue. The tablets would be analyzed and the straw section would not unless information on the RFLE indicates that the straw section was used for a different drug.

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

3.2.5 When multiple residue specimens are submitted within an item (without an item with a measurable quantity), similar residues (e.g., two spoons with residue) may be combined after appropriate screening tests to result in only one GC/MS sample.

3.2.6 Pharmaceutical preparations should be visually examined using pharmaceutical identifiers and appropriate reference compendia.

3.2.6.1 No further analysis is required for misdemeanor offense intact, marked pharmaceutical preparations indicated as non-controlled, Schedule VI, or Schedule V. These may be reported using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual. See ¶ 8.2.6.2 for tamperable capsules.

3.2.6.2 If identical intact, marked pharmaceutical preparations are present in multiple items, analysis is required for only one item. Those preparations not analyzed may be reported as "Not Analyzed" or using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual. See ¶ 8.2.5.3 for tamperable capsules.

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

3.2.6.4 In simple possession cases, if intact, marked pharmaceutical preparations indicated as containing the same controlled substance are present in multiple items, analysis is required for only one item. Those preparations not analyzed will be reported using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual. See ¶ 8.2.5.3 for tamperable capsules.

3.2.6.5 No further analysis is required for Schedule V, VI, and non-controlled pharmaceutical preparations when a Schedule I or II controlled substance is identified in the same case. These may be reported using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual.

3.2.7 For search warrant cases, only those items related to the indicated charge should be submitted and analyzed. If it is subsequently determined that more testing is required, additional items can be submitted following consultation with DFS. In general, residues and syringes will not be analyzed when measurable quantities of the associated drugs are also included among the items submitted.

3.2.8 If items are not analyzed per this procedure, case notes shall indicate this by a notation of "Not Analyzed" or "DIRP".

4 WEIGHING PRACTICES

4.1 Procedures

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

4.1.1.1 Minimum balance loads:

- 5-Place Balance = 0.00450 gram
- 4-Place Balance = 0.0300 gram
- 3-Place Balance = 0.150 gram
- 2-Place Balance = 0.90 gram
- High Capacity (g) Balance (CD-33 and AND) = 27.0 grams
- High Capacity (g) Balance (Kg) = 1.1 Kg

4.1.1.2 For 4-place balances, plastic weigh boats shall not be used for determining net weights.

4.1.1.3 For 5-place balances, plastic beakers and weigh boats shall not be used for determining net weights. Dynamic weighing will be used for 5-place balances whenever possible; static weight measurements are considered two weighing events and therefore double the uncertainty of measurement.

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

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

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

4.1.4 When available balances allow for it, net weights of multiple specimens shall be measured and recorded individually. Measuring and recording the net weight of multiple specimens at the same time shall be avoided whenever it is possible to do so.

4.1.5 When multiple balances are used to record net weights of specimens within one item, the sum of the weights recorded with each balance shall be reported separately (see ¶ 34.4.2.2 for an example).

4.2 Weighing Practices

4.2.1 Weights of evidence will be measured and recorded prior to sampling. The gross weight (GW) of the evidence, including innermost packaging, should be measured whenever possible. At the discretion of the examiner, the net weight (NW) of the material may be measured but only when it is more appropriate to do so than measuring the gross weight.

4.2.1.1 In cases where the container weight is clearly much greater than the sample weight, obtain the net weight (without packaging) of the material and report appropriately.

4.2.1.2 In instances where statutory requirements or state sentencing guidelines designate weight thresholds, sufficient specimens will be weighed and analyzed to exceed the threshold. A list of these instances can be found in the Code of Virginia (thresholds can also be found in ¶ 39). The net weight of the specimens required to exceed the threshold (by a margin greater than

the estimated uncertainty of measurement when possible) will be obtained and designated as such in the case notes.

- The gross weight of the remaining specimens including innermost packaging will be obtained and designated as such in the case notes, if applicable.

- Both the net weight of the analyzed specimens and the gross weight of the remaining specimens will be reported on the Certificate of Analysis.

4.2.2 Resubmissions with Hypergeometric Sampling Plan

4.2.2.1 The net weight or gross weight, as applicable, of the additional samples will be obtained and reported on the Supplemental Certificate of Analysis.

4.2.3 For Extrapolation of Net Weight

4.2.3.1 In cases with a large number of specimens (more than 50) whose gross weight would exceed an established weight threshold but would require the analysis of an excessive number of specimens in order to reach the weight threshold, the extrapolated net weight may be calculated and reported on the Certificate of Analysis using the hypergeometric sampling plan.

4.2.3.2 The appropriate number of specimen units within the population (See ¶ Hypergeometric Sampling Plan) will be randomly selected and weighed.

4.2.3.3 With obtained net weight of the randomly selected specimens, use the Controlled Substances *Net Weight Extrapolation Worksheet* to calculate the extrapolated net weight and associated expanded uncertainty.

4.2.3.3.1 To calculate the extrapolated net weight, the average net weight of the measured specimens will be multiplied by the total number of units in the population.

4.2.3.4 The selected specimens will be considered sufficiently uniform if the Relative Standard Deviation (RSD) of the net weight measurements is 10% or less. The expanded extrapolated uncertainty value (U_T) is considered acceptable if the relative uncertainty (U_T /extrapolated net weight) is 25% or less.

4.2.3.4.1 If a higher RSD value or relative uncertainty is calculated, alternative methods of net weight determination should be considered (increased number of analyzed specimens, separation of specimens into different populations, use of a higher precision balance, etc.).

4.3 References

4.3.1 SWGDRUG Supplemental Document SD-6, “For Part IVC – Quality Assurance/Uncertainty: Measurement Uncertainty for Extrapolations of Net Weight and Unit Count”, July 2017.

4.3.2 *Analysis of Drugs Manual,* .S. Department of Justice, Drug Enforcement Administration, Office of Forensic Sciences, April 2018.

4.3.3 European Network of Forensic Science Institutes Drugs Working Group, *Guidelines on Sampling of Illicit Drugs for Qualitative Analysis*, 2016.

5 SAMPLING

5.1 Introduction

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

5.1.2 For items containing multiple specimens, statistically-based sampling models (e.g., hypergeometric distribution) will allow the analyst to analyze a portion of the specimens and subsequently make statistical inferences about the population. Alternatively, a fixed number of specimens within a population may be analyzed with the purpose in mind of meeting the requirements of a particular criminal charge (e.g., simple possession, distribution). In these instances, an inference to the entire population will not be drawn and the number of specimens that were analyzed will be indicated on the Certificate of Analysis.

5.2 General Sampling

5.2.1 Every effort should be made to avoid handling evidence repeatedly. The material should be sampled and immediately sealed. If necessary, the evidence may be closed and maintained in short term storage until the analysis is complete. Evidence generally will not remain in short term storage for longer than 30 days.

5.2.2 In order to minimize detailed labeling on small items such as very small metal foil packets, plastic bags or plastic bag corners, they may be secured in a bandolier of tape, which is then labeled. If needed, items may be placed in an additional plastic bag that can be sealed, fully labeled and properly documented in the case notes.

5.2.3 For chemical analyses, a representative sample shall be removed from the specimen. When sample size allows, testing should be applied on separate samplings of the material. Taking a small amount of material for use in a color test prior to taking a separate sampling for additional tests is an appropriate method. For suspected Cannabis plant material, performing the microscopic examination serves as a first sampling with additional sample(s) taken for further testing. For pharmaceutical tablets and capsules, the use of pharmaceutical identifiers as a screening test prior to taking a representative sample for confirmatory testing will suffice.

5.3 Multiple Specimens

5.3.1 If all specimens are not analyzed, the number of those that are fully analyzed will be recorded in the case notes.

5.3.2 Sub-numbering of specimen(s) will be documented in case notes.

5.3.3 Net weights, when applicable, and autosampler vial numbers will be associated with specific specimens by the use of sub-numbering in the case notes.

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

5.3.4.1 If samples were chosen randomly (and documented as such) for the first set of observations, those samples may be used for further population sampling. If multiple populations are discovered in the first set of observations, an inference to the entire population cannot be made and additional analysis may be necessary.

5.3.4.2 Subdivision of illicit tablets into separate populations based solely on appearance (e.g., those that vary in color, shape, and markings) is not necessary if a Schedule I or II controlled substance is identified in each of the specimens initially selected under the appropriate sampling plan.

5.3.5 If presumptive testing indicates that no controlled substances are present in the samples chosen, a screening test must be done using the hypergeometric sampling scheme.

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

5.3.6 When multiple balances are required to record weights within one item, the sum of the samples taken and analyzed should meet the requirement of the selected sampling plan.

5.4 Administrative Sampling Plan

The administrative sampling plan will be used in cases to answer a specific legal question. If more specimens than listed below need to be analyzed for prosecution, additional analysis utilizing the hypergeometric sampling plan will be conducted upon written request from the Commonwealth's Attorney. All remaining specimens not analyzed will be left intact in case further analysis is required.

5.4.1 Simple possession

5.4.1.1 One specimen will be selected and fully analyzed.

5.4.2 Possession with intent to distribute or distribution

5.4.2.1 Items containing up to 5 specimens

- Each specimen will be analyzed separately and fully.

5.4.2.2 Items containing greater than 5 specimens

- Five selected specimens will be analyzed separately and fully.

5.4.2.3 In instances where statutory or state sentencing guidelines have weight or plant number thresholds, enough specimens will be weighed/counted and analyzed, separately and fully, to exceed the threshold. A list of these instances can be found in the Code of Virginia (thresholds can also be found in ¶ 39).

5.4.2.4 Samples may be randomly selected, and documented as such in the case notes, using methods listed under ¶ Hypergeometric Sampling Plan.

5.4.3 Pharmaceutical preparations

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

5.4.3.2 For drug substances involving misdemeanor prosecutions in Schedules V and VI, sampling is not normally required. For drug substances involving Schedule IV and above, at least one representative sample must be analyzed fully.

5.4.3.2.1 For tamperable dosage units, screen a sample chosen using the hypergeometric scheme described below utilizing TLC, DART-TOF, and/or color tests.

Hypergeometric screening is not required for Schedule II tamperable pharmaceutical preparations in simple possession cases.

5.4.3.2.2 If tampering or an illegitimate preparation is suspected in simple possession cases, fully analyze one dosage unit from each population. For charges of possession with intent to distribute or distribution, five dosage units from each population shall be fully analyzed.

5.4.3.2.3 If the evidence is resubmitted for further analysis, resample and analyze using either the administrative sampling plan (described above) or the hypergeometric sampling scheme described below) depending on the legal requirements.

5.4.4 Exceptions to this Plan may occur only at the discretion of the Section Supervisors in consultation with the Chemistry Program Manager.

5.5 Hypergeometric Sampling Plan

Hypergeometric sampling is a statistically-based model involving a defined confidence level with an associated probability of finding failures in a population. (¶¶ 5.9.1, 5.9.2, and 5.9.5) The hypergeometric model is used for specimens with no significant markings or labels (e.g., the contents of plastic bags and bag corners, vials, and glassine packets).

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

5.5.2 The appropriate number of specimens within the population will be randomly selected to give a 95% level of confidence that at least 90% of the population contains the analyte in question. Record the number of specimens indicated by the table in ¶ 40 along with an indication of the statistical relevance of the number in the case notes.

5.5.3 For cases utilizing the hypergeometric sampling plan, a random sampling procedure, like one of those listed below, shall be followed and the method recorded in case notes. Random sampling ensures that all units in a population have an equal probability of being selected and avoids selection bias. This allows statistical methods to be used to analyze the results and allows inferences to be made from a sampling of a population.

5.5.3.1 Method 1: Random Number Generator (RNG)

5.5.3.1.1 Arrange all units from the population in a grid or stack pattern.

5.5.3.1.2 Open the RNG (www.random.org, Microsoft Excel, etc.) and generate a random number between 1 and the number of units in the population. A new random number will need to be generated until the number of units to be analyzed has been reached.

5.5.3.1.3 Identify the units to be sampled by following a row or column starting at the upper left corner of the arranged units. If a number is repeated (unit has already been selected), skip and continue to the next number.

5.5.3.2 Method 2: Lottery Method A (for small units such as tablets, capsules, glassines, etc.)

5.5.3.2.1 Place units into one container and mix thoroughly.

5.5.3.2.2 Perform blind selection by reaching into the container and removing one unit at a time.

5.5.3.3 Method 3: Lottery Method B

5.5.3.3.1 Arrange all units from the population in a grid or stack pattern.

5.5.3.3.2 Place numbered pieces of paper (or similarly marked objects) in a container and mix thoroughly.

5.5.3.3.3 Perform blind selection by reaching into the container and removing one piece of paper (or marked object) at a time.

5.5.3.3.4 Identify the units to be sampled by following a row or column starting at the upper left corner of the arranged units.

5.5.3.4 Method 4: Lottery Method C (applies to groupings of units such as multiple bundles each of equal number of glassine envelopes)

5.5.3.4.1 Place numbered pieces of paper (or similarly marked objects), that represent the number of units in each grouping, in a container and mix thoroughly.

5.5.3.4.2 Perform blind selection by reaching into the container and removing one piece of paper (or marked object).

5.5.3.4.3 Select that number unit from each group. Such as the fourth glassine envelope in each bundle if "4" is selected. If the number of bundles is not equal to the number of specimens needed for sampling, another random sampling method must be chosen in order to randomly select which bundles should be used.

5.5.4 Each specimen sampled will be analyzed separately and fully.

5.5.5 For resubmissions, samples previously analyzed during administrative sampling cannot be used as part of the hypergeometric sampling. The population size (*N*) used to calculate the number of units to analyze, or sample size *n*, must therefore reflect the number of previously analyzed samples subtracted from the total submitted in the item. For example, 700 pills were submitted as Item 1, five pills were previously analyzed for administrative sampling, leaving 695 for hypergeometric sampling ($N = 695$).

5.6 Bulk Materials

Bulk materials (e.g., bricks of compressed powder, bales of plant material) should be broken or cored to obtain a representative sample. Depending on the size of the material, samples from several locations may be required to obtain a representative sample. The examiner will record the locations from which the samples were obtained in the case notes.

5.7 Residue Samples

Residues are samples which are either too small to be weighed accurately or that which remains after the bulk has been removed. Residues can be sampled by mechanical means (e.g., shaking or scooping) or chemical means (e.g., rinsing with solvent). Case notes must reflect the method by which the sample was removed.

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

5.7.2 When it is not possible to redeposit and return the residue as received, the extract used in analysis will be returned to the evidence as per the Quality Manual (¶ 14.10.5), regardless of results.

5.7.2.1 Procedure: Evaporate the solvent from the extract in the autosampler vial used in analysis. Seal the autosampler vial (ASV) into a ziplock bag. Label the ziplock bag with the FS Lab #, Item #, initials and a statement similar to "vial and bag added at lab." Record the date in the case notes that the ASV was placed in the evidence.

5.8 Sampling for Quantitative Analysis

5.8.1 Quantitative analyses require homogenized representative samples. Generally, a relatively large sample is homogenized with a mortar and pestle prior to taking the small samples required by the quantitative method to make the solutions. The remainder of the homogenized portion should be returned with the evidence in a suitably labeled plastic bag provided by the laboratory. By their nature, suspected hashish oil samples should require no further homogenization.

5.8.2 Single specimen items

5.8.2.1 Homogenize the entire specimen, take the required number of samples for the quantitation method and return the bulk of the material to the evidence.

5.8.2.2 For large specimens such as kilos of cocaine, six core samples should be taken from multiple locations and homogenized. The locations of samples taken shall be described in the case notes.

5.8.3 Multiple specimen items

5.8.3.1 Items with multiple specimens should be analyzed qualitatively using the administrative sampling plan (up to five specimens) or the hypergeometric sampling plan if necessary.

5.8.3.2 A composite will be formed consisting of the specimens analyzed. Homogenize the composite and take the number of samples required for the quantitation method. The remainder of the composite should be returned to the evidence in a plastic bag provided by the laboratory, clearly marked as a composite.

5.9 References

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

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

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

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

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

5.9.6 ASTM Standard E2548, 2016, "Standard Guide for Sampling Seized Drugs for Qualitative and Quantitative Analysis," ASTM International, West Conshohocken, PA, 2016, DOI: 10.1520/E2548-16, www.astm.org.

6 CANNABIS PLANT MATERIAL

6.1 Introduction

6.1.1 Marijuana is neither a "controlled substance" nor is it "scheduled" under Virginia Law. However, it is defined and covered under separate sections of Virginia's Drug Control Act (§ 54.1-3400 et seq.) and Cannabis Control Act (§ 4.1-600 et seq.) with associated penalties. Marijuana does not include hashish oil (for offense dates prior to July 1, 2020), industrial hemp that is possessed by a person or his agent with the appropriate registration, industrial hemp extracts, or hemp products.

6.1.2 The distinction between marijuana and hemp, in part, is the percent concentration of Tetrahydrocannabinol (THC). As defined, industrial hemp has a concentration of 0.3% delta-9-THC, specifically, or less. As of July 1, 2023, the Code of Virginia's definition of "hemp product" changed such that it contains industrial hemp with a total THC concentration (to include delta-9-THC isomers) of no greater than 0.3% and contains either no more than two milligrams of total THC per package or an amount of Cannabidiol (CBD) that is no less than 25x greater than the amount of total THC per package.

6.1.3 Cannabis plant material generally contains cannabinoids such as CBD, delta-9-THC, Tetrahydrocannabinolic acid (THCA), or a combination thereof.

6.1.4 Gross morphological characteristics in Cannabis may be observed and noted when present, such as the palmate arrangement of the leaflets, the pinnate appearance of the leaflets, the serrated edges of the leaflet, the buds (with or without seeds), and fluted stems and stalks.

6.1.5 For 'home cultivation of marijuana' cases, separately packaged specimens will be counted and analyzed, separately and fully, to exceed the plant number threshold. A list of these instances can be found in the Code of Virginia (thresholds can also be found in ¶ 39).

6.2 Microscopic Identification

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

6.2.2 Cystolithic hairs are unicellular, "bear claw" shaped hairs with a cystolith of calcium carbonate ($CaCO_3$) at the base. They are found in greatest abundance on the upper side of the leaf with longer covering hairs on the underside.

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

6.2.4 The observation of the presence of cystolithic hairs or characteristic seeds is sufficient for a positive test. The observation of additional characteristics is considered supportive. Microscopic examination results (positive or negative) shall be recorded in the analytical notes. A result is considered positive when sufficient characteristics are observed.

6.3 4-Aminophenol (4-AP) Color Test

6.3.1 The 4-aminophenol (4-AP) color test may be used as a screening test to help distinguish between hemp and marijuana and is based on the relative CBD to delta-9-THC concentration ratio.

6.3.2 Reagent preparation is listed in the Color Test section of this manual. (see ¶ 9.3.1)

6.3.3 Procedure:

6.3.3.1 Place a small amount of material (approximately 5 mg or the size of a grain of rice) into a culture tube or spot plate well.

6.3.3.2 Add enough of Reagent A to cover the sample (approximately 1 mL).

6.3.3.3 Add 2-4 drops of Reagent B.

6.3.3.4 Observe color change within one to two minutes.

6.3.3.5 A blank will be run in a separate culture tube or spot plate well. Record the results of the blank in the case notes by using a check mark (√), "ok", or "-" (e.g., Blk √).

- If a color develops in the blank, it should be repeated.
- If the results of the second blank are acceptable, all samples should be re-run.
- If the results of the second blank are unacceptable, the analyst shall take steps to resolve the issue (e.g., replacing the reagents, checking reagents) prior to resampling and any further analysis.

6.3.4 Results:

6.3.4.1 Results must be recorded in the case notes. This shall be accomplished by noting the color(s) observed (e.g., pink, blue, other).

6.3.4.2 The production of a blue color is indicative of a delta-9-THC concentration greater than CBD concentration. A pink color is indicative of a delta-9-THC concentration less than the CBD concentration. Any other observed color or a lack of color change indicates an inconclusive result. An inconclusive result is possible when delta-9-THC and CBD concentrations are nearly equal, sample size is too large, or insufficient levels of cannabinoids are present such as in immature Cannabis plants.

6.3.5 In addition to or in lieu of the 4-AP Color Test, TLC must be performed for Cannabis plant material and extracts.

6.4 Duquenois-Levine

6.4.1 The Duquenois-Levine test can indicate Cannabis, but is unable to distinguish between marijuana and hemp.

6.4.2 Extract sample into a suitable solvent (e.g., hexane, 9:1 MeOH:$CHCl_3$, petroleum ether or methanol). If a large amount of solvent is used, most of it must be evaporated. The solvent used must be recorded in the case notes.

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

6.4.4 Add sufficient $CHCl_3$ to form two discernible layers and mix. For a positive reaction to the Levine portion of the test, the bottom layer turns pink/purple in the presence of THC or other cannabinoids.

6.4.5 Run a solvent blank as a negative control with each batch of samples. The results of the negative control must be documented in the case notes. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., Blk √).

6.4.5.1 If a color develops in the blank, it should be repeated.

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

6.4.5.3 If the results of the second blank are unacceptable, the analyst shall take steps to resolve the issue (e.g., replacing the solvent in the bottle, checking the reagents) prior to re-sampling and any further analysis.

6.4.6 Reagent preparation is listed in the Color Test section of this manual. (see ¶ 9.3.7)

6.4.7 The Rapid Duquenois-Levine Procedure

6.4.7.1 Place a small amount of sample in a culture tube, add Duquenois reagent and concentrated HCl in approximately equal proportions. A positive reaction to the Duquenois portion is a blue/purple color. Add $CHCl_3$. Extraction of a pink/purple color into the $CHCl_3$ layer will occur for a positive reaction.

6.4.7.2 A blank (negative control) will be run in a separate culture tube. The results of the negative control must be documented in the case notes. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., Blk √).

- If a color develops in the blank, it should be repeated.
- If the results of the second blank are acceptable, all samples should be re-run.
- If the results of the second blank are unacceptable, the analyst shall take steps to resolve the issue (e.g., replacing the solvent in the bottle, checking the reagents) prior to re-sampling and any further analysis.

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

6.4.8 Results must be recorded in the case notes and shall include the results of both steps.

6.5 Thin Layer Chromatography (TLC)

6.5.1 TLC is a separation technique that can separate the major cannabinoids present in Cannabis as well as screen for other controlled substances. TLC may help distinguish between hemp and marijuana by comparing relative cannabinoid concentrations.

6.5.2 TLC plates should be silica gel or equivalent, sufficient to resolve CBD, delta-9-THC, and Cannabinol (examples: Analtech Silica Gel GHLF 250um 10 X 20, Analtech Silica Gel 250um GF 10x20, EMD TLC Silica Gel 250um 60 F_{254} 5 X 10, EMD TLC Silica Gel 250um 60 F_{254} 10 X 20).

6.5.3 Procedure:

6.5.3.1 Extract sample into a suitable solvent (e.g., hexane, 9:1 MeOH:$CHCl_3$, petroleum ether or methanol). The solvent used must be recorded in the case notes.

6.5.3.2 Spot sample(s), standard(s) and solvent blank on the plate. The maximum number of spots when using a 10 x 20 cm plate is 32. (See ¶ 10.3 for further information)

6.5.3.3 The results of the blank must be recorded in the case notes. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., "Blk √).

6.5.3.4 Mobile Phase: 4-8% diethylamine in toluene

6.5.4 Visualization Sprays:

6.5.4.1 Fast Blue B Salt (Tetrazotized o-dianisidine zinc chloride salt)

6.5.4.2 Fast Blue BB Salt (4-benzoylamino-2,5-diethoxy-benzenediazonium chloride hemi [zinc chloride] salt)

6.5.4.3 Reagent preparation is listed in the Thin Layer Chromatography section of this manual. (see ¶ 10.5.6)

6.5.5 Results:

6.5.5.1 The three major cannabinoids migrate and develop in the following order:

Top spot - CBD – orange
Middle spot - delta-9-THC – red
Lower spot - Cannabinol – purple

A red spot at the origin may be present in unburned Cannabis due to cannabinolic acid(s).

6.5.5.2 Specific solvent systems and developing sprays utilized in casework will be denoted in the case notes. A result is considered positive for Cannabis when the distance traveled and the reaction with the visualization methods compare favorably with a Cannabis or primary standard (e.g., THC, CBN, and/or CBD). It is not necessary for each of the three major cannabinoids to be present for the results to be considered positive. TLC results shall be recorded in the analytical notes by including an indication of what was observed (e.g., "+THC", "-CBD", "pos CBS").

6.5.6 Samples will be screened for the presence of other commonly encountered drugs such as cocaine or PCP by either overspraying with acidified iodoplatinate or by running a separate plate in an appropriate drug bath (e.g., TLC1 or TLC2.) (see ¶ 10.4).

6.5.7 After the plate is sprayed with Fast Blue B or Fast Blue BB, it shall be scanned and a color hardcopy printed. Each lane shall be labeled (FS Lab# and Item #, standard identifier, blank, etc.) either in the image or on the color copy. Templates may be used to assist with labeling.

6.5.8 There is not a two-system chromatographic requirement for Cannabis when a valid one-system TLC is combined with the semi-quantitative GC-FID-MS technique.

6.6 DART-TOF

6.6.1 DART-TOF can detect the presence of cannabinoids but is unable to distinguish between hemp and marijuana. DART-TOF may be used for general drug screening (see ¶ 16.2).

6.7 Semi-quantitative Gas Chromatography-Flame Ionization Detection-Mass Spectrometry (GC-FID-MS)

6.7.1 This GC-FID-MS method is a semi-quantitative test to establish if the THC concentration is greater than or less than an administrative threshold to assist in the differentiation of hemp from marijuana.

6.7.2 If the total THC administrative threshold is reached or exceeded with the assessment of a single or subset of THC isomers (for example, delta-9-THC), there is no need to assess the contribution from all THC isomers that may be present.

6.7.3 Procedure:

6.7.3.1 All solutions shall be prepared and/or transferred with calibrated mechanical pipettes. Serial numbers or other unique identifiers of calibrated mechanical pipettes shall be recorded in examination documentation or documented in the standard preparation record.

6.7.3.2 Internal Standard Preparation: Prepare a 0.1 mg/mL solution of testosterone in 9:1 MeOH:$CHCl_3$ in an appropriate volumetric flask. This will be used for the extraction solvent.

6.7.3.3 Standard Solution Preparation

6.7.3.3.1 **Delta-9 or delta-8-THC standards**: Pipette 50 µL of delta-8 or delta-9-THC certified reference standard (10 mg/mL in solvent) into a test tube, evaporate to dryness, and reconstitute with 5 mL of internal standard solution. Mix thoroughly. This will serve as the 1% THC reference (0.1 mg/mL) for delta-8 or delta-9-THC. These reference standards may be made as individual standards or as a mixed standard (containing both delta-8-THC and delta-9-THC) based on need at the time of preparation.

- Provided that a 50 mg plant sample containing 1% THC would result in 0.5 mg THC dissolved in 5 mL of internal solvent, this is equivalent to 0.1 mg/mL THC.

6.7.3.3.2 **THC isomer standard (if needed)**: Pipette 100 µL of certified reference standard (1 mg/mL in solvent) into a test tube, evaporate to dryness, and reconstitute with 1 mL of internal standard solution. Mix thoroughly. This will serve as a 1% THC isomer reference (0.1 mg/mL) for delta-6a,10a-THC and both stereoisomers of delta-10-THC. These isomer reference standards may be made as individual standards or mixed isomer standards [containing delta-6a,10a-THC, (6aR,9R)-delta-10-THC, and/or (6a,9S)-delta-10-THC] based on need at the time of preparation.

- The THC isomer standard may be prepared the same as the delta-9/delta-8 standard if the appropriate concentrations become available. Alternatively, the preparation of a single THC isomer standard containing delta-8-THC, delta-9-THC, delta-6a,10a-THC, and delta-10-THC is permissible should the appropriate isomer concentrations become available.

6.7.3.4 Sample Preparation: Weigh 50.0 ± 1.0 mg of material. In a test tube, add 5 mL of testosterone internal standard to the sample. Allow approximately 15 minutes for extraction prior to vortexing/mixing for approximately 30 seconds. As needed, filter the solution into an autosampler vial.

- If the submitted material is insufficient to perform the semi-quantitative GC-FID-MS analysis, analyze as a residue and report as Cannabis with insufficient quantity.

6.7.3.5 Instrument Parameters

- Agilent 6890/5973 (or later) with dual column capability
- Inlet Temperature: 250ºC
- Split Injection, maximum split ratio: 50:1
- Minimum Injection Volume: 1 µL
- Carrier Gas and Flow: high purity Helium; 1.5 mL/min (hold for 4.3 min), ramped to 1.8 mL/min at 2 mL/min per min
- Oven: Initial temperature 210ºC ramped to 235ºC at 30ºC/min; hold at 235ºC for 3 min; ramp to 280ºC at 30ºC/min; hold for at least 0.67 min

MS Parameters:

- Column: DB-5MS (or equivalent); 15 m x 0.25 mm x 0.25 µm
- Solvent Delay: ~ 0.75 min
- Scan Range: 14-400 Da
- Scan Threshold: 200
- MS Source Temperature: 230ºC
- MS Quadrupole Temperature: 150ºC
- Transfer Line Temperature: 280ºC

FID Parameters:

- Column: HP-1-MS (or equivalent); 15 m x 0.25 mm x 0.25 µm
- FID Temperature: 300ºC
- Hydrogen Flow: 40 mL/min
- Air Flow: 450 mL/min
- Nitrogen Makeup Flow: 45 mL/min
- Mode: Constant makeup flow
- Signal Data Rate: 50 Hz

6.7.4 Day of Use:

6.7.4.1 To demonstrate instrument performance, inject the THC standard solution(s). The THC:internal standard peak area ratio for each THC standard solution must fall within the appropriate range:

- Delta-9-THC: 0.85316 – 1.26463
- Delta-8-THC, delta-6a,10a-THC, and delta-10-THC: 0.99987 – 1.26358

6.7.4.2 The THC standard solution data should be maintained in the casefile or its casefile location referenced.

6.7.4.3 Examiners are responsible for entering THC control values into the statewide control chart. THC control chart values shall be verified by another Controlled Substances examiner. The verifier shall denote the completion of the review by entering their initials into the appropriate column in the control chart. For values that have already been verified, additional verifying initials are not necessary.

6.7.4.4 If the ratio falls outside the acceptance criteria range, replace the standard and/or perform instrument maintenance as needed.

6.7.5 An internal standard solution blank shall be run as a negative control.

6.7.6 Inject the sample solution. Use the FID peak area data to calculate the THC:internal standard ratio.

6.7.7 Use the MS total ion chromatogram and FID chromatogram to determine the presence/absence of any interfering compounds that co-elute with delta-9-THC, its isomers, or the internal standard.

6.7.7.1 Delta-9-THC and delta-6a,10a-THC are known to coelute on this FID chromatogram; therefore, the peak area for delta-9-THC will include both isomers. No further separation is necessary for semi-quantitation purposes.

6.7.7.2 Cannabielsoin (CBE) is a degradation product of CBD. CBE has been shown to co-elute with delta-9-THC in further analysis (outlined in ¶ 6.7.7.3). Therefore, if an interferent and CBE are present in the MS total ion chromatogram:

6.7.7.2.1 This sample would not be suitable for analysis (for cases with offense dates prior to July 1, 2023).

6.7.7.2.2 Do not evaluate the peak area of delta-9-THC and rather assess peak areas for isomers that may be present (for cases with offense dates on or after July 1, 2023). If no delta-9-THC isomers are present, this sample would not be suitable for analysis.

6.7.7.3 If an interferent is present and CBE is absent, analyze the GC-FID-MS extract on a GC-FID with the following parameters:

- Column: HP-35MS (or equivalent); 15 m x 0.25 mm x 0.25 µm
- Split: 50:1
- Flow Rate: 1.0 mL/min
- Inlet Temperature: 290ºC
- Oven: Initial temperature 255ºC (hold 5 min) ramped to 300ºC at 30ºC/min; hold for at least 4 mins
- FID Temperature: 300ºC

6.7.7.3.1 A 1% THC standard solution in testosterone would be necessary for comparison, and the THC:internal standard peak area ratio for each THC standard solution must fall within the appropriate range:

- Delta-9-THC: 0.85669 – 1.33295
- Delta-8-THC, delta-6a,10a-THC, and delta-10-THC: 0.99072 – 1.30081

6.7.7.3.2 Delta-8-THC and delta-6a,10a-THC are known to coelute on this FID chromatogram; therefore, the peak area for delta-8-THC will include both isomers. No further separation is necessary for semi-quantitation purposes.

6.7.7.3.3 Examiners are responsible for entering THC control values into the statewide control chart. THC control chart values shall be verified by another Controlled Substances examiner. The verifier shall denote the completion of the review by entering their initials into the appropriate column in the control chart. For values that have already been verified, additional verifying initials are not necessary.

6.7.8 Compare the GC-FID peak area ratio of the sample's THC:internal standard to the respective day-of-use THC standard solution ratio for each isomer evaluated to generate results. "Day-of-use" is defined as an approximate 24 hour period. The GC-FID-MS peak area ratios for delta-9-THC and CBD can also be used to clarify an inconclusive 4-AP color test result.

6.7.8.1 The internal standard response shall be monitored. The peak area of the internal standard in the case sample(s) shall be within ± 20% of the peak area in the delta-9-THC standard solution. If there is greater than ± 20% difference, take appropriate action to reconcile the difference (e.g., refresh the delta-9-THC standard solution, remake and reanalyze the affected sample, etc.). Following reconciliation attempts, if there continues to be more than ± 20% peak area difference or the delta-9-THC standard solution falls outside of the acceptable day of use range, the internal standard solution, delta-9-THC standard solution, and all case samples shall be freshly prepared.

6.7.8.2 For cases with offense dates prior to July 1, 2023:

6.7.8.2.1 If the GC-FID peak area ratio of the sample THC:internal standard is greater than or equal to the respective day-of-use THC standard solution ratio, the amount of total delta-9-THC (delta-9-THC + decarboxylated THCA) is determined to be greater than 0.3% and the result will be reported as Marijuana.

6.7.8.2.2 If the GC-FID peak area ratio of the sample THC:internal standard is less than the respective day-of-use THC standard solution ratio, the amount of total delta-9-THC (THC + decarboxylated THCA) is inconclusive as to the Cannabis type (marijuana/hemp). This sample would require full quantitation to determine the concentration of delta-9-THC and THCA.

6.7.8.2.3 Other tetrahydrocannabinol isomers should be reported if present in higher concentrations than those typically seen in plant material.

6.7.8.3 For cases with offense dates on July 1, 2023, and after:

6.7.8.3.1 If the total THC administrative threshold is reached or exceeded with the assessment of a single or subset of THC isomers (e.g., delta-9-THC), there is no need to assess the contribution from all THC isomers that may be present.

6.7.8.3.2 If the sum total of the GC-FID peak area ratios of the sample THC:internal standard is greater than or equal to the respective day-of-use THC standard solution ratios, the amount of total THC (delta-9-THC + delta-8-THC + delta-10-THC + delta-6a,10a-THC + their decarboxylated acids) is determined to be greater than 0.3% and the result will be reported as Marijuana.

6.7.8.3.3 If the sum total of the GC-FID peak area ratios of the sample THC:internal standard is less than the respective day-of-use THC standard solution ratios, the amount of total THC (delta-9-THC + delta-8-THC + delta-10-THC + delta-6a,10a-THC + their decarboxylated acids) is inconclusive as to the Cannabis type (marijuana/hemp). This sample would require full quantitation to determine the concentration of delta-9-THC and THCA.

6.7.8.4 Retention time comparison and two-system chromatography are not required to identify Cannabis or Marijuana.

6.7.8.5 Quantitative concentrations are not established nor inferred from this semi-quantitative method. Factors such as sampling, sample homogeneity, and source (e.g., lot number) could cause differences between semi-quantitative and quantitative results.

6.8 Quantitative Analysis of Total THC in Plant Material using GC/MS (SIM)

6.8.1 This GC-FID-MS SIM method is a quantitative test to establish the total delta-9-THC (delta-9-THC + decarboxylated THCA), present in Cannabis plant material.

6.8.2 The Department does not routinely perform drug quantitations. The total THC concentration in Cannabis plant material will not be routinely determined, but rather the request evaluated on a case-by-case basis.

6.8.3 Silanization of Glassware

6.8.3.1 Materials

- 5% Dichlorodimethylsilane in toluene solution
  - This silanizing solution can be used 3-4 times before discarding
- Volumetric flask including glass stopper
- Screw-top 5 mL glass tubes
- Cell culture tubes
- Autosampler vial spring inserts
- Beakers and glass storage containers
- Toluene
- Methanol

6.8.3.2 Procedure:

6.8.3.2.1 All glassware used in this procedure shall be silanized, unless otherwise noted.

6.8.3.2.2 Fill appropriate glassware with a 5% dichlorodimethylsilane in toluene solution.

6.8.3.2.3 Allow glassware to silanize under standard laboratory conditions for at least 20 minutes.

6.8.3.2.4 Remove the silanizing solution and perform a series of rinses: first with toluene, then methanol, then toluene again, and finishing with methanol.

6.8.3.2.5 Dry silanized glassware in the oven at approximately 80°C for at least 20 minutes, exercising caution to vent vapors appropriately.

6.8.4 Quantitation

6.8.4.1 Materials

- Silanized glassware (See ¶ 6.8.3)
- delta-9-THC certified reference standard (10 mg/mL in ethanol)
- delta-9-THC-D_9 internal standard (1.0 mg/mL in methanol)
- 80:20 Acetonitrile:Methanol
- BSTFA with 1% TMCS
- Ethyl acetate
- Hemp certified reference material (CRM)
- Calibrated pipettes
- Mill grinder and disposable grinder cups

6.8.4.2 Standards Preparation

6.8.4.2.1 Calibrated pipettes shall be used for all liquid transfers, unless otherwise noted.

6.8.4.2.2 The internal standard solution is a 0.04 mg/mL solution of delta-9-THC-D_9 in 80:20 acetonitrile:methanol. Prepare a sufficient volume of internal standard solution to prepare calibrators, controls, and samples.

- Pipette 1.2 mL of 1.0 mg/mL THC-D_9 standard and 28.8 mL of 80:20 acetonitrile:methanol in appropriate glassware. Other volumes may be pipetted which result in a 0.04 mg/mL solution.

- The expiration date for the prepared internal standard solution shall not exceed the date of expiration of the delta-9-THC-D_9 standard.

6.8.4.2.3 THC working standard (1.0 mg/mL): pipette 100 µL of 10.0 mg/mL THC standard and 900 µL of 80:20 acetonitrile:methanol into an autosampler vial.

- The expiration date for the THC working standard shall not exceed the date of expiration of the delta-9-THC standard.

6.8.4.2.4 Calibration Curve and Controls Preparation

Calibration Level	Calibrator Concentration (mg/mL)	Associated % of delta-9-THC	Volume 10 mg/mL delta-9-THC Standard (µL)	Volume 1.0 mg/mL delta-9-THC Standard (µL)	Volume 0.04 mg/mL Internal Standard Solution (mL)
1	0.008	0.04	-	20	2.5
2	0.016	0.08	-	40	2.5
3	0.060	0.30	15	-	2.5
4	0.120	0.60	30	-	2.5
5	0.252	1.26	63	-	2.5

Control Level	Control Concentration (mg/mL)	Associated % of delta-9-THC	Volume 10 mg/mL delta-9-THC Standard (µL)	Volume 1.0 mg/mL delta-9-THC Standard (µL)	Volume 0.04 mg/mL Internal Standard Solution (mL)
6	0.030	0.15	-	75	2.5
7	0.100	0.50	25	-	2.5
8	0.200	1.00	50	-	2.5

- In addition to the three controls, a hemp CRM shall be processed concurrently and in the same manner as case samples with every batch. The hemp CRM will serve as both a QA check for the process as well as contribute to UoM calculations.

- Calibrators and controls shall be prepared using standards from different manufacturers. If this cannot be obtained, different lot numbers for calibrators and controls must be used. If different lot numbers cannot be obtained, the calibrators and controls must be prepared by different individuals.

- Calibrators and controls shall be prepared and undergo the same procedure concurrently as the samples, with the exception of drying, grinding, and decarboxylation.

- A 2.5 mg/mL derivatized CBD standard shall be freshly prepared as an additional control.

6.8.4.3 Sample Drying and Grinding

6.8.4.3.1 Record the weight of the empty drying vessel. Appropriate vessels include disposable foil or paper weighing containers.

6.8.4.3.2 Weigh approximately 1.0 gram of plant material and hemp CRM into separate vessels and record their weights.

6.8.4.3.3 Place the vessels containing plant material into a 40°C ± 2°C oven for at least 16 hours. The temperature shall be logged in accordance with oven quality assurance procedures.

6.8.4.3.4 Remove the vessels containing plant material from the oven and allow them to cool to room temperature. Weigh the vessels containing plant material and record weights.

6.8.4.3.5 Calculate the percent change in weight of the plant material pre- and post-drying. If the percent change is greater than 10%, place the vessel containing plant material back into the oven in 4 hour increments until the percent change is less than 10%.

% change = [(weight of plant material pre-drying) - (weight of plant material post-drying)] / (weight of plant material pre-drying) * 100

6.8.4.3.6 Once dry, transfer the plant material into a disposable grinding cup and grind the sample with two 1.0 minute grinding sessions at approximately 5000 rpm.

6.8.4.3.7 Using a 4-place balance, weigh and record two 50.0 ± 1.0 mg portions and place into screw-top 5 mL glass test tubes. For the hemp CRM, only one 50.0 ± 1.0 mg portion shall be used.

- All remaining ground sample(s) shall be stored in the freezer until such time as it is determined they are no longer necessary for analysis.

- If further analysis is required, frozen ground samples and hemp CRM stored under the same conditions shall be re-extracted in conjunction with freshly prepared calibrators and controls.

6.8.4.3.8 If unable to continue to decarboxylation on the same day, plant material samples and solutions shall be stored in the freezer, for no more than 24 hours, and allowed to return to ambient temperature prior to continuing with decarboxylation.

6.8.4.4 Plant Material Decarboxylation

6.8.4.4.1 Ensure the plant material is at the bottom of the tube. If needed, centrifuge all capped samples for 2.0 minutes at approximately 1000 rpm.

6.8.4.4.2 Incubate capped samples using a heat block for 20 minutes at approximately 130°C. The temperature shall be logged in accordance with heat block quality assurance procedures.

6.8.4.4.3 Allow tubes to cool to room temperature.

6.8.4.5 Extraction and Derivatization

6.8.4.5.1 To each test tube, add 2.5 mL of internal standard solution.

- A procedure blank (negative control) of 80:20 acetonitrile:methanol and internal standard shall be extracted alongside all extracts (samples, calibrators, and controls).

6.8.4.5.2 Vortex all extracts for approximately one minute.

6.8.4.5.3 Sonicate all extracts for 15 minutes; then vortex for approximately 30 seconds.

6.8.4.5.4 Centrifuge capped extracts for 2.0 minutes at approximately 1000 rpm.

6.8.4.5.5 Transfer 250 µL of the extract into an appropriate glass tube.

- If unable to continue with derivatization on the same day, extracts shall be capped and stored in the freezer, for no more than 24 hours, and allowed to return to ambient temperature prior to continuing with derivatization.

6.8.4.5.6 Evaporate extracts to dryness. During evaporation, ensure that excessive drying does not occur. Heat shall not be applied during the evaporation process.

6.8.4.5.7 Add 125 µL of BSTFA with 1% TMCS and 125 µL of ethyl acetate to each tube.

6.8.4.5.8 Cap and vortex extracts for 15-30 seconds. Centrifuge capped tubes for 2.0 minutes at approximately 1000 rpm.

6.8.4.5.9 Incubate extracts using a heat block at approximately 70°C for 30 minutes. The temperature shall be logged in accordance with heat block quality assurance procedures.

6.8.4.5.10 Remove capped tubes from heat and allow to cool to room temperature.

6.8.4.5.11 Transfer extracts to an autosampler vial with a silanized insert for analysis. The pipettes used for transfer and autosampler vial do not need to be silanized nor calibrated.

6.8.4.6 Instrument Parameters

GC Parameters:
- Inlet Temperature: 250°C
- Split Injection, Split Ratio: 10:1
- Injection Volume: 1 µL
- Carrier Gas and Flow: high purity Helium; 1.5 mL/min (hold for 4.3 min), ramped to 1.8 mL/min at 2 mL/min^2 (hold for at least 0.7 min)
- Oven Temperature Program: Initial temperature 210°C ramped to 235°C at 30°C/min (hold for 3 min), ramped to 280°C at 30°C/min (hold for at least 0.67 min)

MS Parameters:
- Column: DB-5MS (or equivalent); 15 m x 0.25 mm x 0.25 µm
- Solvent Delay: ~ 0.75 min
- MS Source Temperature: 230°C
- MS Quadrupole Temperature: 150°C
- Transfer Line Temperature: 280°C
- Acquisition Mode: SIM
  - Number of SIM groups: 2
  - Resolution: Low
  - Group 1
    - Start Time: 0.75 min
    - Number of Ions: 6
    - Dwell Time: 35 ms

Analyte: delta-9-THC-TMS	
Mass (*m/z*)	**Ion Type**
343	Qualifier
371	Qualifier
386	Quantifier

Analyte: delta-9-THC-D_9-TMS	
Mass (*m/z*)	**Ion Type**
352	Qualifier
380	Qualifier
395	Quantifier

  - Group 2 – Derivatization Check
    - Start Time: 3 min
    - Number of Ions: 2
    - Dwell Time: 50 ms

Mass (*m/z*)	**Analyte**
299	Underivatized delta-9-THC
308	Underivatized delta-9-THC-D_9

FID Parameters:
- The FID data is not necessary for this analysis. Therefore, within the method, the saving of the FID signal may be disabled.

6.8.4.7 Methods

6.8.4.7.1 A 2.5 mg/mL derivatized CBD standard shall be run prior to or at the beginning of a sequence to demonstrate no conversion of CBD to THC. To demonstrate

acceptable conditions, no derivatized delta-9-THC shall be detectable. The TIC shall be included in the casefile.

- If derivatized delta-9-THC is detected, appropriate instrument maintenance shall be performed.

6.8.4.7.2 At a minimum, a solvent blank of ethyl acetate must be run before each sequence of sample runs to demonstrate instrument cleanliness. Additional solvent or procedural blanks may be run at the examiner's discretion, such as after a sample if the potential for carryover is suspected.

6.8.4.7.3 A standard of underivatized THC shall be included in every run to allow for comparison.

6.8.4.7.4 A sample's position relative to a blank shall be documented in the case file. This may be accomplished by consecutive data file numbering.

6.8.4.7.5 Successively run samples, exceeding no more than 10, must be bracketed by positive standards (i.e., calibrators or controls).

6.8.4.7.6 Extracted plant material samples are stable for five days. However, due to instrument response variability, daily tunes, and the ratio nature of this analysis, the entire sequence must be reinjected, including the calibrators and controls, if the need to rerun a sample arises.

- Re-injecting the sequence shall include the previously prepared and injected 2.5 mg/mL derivatized CBD standard. To demonstrate acceptable conditions, no derivatized delta-9-THC shall be detectable. The TIC shall be included in the casefile.
- If derivatized delta-9-THC is detected in the CBD standard, appropriate instrument maintenance shall be performed. If derivatized delta-9-THC remains detectable, all samples shall be re-extracted and a new CBD standard prepared.

6.8.5 Calculations, Acceptance Criteria and Reporting

6.8.5.1 Using ChemStation Data Analysis, ensure the absence of any underivatized delta-9-THC or delta-9-THC-D_9. The TIC shall be included in the case file. If underivatized delta-9-THC and/or delta-9-THC-D_9 are present, repeat the derivatization with new 250 µL aliquots as their presence indicates an incomplete derivatization.

6.8.5.2 If quality assurance criteria (i.e., qualifier ratios, peak shape, retention time), are not met, take appropriate steps (e.g., perform appropriate corrective instrument maintenance, rerun, remake controls, or prepare new samples) to resolve the problem.

6.8.5.3 Using MassHunter, generate Calibration and Quantitative Summary Reports as well as individual sample reports for each injection within the sequence.

6.8.5.4 For comparison purposes, standards must be run using the same method conditions as the samples. Standards used in the comparison must be run on the same day as the samples. "Same day" is defined as an approximate 24 hour period. Retention times for analytes must agree with the standard within 2 seconds (± 2 sec) or 0.033 minutes to be considered a positive result.

6.8.5.5 The calibration model is weighted (1/x) quadratic. The correlation of determination (r^2) value for the curve must be 0.995 or greater.

6.8.5.6 The analytical method utilizes selected ion monitoring mode with the acquisition of three ions for delta-9-THC (386.0 *m/z*, 343.0 *m/z*, and 371.0 *m/z)* and an additional three ions for the internal standard (395.0 *m/z*, 352.0 *m/z*, and 380.0 *m/z*). The quantifier ion for delta-9-THC is 386.0 *m/z* and the quantifier ion for the deuterated internal standard is 395.0 *m/z*. A peak area ratio is established between the quantifier ion and each qualifier ion. The qualifier ratios of the calibrators will be averaged for each batch. The qualifier ratios for the controls and plant samples shall be within ± 15% of this average of the calibrators.

6.8.5.7 If the selected ion chromatogram demonstrates poor peak shape, for example resulting from interference, the data will be considered unacceptable.

6.8.5.8 The signal to noise (S/N) of the quantitation ion must be greater than 10:1 to be considered acceptable.

6.8.5.9 The concentration of the calibrators and controls must be within ± 15% of the theoretical values.

6.8.5.9.1 Only one calibration point or control may be dropped. The reason (e.g., due to poor peak shape or falling outside the accuracy acceptance range) and date a calibrator/control was dropped shall be recorded in examination documentation.

6.8.5.9.2 A new preparation of all extracts would be appropriate if a calibrator remains outside the acceptance criteria, even with a calibrator removed, or more than one control remains outside the acceptance criteria.

6.8.5.10 Using the concentrations generated by MassHunter, use the *SIM Quantitation Worksheet* to calculate the item's % Total THC.

Total THC (%) per sample = [concentration / (sample weight / internal standard volume)] * 100 * dilution factor, if needed

Total THC (%) per item = (Total THC $\%_{sample1}$ + Total THC $\%_{sample2}$) / 2

6.8.5.10.1 The concentration of the hemp CRM must be within ± 20% of the theoretical value calculated from the CRM certificate of analysis.

- Examiners are responsible for entering hemp CRM values into the statewide control chart.
- If the hemp CRM falls outside of this acceptance criteria, new extractions of all samples would be appropriate.

6.8.5.10.2 The relative standard deviation of the two samples must be 15% or less.

6.8.5.11 Negative samples are those that have instrumental response less than 10% of the lowest calibrator concentration peak area.

6.8.5.12 For samples with a concentration greater than the upper limit of quantitation (i.e., highest acceptable calibrator concentration used in the calibration curve), dilute the samples with the internal standard solution using a 1:2 or 1:10 dilution ratio prior to derivatization.

6.8.5.12.1 For a 1:2 dilution, take 125 µL of the extract and dilute with 125 µL of internal standard.

6.8.5.12.2 For a 1:10 dilution, take 25 µL of the extract and dilute with 225 µL of internal standard.

6.8.5.13 If quality assurance criteria are met, the results may be reported and shall include the Uncertainty of Measurement (UoM).

6.8.5.13.1 Two-system chromatography is not required to quantitate total THC if the item was previously analyzed by semi-quantitative GC-FID-MS.

6.8.5.13.2 If the sample's calculated average concentration falls below the limit of quantitation (i.e., the lowest calibrator concentration included in the calibration response curve that meets all quality requirements) and has an instrumental response greater than 10% of the lowest calibrator concentration peak area, the result shall be reported as less than 0.04% total THC (or the lowest calibrator concentration included in the calibration response curve).

6.8.5.13.3 If the sample's calculated average concentration falls above the upper limit of quantitation (i.e., the highest calibrator concentration included in the calibration response curve that meets all quality requirements), an appropriate dilution factor should be used to obtain concentrations within the calibrated range. The resulting total THC concentration shall be reported.

6.8.5.13.4 If quality assurance criteria are not met and the instrumental response for THC is less than 10% of the lowest calibrator concentration peak area, the results will be reported as not detected.

6.8.5.13.5 In instances where appropriate remediation is not possible, such as insufficient sample or the presence of an interfering compound which prevents an accurate quantitative determination, the report will include an explanation for the lack of quantitative results (e.g., insufficient sample quantity, not suitable for analysis).

6.9 Residues

6.9.1 Due to limited sample size present in residues, the Cannabis type (marijuana/hemp) cannot be determined using the GC-FID-MS semi-quantitative test.

6.9.2 Following the microscopic examination, TLC or DART-TOF would be appropriate to screen for the presence of controlled substances. If either is positive for cannabinoids, perform the Duquenois-Levine Test.

6.9.3 If microscopic examination, TLC or DART-TOF, and Duquenois-Levine tests are positive, report the result as Cannabis residue without distinguishing the Cannabis type (marijuana/hemp).

6.9.4 If one of the tests (microscopic examination, TLC, DART-TOF, or Duquenois-Levine tests) is inconclusive, negative, or omitted, Gas Chromatography/Mass Spectrometry (GC/MS) shall be performed. Retention time data from GC/MS may be used if more than one test is inconclusive, negative, or omitted.

6.10 References

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

6.10.2 Schläpfer, M. "Recipe for presumptive test solutions – Cannabis Typification," Forensisches Institut Zürich, 2017.

6.10.3 Lewis, K., Wagner, R., Rodriguez-Cruz, S.E., Weaver, M.J., Dumke, J.C. Validation of the 4-aminophenol color test for the differentiation of marijuana-type and hemp-type cannabis. *Journal of Forensic Sciences*. 2020; 00:1-10. https://doi.org/10.1111/1556-4029.14562.

6.10.4 Hadener, M., Gelmi, T., Martin-Fabritius, M., Weinmann, W., Pfaffli, M. Cannabinoid concentrations in confiscated cannabis samples and in whole blood and urine after smoking CDB-rich cannabis as a “tobacco substitute”. *International Journal of Legal Medicine*. 2019.

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

6.10.6 Hughes, R. B. and Warner, V. J., Jr. "A Study of False Positives in the Chemical Identification of Marihuana", *Journal of Forensic Sciences*, Volume 23 (2), pp. 304-309. (NOTE: Substitute toluene for benzene, a known carcinogen.)

7 CANNABIS EXTRACTS AND PRODUCTS

7.1 Introduction

7.1.1 For cases with offense dates prior to July 1, 2020, hashish oil is an oily extract containing one or more cannabinoids with little, if any, plant material present and containing 12% or more THC (controlled in Schedule I). For cases with offense dates on or after July 1, 2020, what was formerly considered hashish oil is considered marijuana and will be analyzed and reported as such. For cases with offense dates on or after July 1, 2023, a hemp product is defined as containing a total THC concentration (to include delta-9-THC isomers and their decarboxylated acids) no greater than 0.3% and contains either no more than two milligrams of total THC per package or an amount of Cannabidiol (CBD) that is no less than 25x greater than the amount of total THC per package.

7.1.2 Cannabinoid products derived from plant material may contain CBD, delta-9- THC, Tetrahydrocannabinolic acid (THCA), or a combination thereof. Cannabinoid products shall be tested pursuant to the Code of Virginia (§ 54.1-3408.3).

7.1.3 The Department of Forensic Science does not currently have a validated method to quantify delta-9-THC or any of its isomers in food products.

7.2 Cannabis Extracts

7.2.1 Analytical Scheme

7.2.1.1 Weigh or approximate the volume of the material. The weight of the material should be recorded whenever possible.

7.2.1.1.1 The contents of electronic smoking device cartridges can be collected by centrifugation.

7.2.1.2 Remove a representative sample for testing.

7.2.1.3 The 4-AP color test may be used as a screening test and performed as listed for Cannabis plant material.

7.2.1.4 Dilute with appropriate solvent and perform TLC as listed for Cannabis plant material.

7.2.2 For oily extracts with offense dates PRIOR TO July 1, 2020:

7.2.2.1 Dilute with appropriate solvent and run on a GC-FID and GC/MS or GC-FID-MS against a THC standard, for two-system chromatography (¶ 2.7.3), to confirm the presence of THC.

7.2.2.1.1 If no delta-9-THC is present, identify non-naturally occurring tetrahydrocannabinols.

7.2.2.2 Quantitate THC using method ¶ 7.2.4 below.

7.2.2.2.1 If the concentration of delta-9-THC is greater than 12% by weight, there is no need to confirm and report any other indicated tetrahydrocannabinols (e.g., delta-6a(10a)-THC; delta-8-THC; and delta-10-THC).

7.2.2.2.2 If the concentration of delta-9-THC is less than 12% by weight and tetrahydrocannabinols are indicated during analysis (e.g. delta-6a(10a)-THC, delta-8-THC, and delta-10-THC), whenever possible the indicated tetrahydrocannabinols shall be confirmed and reported.

7.2.2.2.3 Hashish oil will be reported per ¶ 34.9.3.

7.2.3 For cannabis extracts with offense dates ON or AFTER July 1, 2020, and non-oily extracts with offense dates PRIOR TO July 1, 2020:

7.2.3.1 Perform the semi-quantitative GC-FID-MS analysis per ¶ 6.7. For samples in which the TLC results indicate high levels of delta-9-THC or a THC isomer (isomers should not be considered for cases with offense dates between 07/01/2020 and 06/30/2023), the following modifications shall be performed:

7.2.3.1.1 Add a tenfold post-extraction dilution following ¶ 6.7.3.4 - In an autosampler vial, dilute 100 µL of the extract with 900 µL of internal standard solution using a calibrated mechanical pipette.

7.2.3.1.2 The GC-FID peak area ratio of the sample's THC:internal standard is determined and shall include a tenfold multiplication factor. The data shall clearly include that a multiplication factor was applied (e.g., macro language).

7.2.3.1.3 If THC is not detectable in the resulting FID data, analyze the extract from ¶ 6.7.3.4 (which is prior to tenfold dilution) using the semi-quantitative GC-FID-MS analysis outlined in ¶ 6.7.

7.2.3.2 If there is insufficient sample to perform either the semi-quantitative GC-FID-MS analysis or the THC quantitation method, analyze as a residue and report as Cannabis with insufficient quantity to distinguish marijuana and hemp per ¶ 34.6.4.1. If the offense date is prior to July 1, 2023, other tetrahydrocannabinol isomers should be reported if present in higher concentrations than those typically seen in plant material.

7.2.4 THC Quantitation

7.2.4.1 This GC-FID method is a quantitative test to establish the purity of delta-9-THC or the total delta-9-THC (delta-9-THC + decarboxylated THCA) present in Cannabis extracts and products. See GC ¶ 11 for general quantitation procedure.

7.2.4.2 Materials

- n-Hexane: High purity
- delta-9-Tetrahydrocannabinol: (10 mg/mL in EtOH)
- n-Triacontane: 98% pure or greater.
- NaOH
- Class A volumetric flasks
- Calibrated mechanical pipettes
- Calibrated volumetric flasks
- Analytical balance

7.2.4.3 Internal Standard Solution

7.2.4.3.1 Prepare a sufficient volume to dilute the delta-9-THC standard solution and all samples.

7.2.4.3.2 Prepare a 1 mg/mL solution of n-triacontane in n-hexane in the appropriate volumetric flask.

7.2.4.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

7.2.4.3.4 Internal standard blank shall be washed with 4N NaOH prior to placing in autosampler vial.

7.2.4.4 Delta-9-THC Standard Solution

7.2.4.4.1 Using a calibrated mechanical pipette, pipette 0.25 mL of the delta-9-THC standard into a culture tube. Evaporate to dryness. Using a calibrated mechanical pipette, pipette 2.5 mL of internal standard solution into the culture tube and mix thoroughly. This results in a 1 mg/mL solution of delta-9-THC in internal standard solution, which will serve as a check standard. Other volumes may be pipetted which result in a 1 mg/mL solution. Add 1 mL 4N NaOH solution to the culture tube, shake vigorously for 30 seconds and let settle. Pipette the hexane layer into an autosampler vial for analysis. If a delta-9-THC standard concentration other than 10 mg/mL is used, adjust pipetted volumes to achieve the desired final concentration.

7.2.4.4.2 Prepare a second 2.5 mg/mL standard solution, which will serve as the calibration standard for the one point calibration. Using a calibrated mechanical pipette, transfer 0.5 mL of the delta-9-THC standard into a test tube. Evaporate to dryness. Using a calibrated mechanical pipette, pipette 2 mL of internal standard solution into the culture tube and mix thoroughly. This results in a 2.5 mg/mL solution of delta-9-THC in internal standard solution. Other volumes may be pipetted which result in a 2.5 mg/mL solution. Add 1 mL 4N NaOH solution to the culture tube, shake vigorously for 30 seconds and let settle. Pipette the hexane layer into an autosampler vial for analysis. If a delta-9-THC standard concentration other than 10 mg/mL is used, adjust pipetted volumes to achieve the desired final concentration.

7.2.4.5 Sample Preparation

7.2.4.5.1 Prepare six separate sample solutions. For each, weigh at least 10 mg of sample into a test tube and add an appropriate amount of internal standard solution via calibrated mechanical pipette. This method provides quantitative results for the total delta-9-THC concentration (delta-9-THC + delta-9-THCA).

7.2.4.5.2 To avoid the conversion of THCA to delta-9-THC in the injection port, add 1 mL 4N NaOH solution to the culture tube as a base wash, shake vigorously for 30 seconds and let settle. Pipette the hexane into an autosampler vial for analysis. This method measures the quantity of delta-9-THC.

7.2.4.6 GC parameters

- Column: 15 meter HP-1 (or equivalent) (0.25 mm i.d., 0.25 µm film thickness)
  - Oven temperature: approximately 240-260 °C

- Column: 15 meter HP-35 (or equivalent) (0.25 mm i.d., 0.25 µm film thickness)
  - Oven temperature: approximately 250-300 °C
  - This column could be used to separate THC isomers, when necessary.

- Column: 30 meter, HP-5 (or equivalent) (0.25 mm i.d., 0.25 µm film thickness)
  - Oven temperature: approximately 200-280 °C
  - This column could be used to separate THC isomers, when necessary; however, delta-9-THC and delta-6a,10a-THC co-elute.

7.2.4.7 Linear Range

7.2.4.7.1 The validated linear range of this GC-FID delta-9-THC quantitative method is 0.5 – 5 mg/mL.

7.2.4.7.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

7.2.4.8 Delta-9-THC elutes before n-triacontane.

7.2.4.9 Delta-9-THC solutions and internal standard solutions should be closed and stored in the refrigerator when not in use.

7.2.4.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 – 11.4.3.11

7.3 Extraction of THC from Food Products (candy, brownies, etc.)

7.3.1 If plant material is visible, remove a sample of plant material and analyze appropriately.

7.3.2 If an extraction is necessary, (Ref. 7.5.3)

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

7.3.2.1 A procedure blank shall be run with the extraction and documented in the examination documentation.

7.4 Cannabinoid Quantitation by High Performance Liquid Chromatography – Diode Array Detection (HPLC-DAD)

7.4.1 This HPLC-DAD method is a quantitative test to establish the delta-9-THC, delta-9-THCA, and/or CBD concentrations. This cannabinoid quantitation is necessary in the analysis of oils when a written certification claim has been established per the Code of Virginia (§ 54.1-3408.3).

7.4.2 Materials

- Chloroform, HPLC grade or higher
- Methanol, HPLC grade or higher
- Acetonitrile, HPLC grade or higher
- Type I water
- Formic acid, HPLC >99.0%
- Cannabidiol (CBD): (1.0 mg/mL)
- delta-9-Tetrahydrocannabinol (THC): (1.0 mg/mL)

- delta-9-Tetrahydrocannabinolic Acid (THCA): (1.0 mg/mL)
- 4-Androsten-3,17-dione (Androstenedione)
- Calibrated mechanical pipettes
- 5-place analytical balance
- Millex-FH13 Millipore syringe filter, or equivalent

7.4.3 Instrument Parameters

- HPLC-DAD Parameters
  - Column: Agilent Zorbax Eclipse XDB-C18, 3.0 x 150 mm, 3.5 µm particle size, or equivalent
  - Column Thermostat: 28 °C
  - Mobile Phase A: 0.1% formic acid in water
  - Mobile Phase B: 0.1% formic acid in acetonitrile
  - Flow Rate: 1 mL/min
  - Injection Volume: 5 µL
  - Stop Time: 13.0 minutes
  - Post Time: 2.5 minutes
  - Gradient:

Time	%B
0.00	60.0
1.00	60.0
2.00	70.0
6.00	77.0
11.00	77.0
12.00	95.0
13.00	95.0

- DAD Signal: 220 nm with reference wavelength 360 nm
- Spectrum: 190 nm to 400 nm
- Elution order: Androstenedione, CBD, THC, THCA

7.4.4 Procedure

7.4.4.1 Prepare mobile phases:

- Mobile Phase A: 1 mL of formic acid in 1 L of Type I water
- Mobile Phase B: 1 mL of formic acid in 1 L of Acetonitrile

7.4.4.2 Prepare a sufficient volume of internal standard solution to prepare calibrators and samples. Internal standard solution is a 1.0 mg/ml solution of androstenedione in 9:1 MeOH:CHCl3.

7.4.4.3 Prepare a working solution containing all analytes of interest. Remove 0.5 ml from each ampoule of 1 mg/ml solutions. Combine all and evaporate to dryness. Add 1 mL of internal standard solution to give a final concentration of 0.5 mg/mL for all analytes (THC, CBD, THCA).

7.4.4.4 Prepare the following calibrators using the volumes in the table below. Pipette the appropriate volume of working solution for each calibrator. Bring to volume using the appropriate volume of internal standard solution, each resulting in the following final concentrations.

Final Concentration	Volume of Working Solution	Volume of Internal Standard Solution

0.5 mg/mL	Remainder of working solution	No dilution needed
0.25 mg/mL	200 µL	200 µL
0.125 mg/mL	200 µL	600 µL
0.008 mg/mL	20 µL	1230 µL

7.4.4.5 Prepare a 0.5 mg/mL positive control working solution. This solution may be stored for one year.

7.4.4.5.1 Prepare a 0.25 mg/mL control using the appropriate volumes from the table above.

7.4.4.6 Six samples shall be prepared. For each, weigh approximately 20 mg of sample and dilute with 10 mL (V1) of internal standard solution. Perform a second dilution by transferring 200 µL (V2) of sample solution V1 and add 700 µL of internal standard solution. Weights and volumes may be adjusted as necessary to ensure final concentrations are within the linear range.

7.4.4.7 Filter calibrators, control, blank and samples prior to injecting on the HPLC.

7.4.5 Calculations, Acceptance Criteria, and Reporting

7.4.5.1 The *CS HPLC Cannabinoid Quantitation Worksheet* shall be used to calculate % purity and mg/dose. Quantitation results are only pursued and reported for cannabinoids that have been qualitatively confirmed.

7.4.5.2 The correlation of determination (r^2) value for the curve must be 0.995 or greater for each analyte of interest.

7.4.5.3 One calibration point may be dropped due to poor peak shape or falling outside the accuracy acceptance range; however, the sample concentrations must fall within the linear range. The reason a calibrator was dropped and the date calibrator was dropped shall be recorded in the examination documentation.

7.4.5.4 The linear range is defined by the lowest acceptable calibrator and the highest acceptable calibrator in the calibration curve for each analyte.

7.4.5.5 The accuracy of the control concentration must be within 15% of the theoretical value. Examiners are responsible for entering control values into the statewide control chart.

7.4.5.6 The relative standard deviation of the six samples must be less than 15%.

7.4.5.7 If the above criteria are met, the purity results may be reported and shall include the Uncertainty of Measurement (UoM). When possible, the mg/dose of analyte (THC, THCA, CBD) will also be reported and shall include the UoM.

7.4.5.8 If an analyte is present and the sample's calculated concentration falls below 0.008 mg/mL, the result shall be reported as less than 2% (0.008 mg/dose) analyte.

7.4.5.9 See ¶ 34.9.5 for report wording.

7.5 References

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

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7.5.5 "The legal status of cannabis (marijuana) and cannabidiol (CBD) under U.S. law", Epilepsy & Behavior 70 (2017) 288-291.

8 PHARMACEUTICAL IDENTIFIERS

8.1 Introduction

Pharmaceutical preparations possess unique identifying information both in the general appearance of the preparation and the inscriptions or markings.

8.2 Procedure

8.2.1 Visually examine the tablets, capsules, etc., to determine that their size, color, shape, and markings are consistent. Record the results of the visual examination in the case notes.

8.2.1.1 Tablet descriptions in case notes should clearly reflect the physical characteristics used in the visual examination.

8.2.1.2 If the appearance of the dosage unit suggests it is not a legitimate preparation, record observation(s) in the case notes.

8.2.1.3 For packaged pharmaceuticals (e.g., Suboxone strips, blister packs of tablets), visual inspection of all dosage units is not required; however, the evidence description and the reported results shall clearly reflect those analyzed vs. not examined.

8.2.2 Check the PDR, Poison Control, DEA Logo Index, Identadrug, Drug ID Bible / Amera-Chem, Inc. CD, Epocrates, webpoisoncontrol.org, NIH-DailyMed, Drugs.com, or manufacturer's resources for information relating to inscriptions on tablets and capsules. Additional references may be used following approval by the Chemistry Program Manager.

8.2.2.1 Only one reference is necessary if the dosage unit is fully analyzed; otherwise, two unrelated references are recommended.

8.2.2.2 Reference information including page number shall be recorded in the case notes and/or a hardcopy of relevant entries from an electronic database shall be included in the case file.

8.2.3 Tablets with incomplete markings may be reported as "visually examined" if they are mixed with intact tablets that are identical in other aspects. If the markings are illegible or not clear enough to compare to the intact tablets, a screening test should be performed.

8.2.4 When the sample is not an identifiable pharmaceutical preparation, it is required that a definitive structural elucidation technique be used within the analytical scheme (to include two-system chromatography), if the substance is to be reported.

8.2.5 Schedule IV and above:

8.2.5.1 At least one dosage unit must be fully tested in those cases involving Schedule IV and above.

8.2.5.2 Per DIRP, in simple possession cases, if intact, marked pharmaceutical preparations indicated as containing the same controlled substance are present in multiple items, analysis is required for only one item.

8.2.5.3 Tamperable capsules shall be screened for tampering using appropriate color tests, TLC, or DART-TOF using the hypergeometric sampling scheme.

8.2.5.3.1 Color tests are only appropriate for screening when the drug expected to be present in the capsules has a known reaction to the color test employed for screening.

8.2.5.3.2 Hypergeometric screening is not required for Schedule II tamperable pharmaceutical preparations in simple possession cases. One capsule may be analyzed fully and reported as such.

8.2.5.4 If all dosage units are visually similar and if tamperable capsules have consistent screening results, take one representative sample for analysis. If all dosage units are not visually similar, take one representative sample per population for analysis.

8.2.5.5 A structural elucidation technique must be used within the analytical scheme. This may be done by comparing the spectrum to a GC/MS NIST purchased library reference, IDDA reference or traceable in-house reference spectrum. Alternatively, an identification scheme using DART-TOF Mass Spectrometry (See ¶ 16.4) or FTIR may be used. There are no chromatographic requirements for pharmaceutical preparations when a valid visual examination is combined with a structural elucidation technique.

8.2.5.5.1 If the results of the analysis are consistent with the manufacturer's specifications with regard to content, the results shall be reported as outlined in the Reporting Guidelines, ¶ 34.8.2.

8.2.5.5.2 If the results of the analysis are inconsistent with the manufacturer's specifications with regard to content:

- The original structural elucidation technique can serve as the confirmatory test and further testing shall be performed to screen (i.e., TLC, GC/MS screen method, DART-TOF) for other compounds if not already performed.
- One dosage unit from each population should be analyzed and reported for charges of simple possession (See Reporting Guidelines ¶ 34.8.3.1).
- Five dosage units from each population should be analyzed and reported for charges of possession with intent to distribute or distribution (See Reporting Guidelines ¶ 34.8.3.2). If the results of the five dosage units are inconsistent, additional analysis may be necessary. If all five are found to contain a Schedule I and/or Schedule II compound or there is a mixture with similarities (e.g., low level absence/presence, apparent batch inhomogeneity), report the results of the five analyzed (See Reporting Guidelines ¶ 34.8.3.3). Treat Schedule III and IV controlled substances similar to Schedule I and II controlled substances for charges of possession with intent to distribute or distribution. If there is a mixture which contains Schedule V, VI, or a non-controlled substance, hypergeometrically sample and fully analyze (See Reporting Guidelines ¶ 34.8.3.4). Despite the use of hypergeometric sampling here, no inference can be made as to the contents of the units that were not analyzed.

8.2.6 Schedule V, VI, and non-controlled:

8.2.6.1 It is normally acceptable to visually examine intact, marked tablets or untamperable, marked capsules in those cases involving misdemeanor prosecutions in Schedules V and VI. Results should be reported as given in the Reporting Guidelines section of this manual. (See ¶ 34.8.1)

8.2.6.2 Tamperable capsules should be screened for tampering using appropriate color tests, TLC, or DART-TOF using the hypergeometric sampling scheme. Color tests are only appropriate for screening when the drug expected to be present in the capsules has a known reaction to the color test employed for screening.

8.2.6.2.1 Tamperable dosage units do not need to be screened if a Schedule I or II compound is identified in the same case (see ¶ DIRP). These may be reported

using the “Visual examination determined…” language delineated in the Reporting Guidelines section of this manual.

8.2.6.3 If tampering is not detected, it may be acceptable to report as visually examined.

8.2.6.4 If tampering is suspected, then a complete analytical scheme including a structural elucidation technique is required for identification.

8.2.6.5 "No controlled substances found" or "No controlled substances identified" may be used for reporting those non-controlled substances not structurally identified.

9 COLOR TESTS

9.1 Introduction

9.1.1 Color tests are used as a screening test at the beginning of an analysis. Most are performed on clean porcelain or disposable plastic spot plates; however, some may be performed in disposable culture tubes (e.g., Scott's, Tannic Acid).

9.1.2 Thin Layer Chromatography visualization sprays may act as color tests when sprayed on a TLC plate or filter paper where a drop of sample solution has been placed.

9.2 Procedures

9.2.1 Generally, the test reagent should be added to the plate or tube first, and then the questioned sample. This practice determines if the plate or tube was clean before the analysis.

9.2.1.1 If a reaction occurs prior to the addition of the sample, move to the next well or use a new tube. If a reaction occurs in the subsequent well prior to the addition of the sample, discard or clean the plate before testing the sample.

9.2.1.2 For some two-part color tests, the development of any color depends on the combination of the reagents in the presence of the sample. Therefore for these color tests (i.e., 4-AP, Cobalt Thiocyanate with HCl addition or any other modification, Dille-Koppanyi, Duquenois-Levine, Fehlings, Fiegel's/Nitroprusside, Mayer's, TBPEE, Weber's), a blank consisting of both reagents should be run. Record that the results of the blank were acceptable by using a check mark (√), "ok", or "-" (e.g., "Blk √"). If the results of the blank are unacceptable, the analyst shall take steps to resolve the issue.

9.2.2 Several of the listed reagents have more than one recipe listed. Any of the listed, referenced recipes may be utilized in casework and should be reflected in the reagent logbook.

9.2.3 The *CS Color Test Reagent Preparation Log* shall be used to record reagent preparation.

9.2.3.1 It is acceptable to make final volumes different from those listed below as long as the amount of each component is recorded.

9.2.4 Reagents should be made in quantities to minimize waste. The shelf life of color test reagents is two years, unless otherwise listed.

9.2.5 Reagents, indicators and solutions listed in the USP-NF may be used for their published purposes.

9.2.5.1 Positive controls and blanks shall be performed when using reagents or tests listed in the USP-NF. The results shall be recorded in the case notes.

9.2.5.2 Case notes shall include the procedure with the appropriate reference and the results of the test.

9.2.6 The color and/or reaction observed must be noted for drugs. Negative reactions should likewise be documented in the case notes.

9.2.7 Most color test reagents are comprised of strong acids and chemicals requiring careful handling. Appropriate safety precautions should be observed. Refer to SDSs for storage and handling.

9.3 Color Tests and Reagents

The following lists the commonly used color test reagents and some examples of reactions with various drugs. The references for each test are in parenthesis.

9.3.1 The 4-aminophenol (4-AP) color test may be used as a screening test to help distinguish between hemp and marijuana and is based on the relative CBD to delta-9-THC concentration ratio.

9.3.1.1 Recipes:

- Reagent A: Dissolve 150 mg of 4-aminophenol in 497.5 mL of 95% ethanol and 2.5 mL of 2N HCl. Store stock Reagent A in a brown bottle and refrigerate.
- Reagent B: Dissolve 3 g of NaOH into 30 mL of water and 70 mL of 95% ethanol.

9.3.1.2 Procedure: See section ¶ 6.4.

9.3.1.3 Results:

- A blue color is indicative of a THC concentration greater than the CBD concentration.
- A pink color is indicative of a THC concentration less than the CBD concentration.
- Any other observed color or a lack of color change indicates an inconclusive result. An inconclusive result is possible when delta-9-THC and CBD concentrations are nearly equal, sample size is too large, or insufficient levels of delta-9-THC are present such as in immature Cannabis plants.

9.3.2 Bates Test (¶ 9.4.5) tests for cocaine base.

9.3.2.1 Procedure: The Bates test is used as the second part of the Cobalt thiocyanate test (¶ 9.3.5). If the Cobalt thiocyanate test is negative, add Marquis reagent to spot well.

9.3.2.2 Results: The formation of a very blue precipitate indicates cocaine base, other compounds give weaker blue or no reaction.

9.3.3 Benedict's Solution (¶ 9.4.2) tests for reducing sugars and some antibiotics.

9.3.3.1 Recipe: 1.73 g of copper sulfate in 10 mL of water. With the aid of heat, dissolve 17.3 g trisodium citrate and 10 g of anhydrous sodium carbonate in 80 mL of H_2O. Pour the two solutions together and let cool. Dilute to 100 mL with water.

9.3.3.2 Procedure: Add 0.5 mL of the reagent to sample and heat.

9.3.3.3 Results:

- Ascorbic acid, strong reducing agents, glucose, tetracycline – red
- Streptomycin - orange/brown

9.3.4 Chen's Test (¶ 9.4.2) tests for phenethylamines.

9.3.4.1 Recipe: 1 g copper sulfate and 1 mL glacial acetic acid in 100 mL H_2O.

9.3.4.2 Procedure: Make an approximate 1% aqueous solution of the sample; add equal volumes of Chen's reagent and 2N NaOH.

9.3.4.3 Results: ephedrine, PPA and pseudoephedrine – purple

9.3.5 Cobalt Thiocyanate reacts with tertiary and quaternary amines to form a blue precipitate and is used for general screening. May be used in conjunction with the Bates test (¶ 9.3.2) or the Stannous Chloride test (¶ 9.3.22).

9.3.5.1 Recipes:

- 2 g cobalt thiocyanate in 100 mL H_2O or methanol (¶ 9.4.1)
- 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine. (¶ 9.4.3)
- 1.4 g $CoCl_2$. $6H_2O$ and 0.9 g NH_4SCN in 100 mL H_2O. (¶ 9.4.7)

9.3.5.2 Procedure: Place reagent in well and add sample.

9.3.5.3 Results:

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

9.3.6 Dille - Koppanyi Test (¶ 9.4.9) reacts with barbiturates.

9.3.6.1 Recipe:

- DK1: 0.1 g cobaltous acetate tetrahydrate in 100 mL methanol plus 0.2 mL glacial acetic acid
- DK2: 5 mL isopropyl amine in 95 mL methanol

9.3.6.2 Procedure: This is a two-part test. Place 2 drops of DK1 reagent in a well. Add sample. Add 1 drop of DK2 reagent. When doing multiple samples, they should be separated to avoid cross-contamination due to reagent spreading.

9.3.6.3 Results:

- barbiturates - blue purple
- theophylline, glutethimide and hydantoins - purple
- ampicillin - brown

9.3.7 Duquenois - Levine Test (¶¶ 9.4.3 and 9.4.4) reacts with Cannabis and Cannabis extracts.

9.3.7.1 Recipe: 4 g vanillin and 2.5 mL fresh acetaldehyde per 200 mL 95% ethanol

9.3.7.2 Procedure: See Cannabis Plant Material section (¶ 6).

9.3.7.3 Results: Cannabis – blue/purple, pink/purple extracts into $CHCl_3$

9.3.8 Ehrlich's Reagent (¶ 9.4.7) reacts with indole moiety and some amines.

9.3.8.1 Recipe: 5 g p-dimethylaminobenzaldehyde to 50 mL of 95% ethanol and 50 mL of conc. HCl

9.3.8.1.1 Alternatively, the TLC visualization reagent recipe may be used: 2 g of p-DMAB in 50 mL 95% ethanol and 50 mL 37% HCl

9.3.8.2 Procedure: Place reagent in well and add sample.

9.3.8.3 Results:

- LSD, psilocyn - purple (beware of leaching of dyes in blotter paper or tablets)
- benzocaine, procaine - yellow

9.3.9 Fehlings Solution (¶ 9.4.8) reacts with reducing compounds such as sugars.

9.3.9.1 Recipe:

- Fehlings1 - 3.46 g copper sulfate per 50 mL H_2O
- Fehlings2 - 86.5 g sodium potassium tartrate and 35 g of NaOH per 250 mL of H_2O

9.3.9.2 Procedure: Dissolve sample in water and mix. Add five drops of Fehlings1 and five drops of Fehlings2 and mix. Heat on steambath for approximately five minutes or until warm.

9.3.9.3 Results: reducing sugars – yellow to red.

9.3.10 Ferric Chloride ($FeCl_3$) tests for phenols and GHB.

9.3.10.1 Recipes:

- 9% aqueous solution (¶ 9.4.14)
- 5% aqueous solution (¶ 9.4.2)

9.3.10.2 Procedure: Place sample into a solution of water or methanol and add a drop of reagent.

9.3.10.3 Results:

- salicylamide - dark purple
- acetaminophen - blue
- hydrolyzed aspirin – purple (to hydrolyze a sample, place in H_2O, add a little acid and heat)
- GHB – red/brown

9.3.11 Fiegel's / Nitroprusside (nitroferricyanide) (¶¶ 9.4.3 and 9.4.8) for secondary amines.

9.3.11.1 Recipe: 1 g of sodium nitroprusside in 100 mL H_2O and 10 mL acetaldehyde

9.3.11.2 Procedure: Dissolve sample in 2N Na_2CO_3 and add reagent.

9.3.11.3 Storage: store in brown bottle and refrigerate.

9.3.11.4 Results: secondary amines - deep blue color.

9.3.12 Froehde's (¶¶ 9.4.1 and 9.4.2) reacts with narcotics and is used for general screening.

9.3.12.1 Recipe: 0.5 g ammonium molybdate per 100 mL H_2SO_4 (conc.)

9.3.12.2 Procedure: Place reagent in well and add sample.

9.3.12.3 Results:

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

9.3.13 GHB Color Test #3 (Smith Test) (¶ 9.4.13) for GHB powders and solutions. This test will not react with GBL or 1,4-butanediol.

9.3.13.1 Recipe:

- Bromocresol Green – 0.03 g bromocresol green in 100 mL of 4:1 methanol:DI water adjusting the pH to 7.0 with 0.1N sodium hydroxide
- Methyl Orange – 0.01 g methyl orange in 100 mL DI water adjusting the pH to 7.0 with 0.1N sodium hydroxide
- Modified Schweppes Reagent: Mix solutions A and B
  - Solution A – 2 g dextrose in 20 mL of DI water
  - Solution B – 2.4 g aniline hydrochloride in 20 mL methanol
- Mix Bromocresol Green and Methyl Orange together in a 1:1 ratio. Then, mix that indicator mixture with the modified Schweppes reagent in a 3:1 ratio.

9.3.13.2 Procedure: Add 0.5 mL of a liquid sample or a small amount of powder to a test tube. Add 2 drops of the mixed reagent and gently swirl.

9.3.13.3 Results:

- GHB – immediate green color
- Negative results – pinkish orange (generally the same or slightly darker than the original test solution)

9.3.14 Marquis (¶¶ 9.4.1 and 9.4.2) reacts with opiates and phenethylamines and is used for general screening.

9.3.14.1 Recipe:

2 mL 37% formaldehyde in 75 mL H_2SO_4 (conc.)

WARNING! 37% formaldehyde is a "particularly hazardous substance", for which OSHA has established very low permissible airborne concentrations and prohibited skin or eye contact. Therefore, it must be handled in an exhaust hood using appropriate PPE (laboratory coat, appropriate forearm length gloves, and face shield).

9.3.14.2 Procedure: Place reagent in well and add sample.

9.3.14.3 Storage: Keep tightly capped.

9.3.14.4 Results:

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

9.3.15 Mayer's Reagent (¶ 9.4.7) reacts with alkaloids

9.3.15.1 Recipe: Dissolve 1 g of mercuric chloride in 100 mL H_2O. Add solid potassium iodide until the reddish precipitate first formed dissolves. Reagent should be clear and pale yellow in color.

9.3.15.2 Procedure: Add 0.1N HCl to a test tube. Add sample to acid and mix. Add Mayer's reagent to the acid solution.

9.3.15.3 Results: alkaloids – a white to yellow precipitate is formed

9.3.16 Mecke's (¶¶ 9.4.1 and 9.4.2) reacts with narcotics and is used for general screening.

9.3.16.1 Recipe: 1 g selenious acid per 100 mL H_2SO_4 (conc.)

9.3.16.2 Procedure: Place reagent in well and add sample.

9.3.16.3 Results:

- heroin - green/blue
- codeine - bright-green/blue green
- PCP - light yellow
- quinine - light yellow

9.3.17 Methylene Blue (¶ 9.4.10) reacts with vitamin C.

9.3.17.1 Recipe: 12.5 mg of methylene blue dissolved in 25 mL of 95% ethanol.

9.3.17.2 Procedure: Add reagent to well and add sample. It may be helpful to run a blank to compare the results.

9.3.17.3 Results: Vitamin C - slowly decolorizes solution from dark blue to light blue.

9.3.18 Nitric Acid (HNO_3) (¶¶ 9.4.1 and 9.4.2) reacts with opiates and mescaline.

9.3.18.1 Recipe: concentrated nitric acid

9.3.18.2 Procedure: Place reagent in well and add sample.

9.3.18.3 Results:

- heroin - yellow green
- morphine - red
- codeine - orange
- mescaline - red
- acetaminophen – fumes, orange brown

9.3.19 Parri (¶ 9.4.11) reacts with barbiturates.

9.3.19.1 Recipe: cobaltous acetate (solid), barium oxide (solid), and methanol

9.3.19.2 Procedure: Mix cobaltous acetate, BaO and powdered sample in equal parts in a spot plate well, add methanol.

9.3.19.3 Results: barbiturates – blue

9.3.20 Scotts - Modified Cobalt Thiocyanate (¶ 9.4.3) reacts with cocaine.

9.3.20.1 Recipe: 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine.

9.3.20.2 Procedure: Add reagent to well or tube and add sample. Dissolve the blue precipitate from the $Co(SCN)_2$ by the addition of HCl. Add $CHCl_3$

9.3.20.3 Results: cocaine - blue color in the lower ($CHCl_3$) layer.

9.3.21 Silver Nitrate (¶ 9.4.15) indicates the presence of chloride ions.

9.3.21.1 Recipe: 5.0% w/v solution of silver nitrate in DI water.

9.3.21.2 Caution: Poison; will cause staining.

9.3.21.3 Storage: Store in the refrigerator in a dark environment.

9.3.21.4 Procedure: Dissolve sample in water. Add silver nitrate solution. A white, curdy precipitate will form in the presence of chloride ions, which will be insoluble in nitric acid. The precipitate will be soluble in 6N ammonium hydroxide.

9.3.22 Stannous Chloride modification for $Co(SCN)_2$ - HCl acidified (¶ 9.4.9) differentiates between "caines".

9.3.22.1 Recipe: 5 g $SnCl_2$ and 10 mL conc. HCl diluted to 100 mL with H_2O

9.3.22.2 Procedure: The Stannous Chloride test is used as the second part of the Cobalt thiocyanate test (¶ 9.3.4). After performing the cobalt thiocyanate test, add a drop of stannous chloride reagent.

9.3.22.3 Results:

- Cocaine salts - blue remains
- Cocaine base - blue color forms (initially negative)
- Other compounds which turned blue initially - blue fades

9.3.23 Sulfuric Acid (H_2SO_4) (¶¶ 9.4.1 and 9.4.2)

9.3.23.1 Recipe: concentrated sulfuric acid

9.3.23.2 Procedure: Add reagent to well and add sample.

9.3.23.3 Results:

- tetracycline - purple turning to yellow upon addition of water
- 2,3-MDMA, 2,3-MDA – rose
- 3,4-MDMA, 3,4-MDA – gray-brown

9.3.24 Tannic Acid (¶ 9.4.3) reacts with xanthines.

9.3.24.1 Recipe: 1% aqueous solution of tannic acid

9.3.24.2 Procedure: Add reagent to test tube then add powdered sample.

9.3.24.3 Results: caffeine and theophylline - positive test will produce a precipitate that develops from "streamers" immediately visible in the solution.

9.3.25 TBPEE Solution (¶ 9.4.8) differentiates between amines.

9.3.25.1 Recipe:

- 0.01 g Tetrabromophenolphthalein ethyl ester (TBPEE) in 100 mL CCl_4
- 10.6 g sodium carbonate in 100 mL H_2O (2N solution)

9.3.25.2 Caution: Carbon tetrachloride is carcinogenic. Use appropriate safety precautions.

9.3.25.3 Procedure: Dissolve suspected amine in 2N Na_2CO_3 solution and add TBPEE solution. Note color change in the bottom TBPEE layer.

9.3.25.4 Results:

- primary amines - violet
- secondary amine - blue
- tertiary amine – red

9.3.26 Van Urk's (¶ 9.4.12) reacts with the indole moiety and some amines.

9.3.26.1 Recipe: 125 mg p-dimethylaminobenzaldehyde, 65 mL of concentrated H_2SO_4, and 2 drops of ferric chloride (USP T. S.) diluted to 100 mL with water.

9.3.26.2 Procedure: Add reagent to well; then add sample.

9.3.26.3 Results: LSD – blue/purple

9.3.27 Weber Test (¶ 9.4.6) reacts with psilocyn.

9.3.27.1 Recipe: 0.01 g of Fast Blue B or Fast Blue BB in 10 mL H_2O

9.3.27.1.1 Alternatively, the following TLC visualization reagent recipe may be used: Approximately 0.05% solution of Fast Blue B salt OR Fast Blue BB salt in water

9.3.27.1.2 The higher concentration recipe has shown to be more useful for unusual matrices, such as chocolate.

9.3.27.2 Shelf life: One month from preparation.

9.3.27.3 Procedure: Add 2 to 3 drops of reagent to a sample of mushrooms. Observe slow color change. Add 1 to 2 drops of conc. HCl; observe color change.

9.3.27.4 Results: Psilocyn – Initially the solution turns red. The solution will turn from red to blue when the acid is added.

9.4 References

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10 THIN LAYER CHROMATOGRAPHY

10.1 Introduction

10.1.1 Thin layer chromatography (TLC) is a useful method for screening, separation and preliminary identification. Both the approximate concentration of the sample and the number of components contained in the sample can be ascertained by TLC. TLC can provide valuable information before proceeding on to instrumental tests. Clues as to the chemical structure of an analyte can be obtained by noting the distance traveled in different solvent systems and noting the reactions to a variety of chemical sprays.

10.1.2 The specificity of TLC is greatly increased by using multiple solvent systems of different polarities or pH. Because many drug compounds (or other organic compounds) have similar R_f values in any one solvent system, at least two solvent systems should be used, except for Cannabis and hashish oil.

10.2 Materials

10.2.1 Solvent tank - Any covered glass container with a level bottom can be used. Rectangular tanks are most common. The developing solvent should be at a depth of approximately 0.5 cm to maintain constant contact with the stationary phase throughout the analysis. Filter paper or some other suitable absorbent paper should line the back inside wall of the tank at a height greater than the plate being used when it is required to maintain an atmosphere saturated with solvent vapor. This can provide for better migration and results that are more consistent. Care should be taken to maintain this atmosphere. The absorbent paper is not required for Cannabis analysis.

10.2.2 Thin layer plates - silica gel (250µm) coated glass plates with a fluorescent indicator, or equivalent (Most drug compounds quench fluorescence when visualized under short wave UV light)

10.2.3 Capillary tubes or Micropipettes

10.2.4 Long wave/short wave UV light source

10.2.5 Solvent baths and visualization reagents

10.2.5.1 The *CS TLC Bath and Visualization Reagent Preparation Log* shall be used to record reagent preparation.

10.2.5.2 It is acceptable to make final volumes different from those listed below as long as the amount of each component is recorded.

10.3 Methods

10.3.1 The sample to be tested is dissolved in $CHCl_3$, MeOH or other suitable solvent. The solvent used must be recorded in the case notes.

10.3.2 The solution is drawn up into a capillary tube and 1-10 µl (depending on concentration) is spotted on a dry plate approximately 0.5 - 1 cm from the bottom, making sure that the spot is above the solvent level in the developing tank. The spot size should be kept to a minimum, as its diameter will increase while the compound migrates up the plate during development. Heavy concentrations should be avoided as this causes streaking and tailing.

10.3.3 A standard is spotted beside the sample(s) for comparison. Care should be taken that the standard and sample(s) are approximately the same concentration. Unequal concentrations may result in unequal rates of advance. This can easily be checked by visualizing the plate under UV light before development.

10.3.4 A blank of the solvent used to dissolve the sample is also spotted on the plate. The results of the blank must be recorded in the case notes. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., "Blk √").

10.3.4.1 If spots are visualized in the blank region, the blank should be run again under the same conditions.

10.3.4.1.1 If the results of the second blank are acceptable, the entire plate should be re-spotted and re-run.

10.3.4.1.2 If the results of the second blank are unacceptable, the analyst shall take steps to resolve the issue (e.g., replacing the solvents in the bottle, checking the baths) prior to re-sampling and any further analysis.

10.3.5 The plate is placed in the tank and allowed to develop until the solvent reaches the top. The plate is then removed, dried, inspected under UV light, and/or sprayed with the appropriate visualizing reagent. Do not allow the plate to stand in the solvent after development is complete as this will cause a gradual diffusion of the compound.

10.3.6 Specific solvent systems, developing sprays utilized, standard identifier(s) used for comparison and/or checking system performance, and results will be denoted in the analytical case notes. A result is considered positive when the distance traveled and the reaction with the visualization methods compare favorably with a standard. At least one visualized standard identifier must be documented in the case notes for objective evidence that the performance check passed.

10.4 TLC Baths

- TLC1: $CHCl_3/CH_3OH$ (9:1)(v/v) – general drug screening (¶ 10.8.1)
- TLC2: Ammonia saturated $CHCl_3/CH_3OH$ - (18:1) (v/v) – general drug screening (¶ 10.8.2)
- TLC3: T-1 Methanol/NH_4OH (100:1.5) (v/v) – general drug screening (¶ 10.8.1)
- TLC4: 8% Diethylamine in Toluene (v/v) – Cannabis / general drug screening (¶ 10.8.3)
- TLC5: 4% Diethylamine in Toluene (v/v) – Cannabis / general drug screening (¶ 10.8.3)
- TLC6: Chloroform/Ethyl Acetate (80:20) (v/v) – steroids (¶ 10.8.5)
- TLC7: Isopropyl Ether – barbiturates (¶ 10.8.8)
- TLC8: Acetone /$CHCl_3$ (2:1)(v/v) – LSD/LAMPA (¶ 10.8.2)
- TLC9: Cyclohexane/Toluene/Diethylamine (75:15:10) (v/v) – MDMA/methamphetamine (¶ 10.8.1)
- TLC10: Ethyl Acetate – GHB, GBL, 1,4-butanediol
- TLC11: Deionized water - vitamins
- TLC12: Acetone – amphetamines and other basic drugs (¶ 10.8.4)
- TLC13: Ethyl acetate/acetone/ammonium hydroxide (25:5:1) (v/v) – ephedrine/pseudoephedrine
- TLC14: Ethyl acetate/hexane (1:1) (v/v) – salvinorin A (¶ 10.8.11)

10.5 Visualization Reagents

10.5.1 All visualization sprays must be used in a fume hood.

10.5.2 Ceric Sulfate (¶ 10.8.10)

10.5.2.1 Used as an overspray to intensify the reaction with iodoplatinate, especially for caffeine

10.5.2.2 Recipe: 5 g $Ce(SO_4)_2$ in 500 mL H_2O and 14 mL H_2SO_4.

10.5.3 Diphenylcarbazone (¶ 10.8.6)

10.5.3.1 Used as an overspray with mercuric sulfate for barbiturates. Can also be freshly mixed 50/50 with the mercuric sulfate reagent (¶ 10.5.12).

10.5.3.2 Recipe: 19 mg diphenylcarbazone in 200 mL (50% acetone/water).

10.5.4 Dragendorff (¶ 10.8.1)

10.5.4.1 General spray that visualizes alkaloids and other nitrogen containing compounds, including methamphetamine and diazepam.

10.5.4.2 Recipe: 1.3 g of bismuth subnitrate in 60 mL water with 15 mL glacial acetic acid. Add this to 12 g potassium iodide in 30 mL H_2O. Dilute with 100 mL of H_2O and 25 mL glacial acetic acid.

10.5.5 Ehrlich's or p-Dimethylaminobenzaldehyde (p-DMAB) (¶ 10.8.7)

10.5.5.1 Visualizes LSD and psilocybin, reacts with indole nucleus of alkaloids. Plate may be heated after spraying to increase intensity of color.

10.5.5.2 Recipe: 2 g of p-DMAB in 50 mL 95% ethanol and 50 mL 37% HCl.

10.5.6 Fast Blue B (¶ 10.8.1) or Fast Blue BB (¶ 10.8.9)

10.5.6.1 Visualizes the three common cannabinoids in Cannabis. They migrate and develop in the following order:

- Top spot - Cannabidiol – orange
- Middle spot - delta-9-Tetrahydrocannabinol – red
- Lower spot - Cannabinol – purple

10.5.6.2 Visualizes psilocyn – red, which then turns blue when acidified with HCl.

10.5.6.3 Recipe: Approximately 0.05% solution of Fast Blue B salt OR Fast Blue BB salt in water

10.5.6.4 Shelf life: One month from preparation.

10.5.7 Fluorescamine (Fluram^R)

10.5.7.1 Visualizes amino acids, primary amines and amino sugars.

10.5.7.2 Recipe: 20 mg Fluram^R in 100 mL acetone.

10.5.7.3 Procedure: Spray plate with reagent, then check under long wave UV light (amphetamine fluoresces green-yellow). Heating the plate may intensify the visualization.

10.5.8 Furfuraldehyde and HCl (¶ 10.8.1)

10.5.8.1 Visualizes meprobamate and other carbamates.

10.5.8.2 Recipe: 10% solution of furfuraldehyde in ethanol. Overspray with concentrated HCl.

10.5.8.3 Procedure: Spray plate and heat, if necessary. Spots are black on a white background.

10.5.9 6N HCl

Used to acidify plates

10.5.10 Iodine Vapors (¶ 10.8.1)

10.5.10.1 Visualizes general unknowns and compounds which are not UV active. Suitable for GHB, 1,4-butanediol and GBL analysis. This is a good method of visualization if further testing is to be done on the sample on the plate, as it is reversible.

10.5.10.2 Procedure: Place iodine crystals in an enclosed chamber. Let TLC plate develop in the chamber. Many organic compounds will produce a brown spot.

10.5.10.3 Results:

- GHB – white spot on yellow background
- GBL, 1,4-butanediol – brown spot on yellow background

10.5.11 Iodoplatinate (¶ 10.8.1)

10.5.11.1 Visualizes nitrogen-containing compounds, may be acidified with HCl to intensify some reactions.

10.5.11.2 Recipes:

- 5 mL of 10% platinic chloride aqueous solution and 10 g of potassium iodide in 500 mL of H_2O.
- 1 g of platinic chloride and 10 g of potassium iodide in 500 mL H_2O.

10.5.11.3 If acidified iodoplatinate is preferred, either overspray the TLC plate with 6N HCl or prepare stock solution with approximately 5% HCl.

10.5.11.4 Results may be intensified with an overspray of ceric sulfate reagent.

10.5.12 Mercuric Sulfate (¶ 10.8.8)

10.5.12.1 Visualizes barbiturates, which appear as white spots on off-white background. The plate may need to be sprayed heavily.

10.5.12.2 Recipe: Suspend 5 g HgO in 100 mL of H_2O. Add 20 mL concentrated H_2SO_4. Cool, dilute with 250 mL H_2O.

10.5.12.3 Mercuric Sulfate can also be freshly mixed 50/50 with the diphenylcarbazone reagent (¶ 10.5.3).

10.5.13 Ninhydrin (¶ 10.8.1)

10.5.13.1 Visualizes amino acid, primary and some secondary amines and amine sugars. (¶ 10.8.1)

10.5.13.2 Recipe: Add 0.5 gram of ninhydrin to 10 mL concentrated HCl. Dilute to 100 mL with acetone.

10.5.13.3 Procedure: Spray with ninhydrin solution and heat the plate (e.g., hotplate, approximately 100°C oven) for 2 minutes. After spraying, the plate may be irradiated under long wave UV light for 2 minutes prior to heating.

10.5.13.4 Results: yields pink-violet or orange-brown spots.

10.5.13.5 Alternatively, the commercially available Chem Print Ninhydrin may be used.

10.5.14 Potassium Permanganate (¶ 10.8.1)

10.5.14.1 Visualizes unsaturated hydrocarbons. $KMnO_4$ is an alternative to Mercuric Sulfate for barbiturates that contain a double bond. $KMnO_4$ may be used as an underspray or overspray with Iodoplatinate.

10.5.14.2 Recipe: Dissolve 1 g $KMnO_4$ in 100 mL H_2O.

10.5.14.3 Results: yields a yellow spot on a purple background.

10.5.15 Sulfuric Acid/Ethanol Reagent for Steroids (¶ 10.8.5)

10.5.15.1 Recipe: Add gradually 10 mL of conc. sulfuric acid to 90 mL of ethanol.

10.5.15.2 Procedure: Spray plate and heat gently on a hot plate to develop.

10.5.15.3 Results:

- Testosterone - green
- Testosterone esters – purple
- Oxymethalone – red
- Nandrolone decanoate – purple

10.5.16 Vanillin Reagent for Salvinorin A (¶ 10.8.11)

10.5.16.1 Recipe: 1 g of vanillin with 50 mL anhydrous ethanol and 0.3 mL concentrated H_2SO_4

10.5.16.2 Procedure: After developing plate, dry thoroughly. For plate developed in the basic TLC2 bath, spray plate with 6N HCl prior to Vanillin. Spray plate generously with reagent and heat with heat gun for approximately 2 minutes or place in oven at 110ºC for several minutes.

10.5.16.3 Results: Salvinorin A - Pink/Purple spot. Marijuana also gives a pink/purple spot, but at a different R_f when using TLC14.

10.5.16.4 Store in refrigerator when not in use.

10.6 Preparative Thin Layer Chromatography

10.6.1 Introduction

10.6.1.1 Frequently, samples contain other organic compounds that interfere with the drug analysis (e.g., heroin and quinine). Preparative TLC can be used to clean up a sample for other methods of testing such as IR or MS.

10.6.1.2 If cleaning up cocaine for a base determination, be careful to use a neutral bath to develop the plate so that the original salt form will not be altered.

10.6.2 Materials

10.6.2.1 Thin layer plates

10.6.2.1.1 A section of 250µm thin layer plate can be used if only a small quantity of pure compound is needed.

10.6.2.1.2 For larger quantities, use a 1000µm plate.

10.6.3 Procedure

10.6.3.1 The sample is dissolved in an appropriate solvent and streaked along the bottom of the plate using a capillary tube, long tipped Pasteur pipette or a commercial streaking device (if available).

10.6.3.2 A standard may be spotted separately at either the beginning or end of the plate in order to identify the desired compound after development.

10.6.3.3 Develop the plate as in regular TLC.

10.6.3.4 After drying the plate, the desired area is located and marked under UV light. (For compounds not UV visible, iodine vapors can be used.)

10.6.3.5 Scrape off the desired area, wash thoroughly with solvent in a small beaker and filter to remove the silica gel. Smaller quantities can be filtered using a disposable Pasteur pipette with a pre-washed glass wool plug.

- After development, most compounds adhere strongly to the deactivated silica gel and therefore must be washed with a fairly polar solvent. Methanol is recommended. For some compounds, an extraction from an aqueous acidic or basic solution may be necessary.

10.6.3.6 If using two-dimensional TLC, first develop the plate as usual, and then develop the plate in a polar solvent system at a 90-degree angle in order to concentrate the sample into a tighter spot. The standard would need to be removed by breaking off the portion of the plate containing the standard prior to this step. The compound is then removed from the silica as described above in ¶ 10.6.3.5.

10.7 Comparative Semi-Quantitative Thin Layer Chromatography

10.7.1 Thin layer chromatography can be used to determine relative concentration between a sample and a standard. This is useful when it is necessary to determine whether a pharmaceutical preparation has been diluted or substituted. In cases where an exact assay is needed, a suitable quantitation should be performed.

10.7.2 Procedure:

10.7.2.1 Obtain or prepare a standard at the concentration expected for the sample.

10.7.2.2 Apply equal amounts of the standard solution and the sample solution to the TLC plate.

10.7.2.3 Develop and visualize the plate as described above for regular TLC.

10.7.2.4 Visually compare the size and color of the spots to determine if the substance has been substituted or diluted.

10.7.2.5 Approximate concentrations can be estimated by bracketing the observed sample concentration within appropriate standard dilutions. Visually compare the sample response to that of the closest standard dilution. This approximation should be recorded in the case notes but not indicated on the report.

10.7.3 Reporting: Any controlled substance present will be initially identified in the usual manner. The concentration or substitution of the sample will normally be addressed in the report with one of the two following statements for suspected tampering cases.

10.7.3.1 Meets label specifications (with regard to concentration and/or contents).

10.7.3.2 Does not meet label specifications (with regard to concentration and/or contents).

10.7.3.3 Any pharmaceutical substitutions or adulterants, properly identified, should be reported as usual. Their concentrations will not normally be required. (e.g., Diazepam substituted into a device labeled to contain morphine would be identified and reported with no concentration value required).

10.8 References

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10.8.3 Hughes, R. B. and Warner, V. J., Jr. "A Study of False Positives in the Chemical Identification of Marihuana", *Journal of Forensic Sciences*, Volume 23 (2), pp. 304-309. (NOTE: Substitute toluene for benzene, a known carcinogen.)

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10.8.7 Sperling, Albert, "Thin-Layer and Gas Chromatographic Identification of LSD", *Journal of Chromromatographic Science*, Vol. 12, May 1974, pp. 265 – 266.

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11 GAS CHROMATOGRAPHY

11.1 Introduction

11.1.1 Gas Chromatography (GC) is a useful method for screening, separation and preliminary identification. GC provides both qualitative and quantitative information about the components of samples. Specificity is dependent on a variety of factors including stationary phase and type of detector. GC can be used to determine such things as isomers along with sufficient structurally definitive information from other techniques.

11.1.2 GC retention times of the analyte are compared to that of a known standard. The specificity of GC is increased by using two columns with stationary phases of different polarities.

11.1.3 Specific column designations, conditions, detectors, and any standard identifier used in casework will be denoted in the examination documentation. GC results shall be recorded in the analytical notes.

11.2 Materials

11.2.1 Capillary Columns:

11.2.1.1 All routine methods employ gas chromatography using flexible fused silica capillary columns of 0.20 to 0.320 mm i.d.

11.2.1.2 The stationary phase is chosen to effect needed resolution. Methylsilicone (e.g., HP-1) and 5% phenylmethyl silicone (e.g., HP-5 and HP-5MS) are utilized in routine casework. The film thickness should be approximately 0.25 microns. The normal general purpose column has a 0.25 µm film thickness and 0.25 mm internal diameter. Columns of greater stationary phase polarity (e.g., HP-35, HP-35MS, DB17, or DB17MS) may be used when beneficial for separating compounds with similar structures, such as cannabimimetic agents or designer drug analogues.

11.2.1.3 All routine methods utilize columns containing a bonded, cross-linked stationary phase.

11.2.1.4 If a stationary phase is required that is outside of these recommended parameters for a specialized analysis or if more resolving power is required, an alternative column may be used or an additional, different diameter and/or phase column can be temporarily attached at the end of the existing column by using an appropriate connector.

- These changes should be made under the guidance of the instrument operator and must be approved by the Chemistry Program Manager.
- In the case of thick films or non-bonded stationary phases, the extra column bleed generated may require more frequent maintenance of the detector.

11.2.2 Additional Instrument Parameters

11.2.2.1 The carrier gas is normally a high purity helium at a flow rate of 0.5 to 3 mL/min.

11.2.2.2 Nitrogen makeup gas is recommended in order to support gas flow at the FID to provide optimal detector sensitivity.

11.2.2.3 Split/splitless liners designed specifically for use with the particular instrument should be used.

- Injection port liners may be reused after appropriate cleaning and deactivation.

11.2.2.4 For “open tubular" liners, a small amount of silanized glass wool shall be inserted in the center of the liner.

- If the liner is to be packed with GC column packing material, the packing material should be sandwiched between layers of silanized glass wool or equivalent. Packed liners may require "on column" silyl treatment when first installed.

- Liner packing material: The solid support should be either Chromasorb W-HP or Gas Chrom Q. Mesh size should be 80/100 or 100/120 mesh. Stationary phases such as OV-1, OV-17, OV-7, and SE 30 series or their equivalent at 2-5% loading may be utilized.

11.2.2.5 The use of a "two hole" capillary ferrule allows two capillary columns of slightly different polarities to be connected into the same injection port. The sample is analyzed on two columns with a single injection of typically less than 5 µL.

11.2.2.6 Detectors most typically used for controlled substances analyses include both flame ionization detectors and mass spectrometers. Other specific detectors such as NPD and ECD may be used in circumstances requiring them in consultation with the Chemistry Program Manager. Retention time comparison may be accomplished with any detector.

11.3 Methods

11.3.1 Analysis conditions are generally set to allow sample elution time to be greater than 3 - 5 times that of the solvent front. This allows the sample to interact sufficiently with the stationary phase.

11.3.2 The maximum allowable temperature program ramp rate for reproducible retention times is 30 degrees centigrade per minute for Agilent GCs.

11.3.3 In most instances, injection is made in the split mode at a split ratio of 5 - 100:1. Splitless injections may be used when required to increase the amount of analyte delivered to the column and the detector.

11.3.4 Normal injection volume and sample size should be sufficient to provide 8 - 160 nanograms of analyte "on column" for the normal setup. This correlates to a 1 µL injection of an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio.

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

11.3.6 Sample concentrations should be approximately the same concentration as the GC standard and should be within the linear dynamic range of the chromatographic system and detector.

11.3.7 For comparison purposes, a standard must be run using the same method conditions as the samples (with the exception of split ratio, injection volume, or hold time at the end of a run). Standards used in the comparison must be run on the same day as the sample. “Same day” is defined as an approximate 24 hour period.

11.3.8 At a minimum, a solvent blank or procedure blank (for extractions) must be run on both the GC and GC/MS systems, when any of the following conditions are met:

- Before each analyst’s series of sample runs, whether manual or autosampler methods are utilized.

- No more than 10 samples can be run before another blank or standard/blank combination is required. A sample’s position relative to the blank shall be documented in the case file. This may be accomplished by several methods, including consecutive data file numbering when using “windows macros”.

- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded (except those that merely change the injection volume, split ratio, or hold time at the end of the run) or run between blank and sample.

- It is strongly suggested that a solvent blank be injected and properly documented immediately prior to a sample known to be extremely weak.

- Additional blanks may be run at the examiner's discretion.

- The injection order when running samples with standards should be either "standard, blank, sample(s)" or "blank, sample(s), standard."

11.3.8.1 The specific solvent(s) or a reference to the specific solvent(s) (e.g., "MeOH blk", "ext blk", lot number) shall be indicated on the data for the blank.

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

11.3.8.3 The solvent blank must be run at the same or lower split ratio as the sample. The solvent blank shall be run directly before samples that are run at a reduced split ratio (e.g., 20:1 when typical methods use 60:1).

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

- If a controlled substance or related compound is present in any concentration, the blanks and associated samples should be re-run.

- If an interfering substance is present, the blanks and associated samples should be re-run.

- Blanks and associated samples should be replaced and re-sampled, respectively, prior to further analysis if the same extraneous peaks are still present.

11.3.8.5 The results of the blank must be recorded in the case notes if the data is not included in the case file. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., "Blk √").

11.3.9 In all instances, the GC standards file may be referred to for chromatographic conditions. Broad screening methods can be surmised from these files.

11.3.10 Sequencing via autosampler should be utilized whenever practical.

11.3.11 Sequences and/or samples (including standards and blanks) shall be recorded in the instrument logbook.

11.3.11.1 Data files should not be overwritten.

11.3.11.2 Sequence files should not be overwritten unless additional data files have been added during the sequence run.

11.3.11.3 Sequences and sequence log files shall be archived along with data files as per ¶ 36.6.4.2.

11.3.12 Integrated retention times for analytes must agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive result.

11.3.13 Derivatization

11.3.13.1 Some compounds, such as amphetamines or barbiturates, do not chromatograph well. Derivatives may need to be made to help effect good chromatographic peak shape.

11.3.13.2 Procedures:

- Acetyl derivatives – appropriate for primary and secondary amines
    - The acetyl derivative of phenethylamines is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. Acetyl derivatives generally have a longer retention time than the underivatized compound and may require a higher temperature than the underivatized compound.
    - These derivatives can also be formed prior to injection by heating the sample and derivatizing reagent (~ 70°C) in a closed vial.
- Alkyl derivatives – appropriate for barbiturates

  The methyl derivative of barbiturates is made by the same procedure as listed above, only using trimethylanilinium hydroxide (TMAH) instead of acetic anhydride. Methyl derivatives often have a shorter retention time and may require a lower temperature than the underivatized compound.
- Silyl derivatives
    - Silyl derivatives are often very helpful in the analysis of compounds that exhibit chromatographic difficulties due to polar functional groups such as alcohols, amines, acids, and phenols (e.g., GHB, morphine). Silyl derivatives exhibit an M-15 and M-57 peak and sometimes do not exhibit a molecular ion peak in electron impact (EI) mass spectrometry.
    - There are several good silylation reagents available from Regis Chemical Co., Pierce Chemical Co. and others that are designed for various applications. Catalogues from these companies are quite useful in determining the most useful derivatizing agent and their application procedure. BSTFA and BSTFA with 1% TMCS work well as silylating reagents for drug compounds.
    - A suitable aprotic solvent (e.g., pyridine, chloroform, toluene) may be used to dissolve the analyte and the manufacturers' directions must be followed carefully to achieve the desired result.

11.3.14 Split/Splitless Liner Cleaning and Preparation Methods

11.3.14.1 Liner Cleaning with aluminum oxide

- Remove any packing material.
- Dip cotton swab in aluminum oxide slurry and twist swab into liners to remove residue.
- Sonicate liners in water with laboratory glassware soap, rinse several times with water, acetone and then methanol.
- Dry thoroughly.
- Immerse liners in silylating reagent (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight.
- Rinse three times with toluene and dry thoroughly.
- Wear nylon gloves when handling clean liners and glass wool.
- Insert packing, such as silanized glass wool, prior to installation in the instrument.
- After installation, put injection port and oven temperatures to 290ºC. Allow to sit for at least five minutes at this temperature before running QA mix.

11.3.14.2 Liner Cleaning with HCl

- Remove any packing or glass wool.
- Soak liners in 1N HCl for at least eight hours (overnight is fine).
- Rinse with distilled water and methanol.
- After thoroughly drying, soak liners in silylating reagent (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight.
- Rinse three times with toluene and dry thoroughly.
- Wear nylon gloves when handling clean liners and glass wool.
- Insert packing, such as silanized glass wool, prior to installation in the instrument.
- After installation, put injection port and oven temperatures to 290ºC. Allow to sit for at least five minutes at this temperature before running QA mix.

11.4 Quantitation

11.4.1 Gas Chromatography utilizing a flame ionization detector is an excellent method for quantitative analysis. The preferred method is the internal standard method.

11.4.2 The Department does not routinely perform drug quantitations. When a quantitation is specifically requested or required by the Code of Virginia, this is the general procedure for a suitable GC quantitation method. Specific examples are found in the sections specific to a particular compound. If the customer does not require a quantitation required by the Code of Virginia, an MFR or email shall be placed in the case file documenting the communication.

11.4.2.1 The purity of evidence found to contain methamphetamine will not be routinely determined unless it is specifically requested by a Commonwealth's Attorney.

11.4.3 General Quantitation Procedure

11.4.3.1 Weights will be measured using an analytical balance with a readability of ± 0.00001 gram. Weights shall be adjusted for purity when solid standards are used in the preparation of the quantitation standards.

11.4.3.2 Certified reference materials shall be used, where available, for all quantitations and include the supplier's Certificate of Analysis. Standard solutions shall be prepared using calibrated volumetric flasks and/or transferred with calibrated mechanical pipettes as specified in the individual method. Internal standard and sample solutions shall be prepared in Class A volumetric flasks, calibrated volumetric flasks, and/or transferred with calibrated mechanical pipettes. Graduated pipettes are not acceptable for quantitative purposes.

11.4.3.3 Make up an internal standard solution of known concentration between 1-2 mg/mL, which will be used in making all standard and sample solutions. Refrigerated solutions should be allowed to return to ambient temperature prior to use.

11.4.3.4 Two standard solutions (1 mg/mL and 2.5 mg/mL) shall be prepared in the internal standard solution as defined in the method. Do not use serial dilutions.

- The 1 mg/mL standard solution will serve as a check standard. The analyst shall prepare the check standard at the same time the samples are prepared.

- The 2.5 mg/mL standard solution will serve as the calibration standard for the one point calibration.

11.4.3.5 All quantitations shall be performed by preparing six separate sample solutions as per the method, unless a historical uncertainty exists for the method. Refer to Quantitative Sampling

(¶ 5.8) for further information. Serial numbers or other unique identifier of calibrated flasks and/or mechanical pipettes used for standard preparation shall be recorded. Multiple-use standards shall be recorded in the standard preparation record with the lot number referenced in examination documentation. Single-use standard preparation may be recorded in examination documentation or in the standard preparation record. Serial numbers or other unique identifier of calibrated flasks and/or mechanical pipettes used for sample preparation will be recorded in examination documentation. Standards and samples shall be prepared in the same manner (use of flask vs. pipette).

11.4.3.6 Run the two standards, a blank of the internal standard solution, and the samples using the appropriate GC method. The standards will be injected three times and the ratios will be averaged to calculate the concentration. Samples will be injected once each and one injection of the blank is sufficient. The injection volume should be 1-2 µl.

11.4.3.7 Control charts are used to establish the historical standard deviation for quantitative procedures. Results from quantitative quality control materials are recorded in control charts to readily detect trends such as deterioration of reagents, calibrators and controls.

- All staff are responsible for entering the 1 mg/mL check standard values into the statewide control charts.

- The DTS Research Section Supervisor or designee should review the statewide control charts each month to ensure consistency between laboratories and communicate results to the Chemistry Program Manager as necessary.

11.4.3.8 Using the equation listed below, calculate the % purity of both the check standard and the samples. The *Drug Quantitation Worksheet* shall be used for amphetamine/methamphetamine, cocaine, heroin, PCP and MDMA quantitation calculation purposes. The *THC Quantitation Worksheet* shall be used for THC quantitation calculation purposes.

- For THC quantitations, the concentration of the check standard must be within 7% of the theoretical value. For other drugs, the concentration of the check standard must be within 5% of the theoretical value.

- The precision of the check standard must be within 3%.

- For THC quantitations, the relative standard deviation of the six samples must be 15% or less. For other drugs, the relative standard deviation of the six samples must be 10% or less.

- If the above criteria are met, the results may be reported and shall include the Uncertainty of Measurement (UoM).

- If any of the above criteria are not met, take appropriate steps (e.g., perform appropriate corrective instrument maintenance, rerun, remake one or both of the standard solutions, or prepare new samples) to resolve the problem.

11.4.3.9 Standard Salt Form Calculation

- If the purity is calculated as the salt, the salt form of the drug should be confirmed by FTIR/ATR and reported.

- Unless the salt form of the drug is to be reported, the concentration of the analyte in its base form will be calculated and reported. If the calibration standard is not in free base form, it will need to be corrected.

Example: Heroin hydrochloride monohydrate is used to quantitate a sample containing heroin. The sample will be reported as "Heroin" without a specified salt form. The calibration standard is made up using 22.4 mg of heroin hydrochloride in 10.0 mL of internal standard solution. The corrected concentration will be:

[Heroin base] = (22.4 mg ÷ 10.0 mL) * (369.4 g/mol ÷ 423.9 g/mol)
[Heroin base] = 1.95 mg/mL

11.4.3.10 Purity Calculation

$$\% \text{ Drug} = \frac{[\text{STD}] \times R_2 \times V \times 100}{R_1 \times W}$$

[STD] = concentration of calibration standard in mg/mL

R_2 = peak area (height) of sample / peak area (height) of internal standard

R_1 = peak area (height) of standard / peak area (height) of internal standard

V = volume of internal standard solution used in mL

W = sample weight in mg

Take the average of the replicates and round to one (1) decimal place.

12 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

12.1 Introduction

12.1.1 High Performance Liquid Chromatography (HPLC) is a useful method for separation and quantification. Specificity is dependent on a variety of factors including stationary phase and type of detector.

12.1.2 HPLC retention times of the analyte are compared to that of a known standard.

12.1.3 Specific column designations, conditions, detectors, and any standard identifier used in casework will be denoted in the examination documentation. HPLC results shall be recorded in the analytical.

12.2 Materials

12.2.1 Columns:

12.2.1.1 There is a wide variety of column choices available for use. The stationary phase is chosen to affect needed resolution.

12.2.1.2 Columns and chromatographic conditions will be dependent on the currently used instrument and technology available. Literature resources are useful for specific separations. If a specialized analysis is required, consult the primary operator for the HPLC.

12.2.2 Additional Instrument Parameters

12.2.2.1 Mobile phases will be selected based on the target analytes.

12.2.2.2 Detectors most appropriate for normal drug analyses include both diode array detectors (DAD) and mass spectrometers. Retention time comparison may be accomplished with any detector. Quantitative analyses shall use the diode array detector.

12.3 Methods

12.3.1 Analysis conditions should allow the sample to interact sufficiently with the stationary phase.

12.3.2 Normal injection volumes range from 1 to 5 microliters but may be adjusted depending on the sample concentration.

12.3.3 Samples should be prepared in an appropriate solvent based on the chosen method. Depending on the nature of the samples, an extraction may be required; however, most may be directly dissolved in the solvent. Samples may require filtration using syringe filters, or similar, before injection onto the HPLC system.

12.3.4 Sample concentrations should be within the linear dynamic range of the chromatographic system and detector.

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

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

- Before each analyst’s series of sample runs.
- No more than 10 samples can be run before another blank or standard/blank combination is required. A sample’s position relative to the blank shall be documented in the case file. This may be accomplished by consecutive data file numbering.

- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded or run between blank and sample.
- It is strongly suggested that a solvent blank be injected and properly documented immediately prior to a sample known to be extremely weak.
- Additional blanks may be run at the examiner's discretion.
- The injection order when running samples with standards should be either "standard, blank, sample(s)" or "blank, sample(s), standard."

12.3.6.1 The blank solvent shall be the same solvent utilized for samples. In addition, the injection volume shall be equal to, or greater than, the volume injected for the samples.

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

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

12.3.7 Sequences and/or samples (including standards and blanks) shall be recorded in the instrument logbook.

12.3.7.1 Data files should not be overwritten.

12.3.7.2 Sequence files should not be overwritten unless additional data files have been added during the sequence run.

12.3.7.3 Sequences and sequence log files shall be archived along with data files as per ¶ 36.7.3.1.

12.3.8 Retention times for analytes must agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive result.

12.4 Quantitation

12.4.1 High Performance Liquid Chromatography utilizing a diode array detector is commonly used to perform quantitative analyses.

12.4.2 The Department does not routinely perform drug quantitations. When a quantitation is specifically requested or required by the Code of Virginia, this is the general procedure for a suitable HPLC quantitation method. Specific examples are found in the sections specific to a particular compound. If the customer does not require a quantitation required by the Code of Virginia, an MFR or email shall be placed in the case file documenting the communication.

12.4.3 General Quantitation Procedure

12.4.3.1 Weights will be measured using an analytical balance with a readability of ± 0.00001 gram. Quantities used in the preparation of primary standards and samples for quantitative purposes shall be at or above 10 milligrams (0.01000 gram). Weights shall be adjusted for purity when solid standards are used in the preparation of quantitation standards.

12.4.3.2 Certified reference materials shall be used, where available, for all quantitations and include the supplier's Certificate of Analysis. Standard solutions shall be prepared in calibrated volumetric flasks and/or transferred with calibrated mechanical pipettes. Serial numbers of

calibrated flasks and/or mechanical pipettes shall be recorded in examination documentation or documented in the standard preparation record with a lot number reference. Internal standard and sample solutions shall be prepared using Class A volumetric flasks and/or transferred with calibrated mechanical pipettes. Graduated pipettes are not acceptable for quantitative purposes.

12.4.3.3 Prepare an internal standard solution of known concentration, which will be used in preparing all standard and sample solutions. Refrigerated solutions should be allowed to return to ambient temperature prior to use.

12.4.3.4 Prepare an appropriate number of calibrators to create a calibration curve as defined in the method.

12.4.3.5 Prepare a positive control as defined in the method.

12.4.3.6 All quantitations shall be performed by preparing six separate sample solutions as per the method, unless a historical uncertainty exists for the method. Refer to Quantitative Sampling (¶ 5.8) for further information.

12.4.3.7 Run the calibrators, control, a blank of the internal standard solution, and the six samples using the appropriate HPLC method. The standards, control and samples shall be injected once each in addition to a sufficient number of blanks.

12.4.3.8 Control charts are used to establish the historical standard deviation for quantitative procedures. Results from quantitative quality control materials are recorded in control charts to readily detect trends such as deterioration of reagents, calibrators and controls.

- All staff are responsible for entering control values into the statewide control charts as necessary.
- The DTS Research Section Supervisor or designee shall review the statewide control charts monthly to ensure consistency between laboratories and communicate results to the Chemistry Program Manager as necessary.

12.4.3.9 For reporting Cannabis Oil, see examples in ¶ 34.9.5.

13 ANALYSIS OF PHARMACEUTICAL INJECTABLE DOSAGE FORMS

13.1 Introduction

13.1.1 The analysis of cases involving pharmaceutical injectable dosage forms may require more than just the identification of the contents.

13.1.2 Tampering can involve the dilution, contamination, or removal of the contents of a Tubex®, Carpuject®, injection vial, pharmacy intravenous (IV) preparation, IV stock solution, or IV supplies and apparatus.

13.2 Procedure

13.2.1 Carefully visually inspect the item. Note stopper condition (for small punctures), plunger location and condition, fill volume, appearance, color and consistency of the contents.

13.2.2 If possible, a standard of the same brand and lot number should be requested from the submitting agency or a licensed pharmacy for comparison of the visual characteristics, chemical contents and concentration.

13.2.3 Any controlled substance present will be identified in the usual manner.

13.2.4 Analyses for concentration are normally run with semi-quantitative TLC, comparative UV quantitation, or standard GC quantitation methods.

13.3 Reporting

The concentration of the sample will normally be addressed in the report with one of the two following statements for suspected tampering cases. Additional clarifying wording may be used at the discretion of the Section Supervisor, such as “Does not meet label specifications with regard to concentration” or “Does not meet label specifications with regard to concentration and contents.”

13.3.1 Meets label specifications.

13.3.2 Does not meet label specifications.

13.3.3 Any pharmaceutical substitutions or adulterants, properly identified, should be reported as usual. Their concentrations will not normally be required. (e.g., Diazepam substituted into a device labeled to contain morphine would be identified and reported with no concentration value required).

14 INFRARED SPECTROSCOPY

14.1 Introduction

14.1.1 Infrared spectroscopy (IR) is a specific method of identification in most instances and is therefore a desirable analytical tool for the forensic drug chemist. IR may be used to obtain semi-quantitative data on known mixtures to express relative percentages, but is not normally used for quantitation.

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

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

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

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

14.1.6 Spectra may be collected using an Attenuated Total Reflectance (ATR) accessory and compared to standards also collected utilizing the ATR. These standards may be stored in a user-generated library. For unknown compounds, an ATR correction may be utilized in order to search a library of transmission spectra. The uncorrected unknown spectrum would then be compared to that of a known uncorrected standard spectrum.

14.1.7 Spectra may be collected using the DiscovIR Gas Chromatograph-Solid State Infrared Spectrophotometer and compared to standards also collected on the DiscovIR. These standards may be stored in a user-generated library.

14.1.8 If unique sample preparation or data reduction techniques are required, consult the Primary Operator for the FTIR.

14.2 Sample Preparation

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

14.2.2 Pure liquid organics can be run neat between two salt (NaCl) plates or using the ATR accessory.

14.2.3 Pure solids can be dissolved in a suitable organic solvent and run in solution cells, mixed with KBr and pressed into a pellet, mixed with a saturated long chain hydrocarbon oil (mulled) or run using the ATR accessory.

14.2.4 Solution Technique

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

14.2.4.2 Oils or dissolved solids may be deposited or "cast" on a salt plate (e.g., standard NaCl window) and placed in the sample beam. (Care must be taken to drive off all residual solvent).

14.2.4.3 The solvent absorption bands may be subtracted from the spectrum. Either a pair of salt plates with the solution solvent or a solution cell (of the same pathlength) containing only solvent can be scanned into the background spectrum.

14.2.5 Gas Techniques

Standard 10 cm gas cells or other similar hardware (e.g., multiple internal reflectance units) can be used.

14.2.6 Mull Technique

The sample is finely ground and suspended in mineral oil (Nujol). A thin film of the suspension is placed between two salt plates.

14.2.7 Pellet Technique

14.2.7.1 Infrared grade KBr should be kept dry by storing it in a suitable location such as a desiccator.

14.2.7.2 Infrared grade KBr and the sample each must be finely ground. The KBr and sample are mixed by grinding with a mortar and pestle in an approximate ratio of 100 parts KBr to 1 part sample.

14.2.7.3 The mixture is placed in a pellet press to prepare the pellet. A hand press with a 7 mm die or the Hydraulic 13 mm die set may be used. The 7 mm hand press KBr pellet is the preferred preparation technique.

14.2.8 ATR Accessory

14.2.8.1 Clean the diamond crystal and anvil surface before and after analysis with acetone or methanol soaked wipes. Methanol takes a slightly longer time to evaporate.

14.2.8.2 A background is collected prior to each sample.

- An acceptable background shall be noted on the data.
- If unexpected peaks are present in the background, the ATR crystal and anvil shall be cleaned and the background repeated.
- If the results of the second background are unacceptable, the analyst shall take steps to resolve the issue prior to any analysis.

14.2.8.3 For ATR, the background run prior to consecutive samples serves as the blank for that case or sequences of cases. This background/blank shall be printed and stored in the corresponding case file(s).

14.2.8.4 For solid samples, cover the center of the crystal with sample. Press the anvil against the sample by turning the anvil screw clockwise until it spins without further tightening.

14.2.8.5 For liquid samples, place a drop or two of liquid directly onto the ATR crystal. Use enough sample to cover the crystal completely. If the sample is volatile, place the cover over the sampling area to prevent evaporation during analysis.

14.2.8.6 If the sample requires an extraction, the sample in an organic solvent may be dropped on a crystal and allowed to evaporate to form a film. An o-ring may be used to contain the liquid as it is placed on the crystal.

14.3 Solid State FTIR via Gas Chromatography

14.3.1 The DiscovIR instrument deposits the eluent from a gas chromatograph onto a liquid nitrogen cooled disk allowing for collection of solid phase transmission spectra. The MCT-A detector scans from 4000 – 680 cm^{-1} and requires cooling with liquid nitrogen prior to analysis.

14.3.2 The spectra produced must be compared to known spectra taken under similar conditions. Spectra collected with the DiscovIR may differ from traditional FTIR libraries due to the water content or salt form of the material.

14.3.3 DiscovIR analysis is especially useful for positional isomer differentiation, which may be required with cannabimimetic agents and research chemical analysis. The solid phase spectra collected with the DiscovIR have a higher resolution (4 cm^{-1}) than the gas phase spectra collected with the Gas Phase FTIR (8 cm^{-1}).

14.3.4 Procedure:

14.3.4.1 Liquid nitrogen is added to the MCT-A detector during the daily QA check. While creating the sequence table, liquid nitrogen is added to the Dewar. The large Dewar holds up to 10 L of liquid nitrogen where each liter allows for approximately 1.5 hours of instrument operation. The MCT-A detector holds approximately ¾ L of liquid nitrogen, which allows for approximately 15 hours of instrument operation.

14.3.4.2 Method parameters for the instrumentation are as follows:

- Column HP-35 15 m x 0.25 mm i.d. x 0.25 µm film thickness (or equivalent)
- Flow 1.5 – 1.8 mL/min.
- Resolution 4.0
- Transfer line 280ºC
- Oven temp. 280ºC
- Restrictor temp. 280ºC
- Disk temp. -40ºC
- Dewer cap temp. 35ºC

14.3.4.3 Methods selected from those listed below can be documented in the case notes by name. Temperature Range, Ramp Rate, Split Ratio, Flow Rate, and Inlet Temperature shall remain the same but the Disk Speed may be adjusted for increased chromatographic separation. The Disk Speed can range from 3 – 6 mm/min with 3 mm/min being used for routine analysis. If alternate method parameters are utilized, the parameters must be recorded in the case notes.

Method	Injection Volume (µL)	Temperature Range (ºC)	Ramp Rate (ºC/min.)	Split Ratio	Flow Rate (mL/min.)	Inlet Temperature (ºC)	Disk Speed (mm/min.)
LOW	1	90 – 190	30	10:1	1.5	250	3
LOWRESIDUE	1.5	90 – 190	30	5:1	1.5	250	6
MID1	1	170 – 270	30	10:1	1.5	250	3-6
MIDRESIDUE	1.5	170 - 270	30	5:1	1.5	250	3-6
HIGH1	1	225 – 315	30	10:1	1.8	270	3-6
HIGHRESIDUE	1.5	225 – 315	30	5:1	1.8	270	3-6
SCREEN1	1	105 – 315	40	10:1	1.8	270	3-6
SCREENRESIDUE	1.5	105 - 315	40	5:1	1.8	270	3-6

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

14.3.6 An amount of sample (1 – 1.5 µL) is injected utilizing an autosampler.

14.3.7 Sample concentrations should be approximately the same concentration as the standard. Concentrations of approximately 1-2 mg/mL are recommended.

14.3.8 Basic extractions are recommended for suspected clandestine laboratory samples or other phenethylamine type compounds.

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

14.3.10 Solvent blanks shall be run prior to each sample in the same location where the sample is to be deposited on the disk.

14.3.11 After the data is processed, the sample spectrum will be compared to a known solid phase spectrum. For unknown compounds, a search of a library of solid state transmission spectra may be conducted, however for an identification, the spectrum produced must be compared to a known spectrum taken under similar conditions.

14.3.12 Integrated retention times for analytes must agree with the standard within the appropriate acceptance range below to be considered a positive GC result.

Sample Retention Time (min)	RT Acceptance Range vs. Reference Standard (min)
< 1.50	± 0.03
1.50 to 2.50	±0.04
> 2.50	±0.05

14.4 Acceptance Criteria

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

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

14.4.3 Data that supports the analyst's conclusion shall be printed and included in the case file.

14.4.3.1 At a minimum, the data shall include:

- FS Lab number and Item number
- Date
- Instrument name
- Sampling information (e.g., DiscovIR, ATR, KBr pellet, pentane extract)
- Method parameters (e.g., # scans, resolution, sample gain, mirror velocity, aperture) (for DiscovIR see ¶¶ 14.3.4.2 – 14.3.4.3)

15 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

15.1 Introduction

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

15.1.2 Depending on the structure of the molecule, the amount and type of fragmentation will vary. Due to this, some drugs do not exhibit a molecular ion using electron impact mass spectrometry. Examples include barbiturates, lorazepam and methylphenidate.

15.1.3 Following evaluation of an unknown spectrum, confirmation of the unknown spectrum is done by direct comparison with a known or suitable reference spectrum or through use of interpretation methods. Mass spectral results shall be recorded in the analytical notes by listing the drug identified. It is not required to record the analyst's disagreement with library search results on the data.

15.1.4 Specific column designations, conditions, detector conditions, and any standard identifier used in casework will be denoted in the examination documentation. GC-MS results shall be recorded in the analytical notes.

15.2 Procedure

15.2.1 Samples will be dissolved in a suitable solvent, preferably methanol.

15.2.2 The general concentration should be determined by TLC or GC before being run on the GC/MS. The usual amount of sample delivered to the ion source for good qualitative results should be 8 - 160 ng. This correlates to an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio with a 1 µL injection volume. In any case, sufficient abundance of the total ion chromatogram peaks needs to be achieved in order to produce acceptable spectra, without overloading the chromatographic system.

15.2.3 For analysis of volatile organics, such as amyl nitrite, the headspace may be injected. An air blank must be run prior to headspace analysis.

15.2.4 Chromatographic conditions may be determined by the chemist from the GC/MS standards file.

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

15.2.6 At a minimum, a solvent blank or procedure blank (for extractions) must be run on the GC/MS system, when any of the following conditions are met:

- Before each analyst's series of sample runs, whether manual or autosampler methods are utilized.
- No more than 10 samples can be run before another blank or standard/blank combination is required. A sample's position relative to the blank shall be documented. This may be accomplished by several methods, including consecutive data file numbering when using "windows macros".

- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded (except those that merely change the injection volume, split ratio, or hold time at the end of a run) or run between blank and sample.

- It is strongly suggested that a solvent blank be injected immediately prior to a sample known to be extremely weak.

- Additional blanks may be run at the examiner's discretion.

- The injection order when running samples with standards should be either "standard, blank, sample(s)" or "blank, sample(s), standard."

15.2.6.1 The specific solvent(s) or a reference to the specific solvent(s) (e.g., "MeOH blk", "ext blk", lot number) shall be indicated on the data for the blank.

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

15.2.6.3 The solvent blank must be run at the same or lower split ratio as the sample. The solvent blank shall be run directly before samples that are run at a reduced split ratio (e.g., 20:1 when typical methods use 60:1).

15.2.6.4 Any significant peaks in blank chromatograms and any peaks within 0.033 mins of an analyte of interest must be properly investigated and documented in the referenced case file.

- If a controlled substance or related compound is present in any concentration, the blanks and associated samples should be re-run.

- If an interfering substance is present, the blanks and associated samples should be re-run.

- Blanks and associated samples should be replaced and re-sampled, respectively, prior to further analysis if the same extraneous peaks are still present.

15.2.6.5 The results of the blank must be recorded in the case notes if the data is not included in the case file. This may be done by using a check mark (√), "ok", or "-" to record that the results of the blank were acceptable (e.g., "Blk √").

15.2.7 Sequencing via autosampler should be utilized whenever practical.

15.2.8 Sequences and/or samples (including standards and blanks) shall be recorded in the instrument logbook.

15.2.8.1 Data files should not be overwritten.

15.2.8.2 Sequence files should not be overwritten unless additional data files have been added during the sequence run.

15.2.8.3 Sequences and sequence log files shall be archived along with data files as per ¶ 36.8.5.

15.2.9 Data that supports the analyst's conclusion shall be printed and included in the case file.

15.2.9.1 At a minimum, the data shall include:

- Data file name
- Date and time
- Instrument name
- Method name

- Sample name
- Barcode number (may be handwritten) and vial number, if applicable
- Integrated total ion chromatogram
- A background subtracted mass spectrum and normalized tabulation for peaks

15.2.10 It is permissible to use GC/MS integrated retention times for GC retention time data. For comparison purposes, a standard must be run using the same method conditions as the samples (with the exception of split ratio, injection volume, or hold time at the end of a run). Standards used in the comparison must be run on the same day as the sample. “Same day” is defined as within an approximate 24 hour period.

15.2.11 Compare spectra to standard spectra run under the same conditions (with the exception of split ratio, injection volume, or hold time at the end of a run), in-house or reputable "library" spectra or published standard spectra to verify sample identification. Due caution should be exercised when using the PBM similarity index generated by the library search algorithm. Spectra may also be identified through the use of interpretation methods in conjunction with data generated from additional testing with the approval of the Section Supervisor.

15.3 Data Interpretation and Acceptance Criteria

15.3.1 Integrated retention times for analytes are expected to agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive GC result.

15.3.2 Autosampler vials used for standards, blanks, and case samples shall have a barcode attached. The barcode number shall be printed on the data. In addition, barcode numbers for case samples shall be documented in the case notes. If the barcode is not printed on the data during data analysis, it must be handwritten and initialed after checking the vial’s tray location.

15.3.3 In order for a mass spectrum to be considered definitive, all major peaks must have associated ^{13}C isotope peaks present.

15.3.4 For compounds such as cocaine, heroin and LSD, a molecular ion peak with associated ^{13}C isotope peak must be present in order for the result to be considered definitive.

15.3.5 For compounds, such as methamphetamine, amphetamine and related compounds, the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular ion shall be present for the result to be considered definitive (e.g., methamphetamine must have a 148 and 149 *m/z* ion). In the absence of the $[M-H]^+$ ion and/or its associated ^{13}C isotope peak/molecular ion, the DART-TOF may be used to supplement mass spectral data for identification purposes. A molecular ion peak with associated ^{13}C isotope peak shall be present in order to confirm ephedrine/pseudoephedrine. In the absence of the molecular ion peak and/or its associated ^{13}C isotope peak the DART-TOF may be used to supplement the GC/MS data to satisfy this requirement.

15.3.6 The DART-TOF can be used to confirm the molecular weight of compounds, such as methylphenidate and fentanyl analogues, which do not typically exhibit a molecular ion in their mass spectra. If the molecular ion is not observed and the DART-TOF is not utilized, low abundance ions that can be useful in the discrimination of closely related compounds must be present (e.g., fentanyl must have a 334 m/z ion) when making an identification.

15.3.7 Compounds such as barbiturates and some benzodiazepines should be derivatized to improve chromatographic performance or confirm the predicted molecular ion. Techniques of derivatization include silylation, alkylation and acetylation (see ¶ 11.3.13). DART-TOF may be used to confirm the predicted molecular ion. Alternate ionization methods for mass spectrometry can also be used via Instrument Support with the approval of the Section Supervisor and Chemistry Program Manager.

15.3.8 For compounds identified and reported, anomalous mass peaks occurring above the molecular ion must be explained with data documentation in the case file. This may be accomplished using the ion reconstruct function of the ChemStation software.

15.3.8.1 Easily recognizable column/septum bleed peaks, e.g., 207, 221, 267, 281, 327, 341, 355, 385, 415 and 429 *m/z*, occurring above the molecular ion may be labeled as such on the spectrum without further data documentation.

15.3.8.2 Anomalous ions related to interference from tailing compounds may be explained by labeling on the spectrum without further data documentation.

15.3.9 The strength of the sample/sensitivity of the instrument can be enhanced in the following ways:

- Up to 4 µL of solution may be injected.
- The sample can be concentrated and placed into an autosampler vial insert.
- The split can be lowered to 10:1 for split methods
- Splitless methods may be employed for samples containing small amounts of drugs including, for example, residues, LSD and fentanyl.

15.3.9.1 If the spectrum still does not meet the criteria, it should be reported as "Insufficient for Identification".

15.3.10 Chromatographic and mass spectrometer conditions will be dependent on the currently used instrument and technology available. If there is any question as to either, consult with the primary operator of the instrument being utilized.

16 DART-TOF MASS SPECTROMETRY

16.1 Introduction

16.1.1 Direct Analysis in Real Time (DART) is an atmospheric pressure ionization technique that can analyze solids, liquids and gases by placing the test material into a heated gas flowing through the sampling area. Ionization occurs from the surface of the sampling medium. Coupling of this ion source with an accurate mass time-of-flight mass spectrometer (TOF) gives quick and simple analyses with little to no sample preparation.

16.1.2 While ionization can be done in both positive and negative mode, the large majority of drugs give usable spectra in positive ion mode. Ionization in positive ion mode is accomplished by charging a heated helium gas stream, forming metastable helium ions, which react with ambient water vapor, producing hydronium ions that subsequently react with the sample molecules to induce ionization. The mechanism of positive and negative ion production with the DART is discussed by Cody, *et al*. (See Reference 16.6.1)

16.1.3 In general, DART ionization produces spectra with a characteristic peak at the protonated or deprotonated molecule. These ions are measured at their exact mass in the TOF mass spectrometer. Elemental composition calculations, based on empirical formulas, can be performed on these ions to determine whether they fall within a specified range, usually measured in milliDalton units (mDa), of a known compound. While accurate-mass spectra have an inherent specificity, full identification is difficult if the possibility of an isomer exists. Collision induced dissociation (CID) (either up-front or via a collision cell) can be utilized to produce spectra with extensive fragmentation, thus increasing the specificity of the technique. If a JEOL AccuTOF mass spectrometer is used, this can be accomplished by utilizing the function switching mode of the operating software. Function switching allows for simultaneous collection of spectra at several different Orifice1 voltages (See References 16.6.3, 16.6.5, and 16.6.7). Higher Orifice1 voltages generally result in more characteristic ions being produced. The combination of accurate mass measurement of the protonated molecule and characteristic CID fragmentation allows for the production of spectra that can be used as part of an identification scheme for drugs of abuse.

16.2 General Drug Screening Method

16.2.1 Instrumentation, Instrument Parameters and Materials

16.2.1.1 While the original DART ion source was marketed to be sold only with a JEOL AccuTOF mass spectrometer, this is no longer the case. IonSense, the manufacturer of the DART source, makes interfaces for all ambient ionization-capable mass spectrometers currently on the market. Instrument parameters will be determined by the specific model of mass spectrometer upon which the DART source is mounted. (Instructions for operation of the DART SVP-AccuTOF can be found in Reference 16.6.3.)

16.2.1.2 Internal mass calibration is accomplished using a dilute solution of polyethylene glycol (PEG) 600 in methanol. Drift compensation and calibration checks are accomplished with a solution containing methamphetamine, cocaine, and nefazodone.

16.2.1.3 Cleaned glass melting point tubes

16.2.1.3.1 It is necessary to clean the melting point tubes prior to use as a sampling device. The procedure below removes the majority of the dioctyladipate contaminant from the tubes. It does not, however, remove ALL of this contaminant from every tube. A more exhaustive cleaning method may need to be employed if this peak interferes with analyte peaks of interest.

16.2.1.3.2 Capillary Tube Cleaning Procedure:

- Remove tubes from plastic container and place into beaker, closed end down.
- Rinse the tubes with acetone. Discard acetone.
- Rinse the tubes with methanol. Discard methanol.
- Repeat methanol wash.
- Place tubes, closed end UP, in another beaker. Dry tubes in an oven until methanol is evaporated.
- Place tubes into a clean, screw-top vial until ready for use.

16.2.2 Procedure

16.2.2.1 Although samples may be run in any chemical state, it is recommended that powders, plant materials, tablets and capsules be dissolved in a suitable solvent (e.g., methanol, methylene chloride, ammonia saturated chloroform).

16.2.2.2 In general, samples are run by dipping the closed end of the glass melting point tube into the sample solution and then immediately inserting the tube into the DART gas stream for several seconds. Replicate samplings (2-3) are recommended within the data file to more fully represent spectra of the analytes of interest. Let the melting point tube cool briefly between samplings in order to achieve better consistency when sampling volatile solvents. Other sampling methods (e.g., solids, plant materials, gases, dried liquids) may be run after consultation with primary instrument operator.

16.2.2.3 Instructions for operation of the DART SVP-AccuTOF can be found in References 16.6.3 and 16.6.7.

16.2.2.4 PEG 600 calibrant solution shall be run within each data file. Replicate samplings (2-3) are recommended for proper internal mass calibration.

16.2.2.5 A mixture of cocaine, methamphetamine and nefazodone shall be run within each data file as a series of positive controls. Where applicable, the cocaine protonated molecule will be used for drift compensation. The protonated molecules of methamphetamine, cocaine, and nefazodone shall be within ± 5mDa of their calculated values of 150.1283 Da, 304.1549 Da, and 470.2323 Da, respectively. The spectrum demonstrating that the cocaine, methamphetamine and nefazodone protonated molecules are within the above acceptance criteria shall be included in the case file.

16.2.2.6 For routine case analysis, the following instrument parameters will be used:

- DART source temperature: 350ºC
- Orifice1 temperature: 100-120ºC
- Scan range: approximately 66 - 600 Da
- Sampling interval: 0.25ns, 0.5ns (MassCenter Main only)
- Function switching cycling: 20V, 30V, 60V, 90V

Any deviation(s) from the above parameters deemed necessary for casework, shall be noted on the data.

16.2.3 Data Interpretation

16.2.3.1 Comparison of background subtracted mass spectra to in-house library search results should be included in the case file. Results shall be recorded in the case notes.

16.2.3.2 To report the identity of a drug indicated by this screening method, confirmation utilizing the normal analytical scheme is required.

16.3 Negative Ion Screening Method including GHB

16.3.1 The Negative Ion Screening Method must be run in consultation with the DART operator or designated backup.

16.3.2 Instrumentation, Instrument Parameters and Materials

16.3.2.1 If using a DART SVP-AccuTOF system, refer to Reference 16.6.4 for operating parameters.

16.3.2.2 Internal mass calibration solution: polyethylene glycol (PEG) 600 in methanol.

16.3.2.3 QC check solution: malic acid (calculated mass: 133.0137 Da) in methanol.

16.3.2.4 GHB (calculated mass: 103.0395 Da) solution in methanol.

16.3.3 Procedure

16.3.3.1 Liquid samples may be run neat or diluted.

16.3.3.2 Data files should contain PEG600 calibration, malic acid QC check, GHB standard and samples.

16.3.3.3 If the sample is a drink, refer to Reference 16.6.4 to determine administrative cutoffs for various drink matrices and the reasoning behind these.

16.3.3.4 For samples that turn out to be negative, and if sample size permits, spike a milliliter of sample with one milligram of GHB standard and run on the DART-TOF to demonstrate that if GHB were present in the sample, it would have produced a spectrum under the conditions used for that matrix.

16.4 Pharmaceutical Confirmation via DART-TOF

For licit pharmaceutical preparations, the combination of accurate mass measurement of the protonated molecule and characteristic CID fragmentation allows for the production of spectra that can be used as part of an identification scheme.

16.4.1 Refer to section 8 for information on pharmaceutical identifiers.

16.4.2 Procedure

16.4.2.1 Settings for the DART and TOF can be found in References 16.6.3 and 16.6.7.

16.4.2.2 Based on indicated concentrations obtained from the visual examination, a portion of the pharmaceutical preparation shall be placed in a suitable solvent and diluted to approximately 0.5 to 2.0 mg/mL.

16.4.2.3 The sample is run on the DART-TOF system in the same manner as described for general screening in ¶ 16.2.2.

16.4.2.4 A solvent blank shall be screened using the DART-TOF system. A blank screen using the Spectrum Monitor (JEOL system) either prior to or after sample analysis is sufficient for this purpose. The results of the blank must be recorded in the case notes. This may be done by using a check mark (√), “ok”, or “-“ to record that the results of the blank were acceptable (e.g., Blk √). If the results of the blank are unacceptable, the analyst shall take steps to resolve the issue.

16.4.2.5 For data collected on a JEOL system, case file data shall include the 30V total ion chronogram, drift compensation/QC data and at a minimum, the 20V or 30V spectrum for comparison of the protonated molecule and data generated at either 60V or 90V, as appropriate to the compound(s), for confirmatory fragment ions. Sample spectra shall have all calibrations applied for any data included in a case file.

- Protonated molecule data shall be within ± 5 mDa of calculated mass for the compound(s) being confirmed.
- Appropriate CID spectra shall compare favorably with library spectra generated on a DART-TOF system.

16.4.2.6 If the DART-TOF data does not agree with the visual examination, other analytical techniques shall be employed for further identification of any controlled substances present.

16.5 Documentation

16.5.1 Data that supports the analyst's conclusion shall be printed and included in the case file.

16.5.2 At a minimum, acquisition data shall include:

- Data file name
- Sample name
- Date and time
- Instrument name, if the lab has more than one

16.6 References

16.6.1 Cody RB, Laramee JA, Nilles JM, Durst HD. "Direct Analysis in Real Time (DART) mass spectrometry" *JEOL News* 2005; 40(1): 8-12.

16.6.2 JEOL, Inc. Application note: *LC/MS: Identification of unknowns by combining exact mass measurement with the NIST 02 Mass Spectral Database Similarity Search*, www.JEOL.com/ms/accutof.html, January 2003.

16.6.3 221-D102 Controlled Substances Instructions for the AccuTOF-DART SVP.

16.6.4 Bennett MJ and Steiner RR, "Detection of Gamma-Hydroxybutyric Acid in Various Drink Matrices via AccuTOF-DART", *J Forensic Sci*, 2009, 54(2), 370-5.

16.6.5 Steiner RR, Larson RL. "Validation of the Direct Analysis in Real Time source for use in forensic drug screening." *J Forensic Sci* 2009; 54(3):617-22.

16.6.6 Easter JL, Steiner RR. "Pharmaceutical identifier confirmation via DART-TOF." *Forensic Science International,* 2014, 240, 9-20.

16.6.7 221-D104 Acquiring Mass Spectral Data – DART msAxel.

17 GENERAL ANALYTICAL METHODOLOGY

17.1 Introduction

The following sections include the general methodology for drug groups and drug compounds. At various times, a drug chemist will encounter drug substances for analysis that do not fall under the following categories or that require specialized analysis. For these cases, a general guideline for analysis can be followed based on the general unknown/powder flow chart. It should be noted that sample size or other circumstances may require a rearrangement or modification of one or more steps. These modifications must be documented in the case file.

17.2 Techniques

17.2.1 Positive color test reactions are denoted in each drug section.

17.2.2 Recommended extraction solvents and procedures are listed in each drug section. For compounds not individually listed, extraction and solubility information are found in references such as *Clarke's Isolation and Identification of Drugs* and the *Merck Index*. Extraction solvents used in casework will be recorded in the case notes. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

17.2.3 Unless otherwise noted in the following sections, the chemist should consult the MS file for GC/MS method conditions and the GC file for GC method conditions.

17.2.4 All efforts should be made to utilize the automated data acquisition and reduction functions on the mass spectrometers and other instruments.

17.2.5 In the presence of controlled substances, minor or inconsequential GC peaks or TLC spots may, at the examiner's discretion, be ignored (e.g., cis- and/or trans-cinnamoylcocaine in the presence of cocaine).

17.2.6 In the presence of an identifiable controlled substance, subsequent identification and reporting of minor constituents (weak samples/common cutting agents) is not required when the initial data generated does not meet the identification criteria for the technique. Note: controlled substances of a higher schedule should be confirmed within reason, however, this is not required if the instrument must be overloaded with the major constituent.

17.3 Reference Collections

17.3.1 Reference collections of data or materials used for identification, comparison or interpretation shall be fully documented, uniquely identified and properly controlled.

17.3.2 Purchased data libraries (reference collections) are fully documented and uniquely identified. No changes may be made to purchased reference collections. Examples of such libraries include:

- GC/MS NIST
- GC/MS Wiley
- FTIR Aldrich
- FTIR Georgia State ATR
- FTIR Thermo/Nicolet White Powders
- FTIR Thermo/Nicolet Chemical Warfare Agents
- FTIR Georgia State Crime Lab
- FTIR Georgia Forensic Sample Library
- FTIR DEA Full 4cm-1 resolution KBr collection
- FTIR DEA Full 4 cm-1 resolution KBr Liquids

17.3.3 Data libraries (reference collections) obtained from reputable forensic sources are fully documented and uniquely identified. No changes may be made to these reference collections. The addition or removal of

forensic libraries must be approved by the Chemistry Program Manager. Current forensic libraries approved for use:

- GC/MS AAFS
- GC/MS ENFSI
- GC/MS TIAFT
- Synthetic Cannabinoid MS Library (syncann)
- SWGDRUG Mass Spectral Library
- FTIR Durascope ATR (NCIS)
- FTIR Mills (Georgia)
- SWGDRUG FTIR Library
- Cayman Chemicals Spectral Library (GC/MS)
- Center for Forensic Science Research and Education (CFSRE) Mass Spectral Library

17.3.4 In-house data libraries include:

- GC/MS VAL
- FTIR DFS ATR
- FTIR NOVA DRUGS ATR
- FTIR VCU General Chemicals
- DART-TOF Drug Standard Library (_ori20, _ori30, _ori60 and _ori90)
- DART-TOF Prep Library (_ori20, _ori30, _ori60 and _ori90)
- GC/IR Library

17.3.5 At a minimum the following information shall be included with each new entry into in-house data libraries:

- Compound name
- Pharmaceutical preparation or drug standard identifier
- Date
- Initials of person entering data

17.3.6 For in-house libraries, each entry is automatically identified by a unique tracking number generated by the instrument software.

17.3.7 In-house libraries shall be generated or modified either by an instrument operator or by a designee of the Section Supervisor.

17.3.8 A reference collection of pharmaceutical preparations and drug standards is maintained for use as reference standards. Receipt and use of these standards is recorded and maintained as required by § 54.1-3404. These standards are uniquely identified through the use of laboratory lot/tracking numbers in addition to the manufacturer's lot number. Access to drug standards and records is limited to section members.

17.4 Structural Similarity Evaluation

Structural similarity with regard to possible controlled substance analogs (as defined in § 54.1-3401) shall be evaluated by completing the structural similarity *Compound Evaluation Worksheet*. The *Compound Evaluation Worksheet* shall be included in any case file for which the structural similarity was evaluated. Compounds determined not to be substantially similar to a Schedule I or II compound shall be reported as "No controlled substances found" or "No controlled substances identified".

17.4.1 A compound is reported as "substantially similar" to a listed controlled substance if the following requirements are met.

17.4.1.1 The chemical structure has no more than 2 minor substituents replaced, added, removed, or extended within the chemical structure.

17.4.1.1.1 Minor substituents are defined as: a carbon, a halogen, an oxygen, a sulfur, a hydroxyl group, a methoxy group, an acetyl group, a cyano group, a nitro group, and alkyl group with no more than two carbon units added or deleted as a side chain to/from a molecule or added or deleted from a side chain of a molecule; including a haloalkyl and thioalkyl side chains with 5 carbons or less.

17.4.2 Structural changes involving substituents beyond those described in ¶ 17.4.1.1.1 may be allowed following unanimous approval by the Technical Resource Team (TRT).

17.5 Guidelines for Quantitative Method Validation and Verification

17.5.1 Method Validation Procedure

17.5.1.1 When a compound or compounds are to be added to an existing method for quantitative purposes or a new quantitative method is to be validated, a written validation plan (MFR) shall be proposed to the Chemistry Program Manager for approval after review by the TRT (or designees). This plan should address the following areas and appropriate acceptance criteria, if applicable (Note: criteria may be methodology-dependent, exceptions may be approved by the Chemistry Program Manager):

- Accuracy and Precision
- Sensitivity
- Calibration Model
- Ion Suppression/Enhancement
- Recovery
- Carryover
- Interferences (endogenous, internal standard, commonly encountered analytes)
- Dilution Integrity
- Stability
- Robustness

17.5.1.2 Upon completion of the validation plan, a validation summary document shall be written to summarize data from the validation plan. The validation summary shall be proposed to the Chemistry Program Manager, TRT, and the Research Section Supervisor for review.

17.5.1.3 A binder of the finalized validation project shall be created to contain the method development documents, approved validation plan, approved validation summary, draft SOP, worksheets, and any other relevant documentation. The binder will then be sent to the Chemistry Program Manager.

17.5.2 Quantitative Method Verification

17.5.2.1 Upon final approval of quantitative method validations, method verification is required in each laboratory before the method can be used. Consult the Chemistry Program Manager for a verification plan.

18 STIMULANT METHODOLOGY

18.1 Brief Pharmacology

Central nervous system stimulants and appetite suppressants, which are commonly referred to as "uppers".

18.2 Drug Group Examples

Amphetamine, methamphetamine, phentermine, phendimetrazine, phenmetrazine, methcathinone and methylphenidate.

18.3 Types of Samples

18.3.1 Many stimulants are found in pharmaceutical preparations.

18.3.2 Methamphetamine, in particular, is often clandestinely manufactured.

18.4 Scheduling

- Schedule I - methcathinone
- Schedule II - amphetamine, methamphetamine, phenmetrazine and methylphenidate
- Schedule III - phendimetrazine
- Schedule IV - phentermine
- Non-scheduled, but listed in Code of Virginia § 18.2-248(K) as “methamphetamine precursor drugs” – ephedrine, pseudoephedrine and phenylpropanolamine

18.5 Extraction

18.5.1 May be extracted from basic aqueous solutions with organic solvents. This is routinely necessary to obtain good chromatographic results with the phenethylamine-type compounds. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

18.5.2 May be dry extracted with methanol or other organic solvents.

18.6 Color Tests Results

18.6.1 Marquis Results

- Most phenethylamines - Orange → Brown
- Phentermine, phenmetrazine and phendimetrazine - do not give an orange color
- Add water to the well after noting color results and place under longwave UV. Methamphetamine fluoresces blue while MDMA will not.

18.6.2 Nitroprusside (Fiegel's Test) Results

- Secondary amines - dark blue

18.6.3 TBPEE Results

- Primary amines - purple
- Secondary amines - blue
- Tertiary amines - red

18.7 TLC

18.7.1 Extraction of the sample may be necessary to get good TLC results.

18.7.2 Baths:

- TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.
- TLC9 separates methamphetamine from MDMA.
- TLC13 separates ephedrine from pseudoephedrine.

18.7.3 Detection sprays, including but not limited to,:

18.7.3.1 Fluorescamine (Fluram) for primary amines.

18.7.3.2 Iodoplatinate for secondary and tertiary amines. Iodoplatinate results may be enhanced by overspraying with ceric sulfate.

18.7.3.3 Dragendorff

18.7.3.4 Ninhydrin is recommended for ephedrine and pseudoephedrine.

18.8 GC

18.8.1 Extraction of the sample may be necessary to obtain good chromatography.

18.8.2 Acetyl Derivative, to improve chromatographic performance, if necessary: The acetyl derivative of phenethylamines is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. The acetyl derivative should have a longer retention time than the underivatized compound and may require a higher chromatographic temperature than the underivatized compound.

18.9 GC/MS

18.9.1 The concentration of the sample must be strong enough to detect the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular in order for the result to be considered definitive (e.g., methamphetamine must have a 148 and 149 *m/z* ion). In the absence of the $[M-H]^+$ ion and/or its associated ^{13}C isotope peak/molecular ion, the DART-TOF may be used to supplement mass spectral data for identification purposes.

18.9.2 Ephedrine and pseudoephedrine cannot be differentiated by their mass spectra. In the absence of the 166 ion, an acetyl derivative or DART-TOF is required for identification.

18.10 FTIR

18.10.1 Extraction from excipients may be necessary to obtain a good spectrum or chromatographic performance.

18.10.2 GC-FTIR is a useful technique to differentiate between phenethylamine-type compounds.

18.11 Amphetamine/Methamphetamine Quantitation

18.11.1 See GC ¶ 11 for general quantitation procedure. The purity of evidence found to contain methamphetamine will not be routinely determined unless it is specifically requested by a Commonwealth's Attorney.

18.11.2 Materials

- Methylene Chloride or Chloroform
- Amphetamine Sulfate
- Methamphetamine HCl
- Tridecane
- 4N NaOH solution

- Class A volumetric flasks
- Calibrated mechanical pipettes
- Analytical balance

18.11.3 Internal Standard Solution

18.11.3.1 Prepare a sufficient volume to dilute the standard solutions and all samples.

18.11.3.2 Prepare a 1 mg/mL solution of tridecane in methylene chloride or chloroform in the appropriate volumetric flask.

18.11.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

18.11.4 Standard Solutions

18.11.4.1 Prepare a 2.5 mg/mL standard solution, which will serve as the calibration standard for the one point calibration.

- Weigh approximately 25 mg of the desired standard
- Dilute to approximately 5 mL with D.I. or R.O. water
- Add five drops of 4N sodium hydroxide
- Use a calibrated mechanical pipette to deliver 10 mL of the internal standard solution. Use a vessel large enough to allow thorough mixing.
- Mix by either vortexing for at least 20 seconds following the formation of the tornado or rocking/inverting for at least 30 seconds.
- Centrifuge if necessary, then remove the organic layer for further analysis
- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the concentration of the standard solution must remain the same as directed

18.11.4.2 Prepare a 1 mg/mL standard solution, which will serve as a check standard. Weigh at least 10 mg of standard and prepare as outlined above.

- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the concentration of the standard solution must remain the same as directed

18.11.4.3 Mathematical Conversion:

Unless amphetamine sulfate or methamphetamine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated.

To convert amphetamine sulfate to free base, multiply the weight amount of amphetamine sulfate by 0.7338 (270.42 F.B.$_2$/368.48 SO_4). Amphetamine sulfate is dibasic. This will give the free base weight of amphetamine in the standard solution.

To convert methamphetamine HCl to free base, multiply the weight amount of methamphetamine HCl by 0.8037 (149.24 F.B./185.70 HCl). This will give the free base weight of methamphetamine in the standard solution.

18.11.5 Sample Solution

Prepare six separate sample solutions for analysis. Refer to Quantitative Sampling (¶ 5.8) for further information.

- Weigh approximately 20 mg of the sample

- Dilute to approximately 5 mL with D.I. or R.O. water
- Add five drops of 4N sodium hydroxide
- Use a calibrated mechanical pipette to deliver 10 mL of the internal standard solution. Use a vessel large enough to allow thorough mixing.
- Mix by either vortexing for at least 20 seconds following the formation of the tornado or rocking/inverting for at least 30 seconds.
- Centrifuge if necessary, then remove the organic layer for further analysis
- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the sample weight shall be adjusted proportionally

18.11.6 GC parameters

- Column: 15 m HP-1 capillary or 30 m DB-5 column (0.25 mm i.d, 0.25 µm film thickness)
- Oven temperature: approximately 70 - 210ºC
- FID temperature : 280ºC

18.11.7 Linear Range

18.11.7.1 The validated linear range of both the amphetamine and methamphetamine method is 0.5 – 5 mg/mL.

18.11.7.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

18.11.8 Amphetamine elutes prior to Methamphetamine, which elutes prior to tridecane.

18.11.9 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 – 11.4.3.11

18.12 Differentiation of the Stereoisomers of Methamphetamine using GC Derivatization (Determination of "ICE")

18.12.1 "Ice" is a crystalline form of nearly pure d -methamphetamine.

18.12.2 Isomer determination is not required for normal analysis, but may be requested by an agency to provide information as to the manufacturing process.

18.12.3 Procedure

18.12.3.1 Samples of methamphetamine should be dissolved in $CHCl_3$ or CH_2Cl_2 for GC analysis. Extraction is not usually necessary.

18.12.3.2 Standards, consisting of d- or l- (optically pure) methamphetamine and the d, l-racemate should be prepared in $CHCl_3$ or CH_2Cl_2 at concentrations of approximately 1-2 mg/mL. It is not necessary to use both optically pure isomer standards.

18.12.3.3 n-Trifluoroacetyl-l-prolylchloride (l-TPC) may be obtained from Regis Chemical Co. (Chicago, Il.) or Sigma/Aldrich. l-TPC is supplied as 0.1M in $CHCl_3$ with 1-2% of the d isomer (d-TPC).

18.12.3.4 GC parameters:

- Injection port: 270ºC
- Detector: 280ºC;

- Oven: 215ºC isothermal
- Split flow: approx. 100:1 (standard split liner)
- Columns:
- HP-1 (Methyl silicone) 0.25 mm x 15 m x 0.25 µm (i.d. x length x film thickness)
- HP-5 (5% Phenyl methyl silicone) 0.25 mm x 15 m x 0.25 µm
- Carrier gas: helium

18.12.3.5 Both the optically pure and the racemate standards need to be injected. The racemate will check the resolution of the chromatographic system and the optically pure standard will determine the peak of interest. Baseline resolution should occur with the racemate/ l-TPC derivatives.

18.12.3.6 Load a 10 µL syringe with 1.0 µL l-TPC, 0.5 µL air and 1.0 µL methamphetamine solution (sample or std). Inject directly into the GC.

18.12.3.7 On both columns, the l-methamphetamine / l-TPC derivative elutes first.

18.12.3.8 Several additional peaks may be seen in the chromatogram. One such peak, occurring at a retention time approximately one minute prior to the l-methamphetamine / l-TPC peaks, is due to excess l-TPC. As methamphetamine concentration increases, this peak will decrease in height. Other peaks, very close to the solvent front, appear to be due to decomposition of the l-TPC reagent.

18.12.4 Methamphetamine isomers are not to be routinely reported on the certificate of analysis.

18.12.5 References

18.12.5.1 Fitzgerald, R.L., et al., “Resolution of Methamphetamine Stereoisomers in Urine Drug Testing: Urinary Excretion of R(-)-Methamphetamine Following use of Nasal Inhalers”, *J. Anal. Tox*., Vol 12, Sept/Oct 1988, pp. 255-259.

18.12.5.2 Fitzgerald, R.L., et al., “Determination of 3,4-Methylenedioxyamphetamine and 3,4-Methylenedioxymethamphetamine Enantiomers in Whole Blood”, *J. Chromatogr*., 490 (1989), pp. 59-69.

19 COCAINE AND LOCAL ANESTHETIC METHODOLOGY

19.1 Brief Pharmacology

Depresses sensation of pain, may cause CNS stimulation producing excitement and erratic behavior.

19.2 Drug Group Examples

Cocaine, procaine, benzocaine, tetracaine, lidocaine, as well as the isomers of cocaine such as pseudococaine, allococaine, pseudoallococaine.

19.3 Scheduling

- Schedule II – cocaine
- Schedule VI or non-controlled, depending on their packaging – procaine, lidocaine, benzocaine and tetracaine

19.4 Extraction

19.4.1 May be extracted from basic aqueous solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

19.4.2 May be dry extracted with methanol or other organic solvents.

19.5 Color Test Results

19.5.1 $Co(SCN)_2$ Results

- Cocaine HCl, lidocaine, procaine, tetracaine, benzocaine – blue precipitate
- PCP, heroin and other compounds, including flour – weak blue

19.5.2 Acid Modification to $Co(SCN)_2$ Results

This test may also aid in distinguishing cocaine base.

- Cocaine base + $Co(SCN)_2$ – no reaction
- Upon the addition of the HCl, a blue precipitate readily forms and remains.

19.5.3 $SnCl_2$ Modification to $Co(SCN)_2$ Results

19.5.3.1 This test can help to distinguish between some “caines”.

- Cocaine salt + $Co(SCN)_2$ – blue precipitate forms
- If $SnCl_2$ is added to the spot well, the blue color remains in the presence of cocaine salt, but the blue color will fade with some other “caines”.

19.5.3.2 This test may also aid in distinguishing cocaine base.

- Cocaine base + $Co(SCN)_2$ – no reaction
- Upon the addition of the $SnCl_2$ reagent that contains HCl, a blue precipitate readily forms and remains.

19.5.4 Scott’s Modification of Ruybal’s test for Cocaine Results

19.5.4.1 May get false positive with lidocaine and diethylpropion.

19.5.4.2 Sample is placed in $Co(SCN)_2$ solution to give blue precipitate. Concentrated HC1 is added (1 drop) to make the precipitate disappear and give a pink solution. $CHCl_3$ is added, and the mixture is shaken. The $CHCl_3$ layer turns blue in the presence of cocaine.

19.5.5 Bate's Modification to $Co(SCN)_2$ Results

This test may aid in distinguishing cocaine base from its salts.

- Cocaine base + $Co(SCN)_2$ – No Reaction
- Upon the addition of Marquis reagent, a blue precipitate readily forms and remains.

19.6 TLC

19.6.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

- PCP migrates similar to cocaine if the baths are not fresh or if samples are very concentrated.
- Cocaine and tetracaine separate if baths are fresh.
- Lidocaine migrates close to cocaine in TLC2 (18:1) but not in TLC1 (9:1).

19.6.2 Detection sprays, including but not limited to,:

19.6.2.1 Iodoplatinate, results may be enhanced by overspraying with ceric sulfate.

19.6.2.2 Ehrlich's: Procaine and benzocaine are yellow if the plate is oversprayed.

19.7 FTIR

19.7.1 FTIR is the most easily performed and definitive method for distinguishing cocaine base from its salts.

19.7.2 Base determinations will be routinely performed in the following types of cases:

- The weight of cocaine is near (inclusive of UoM) or between 250 and 500 grams or greater than 2.5 kilograms
- The officer has requested cocaine base analysis for possible federal prosecution
- Task Force or Interdiction cases, when required
- Cases from certain jurisdictions involving firearms

19.7.3 Sample preparation

- KBr pellet
- ATR

19.7.4 Dry extraction with high purity n-pentane or n-hexane will distinguish cocaine base from its salts.

19.7.5 Further extractions based on solubility differences between the cocaine and excipients may be required.

19.7.6 Reporting

19.7.6.1 Materials containing cocaine base (including mixtures of cocaine base and cocaine hydrochloride) will be reported as "Cocaine base."

19.7.6.2 Materials containing cocaine hydrochloride (unless mixed with cocaine base) will be reported as "Cocaine Hydrochloride."

19.8 Cocaine Quantitation

19.8.1 See GC ¶ 11 for general quantitation procedure.

19.8.2 Materials

- Methylene Chloride or Chloroform
- Dicyclohexylphthalate (DCHP)
- Cocaine HCl
- Class A volumetric flasks
- Analytical balance
- Calibrated volumetric flasks
- Calibrated mechanical pipette (optional)

19.8.3 Internal Standard Solution

19.8.3.1 Prepare a sufficient volume to dilute the cocaine standard solutions and all samples.

19.8.3.2 Prepare a 1.5 - 2 mg/mL solution of DCHP in methylene chloride or chloroform in the appropriate volumetric flask.

19.8.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

19.8.4 Cocaine Standard Solutions

19.8.4.1 Weigh approximately 10 mg of cocaine HCl and quantitatively transfer to a calibrated 10 mL volumetric flask. Fill to mark with internal standard solution. Alternatively, weigh approximately 10 mg of cocaine HCl and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette. This results in a solution of approximately 1 mg/mL cocaine HCl in internal standard solution, which will serve as a check standard.

19.8.4.2 Prepare a second 2.5 mg/mL standard solution as outlined above, which will serve as the calibration standard for the one point calibration.

19.8.5 Standard Salt Form Conversion

Unless cocaine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of cocaine HCl to free base, multiply the concentration (mg/mL) of cocaine HCl by 0.8929 (303.4 F.B./339.8 HCl). This will give the concentration of free base in the standard solution.

19.8.6 Sample Preparation

Prepare six separate sample solutions. Refer to Quantitative Sampling (¶ 5.8) for further information. For each replicate, weigh approximately 20 mg of sample into a 10 mL volumetric flask. Dilute with internal standard to the mark. Alternatively, weigh approximately 20 mg of sample and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette.

19.8.7 GC parameters

- Column: 15 m HP-1 (0.25 mm i.d, 0.25 µm film thickness)
- Oven temperature: approximately 220 – 245ºC
- FID temperature: 280ºC

19.8.8 Linear Range

19.8.8.1 The validated linear range of the cocaine method is 0.5 – 5 mg/mL.

19.8.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

19.8.9 Cocaine elutes prior to DCHP.

19.8.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 - 11.4.3.11

20 BARBITURATE METHODOLOGY

20.1 Brief Pharmacology

Central nervous system depressants commonly known as "Downers"

20.2 Drug Group Examples

Butalbital, pentobarbital, secobarbital, allobarbital, amobarbital, butabarbital, barbital, and phenobarbital

20.3 Types of Samples

Most barbiturates are found in pharmaceutical preparations.

20.4 Scheduling

- Schedule II — Amobarbital, secobarbital, and pentobarbital
- Schedule III — Most barbiturates
- Schedule IV — Phenobarbital
- Schedule VI or non-controlled — Some preparations of phenobarbital, butalbital, and other such barbiturates are specifically exempted from control.
- Appropriate caution must be exercised when determining their control status. Any questions should be answered by consulting appropriate compendia references such as the PDR, Poison Control, DEA Logo Index and the appropriate state or federal codes, as well as, informing the Section Supervisor. If any question remains, DO NOT include the schedule in your report.
- Code of Virginia - § 54.1-3445 – 54.1-3455 – Searchable using the online Legislative Information System (https://law.lis.virginia.gov/vacode/)
- Federal Controlled Substances Schedules - www.deadiversion.usdoj.gov/schedules/index.html#list

20.5 Extraction

Barbiturates may be extracted from either acidic or weak basic aqueous solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

20.6 Color Tests Results

20.6.1 Dille-Koppanyi - This is a two part test. Place 2 drops of DK1 reagent in a well. Add sample. Add 1 drop of DK2 reagent. When doing multiple samples, they should be separated to avoid cross-contamination due to reagent spreading. Barbiturates give a purple color. False positives from: glutethimide, theophylline and hydantoins.

20.6.2 $Co(SCN)_2$ - faint blue on barbiturates with an unsaturated side chain (i.e., butalbital).

20.6.3 Parri - blue

20.7 TLC

20.7.1 Baths: The isopropyl ether bath (TLC7) will separate most of the barbiturates from one another.

20.7.2 Detection sprays, including but not limited to,:

20.7.2.1 $KMnO_4$ reacts with barbiturates with an unsaturated side chain to yield a yellow spot on a purple background.

20.7.2.2 $HgSO_4$ - spray very heavily to give light spots on an off-white background.

20.7.2.3 Diphenylcarbazone - overspray for $HgSO_4$ gives pink spots for barbiturates.

20.8 GC

20.8.1 Extraction or derivatization of the sample may be necessary to get good chromatographic peak shape.

20.8.2 Alkyl Derivative: trimethylanilinium hydroxide (TMAH)

20.8.2.1 See GC section 10 for procedure.

20.8.2.2 Formation of the methyl derivative will generally decrease the retention time significantly.

20.9 GC/MS

Barbiturates most often do not exhibit a molecular ion peak and require derivatization.

20.10 FTIR

Extraction may be necessary to obtain a useful FTIR spectrum.

21 NARCOTIC METHODOLOGY

21.1 Brief Pharmacology

Analgesic, sedative effects and causes constipation.

21.2 Drug Group Examples

Morphine, heroin, hydromorphone, pentazocine, codeine, hydrocodone, oxycodone, methadone, propoxyphene, pethidine (meperidine), and fentanyl

21.3 Scheduling

- Schedule I – heroin
- Schedule II – morphine, oxycodone, hydromorphone, methadone, pethidine (meperidine), fentanyl, codeine (pure), hydrocodone, levomethorphan, 6-monoacetylmorphine (morphine derivative)
- Schedule III – Some preparations of codeine
- Schedule IV – pentazocine, tramadol
- Schedule V – Some preparations of codeine (usually cough preparations)
- Non-controlled – dextromethorphan
- Appropriate caution must be exercised when determining the control status of compounds listed in multiple schedules. Any questions should be answered by consulting appropriate compendia references such as the PDR, Poison Control, DEA Logo Index and the appropriate state or federal codes. Marked capsules or tablets need not be quantitated. Questionable samples require at least a semi-quantitative workup to determine the schedule.

21.4 Extraction

21.4.1 Most narcotics may be extracted from basic solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

21.4.2 Morphine may be extracted from aqueous solution by the addition of a carbonate/bicarbonate buffer and extracting with $CHCl_3$ or $CHCl_3$/isopropanol (8:2). Morphine sulfate is not soluble in chloroform.

21.4.3 Methadone is often found in orange juice or Tang-type orange drink. The solution should be made basic with sodium carbonate and extracted into $CHCl_3$ or CH_2Cl_2.

21.4.4 Narcotics in cough syrups may be extracted from basic solutions with organic solvents.

21.5 Color Test Results

Most narcotics give colors with Marquis, Meckes and Froehdes reagents (see Table 2). Numerous other materials give similar colors, such as methapyrilene, glycerol guaiacolate (guaifenesin), and pyrilamine, and are also included.

TABLE 2: Positive Color Test Reactions

Compound	Marquis	Meckes	Froehdes	HNO_3
Morphine	Purple	Green	Purple	Red
Heroin	Purple	Green	Purple	Yellow
Codeine	Purple	Blue-green	Green	Yellow
Propoxyphene	Black	Orange/brown	Brown	No reaction
Meperidine	Orange	Yellow-green	Grey	
Pentazocine	Red→olive green	Olive green	Blue	Yellow
Hydromorphone	Yellow→red	Yellow-orange	Blue→purple	Yellow-orange
Hydrocodone	Purple	Green	Lt. yellow	No reaction

Compound	Marquis	Meckes	Froehdes	HNO_3
Oxycodone	Yellow→purple	Yellow→olive	Yellow	No reaction
Methadone	Slow pink	Yellow-green→green		
Methapyrilene	Purple	Purple	Purple	No reaction
Guaifenesin	Reddish purple	Green/purple	Green with purple streaks	Yellow
Pyrilamine	Purple	Purple	Purple	

21.6 TLC

21.6.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

21.6.2 Detection sprays, including but not limited to,:

21.6.2.1 Iodoplatinate

21.6.2.2 Ceric Sulfate - Fentanyl may require the use of ceric sulfate as an overspray due to the minute amounts of this material found in most pharmaceutical preparations.

21.7 Dextromethorphan Enantiomer Determination

21.7.1 Because of the scheduling differences between levomethorphan (Schedule II) and dextromethorphan (non-controlled), an enantiomer determination should be performed for differentiation via microcrystal test or derivatization with (-)-menthyl chloroformate. If found in a recognizable pharmaceutical preparation or another controlled substance is present, an enantiomer determination is not necessary.

21.7.2 Microcrystal Test

21.7.2.1 Materials:

- Glass slides and coverslips
- Polarizing microscope
- 10% Platinic Chloride solution in water (w/v)
- 1% acetic acid solution in water
- Dextromethorphan reference standard

21.7.2.2 Procedure

21.7.2.2.1 As a negative control/blank, place one drop of 1% acetic acid in water on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and cover. No crystals should form within 2 minutes.

- If crystals form within 2 minutes, discard slide and cover slip, if used, and repeat.

- If the results of the second blank are unacceptable, the analyst shall take steps to resolve the issue (e.g., remaking reagents) prior to any analysis.

21.7.2.2.2 Place one drop of 1% acetic acid in water to a small portion (less than 1 mg of pure dextromethorphan is needed) of the dextromethorphan standard on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and cover. Feathery dendrites will form within 2 minutes.

21.7.2.2.3 Place one drop of 1% acetic acid in water to a small portion of the sample on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and

cover. Feathery dendrites will form when the sample is a pure enantiomer. A racemic mixture will not form these microcrystals.

21.7.2.2.4 To verify the identity as the dextro isomer, mix a small amount of sample with approximately the same amount of dextromethorphan reference standard on a glass slide. Add the acetic acid and platinum chloride solutions as stated above. Observe any microcrystalline formation. If the sample is dextromethorphan, the feathery dendrites will form; if the sample is levomethorphan or racemethorphan, no dendrite-shaped crystals will form.

21.7.2.2.5 The microcrystal formations should be contemporaneously verified or photographed. Verification must be documented in the case notes with the initials of the verifier and the date.

21.7.2.2.6 The sample may need to be purified to allow for crystal formation. Mixtures of dextromethorphan and MDMA will need to be separated before the crystal test, as pure MDMA reacts with platinum chloride to form similar, but not the same, microcrystals.

21.7.2.2.7 Reference: Fulton, Charles C. *Modern Microcrystal Tests for Drugs*, New York: Wiley-Interscience, 1969, pp. 58-59.

21.7.3 (-)-Menthyl Chloroformate Derivatization

21.7.3.1 Take precautions when handling (-)-menthyl chloroformate to prevent skin/eye contact and the inhalation of fumes.

21.7.3.2 Standard Preparation

21.7.3.2.1 Prepare a 1-2 mg/mL solution of the appropriate standard (dextro or levo methorphan) in an aprotic solvent (e.g., chloroform, methylene chloride, ammonia saturated chloroform, hexane).

21.7.3.2.2 Add approximately 5-7 drops of (-)-menthyl chloroformate to the standard solution in an appropriately ventilated area (e.g., fume hood).

21.7.3.2.3 Heat the solution, in a securely closed vessel, using a heat block set to 110°C for at least 15 minutes.

21.7.3.2.4 Derivatized standard solutions can be stored in the freezer for future use. For each use of the standard solution, the data shall be compared to data present in standard verification files to ensure the standard is performing as expected. If there are differences in the chromatography or there are extra or missing peaks, the solution will be discarded and a new solution prepared.

21.7.3.3 Sample Preparation

21.7.3.3.1 Dissolve the sample in a suitable aprotic solvent (e.g. chloroform, methylene chloride, ammonia saturated chloroform, hexane).

21.7.3.3.2 Add approximately 5-7 drops of (-)-menthyl chloroformate to the sample solution in an appropriately ventilated area (e.g., fume hood).

21.7.3.3.3 Heat the sample solution, in a securely closed vessel, using a heat block set to 110°C for at least 30 minutes.

21.7.3.3.4 A procedure blank shall also be prepared, injected with the sample(s), and documented in examination documentation.

21.7.3.3.5 Instrument Parameters

- Oven: 260°C isothermal
- Flow Rate: 1.8mL/min
- Column: DB5-MS (or equivalent)

21.7.3.3.6 It is not necessary to run standards of both enantiomers.

21.7.3.4 Reference: Koo C, Cox M, Klass G, Johnston M. Stereochemical analysis of methorphan using (-)-menthyl chloroformate. J Forensic Sci. 2012 Nov; 57(6):1549-55.

21.8 Heroin Quantitation

21.8.1 See GC ¶ 11 for general quantitation procedure.

21.8.2 Reagents:

- Methylene Chloride or Chloroform
- Heroin HCl or Heroin HCl monohydrate Standard
- Dicyclohexylphthalate (DCHP)
- Class A volumetric flasks
- Analytical balance
- Calibrated volumetric flasks
- Calibrated mechanical pipette (optional)

21.8.3 Internal Standard Solution:

21.8.3.1 Prepare a sufficient volume to dilute the heroin standard solution and all samples.

21.8.3.2 Prepare a 1.5 - 2 mg/mL solution of DCHP in methylene chloride or chloroform in an appropriate volumetric flask.

21.8.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

21.8.4 Heroin Standard Solutions:

21.8.4.1 Weigh approximately 10 mg of Heroin HCl and quantitatively transfer to a calibrated 10 mL volumetric flask. Fill to mark with internal standard solution. Alternatively, weigh approximately 10 mg of Heroin HCl and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette. This results in a solution of approximately 1 mg/mL Heroin HCl in internal standard solution, which will serve as a check standard.

21.8.4.2 Prepare a second 2.5 mg/mL standard solution as outlined above, which will serve as the calibration standard for the one point calibration.

21.8.5 Standard Salt Form Conversion

Unless heroin hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of heroin hydrochloride monohydrate to free base, multiply the concentration (mg/mL) of heroin HCl by 0.8714 (369.4 F.B./423.9 salt). This will give the concentration of free base in the standard solution.

21.8.6 Sample Preparation

Prepare six separate sample solutions. Refer to Quantitative Sampling (¶ 5.8) for further information. For each replicate, weigh 10-40 mg of sample into a 10 mL volumetric flask. Dilute with internal standard to the mark. Alternatively, weigh 10-40 mg of sample and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette.

21.8.7 GC parameters:

- Column: 15 m HP-1 capillary (0.25 mm i.d., 0.25 µm film thickness)
- Oven temperature: approximately 260-280°C
- FID temperature: 280ºC

21.8.8 Linear Range

21.8.8.1 The validated linear range of the heroin method is 0.5 – 5 mg/mL.

21.8.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

21.8.9 DCHP elutes prior to Heroin. Peaks between DCHP and Heroin are often due to monoacetylmorphine or acetylcodeine.

21.8.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 - 11.4.3.11

22 PHENCYCLIDINE (PCP) AND ANALOG METHODOLOGY

22.1 Brief Pharmacology

PCP is classified as a dissociative anesthetic. PCP is used as an animal tranquilizer. It is sometimes called angel dust, crystal, or hog, and known as boat or loveboat when placed on marijuana.

22.2 Scheduling

- Schedule I TCP thienylcyclohexylpiperidine (thiophene analog of PCP)
- Schedule I PHP 1-(1-phenylcyclohexyl) pyrrolidine (pyrrolidine analog of PCP)
- Schedule I PPP 1-(-phenylcyclopentyl) piperidine
- Schedule I PCE N-(1-phenylcyclohexyl) ethylamine (N-ethyl analog of PCP)
- Schedule II PCP phencyclidine; 1-(1-phenylcyclohexyl) piperidine
- Schedule II PCC 1-piperidinocyclohexane carbonitrile (precursor)

22.3 Extraction

22.3.1 May be extracted from basic or acidic aqueous solution with organic solvents.

22.3.2 May be dry extracted with methanol or other organic solvents.

22.3.3 Plant material samples may be extracted with a suitable solvent (e.g., hexane, methanol) and the extract de -colorized by passing it through a pre-washed Pasteur pipette in which activated charcoal has been placed over a plug of glass wool. The resulting solution may be concentrated and used for further testing. A procedure blank shall be run and documented in the examination documentation.

22.4 Color Test Results

$Co(SCN)_2$ Results – blue

22.5 TLC

22.5.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

22.5.1.1 TLC1 is recommended for separating the PCP analogs.

22.5.2 Detection methods:

22.5.2.1 Does not show well under UV light due to weak quenching.

22.5.2.2 Recommended detection spray - iodoplatinate

22.6 GC

Analogs can be separated by GC at appropriate temperatures. See GC file for conditions.

22.7 FTIR

Basic extract often results in an oil that may be run as a smear between salt plates.

22.8 PCP Quantitation

22.8.1 See GC ¶ 11 for general quantitation procedure.

22.8.2 Materials

- Chloroform
- Docosane
- PCP or PCP HCl (quantitative standard)
- Class A volumetric flasks
- Analytical balance
- Calibrated volumetric flasks
- Calibrated mechanical pipette (optional)

22.8.3 Internal standard solution:

22.8.3.1 Prepare a sufficient volume to dilute the PCP standards and all samples.

22.8.3.2 Prepare a 1 mg/mL solution of docosane in chloroform in an appropriate volumetric flask.

22.8.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

22.8.4 PCP standard solutions

22.8.4.1 Weigh approximately 10 mg of PCP and quantitatively transfer to a calibrated 10 mL volumetric flask. Fill to mark with internal standard solution. Alternatively, weigh approximately 10 mg of PCP and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette. This results in a solution of approximately 1 mg/mL PCP in internal standard solution, which will serve as a check standard.

22.8.4.2 Prepare a second 2.5 mg/mL standard solution as outlined above, which will serve as the calibration standard for the one point calibration.

22.8.5 Standard Salt Form Conversion

Unless phencyclidine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of phencyclidine hydrochloride to free base, multiply the concentration (mg/mL) of PCP HCl by 0.8696 (243.4 F.B./279.9 salt). This will give the concentration of free base in the standard solution.

22.8.6 Sample preparation

Prepare six separate sample solutions. Refer to Quantitative Sampling (¶ 5.8) for further information. For each replicate, weigh approximately 20 mg of sample into a 10 mL volumetric flask. Fill to the mark with internal standard solution. Alternatively, weigh approximately 20 mg of sample and, in a test tube, deliver 10 mL of the internal standard solution using a calibrated mechanical pipette.

22.8.7 GC Parameters

- Column: 15 m HP-1 capillary or equivalent (0.25 mm i.d., 0.25 µm film thickness)
- Oven temperature: approximately 210ºC isothermal
- FID temperature: 280ºC

22.8.8 Linear Range

22.8.8.1 The validated linear range of the PCP method is 0.5 – 5.0 mg/mL.

22.8.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

22.8.9 PCP elutes prior to docosane.

22.8.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 - 11.4.3.11

23 LYSERGIC ACID DIETHYLAMIDE (LSD) METHODOLOGY

23.1 Scheduling

Schedule I – LSD

23.2 Analog

LAMPA (Lysergic acid methyl propyl amide) is a positional isomer of LSD and is included in Schedule I.

23.3 Extraction

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

23.3.2 LSD can be extracted from basic aqueous solution with organic solvents.

23.3.3 It may be necessary to dissolve the samples in a MeOH/$CHCl_3$ mixture to extract LSD out of samples like plastic "window panes."

23.3.4 If samples are in a matrix that is impervious to organic solvents, LSD may be extracted by creating the tartrate salt, followed by base extraction. A procedure blank shall be run and documented in the examination documentation.

23.3.4.1 Procedure (*Analysis of Drugs* - DEA publication):

- Soak sample in 1% aqueous tartaric acid solution.
- Extract acid portion with $CHCl_3$. (Discard $CHCl_3$)
- Make basic with sodium bicarbonate powder.
- Extract into $CHCl_3$ for further analysis.

23.4 Color Test Results

p-DMAB (Ehrlich's or Van Urk's) – purple or blue

23.5 TLC

23.5.1 Baths: The TLC8 system is useful to distinguish LSD from LAMPA.

23.5.2 Detection Methods

23.5.2.1 LSD fluoresces blue under long wave UV light.

23.5.2.2 Recommended detection spray - p-DMAB (Ehrlich's or Van Urk's), it may be necessary to heat the plate to get good visualization (Blue spot on a white background).

23.6 GC

23.6.1 Very small samples may require reduced split ratio or splitless injection techniques.

23.6.2 LSD and LAMPA separate well on GC.

23.7 FTIR

Not usually possible because of the small quantity of LSD present in samples.

24 MESCALINE METHODOLOGY

24.1 Scheduling

Schedule I – Mescaline, Peyote

24.2 Extraction

Dry button and soak in methanol. Filter off plant material prior to analysis.

24.3 Color Test Results

24.3.1 Marquis- orange

24.3.2 Meckes – green → dark brown

24.3.3 Froehdes- green → blue

24.3.4 HNO_3 - bright red

24.4 TLC

24.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

24.4.2 Recommended detection spray – iodoplatinate

25 PSILOCYBIN AND PSILOCYN METHODOLOGY

25.1 Scheduling

25.1.1 Schedule I – psilocybin and psilocyn, which are found in mushrooms.

25.1.2 The chemicals, rather than the botanical, are controlled.

25.2 Extractions

25.2.1 Methanol Extraction: (allows for the analysis of either psilocyn or psilocybin)

Sample may be dried in an oven or microwave. Grind and soak in methanol for a period of 1 – 24 hours. Filter off mushroom material prior to analysis.

25.2.2 Acetic Acid Extraction Technique (recommended for mushrooms in chocolate or other matrices). This extraction will allow for the analysis of psilocyn; however, Weber's color test and/or TLC using a solvent extract must demonstrate the presumptive presence of psilocyn prior to conducting this extraction. A procedure blank shall be run and documented in the examination documentation.

- Sample up to approximately 3 grams of material, as sample allows.
- Sample may subsequently be dried and/or ground to increase extraction efficiency. (Easier to grind when dry.)
- Let soak in 6% acetic acid for approximately 30 minutes - 1 hour.
- Filter off insoluble material.
- Extract acid portion with three aliquots of $CHCl_3$* (Discard $CHCl_3$.)
- Basify acid portion with concentrated NH_4OH to pH 8 - 10.
- Extract basic solution with three aliquots of $CHCl_3$*
- Combine aliquots of $CHCl_3$.
- Evaporate $CHCl_3$ with air (low heat).
- Resultant residue will yield psilocyn.

* Do not mix vigorously as an emulsion will probably form.

25.3 Color Test Results

25.3.1 Ehrlich's- purple (positive for psilocyn and psilocybin).

25.3.2 Weber Test- psilocyn - Fast Blue B or Fast Blue BB gives red color; addition of conc. HCl gives blue color.

25.4 TLC

25.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

25.4.2 Detection sprays, including but not limited to,:

25.4.2.1 p-DMAB and HCl - reddish violet area for psilocybin, blue for psilocyn.

25.4.3 Weber Test (Fast Blue B or Fast Blue BB) can also be used as a TLC spray to detect psilocyn.

25.5 GC

25.5.1 GC and GC/MS will give only psilocyn due to the dephosphorylation of the psilocybin caused by the GC injection port temperatures.

25.5.2 After methanol extraction and drying, silylation with BSTFA prior to GC or GC/MS will allow differentiation of psilocybin from psilocyn. If psilocybin is to be confirmed, derivatization is required.

25.6 FTIR

KBr pellet/ATR on extract from acetic acid extraction yields psilocyn.

26 CATHINONE METHODOLOGY

26.1 Scheduling

26.1.1 Schedule I – cathinone, which is found in *Catha edulis* (Khat)

26.1.2 Schedule IV – cathine (also known as: (+)-norpseudoephedrine).

26.1.3 The chemical components, rather than the botanical, are controlled.

26.2 Sample Handling

26.2.1 Suspected freshly harvested Khat should be refrigerated upon receipt into the laboratory and subsequently analyzed as soon as possible.

26.2.2 Dried or freeze-dried samples may be refrigerated, but refrigeration is not required.

26.2.3 Cathinone can enzymatically convert to Cathine.

26.3 Extraction

A procedure blank shall be run with either extraction and documented in the examination documentation.

26.3.1 Suggested Khat extraction technique for freshly harvested plant material

- Cut up the leaves and stems to obtain the sample. The sample size should be based upon the amount submitted, and the need to retain material for reanalysis if requested.
- Homogenize or sonicate the material for approximately 5 minutes in enough 0.1N HCl to cover the sample
- Filter off insoluble material
- Basify the solution with 1N NaOH to pH 11-12
- Extract basic solution with aliquots of $CHCl_3$.
- Evaporate combined $CHCl_3$ aliquots with air
- Reconstitute sample with an appropriate solvent, if necessary
- Analyze residue as soon as possible or refrigerate to avoid degradation.
- Resulting sample should contain Cathinone and Cathine.

26.3.2 Suggested Khat extraction technique for dried plant material

- The sample size should be based upon the amount submitted, and the need to retain material for reanalysis if requested. Dried material need not be cut up.
- Sonicate or soak dried material in enough 0.1N HCl to cover the sample, for approximately 5 minutes
- Filter off insoluble material
- Basify the solution with 1N NaOH to pH 11-12
- Extract basic solution with aliquots of $CHCl_3$.
- Evaporate combined $CHCl_3$ aliquots with air
- Reconstitute sample with an appropriate solvent, if necessary
- Analyze residue as soon as possible or refrigerate to avoid degradation.
- Resulting sample should contain Cathinone and Cathine.

26.4 TLC

26.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

26.4.2 Cathinone and Cathine reference standards shall be run on the plate for comparison.

26.4.3 Detection sprays, including but not limited to,:

26.4.3.1 Ninhydrin/heat gives a red-brown color

26.4.3.2 Fluram

26.5 GC/MS

Consult standard verification files for conditions.

26.5.1 It is recommended that the Cathine standard be run along with the Cathinone standard to illustrate spectral differences.

26.5.2 Derivatization may also be useful in increasing retention time difference between Cathinone and Cathine, as well as providing additional spectral information. Acetylation and Silylation are recommended.

26.5.3 To identify Cathine, it must be differentiated from its non-scheduled stereoisomers, (-)-norpseudoephedrine and Phenylpropanolamine (a racemic mixture of (+)-norephedrine and (-)-norephedrine).

26.5.3.1 Chiral derivatization using l-TPC is required to differentiate Cathine from (-)-norpseudoephedrine (e.g., 215ºC isothermal method on HP-1 and HP-5 columns).

26.5.3.2 Cathine can be differentiated from Phenylpropanolamine using GC retention time data (e.g., 100ºC isothermal method on HP-1 and HP-5 columns).

26.6 References

26.6.1 Morselli *et al.*, "Gas-Chromatography/Mass Spectrometry Determination of the Active Principles of (Catha Edulis) African Vegetable**,"** *Microgram*, Vol. XXV, No. 11, November 1992, pp. 290-294.

26.6.2 Lee, M. M., "The Identification of Cathinone in Khat (Catha edulis): A Time Study," *Journal of Forensic Sciences*, JFSCA, Vol. 40, No. 1, January 1995, pp. 116-121.

26.6.3 Chappell, John S., Lee, Marsha M., "Cathinone preservation in khat evidence via drying," *Forensic Science International,* 195 (2010), pp. 108-120.

26.6.4 LeBelle et al., "Gas chromatographic-mass spectrometric identification of chiral derivatives of the alkaloids of Khat," *Forensic Science International*, 61 (1993), pp. 53-64.

26.6.5 Lehmann et al., "Rapid TLC Identification Test for Khat (*Catha Edulis*)," *Forensic Science International*, 45 (1990), pp. 47-51.

26.6.6 Ripani et al., "GC/MS identification of Catha edulis stimulant-active principles," *Forensic Science International*, 78 (1996), pp. 39-46.

27 MDA/MDMA METHODOLOGY

27.1 Scheduling

- Schedule I - 3,4-methylenedioxyamphetamine (MDA)
- Schedule I - 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy)
- Schedule I - 3,4-methylenedioxy-N-ethylamphetamine (MDEA, Eve)
- Schedule I - 4-bromo-2,5-dimethoxyphenethylamine (2C-B, Nexus)

27.2 Color Tests Results

27.2.1 The sulfuric acid series of color tests generally give intense colors that undergo vivid transitions with MDA and MDMA. These may all appear black with very concentrated samples.

27.2.2 Marquis

- MDA/MDMA - dark violet → black
- Nexus – light green → green

27.2.3 Meckes

- MDA/MDMA - green → dark blue/violet → black
- Nexus - yellow

27.2.4 Froehdes

- MDA/MDMA - brown → dark blue/violet → black
- Nexus – yellow

27.2.5 TBPEE

- MDA – purple
- MDMA – blue
- MDEA - blue
- Nexus – purple

27.3 TLC

27.3.1 Baths:

- TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.
- TLC9 separates methamphetamine from MDMA.

27.3.2 Detection sprays, including but not limited to,:

- Iodoplatinate, results may be enhanced by overspraying with Ceric Sulfate.
- Dragendorff
- Fluram visualizes MDA, Nexus and other primary amines.

27.4 GC

27.4.1 Extraction of the sample may be necessary to get good chromatography.

27.4.2 Acetyl Derivative: The acetyl derivative of MDMA-type compounds is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. The acetyl derivative should

have a longer retention time than the underivatized compound and may require a higher temperature than the underivatized compound.

27.5 FTIR

27.5.1 Extraction from excipients may be necessary to obtain a good spectrum.

27.5.2 GC-FTIR is a useful tool to differentiate MDMA-type compounds.

27.6 MDMA Quantitation

27.6.1 See GC ¶ 11 for general quantitation procedure.

27.6.2 Materials:

- Methylene Chloride or Chloroform
- Octadecane
- 3,4-MDMA HCl
- 4N NaOH
- Calibrated mechanical pipettes
- Class A volumetric flasks
- Analytical balance

27.6.3 Internal Standard Solution:

27.6.3.1 Prepare a sufficient volume to dilute the standard solutions and all samples.

27.6.3.2 Prepare a 1 mg/mL solution of octadecane in methylene chloride or chloroform in the appropriate volumetric flask.

27.6.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

27.6.4 MDMA Standard Solutions:

27.6.4.1 Prepare a 2.5 mg/mL standard solution, which will serve as the calibration standard for the one point calibration.

- Weigh approximately 25 mg of the standard
- Dilute to approximately 5 mL with D.I. or R.O. water
- Add five drops of 4N sodium hydroxide
- Use a calibrated mechanical pipette to deliver 10 mL of the internal standard solution. Use a vessel large enough to allow vigorous vortexing.
- Vortex, centrifuge (if necessary), then remove organic layer for further analysis
- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the concentration of the standard solution must remain the same as directed

27.6.4.2 Prepare a 1 mg/mL standard solution, which will serve as a check standard. Weigh at least 10 mg of standard and prepare as outlined above.

- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the concentration of the standard solution must remain the same as directed

27.6.5 Standard Salt Form Conversion

Unless MDMA HCl is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of MDMA HCl to free base, multiply the concentration (mg/mL) of MDMA HCl by 0.8413 (193.25 F.B./229.71 HCl). This will give the concentration of free base in the standard solution.

27.6.6 Sample Preparation:

Prepare six separate sample solutions for analysis. Refer to Quantitative Sampling (¶ 5.8) for further information.

- Weigh approximately 20 – 40 mg of the sample
- Dilute to approximately 5 mL with D.I. or R.O. water
- Add five drops of 4N sodium hydroxide
- Use a calibrated mechanical pipette to deliver 10 mL of the internal standard solution
- Vortex, centrifuge (if necessary), then remove organic layer for further analysis
- The volume of internal standard solution may be modified from the volume indicated in the method herein, however the sample weight shall be adjusted proportionally

27.6.7 GC parameters:

- Column: 15 m HP-1 capillary (0.25 mm i.d, 0.25 µm film thickness)
- Oven temperature: approximately 170 - 240ºC
- FID temperature: 280ºC

27.6.8 Linear Range

27.6.8.1 The validated linear range of the MDMA method is 0.5 – 5 mg/mL.

27.6.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

27.6.9 Octadecane comes out after MDMA.

27.6.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 11.4.3.8 - 11.4.3.10

28 ANABOLIC STEROID METHODOLOGY

28.1 Brief Pharmacology

Promotes muscle growth (anabolic effect)

28.2 Drug Group Examples

Testosterone (associated esters), stanozolol, boldenone

28.3 Scheduling

Anabolic steroids are listed in Schedule III.

28.4 Extraction

28.4.1 May be dry extracted into methanol or other organic solvents.

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

28.5 Color Test Results

There are no good screening tests for steroids.

28.6 Pharmaceutical Identifiers

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

28.7 TLC

28.7.1 Baths: TLC6 separates many anabolic steroids. TLC1, TLC2, TLC3, TLC4 and TLC5 are also recommended.

28.7.2 Detection methods, including but not limited to,:

- UV
- Sulfuric Acid/Ethanol Reagent for steroids
- Iodoplatinate will visualize Stanozolol
- $KMnO_4$ will visualize steroids with unsaturated bonds

28.8 GC

Some of these materials will require elevated temperatures and have long retention times. Special derivatizing techniques may assist chromatographic performance.

28.9 FTIR

May need additional extraction to eliminate oils.

28.10 GC/MS

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

28.11 References

28.11.1 Chiong *et al*. "The Analysis and Identification of Steroids", *Journal of Forensic Sciences*, March 1992.

28.11.2 Walters *et al.* "Analysis of Illegally Distributed Anabolic Steroid Products", *JAOAC*, Vol. 73, No. 6, 1990.

29 GHB METHODOLOGY

29.1 Brief Pharmacology

Central nervous system depressant

29.2 Drug Group Examples

29.2.1 Gamma hydroxybutyric acid (GHB), gamma-butyrolactone (GBL) and 1,4-butanediol (BD)

29.2.2 Other names for gamma hydroxybutyric acid include gamma hydroxybutyrate; 4-hydroxybutyrate; 4-hydroxybutanoic acid; sodium oxybate; and sodium oxybutyrate.

29.3 Scheduling

- Schedule I : GHB (not found in an approved drug product)
- Schedule III : Any drug product containing gamma hydroxybutyric acid, including its salts, isomers, and salts of isomers, for which an application is approved under section 505 of the Federal Food, Drug, and Cosmetic Act
- Enhanced penalty: Possession and distribution of gamma-butyrolactone and 1,4-butanediol when intended for human consumption is a Class 3 felony.

29.4 Chemical Properties

29.4.1 GHB: Pure GHB is a white powder. It is encountered dissolved into various liquids.

29.4.2 GBL: Pure GBL is a clear liquid. It is encountered dissolved into various liquids.

29.4.3 BD: Pure BD is a viscous clear liquid.

29.5 pH

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

29.6 Color Test Results

29.6.1 Ferric Chloride – GHB will turn red-brown (Results can vary depending on sample pH and liquid matrix. Therefore, further screening is necessary.)

29.6.2 GHB Color Test #3 (Smith Test) – GHB – immediate green

29.7 TLC

29.7.1 Bath: TLC3 and TLC10 (Ethyl Acetate) are recommended.

29.7.2 Detection method:

29.7.2.1 Iodine Vapors (in TLC10), results:

- GHB off-white spot at origin
- GBL brown spot near solvent front
- BD off-white to brown bearding spot midway up plate

29.8 GC

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

29.8.1.1 If the solution contains a mixture of GBL and GHB, perform multiple chloroform rinses of the solution to remove the GBL prior to derivatization. GHB is not soluble in chloroform.

- This chloroform extract containing GBL can be used for GC retention time comparison with a GBL standard.

29.8.1.2 Monitor the chloroform extracts by GC/MS to see when the majority of the GBL has been removed. After performing the extracts, dry down your sample under an air stream and/or in a vacuum oven without heat. Then, derivatize your sample with BSTFA with 1% TMCS.

29.8.2 BD does not chromatograph well and may breakdown (lose water) in the heated injection port.

29.9 GC/MS

29.9.1 GHB and BD - Derivatize dry sample with BSTFA with 1% TMCS. See MS file for conditions.

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

29.10 FTIR

29.10.1 GHB: Direct KBr pellet or ATR on powder sample for GHB.

29.10.2 BD: Light liquid smear on prepared KBr pellet or ATR.

29.10.3 GBL: Light liquid smear on prepared KBr pellet or ATR. If results are unfavorable, perform a chloroform extract of liquid. Use this extract to either prepare a liquid smear on a prepared KBr pellet or dry it on KBr prior to making a pellet. Alternatively, the ATR may be used.

29.10.4 GHB/GBL Mixtures:

For liquid mixture samples of GHB and GBL, it may be necessary to separate the GHB from the matrix and/or accompanying GBL using preparatory TLC. Streak the origin of a TLC plate with the liquid sample, run in TLC10, vacuum/scrape off silica gel at origin, and elute GHB from silica gel with multiple rinses of the same methanol extract. Dry down the methanol extract and prepare a KBr pellet of the resultant powder.

29.11 References

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

29.11.2 Bommarito, C. “Analytical Profile of Gamma-Hydroxybutyric Acid (GHB*)” Journal of the Clandestine Laboratory Investigating Chemists Association*, Vol. 3, No. 3, 1993.

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

30 SALVINORIN A METHODOLOGY

30.1 Scheduling

30.1.1 Schedule I – Salvinorin A, which is found in *Salvia divinorum*

30.1.2 The chemical, rather than the botanical, is controlled.

30.2 Extractions

30.2.1 Dry sample (in drying oven or microwave) if not already in a dried form.

30.2.2 Soak approximately 50-100 mg of plant material in CH_2Cl_2 for at least 30 minutes. CH_2Cl_2 is the recommended solvent as it is the most efficient extraction solvent of Salvinorin A and the least efficient extraction solvent of other plant component interferences. Chloroform and methanol may also be used. Hexane does not effectively extract Salvinorin A and basic extractions may hydrolyze the ester groups on other salvinorins present and should be avoided. A procedure blank shall be run for multi-step extractions and documented in the examination documentation.

30.2.3 For smaller samples or suspected weak samples, sonicating the plant material in the solvent may help increase the efficiency of the extraction.

30.2.4 Filter off plant material prior to analysis.

30.2.5 Concentrate the extraction solvent into an autosampler vial for analysis.

30.2.6 Residues

30.2.6.1 Rinse suspected residues with CH_2Cl_2 and concentrate into an autosampler vial insert.

30.2.6.2 A reduced split of 20:1 on GC and GC/MS may be necessary to concentrate the sample sufficiently for identification. Vials should be returned with the evidence as outlined in ¶ 5.7.2.

30.3 TLC

30.3.1 Baths: TLC14 (see ¶ 10.4) separates Salvinorin A from Salvinorin B/D and C (see reference 30.5.4). Use TLC1 or TLC2 for the additional bath.

30.3.2 Detection spray, including but not limited to,:

30.3.2.1 Vanillin spray (see ¶ 10.5.16)

- For plate developed in the basic TLC2 bath, spray plate with 6N HCl prior to Vanillin.
- Generously spray Vanillin on developed and dried plate
- Heat with heat gun or in oven to 110ºC for several minutes
- Pinkish purple spots will develop for salvinorins

30.4 GC and GC/MS

30.4.1 GC and GC/MS

- Split 50:1 or less
- HP-5 or HP-1 or equivalent
- Approximate temperature range - 240-300 degrees C at 30/min
- MS scan range - 500-14 Da.

30.4.2 Salvinorin A is the most abundant salvinorin in *Salvia divinorum*. The other salvinorins are much less concentrated and will elute on either side of Salvinorin A.

30.5 References

30.5.1 Giroud C, Felber F, Augsburger M, Horisberger B, Rivier L, Mangin P. "*Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland". Forensic Science International 112(2000) 143-150.

30.5.2 Jermain JD. "Analyzing *Salvia divinorum* and its active ingredient Salvinorin A utilizing thin layer chromatography and gas chromatography/mass spectrometry". Permission from author March 2008, CLIC list.

30.5.3 Medana C, Massolino C, Pazzi M, Baiocchi C. "Determination of salvinorins and divinatorins in *Salvia divinorum* by liquid chromatography/multistage mass spectrometry". Rapid Commun. Mass Spectrum 2006; 20:131-136.

30.5.4 Siebert DJ. "Localization of Salvinorin A and Related Compounds in Glandular Trichomes of the Psychoactive Sage, *Salvia divinorum*". *Annuals of Botany* 2004;93:763-771.

30.5.5 Wolowich WR, Perkins AM, and Cienki JD. "Analysis of the Psychoactive Terpenoid Salvinorin A content in five *Salvia divinorum* herbal products". *Pharmacotherapy* 2006;26(9): 1268-1272.

31 CANNABIMIMETIC AGENT METHODOLOGY

31.1 Scheduling

31.1.1 Code of Virginia, § 54.1-3446(6) defines synthetic cannabinoids as “cannabimimetic agents” that includes compounds that are either listed specifically or fall within one of the defined structural classes.

31.1.1.1 Structural classes

- 2-(3-hydroxycyclohexyl)phenol with substitution at the 5-position of the phenolic ring by alkyl or alkenyl, whether or not substituted on the cyclohexyl ring to any extent
- 3-(1-naphthoyl)indole or 1H-indol-3-yl-(1-napthyl)methane with substitution at the nitrogen atom of the indole ring, whether or not further substituted on the indole ring to any extent, whether or not substituted on the naphthoyl or naphthyl ring to any extent
- 3-(1-naphthoyl)pyrrole with substitution at the nitrogen atom of the pyrrole ring, whether or not further substituted in the pyrrole ring to any extent, whether or not substituted on the naphthoyl ring to any extent
- 1-(1-naphthylmethyl)indene with substitution of the 3-position of the indene ring, whether or not further substituted in the indene ring to any extent, whether or not substituted on the naphthyl ring to any extent
- 3-phenylacetylindole or 3-benzoylindole with substitution at the nitrogen atom of the indole ring, whether or not further substituted in the indole ring to any extent, whether or not substituted on the phenyl ring to any extent
- 3-cyclopropoylindole with substitution at the nitrogen atom of the indole ring, whether or not further substituted on the indole ring to any extent, whether or not substituted on the cyclopropyl ring to any extent
- 3-adamantoylindole with substitution at the nitrogen atom of the indole ring, whether or not further substituted on the indole ring to any extent, whether or not substituted on the adamantyl ring to any extent
- N-(adamantyl)-indole-3-carboxamide with substitution at the nitrogen atom of the indole ring, whether or not further substituted on the indole ring to any extent, whether or not substituted on the adamantyl ring to any extent
- N-(adamantyl)-indazole-3-carboxamide with substitution at a nitrogen atom of the indazole ring, whether or not further substituted on the indazole ring to any extent, whether or not substituted on the adamantyl ring to any extent.

31.1.2 The compounds themselves, rather than the herbal blends in which they are commonly found, are controlled.

31.1.3 Under § 54.1-3456, analogs of the listed cannabimimetic agents are also subject to the same criminal penalties.

31.2 Extractions

Extract sample into a suitable solvent (e.g., methanol, $CHCl_3$ or hexane).

31.3 TLC

31.3.1 Baths: TLC1, TLC2, or TLC5 are recommended.

31.3.2 Detection sprays, including but not limited to,:

- $KMnO_4$ (Ceric Sulfate or 6N HCl may be used as an overspray)
- Fast Blue B or BB overspray with 6N HCl

31.3.3 Due to the limitations of TLC in distinguishing isomers, two system GC must be run to determine if more than one isomer is present in the sample.

31.4 GC and GC/MS

31.4.1 Two system GC must be utilized.

31.4.2 GC and GC/MS

- Columns: HP-5, HP-35 ((35% phenyl)-methylpolysiloxane) and HP-1 or equivalent
- Approximate temperature range 225-300 degrees C at 30/min, although broader temperature ranges may be indicated
- MS scan range, 500-14 Da or 600-14 Da

31.5 References

31.5.1 Rainer Lindigkeit, Anja Boehme, Ina Eiserloh, Maike Luebbecke, Marion Wiggermann, Ludger Ernst, Till Beuerle, "Spice: A Never Ending Story?", *Forensic Science International,* 191 (2009), pp. 58-63.

31.5.2 *Journal of Mass Spectrometry* (2009), JMS Letter via www.interscience.wiley.com/journal/jms.

31.5.3 National Forensic Laboratory Information System (NFLIS), Drug Enforcement Administration (DEA), *Year 2008 Annual Report*, DEA Update "Spice – Request for Information", pg. 5.

31.5.4 "Identification of Synthetic Cannabinoids in Herbal Incense Blends by GC/MS" Application Compendium, Agilent Technologies, 5990-7967EN, 2011.

31.5.5 Nahoko Uchiyama, Maiko Kawamura, Ruri Kikura-Hanajiri, Yukihiro Goda, "Identification and quantitation of two cannabimimetic phenylacetylindoles JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products", *Forensic Toxicology*, DOI 10.1007/s11419-010-0100-3 (Nov 2010).

31.5.6 Malinda Combs and Jeremiah A. Morris, "Analytical Profile of Two Synthetic Cannabinoids – JWH–018 and JWH–073", *Journal of the Clandestine Laboratory Investigating Chemists Association*, Vol. 20(2) (April 2010), pp. 2-7.

31.5.7 N. Uchiyama, et al., "Chemical analysis of synthetic cannabinoids as designer drugs in herbal products", Forensic Sci. Int. (2010), doi:10.1016/j.forsciint.2010.01.004

31.5.8 Journal of Mass Spectrometry Letter "'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs?" (www.interscience.com) DOI 10.1002/jms.1558, (2009).

31.5.9 Code of Virginia, § 54.1-3446(6).

32 CLANDESTINE LABORATORIES

32.1 Introduction

32.1.1 Analysis of samples collected from clandestine labs may involve the use of both routine and non-routine analytical techniques. Analysis and subsequent identification of inorganic compounds, including acids and bases, may require the transfer of certain items to Trace Evidence. For examiners qualified in both the Controlled Substances and Trace Evidence General Chemical disciplines, the results from both should be reported on one Certificate of Analysis.

32.1.2 Due to the nature of clandestine laboratories, it is not uncommon for a relatively large number of items to be submitted. In order to determine the best analytical approach, it may be necessary to confer with a supervisor, the investigating officer, and the prosecuting attorney to assess the probative value of each piece of submitted evidence. Refer to the DFS "Evidence Handling and Laboratory Capabilities Guide" under the "Controlled Substances – Clandestine Laboratories" section for guidelines on evidence collection, evidence packaging, and evidence submission.

32.1.3 Occasionally, clandestine labs may be processed by a federal agency or in conjunction with a federal agency. Evidence collected by federal agencies for clandestine lab cases that will be prosecuted in a Federal venue should be submitted to a federal laboratory for analysis.

32.1.4 The evidence submitted for a clandestine laboratory investigation can pose significant health hazards that are not commonly encountered with routine controlled substance examinations. These hazards may include but are not limited to: corrosives, caustic materials, explosives, toxic gases, and flammable solvents. Due caution should be exercised when opening and examining evidence of this nature by utilizing appropriate personal protective equipment and sampling in appropriately ventilated areas (e.g., fume hood). Every effort should be made to prevent exposure of other employees to potentially hazardous materials. Special storage precautions may be necessary.

32.2 Analytical Approach

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

32.2.1.1 When the suspected target product is methamphetamine, methcathinone, or amphetamine, it is important to attempt to identify at least two of the substances listed in § 18.2-248(J). The identification of many of the chemicals on this list will require analysis by the Trace Evidence section.

32.2.2 The evidence that has been determined to have the greatest probative value should be sampled and analyzed first. The Drug Item Reduction Policy should be followed once sufficient analysis has been performed to support the charge on the RFLE.

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

32.3 Procedure

32.3.1 Solid Materials and Powders

32.3.1.1 Solid materials and powders should be sampled and analyzed following the schematic illustrated in ¶ 2.2 of the Procedures Manual. Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

32.3.1.2 If the sample is soluble in water, cation and anion analysis may be performed for screening purposes by using the assays for specific ions found in the U.S. Pharmacopeia.

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

32.3.1.4 Solid materials and powders that are most likely an inorganic essential chemical (e.g., iodine, red phosphorus) should be transferred to the Trace Evidence section for identification.

32.3.2 Liquid Samples

32.3.2.1 Liquids should be sampled and analyzed following the Liquids procedure in ¶ 2 of the Procedures Manual. Analysis of liquids from clandestine laboratory investigations shall include measurement of the sample weight and pH (if aqueous). Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

32.3.2.2 Presumptive identification of suspected ionized species in aqueous solution may be achieved by using the assays for specific cations and anions found in the U.S. Pharmacopeia.

32.3.2.3 The miscibility of liquid samples with both water and a water insoluble solvent (e.g., $CHCl_3$, Hexane) should be determined.

32.3.2.4 For liquids with multiple layers, care should be taken to note the number of layers, the location of each layer relative to the others, the color and clarity of each layer. When liquid samples with multiple layers are encountered, each layer shall be sampled and analyzed.

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

32.3.2.6 Liquid samples that are most likely an essential chemical (e.g., a brown liquid in a labeled "Tincture of Iodine" bottle) should be treated as a Trace Evidence General Chemical examination or transferred to the Trace Evidence section for identification.

32.3.3 A definitive instrumental analysis technique must be employed to identify a drug, precursor or essential chemical. The relevant section of the Procedures Manual for the suspected target drug may help to clarify potential analytical issues that arise during the course of the clandestine laboratory analysis. Significant deviations from routine analytical procedures must be documented in accordance with QM ¶ 5.3.10. Non-routine analytical procedures shall be clearly documented in the examination documentation along with proper approval from the Section Supervisor and the Chemistry Program Manager, if appropriate.

32.4 References

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

32.4.2 U.S. Pharmacopeia National Formulary

33 ESTIMATION OF THE UNCERTAINTY OF MEASUREMENT (UoM)

33.1 Scope

33.1.1 An estimation of the Uncertainty of Measurement of weight measurements shall be calculated for sample weights that are reported on the Certificate of Analysis with the exception of gross weights.

33.1.2 An estimation of UoM shall be determined for quantitative results that are reported on the Certificate of Analysis. Current analyses that fall into this category involve methamphetamine, amphetamine, phencyclidine (PCP), cocaine, heroin, MDMA, some cannabinoids in cannabis oil, and delta-9-Tetrahydrocannabinol in hashish oil or Cannabis.

33.1.3 The expanded uncertainty shall be reported to a 95.45% level of confidence.

33.1.4 Surrogate weights shall be weighed and recorded weekly on all balances as an ongoing component of measurement assurance. Occasional lapses in weekly measurements for personal balances (e.g., due to absences from the laboratory) are anticipated and do not require compensatory measurements. These measurements shall be evaluated annually and included in the uncertainty of measurement program.

33.2 Uncertainty Elements

33.2.1 Uncertainty Budget

33.2.1.1 Estimations of the uncertainty of measurement shall be conducted and documented using an uncertainty budget.

33.2.1.2 The uncertainty budget for a given procedure shall include both Type A standard uncertainty and Type B standard uncertainties.

33.2.1.3 Calculations used to estimate the uncertainty and the final combined uncertainty shall be rounded using conventional rounding rules (see Quality Manual ¶ 5.4.6.3).

33.2.1.4 In order to combine the uncertainty, the units of uncertainty values should be measured in the same units.

33.2.1.5 Uncertainty budgets shall be re-evaluated on an annual basis.

33.2.2 Type A Standard Uncertainty

33.2.2.1 Type A standard uncertainty results from measurement values being scattered in a random fashion due to laws of chance, thus has a normal or Gaussian shaped distribution.

33.2.2.2 Type A standard uncertainty is best determined by historical data of a large number of repeated measurements.

33.2.3 Type B Standard Uncertainties

33.2.3.1 Type B standard uncertainty results from the inherent biases in measuring systems and quantitative analytical methods. These uncertainties may be reduced by optimizing the method or measuring system, but can never be completely eliminated.

33.2.3.2 Type B standard uncertainties resulting from measurement bias typically have an equal chance of falling within a range and therefore follow a rectangular or random distribution.

- With rectangular distribution, the range (± a) of the outer limits is used to estimate the standard deviation (σ) using the equation $\sigma = a/\sqrt{3}$.

- For example, a 10 mL volumetric flask has a tolerance of ± 0.2 mL. The calculated uncertainty associated with this measurement is 0.2/√3 or 0.115. To maintain only 2 significant figures, the uncertainty for this measurement used in the uncertainty budget is 0.12 (after rounding).

33.2.4 Determination of Combined Uncertainty

Uncertainties are combined using the Root Sum Squares technique

$$U_{combined} = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 ....)}$$

33.2.5 Determination of expanded uncertainty and level of confidence

33.2.5.1 In order to determine the expanded uncertainty, the combined uncertainty is multiplied by the coverage factor (k) using the equation $U_{expanded} = U_{combined}$ x k

33.2.5.2 For routine measurements with a large amount of historical data (n ≥ 100), the coverage factor for 95.45% level of confidence is k=2

33.2.5.3 For analysis with reduced level of confidence due to lack of historical data, a corrected coverage factor (k_{corr}) is used based on the Student's t table.

- For example, for an analysis with no historical control data, a sample is analyzed 6 times (degrees of freedom or df = n-1, or 5 in this example).
- Using the Student's t table, k_{corr} value of 2.65 would be used to calculate the expanded uncertainty at 2σ or 95.45% level of confidence.

33.2.6 See Appendices A and B for records pertaining to each evaluation and estimation of measurement uncertainty.

33.3 Weights

33.3.1 An uncertainty budget shall be completed which will include both random (Type A) uncertainty and systematic (Type B) uncertainty for each balance.

33.3.1.1 Random uncertainty will be the standard deviation (σ) of the weight with the largest standard deviation for each balance type from the surrogate weight data.

33.3.1.2 Systematic uncertainty will include consideration of digital balance resolution, corner loading (shift test) and uncertainty of the calibration check weight. Systematic uncertainties given without a level of confidence should be treated as rectangular distributions, and as such will be divided by the √3 prior to combining. (see Reference ¶ 37.32)

33.3.2 The combined uncertainty for the balance will be calculated using the Root Sum Squares.

33.3.3 Calculate the expanded uncertainty with a level of confidence of 95.45% by using a value of k=2. This final uncertainty value will be used for the calculating the uncertainty of weights in casework.

33.3.4 For weight measurements, the expanded uncertainty shall be reported to the same number of decimal places as the readability of the balance used.

33.3.5 Items with Single Specimens

The calculated expanded uncertainty is the uncertainty for that measurement.

33.3.6 Items with Multiple Specimens

When weights are added to calculate a total net weight, the uncertainties associated with each individual value must be taken into account in the total uncertainty. The estimated expanded uncertainty is multiplied by the number of weighing events to determine the total expanded uncertainty. The Controlled Substances *Weighing Event UoM Calculation Worksheet* shall be used to calculate the total expanded uncertainty.

33.4 Determination of Uncertainty of Measurement for Extrapolation Cases

33.4.1 The Controlled Substances *Net Weight Extrapolation Worksheet* shall be used to calculate the extrapolated net weight and associated extrapolated expanded uncertainty (U_T) of a population based on the net weight measurements of the randomly selected specimens.

33.4.2 Two sources of uncertainty shall be considered for the extrapolated total net weight of a population: the uncertainty associated with the calculated average net weight (U_X) and the unexpanded uncertainty associated with the balance used (U_w).

33.4.2.1 The uncertainty associated with the calculated average net weight (U_X) is calculated by taking the ratio of the standard deviation (*s*) of the measured net weights to the square root of the number of specimens weighed (*n*), using the equation $U_X = s/\sqrt{(n)}$.

33.4.2.2 The uncertainty for each balance (U_w) is equivalent to the $U_{combined}$ (see ¶ 33.2.4) for that balance which has not yet been expanded using the coverage factor (k).

33.4.2.3 These two uncertainty considerations are combined using the Root Sum Squares method to obtain the combined uncertainty per unit (U_C).

33.4.3 The total uncertainty value (U_T) that will be reported on the Certificate of Analysis will be the expanded combined uncertainty per unit multiplied by the total number of specimens in the item.

33.4.3.1 In order to determine the expanded uncertainty, the combined uncertainty per unit (U_C) is multiplied by the appropriate coverage factor (k) using the equation $U_{expanded} = U_{combined} \times k$.

33.4.3.2 For weight extrapolation cases, the expanded uncertainty will be rounded to two significant figures using conventional rounding rules. The extrapolated net weight will be rounded to the same resulting number of decimal places.

33.4.4 In cases where the number of specimens analyzed (*n*) is greater than 10% of the population (*N*), a finite correction factor (Q) is typically used where $Q = \sqrt{[(N-n)/N]}$. However, this factor is always less than 1 and will always reduce the reported uncertainty. For this reason, the correction will not be applied in order to result in a more conservative final estimate of uncertainty.

33.5 Case File Records and Reporting for Weights

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

33.5.2 Expanded uncertainty values shall be derived from the appropriate budget worksheet and be reported to the same number of decimal places as the class/readability of balance used.

33.5.3 The Controlled Substances *Weighing Event UoM Calculation Worksheet* shall be used to calculate the reported total expanded uncertainty when two or more specimen weights are added together and will be stored in the case file. The worksheet shall be included in all case files for which a weighing was performed and UoM is required including single weighing events.

33.5.3.1 Calculations used to estimate measurement uncertainty shall be rounded using conventional rounding rules (see Quality Manual ¶ 5.4.6.3).

33.5.3.2 Whenever possible, the measurement result, rounded expanded uncertainty, and associated ounce conversion, if applicable, shall be reported to the same level of significance.

- Example:

  The contents of five were analyzed separately and each was found to contain Marijuana; total net weight of the five: 12.312 ± 0.055 grams (0.434 ± 0.002 ounce) of plant material. The gross weight of the remainder was 10.314 gram(s) including innermost packaging.

33.5.3.3 For 5-place balances, static weighing is considered two weighing events and therefore the uncertainty of measurement doubles. When static weighing is required for net weight reporting, the number of weighing events shall be adjusted appropriately and entered into the *Weighing Event UoM Calculation Worksheet.*

33.6 Measurement Traceability

Traceability is an essential element of the Department's measurement assurance program and is required for all measurements where uncertainty of measurement is reported. The estimation of measurement uncertainty for common controlled substances analyses is accomplished in the following manner.

33.6.1 Measurand: The weight of evidentiary samples submitted for controlled substances analysis.

33.6.2 Traceability of measurement:

33.6.2.1 Balance Calibration: Traceability is established by the annual, external calibration of balances by an ISO/IEC 17025 accredited calibration provider. Calibration certificates are maintained in the laboratory.

33.6.2.2 Steel Weights: Balances are checked weekly using traceable check weights. Check weights are calibrated every three years by an external, ISO/IEC 17025 accredited calibration provider. Calibration certificates are maintained in the laboratory.

33.6.2.3 The "Auto Cal" function of balances is approved due to the measurement traceability afforded by the intermediate checks with calibrated weights.

33.6.3 Equipment used:

33.6.3.1 Calibrated balances (5-Place Analytical, 4-Place Analytical, 3-Place Top Loading, 2-Place Top Loading, and High Capacity).

33.6.3.2 Calibrated, traceable steel check weights.

33.6.3.3 Bags of sand for measurement assurance.

33.6.3.4 Logbook for calibration checks.

33.6.3.5 Spreadsheet for measurement assurance.

33.7 Quantitations

33.7.1 Uncertainty shall be calculated using the *Drug Quantitation Worksheet, HPLC Cannabinoid Quantitation Worksheet* or *THC Quantitation Worksheet.*

33.7.2 Calculate the expanded uncertainty with a level of confidence of 95.45% by using a value appropriate to the number of measurements.

33.7.3 Purities and associated uncertainties will be rounded, using conventional rounding rules, to one decimal place. Concentrations or % Total THC and associated uncertainties may be reported with more than one decimal place, but the associated expanded uncertainty shall not exceed two significant figures.

33.7.3.1 If rounding the uncertainty results in no reportable uncertainty (i.e., ± 0.0), the number of decimal places will increase such that the uncertainty will be reported to no more than two significant figures. The rounded purity value would be reported to the same number of decimal places as the expanded uncertainty.

33.7.4 Calculations to arrive at the reported quantitative result shall be recorded in the case notes.

33.8 References

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

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

34 REPORTING GUIDELINES

34.1 Item Descriptions

34.1.1 The condition of the outer packaging shall be on the Certificate of Analysis (CoA).

34.1.2 Reported item descriptions shall be consistent with the submitting agency's description unless it requires clarification, correction, is too long, or has been reported previously. Item descriptions shall be identical in all reports within a single case file.

34.1.2.1 When there is a significant difference between the item submitted for testing and the description provided by the customer, customer consultation with further instructions is required before proceeding with analysis. Given the nature of Controlled Substances, there are instances where the item description differs from the evidence submitted.

34.1.2.2 Consultation with the customer is required prior to proceeding with analysis if the following types of differences between the item description and submitted evidence are found:

RFLE Description	Submitted Evidence
Plant material	White oblong pills
12 plastic bags with powder	10 plastic bags with powder
One capsule	Capsule, smoking device, and syringe
Tablets and straw with residue	Tablets

34.1.2.3 Consultation with the customer is not required prior to proceeding with analysis if the following types of differences between the item description and submitted evidence are found:

RFLE Description	Submitted Evidence
Powder	Solid material or substance
Pipe with white material	Pipe with residue
White tablet	Off-white tablet
Plastic bag → tan powder	Plastic bag → two plastic bags → tan powder
Green tablet marked "5 90 3"	Green tablet marked "S 90 3"
Round tablet	Five-sided tablet
One capsule	Capsule, lighter, and tissue
100 plastic bags with powder	105 plastic bags with powder
White material	White powder and plant material residue

34.1.2.4 Differences that require expertise or instrumentation (including, but not limited to, magnification and optimal lighting conditions) to distinguish, do not require consultation with the customer prior to proceeding with analysis.

34.2 Methods

The methods used to perform analyses shall be reported on the CoA as generated by LIMS.

34.2.1 Example: Total weight: 5.123 grams of powder including innermost packaging. The contents of four were analyzed separately and each was found to contain Cocaine (Schedule II). [Methods: WM, CT, TLC, GC-FID-MS]

Methods: Weight Measurement (WM), Color Tests (CT), Thin Layer Chromatography (TLC), Gas Chromatography-Flame Ionization Detection-Mass Spectrometry (GC-FID-MS)

34.2.2 Methods available in LIMS:

Weight Measurement (WM), Pharmaceutical Identifiers (PI), Microscopic Examination (ME), Color Tests (CT), Microcrystal Tests (MT), Thin Layer Chromatography (TLC), Gas Chromatography-Flame Ionization Detection (GC-FID), High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD), Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART-TOF), Gas Chromatography-Flame Ionization Detection-Mass Spectrometry (GC-FID-MS), Gas Chromatography-Mass Spectrometry (GC-MS), Infrared Spectroscopy (IR) and Gas Chromatography-Infrared Spectroscopy (GC-IR)

34.2.3 "Weight Measurement" should be included on the CoA (and in case notes) when a balance was used for a weight measurement, including cases in which the weight is not reported.

34.2.4 "Microscopic Examination" should be included on the CoA (and in case notes) if a stereomicroscope was used during examination to make observations that affect or guide the analytical approach. Examples include when the stereomicroscope is used to:

- observe a capsule given the analytical scheme may differ depending on its contents
- better visualize pharmaceutical identifier because the pill is abraded

34.3 General Reporting Guidelines

34.3.1 Controlled substances are to be reported with wording consistent with the Code of Virginia.

34.3.2 The identity and schedule will be reported whenever clearly known.

34.3.3 The "show form" option will be utilized for most types of evidence including plant material, powders and solid material.

34.3.4 Results shall be specific to the item tested. Additional language should be added to the results to clarify which specimen was tested when more than one specimen is present in the item (e.g., the item consists of two pipes so the results clarify that only one was tested).

34.3.5 Non-pharmaceutical compounds shall be reported using their common names as generated by LIMS. If a compound is being reported that is not currently controlled, report the name as approved by the Chemistry Program Manager. Approved names for substantially similar compounds can be found on the shared drive.

34.3.6 "Not analyzed" will be used for items not examined chemically or visually.

34.3.7 "No controlled substances found" or "No controlled substances identified" will be used to describe items in which no compounds that are controlled substances were found or identified, respectively.

34.3.8 "Insufficient for identification" will be used to describe items with too little sample for a complete identification.

34.3.9 "Preliminary testing was performed with no further analysis" will be used to describe items in which limited analysis was performed (e.g., screening). Methods used shall be included on the CoA.

34.3.10 Weights will be routinely reported for controlled substances and Cannabis.

34.3.10.1 Total weights that have been approximated will be reported as "approximate total weight". Measurement uncertainty will not be reported for approximate total weights. Approximated volumes should not be reported.

34.3.10.2 Net weights will not include any packaging and shall be reported to include the expanded uncertainty at a 95.45% level of confidence. In addition, the expanded uncertainty shall be reported to the same number of decimal places as the readability of the balance used and a

statement of the level of confidence included. The level of confidence statement shall be placed on Certificates of Analysis when reporting net weights.

- Example 1: The contents of three were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the three: 0.476 ± 0.033 gram of powder. Measurement uncertainty of weight measurements is reported at a 95.45% level of confidence.

- Example 2: 25.93 ± 0.07 grams of solid material, found to contain Cocaine (Schedule II), 35.6 ± 8.2% pure.
  Measurement uncertainty of weight and purity measurements is reported at a 95.45% level of confidence.

34.3.10.3 Gross weights will include innermost packaging and be clearly delineated when they are reported. Measurement uncertainty will not be reported for gross weights.

34.3.11 When residue samples contain controlled substances or Cannabis, the results section must reflect the term "residue." Examples:

- Cannabis, residue. Marijuana/hemp not determined.
- Heroin (Schedule I), residue

34.3.12 Different reporting options consistent with applicable laws may be used at the discretion of the Section Supervisor.

34.4 Reporting Examples For Administrative Sampling Plan

34.4.1 Simple possession

34.4.1.1 Items with one specimen

- 0.501 gram of solid material including innermost packaging, found to contain Heroin (Schedule I).
- 0.254 ± 0.011 gram of solid material, found to contain Heroin (Schedule I).

34.4.1.2 Items with more than one specimen

- Total weight: 5.14 grams (0.18 ounce) of plant material including innermost packaging. The contents of one were analyzed and found to contain Marijuana.
- Total weight: 0.954 gram of solid material including innermost packaging. The contents of one were analyzed and found to contain Cocaine (Schedule II).

34.4.2 Possession with intent to distribute or distribution

34.4.2.1 Five specimens or less

- The contents of three were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the three: 0.476 ± 0.033 gram of powder.
- The contents of two were analyzed separately and each was found to contain Marijuana; total net weight of the two: 16.31 ± 0.14 grams (0.58 ± 0.01 ounce) of plant material.
- Total weight: 6.31 grams (0.22 ounce) of plant material including innermost packaging. The contents of four were analyzed separately and each was found to contain Marijuana.
- Total weight: 5.123 grams of powder including innermost packaging. The contents of four were analyzed separately and each was found to contain Cocaine (Schedule II)

34.4.2.2 More than five specimens

- The contents of five were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the five: 0.4732 ± 0.0135 gram of powder. The gross weight of the remainder was 2.3314 gram(s) including innermost packaging.
- Total weight: 6.31 grams (0.22 ounce) of plant material including innermost packaging. The contents of five were analyzed separately and each was found to contain Marijuana.
- The contents of fourteen were analyzed separately and each was found to contain Marijuana; total net weight of eleven: 13.30 ± 0.57 grams (0.469 ± 0.020 ounce) of plant material; total net weight of three: 2.8339 ± 0.0095 grams (0.0999 ± 0.0003 ounce) of plant material.
- Total weight: 5.1234 grams of powder including innermost packaging. The contents of five were analyzed separately and each was found to contain Cocaine (Schedule II).

34.4.2.3 More than five specimens, meeting a weight threshold

- The contents of eight were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the eight: 30.51 ± 0.56 grams of solid material. The gross weight of the remainder was 12.32 gram(s) including innermost packaging.

34.5 Reporting Examples For Hypergeometric Sampling Plan

34.5.1 For Initial Submissions:

- Gross weight:
  Total weight: 15.1234 grams of powder including innermost packaging. Utilizing a hypergeometric sampling plan, the contents of twenty-nine bags were analyzed separately and each was found to contain Cocaine (Schedule II). Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine.

- Net weight:
  Utilizing a hypergeometric sampling plan, the contents of twenty-nine bags were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the twenty-nine: 2.427 ± 0.319 grams of powder. Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine. The gross weight of the remainder was 12.332 gram(s) including innermost packaging.

- Extrapolated net weight:
  Utilizing a hypergeometric sampling plan, the contents of twenty-nine bags were analyzed separately and each was found to contain Cocaine (Schedule II). Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine. The extrapolated total net weight of the powder, 55.3 ± 2.0 grams, was calculated using the average net weight of the bags analyzed and considering a population of 2002 bags.

- "No controlled substances" when screening using hypergeometric sampling:
  Utilizing a hypergeometric sampling plan, the contents of fifteen capsules were analyzed separately and each was found to contain no controlled substances. Based on these results, there is a 95% level of confidence that at least 90% of the capsules contain no controlled substances.

34.5.2 For Resubmissions:

- Gross weight:
  Utilizing a hypergeometric sampling plan, the contents of twenty-two additional bags were analyzed separately and each was found to contain Cocaine (Schedule II). Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine.

- Net weight:

Utilizing a hypergeometric sampling plan, the contents of fourteen additional bags were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the fourteen: 1.312 ± 0.154 grams of powder. Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine.

- Extrapolated net weight (Note: the population should remove the number of units analyzed in the initial submission):
  Utilizing a hypergeometric sampling plan, the contents of twenty-nine additional bags were analyzed separately and each was found to contain Cocaine (Schedule II). Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Cocaine. The extrapolated total net weight of the powder, 55.3 ± 2.0 grams, was calculated using the average net weight of the bags analyzed and considering a population of 1997 bags. Five bags from Item 1 were previously analyzed and addressed in the Certificate of Analysis dated October 24, 2019.

34.6 Reporting Cannabis Extracts (for offenses on/after July 1, 2020) and Cannabis Plant Material

34.6.1 If the GC-FID peak area ratio of the sample:internal standard is greater than or equal to the respective day-of-use THC standard solution ratio(s), the amount of total THC (THC + decarboxylated THCA) is determined to be greater than 0.3% and the result will be reported as Marijuana.

Report Examples:

- Marijuana, 5.14 grams (0.18 ounce) of plant material including innermost packaging. The delta-9-*Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. The resulting THC concentration was greater than 0.3% by weight.

- Total weight: 5.14 grams (0.18 ounce) of plant material including innermost packaging. The contents of two were analyzed separately and each was found to contain Marijuana. The delta-9-*Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. The resulting THC concentration was greater than 0.3% by weight.

- Marijuana, 27.95 ± 0.05 grams (0.985 ± 0.002 ounce) of (optional: oily, waxy, appropriate adjective) material. The delta-9-*Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. The resulting THC concentration was greater than 0.3% by weight.

  **** If the date of offense is between 07/01/2020 – 06/30/2023, "delta-9-Tetrahydrocannabinol (THC)" should be specified in the wording. If the date of offense is on or after 07/01/2023, remove the underlined "delta-9-*" specification.***

34.6.2 If the GC-FID peak area ratio of the sample:internal standard is less than the respective day-of-use THC standard solution ratio, the result will be reported as Cannabis. The amount of total THC (THC + decarboxylated THCA) is inconclusive as to the Cannabis type (marijuana/hemp) and would require quantitation to determine the concentration of THC.

Report Examples:

- Cannabis, 13.30 ± 0.57 grams (0.469 ± 0.020 ounce) of plant material. The concentration of delta-9-*Tetrahydrocannabinol (THC) was determined to be below the administrative threshold established for the identification of Marijuana. If a reported THC percentage is required, the evidence should be resubmitted for additional quantitative analysis.

- Total weight: 5.14 grams (0.18 ounce) of plant material including innermost packaging. The contents of two were analyzed separately and each was found to contain Cannabis. The concentration of

delta-9-*Tetrahydrocannabinol (THC) was determined to be below the administrative threshold established for the identification of Marijuana. If a reported THC percentage is required, the evidence should be resubmitted for additional quantitative analysis.

- Cannabis, 27.95 ± 0.05 grams (0.985 ± 0.002 ounce) of (optional: oily, waxy, appropriate adjective) material. The concentration of delta-9-*Tetrahydrocannabinol (THC) was determined to be below the administrative threshold established for the identification of Marijuana.

**** If the date of offense is between 07/01/2020 – 06/30/2023, "delta-9-Tetrahydrocannabinol (THC)" should be specified in the wording. If the date of offense is on or after 07/01/2023, remove the underlined "delta-9-*" specification.***

34.6.3 If multiple listed items contain the same type of plant material, the administrative statements may be removed from the results of each item and reported following this example:

Item 2 Marijuana, 1.85 grams (0.07 ounce) of plant material including innermost packaging.
Item 3A Marijuana, 2.57 grams (0.09 ounce) of plant material including innermost packaging.
Item 3B Not analyzed.

For Items identified as Marijuana, the delta-9-*Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. The resulting THC concentration was greater than 0.3% by weight.

**** If the date of offense is between 07/01/2020 – 06/30/2023, "delta-9-Tetrahydrocannabinol (THC)" should be specified in the wording. If the date of offense is on or after 07/01/2023, remove the underlined "delta-9-*" specification.***

34.6.4 Insufficient to perform the semi-quantitative GC-FID-MS

34.6.4.1 If the submitted plant material/Cannabis extract is insufficient to perform the semi-quantitative GC-FID-MS analysis or the submitted Cannabis extract is insufficient to perform the quantitative GC-FID analysis, analyze as a residue and report as Cannabis with insufficient quantity.

Report Example:

Cannabis, 0.0412 ± 0.0033 gram (0.0015 ± 0.0001 ounce) of plant material. Marijuana/hemp not determined due to insufficient sample quantity.

34.6.4.2 If the submitted Cannabis extract is insufficient to perform the semi-quantitative GC-FID-MS analysis and the total delta-9-THC was quantitated using GC-FID, report following this example:

Marijuana, 0.041 ± 0.007 gram (0.0014 ± 0.0002 ounce) of material found to contain 10.1 ± 1.2% delta-9-Tetrahydrocannabinol by weight. The delta-9-Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. Measurement uncertainty of weight and purity measurements is reported at a 95.45% level of confidence.

34.6.5 For Cannabis plant material where total THC quantitation was pursued via GC/MS (SIM):

34.6.5.1 With quantitation results, report the concentration without identifying the Cannabis type (marijuana/hemp).

34.6.5.1.1 When within the calibration range:

- Material found to contain 0.9 ± 0.2% delta-9-Tetrahydrocannabinol (THC) by weight. The THC concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. Measurement uncertainty of purity measurements is reported at a 95.45% level of confidence.

- The contents of {number} were analyzed separately:

  One previously weighed plastic package with 1.35 ± 0.05 grams (0.047 ± 0.002 ounce) of plant material was found to contain 0.30 ± 0.06% delta-9-Tetrahydrocannabinol (THC) by weight.

  A second previously weighed plastic package with 2.66 ± 0.05 grams (0.093 ± 0.002 ounce) of plant material was found to contain 0.7 ± 0.1% delta-9-Tetrahydrocannabinol (THC) by weight. …

  The results included on the Certificate of Analysis dated Month XX, YYYY, contained a combined total net weight for Item 1A. This Supplemental Certificate of Analysis contains the individual net weights for this Item that were originally measured by the previous examiner.

  The THC concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. Measurement uncertainty of purity measurements is reported at a 95.45% level of confidence.

  Measurement uncertainty of weight and purity measurements is reported at a 95.45% level of confidence.

34.6.5.1.2 When below the calibration range:

The concentration of delta-9-Tetrahydrocannabinol (THC) was determined to be less than 0.04% by weight. The THC concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration.

34.6.5.1.3 For multiple listed items, the administrative statements may be removed from the results of each item and reported following this example:

Item 3A Material found to contain 0.9 ± 0.2% delta-9-Tetrahydrocannabinol by weight.
Item 3B Material found to contain 1.1 ± 0.2% delta-9-Tetrahydrocannabinol by weight.
Item 3C The concentration of delta-9-Tetrahydrocannabinol was determined to be less than 0.04% by weight.

The delta-9-Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. Measurement uncertainty of purity measurements is reported at a 95.45% level of confidence.

34.6.5.2 When no THC was detected:

No delta-9-Tetrahydrocannabinol (THC) was detected.

34.6.5.3 If the submitted plant material is insufficient to perform the quantitative SIM analysis:

The total delta-9-Tetrahydrocannabinol (THC) concentration was not determined due to insufficient sample quantity.

34.6.6 Resubmissions of plant material cases that were previously identified as Marijuana prior to the January 2020 implementation of the semi-quant method that have been resubmitted for supplemental testing, will be reported as per the following examples:

34.6.6.1 For samples with semi-quant greater than 1% standard:

The previously examined plant material was further analyzed and was confirmed to be Marijuana. The delta-9-Tetrahydrocannabinol (THC) concentration was evaluated after first converting THC acid, if present, to THC and then measuring the total resulting THC concentration. The resulting THC concentration was greater than 0.3% by weight. [Methods: GC-FID-MS]

34.6.6.2 For samples with semi-quant less than 1% standard:

After further analysis, the plant material was found to be Cannabis. The concentration of delta-9-Tetrahydrocannabinol (THC) was determined to be below the administrative threshold established for the identification of Marijuana. If a reported THC percentage is required, the evidence should be resubmitted for additional quantitative analysis. [Methods: GC-FID-MS]

34.6.7 In cases with a date of offense prior to 07/01/2023, if THC is indicated in the presence of a controlled substance and differentiation between THC and Cannabis was attempted:

34.6.7.1 THC shall be confirmed and reported (with no schedule) if there is no indication of other cannabinoids or terpenes.

34.6.7.2 Cannabis will be reported if there are other cannabinoids or terpenes present, indicating plant material origin.

34.7 Reporting Food Products

For food products, report weight, identify the cannabinoid(s) present with no schedule, and include an administrative statement.

34.7.1 Report Example:

35.45 ± 0.02 grams of material, found to contain Cannabidiol and delta-9-Tetrahydrocannabinol.

The Department of Forensic Science does not currently have a validated method to quantify delta-9-Tetrahydrocannabinol in food products.

34.8 Reporting Pharmaceutical Preparations

34.8.1 For substances in Schedule V – VI involving misdemeanor prosecutions or non-controlled substances, tablets and capsules visually examined using pharmaceutical identifiers will be reported following these examples.

34.8.1.1 “Visual examination of the physical characteristics, including shape, color and manufacturer’s markings, was consistent with Zoloft, a Schedule VI pharmaceutical preparation containing Sertraline. There was no apparent tampering of the dosage units.”

34.8.1.2 “Visual examination of the physical characteristics, including shape, color and manufacturer’s markings, was consistent with a Schedule VI pharmaceutical preparation containing Sertraline. There was no apparent tampering of the dosage units.”

34.8.1.3 ‘Sealed packaging containing twenty foil packets marked "Azithromycin", one of which was opened and found to contain one white oval tablet marked "787PLIVA".’

“Visual examination of the physical characteristics, including shape, color and manufacturer’s markings, of one dosage unit was consistent with a Schedule VI pharmaceutical preparation containing Azithromycin. There was no apparent tampering of the dosage unit.”

34.8.2 For substances and preparations in Schedules IV and above, tablets and capsules will be reported following these examples.

34.8.2.1 For one specimen, where the analytical results are consistent with the manufacturer’s specifications with regard to content:

34.8.2.1.1 “Tablet, found to contain Oxycodone (Schedule II) and Acetaminophen.”

34.8.2.1.2 “The tablet was found to contain Oxycodone (Schedule II) and Acetaminophen.”

34.8.2.2 For multiple specimens, where the analytical results of those tested are consistent with the manufacturer’s specifications with regard to content:

34.8.2.2.1 “One dosage unit was analyzed and found to contain Diazepam, Schedule IV. Visual examination of the physical characteristics, including shape, color and manufacturer’s markings of both the analyzed and remaining dosage units, was consistent with Valium, a pharmaceutical preparation containing Diazepam. There was no apparent tampering of the dosage units.”

34.8.2.2.2 “One dosage unit was analyzed and found to contain Diazepam, Schedule IV. Visual examination of the physical characteristics, including shape, color and manufacturer’s markings of both the analyzed and remaining dosage units, was consistent with a pharmaceutical preparation containing Diazepam. There was no apparent tampering of the dosage units.”

34.8.2.2.3 For packaged pharmaceuticals where the other specimens are not examined:

‘Sealed packaging containing twenty foil packets marked "Suboxone", one of which was opened and found to contain one orange rectangular film marked "N8".’

“One dosage unit was analyzed and found to contain Buprenorphine and Naloxone, a Schedule III pharmaceutical preparation.”

34.8.2.2.4 Schedule II tamperable pharmaceuticals in simple possession cases that were not hypergeometrically screened:

“One dosage unit was analyzed and found to contain Amphetamine, Schedule II. Visual examination of the physical characteristics, including shape, color and manufacturer’s markings of the analyzed dosage unit, was consistent with a pharmaceutical preparation containing Amphetamine.”

34.8.2.2.5 Schedule II tamperable pharmaceuticals in simple possession cases that were not analyzed due to the presence of the same controlled substance being confirmed in another intact, marked pharmaceutical preparation:

“Visual examination of the physical characteristics, including shape, color and manufacturer’s markings, was consistent with a Schedule II pharmaceutical preparation containing Amphetamine.”

34.8.3 Remove "There was no apparent tampering of the dosage units." when appropriate for the evidence type.

34.8.3.1 For pharmaceutical preparations when the expected controlled substance(s) is identified, but an additional potential contaminant is present that cannot be confirmed:

One dosage unit was analyzed and found to contain Alprazolam (Schedule IV). Visual examination of the physical characteristics, including shape, color and manufacturer's markings of both the analyzed and remaining dosage units, was consistent with a pharmaceutical preparation containing Alprazolam.

34.8.4 For tablets and capsules with substances and preparations in Schedules IV and above, where the analytical results are inconsistent with the manufacturer's specifications with regard to content, report following these examples.

34.8.4.1 For one specimen:

34.8.4.1.1 "Tablet, found to contain Oxycodone (Schedule II) and Acetaminophen. Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule IV pharmaceutical preparation containing Alprazolam; therefore, the contents were inconsistent with dosage unit labeling."

34.8.4.1.2 "The tablet was found to contain Oxycodone (Schedule II) and Acetaminophen. Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule II pharmaceutical preparation containing Amphetamine; therefore, the contents were inconsistent with dosage unit labeling."

34.8.4.2 For multiple specimens:

34.8.4.2.1 "One dosage unit was analyzed and found to contain Acetaminophen (non-controlled). Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule II pharmaceutical preparation containing Hydrocodone and Acetaminophen; therefore, the contents were inconsistent with dosage unit labeling."

34.8.4.2.2 "Five dosage units were analyzed separately and each was found to contain Diazepam (Schedule IV). Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule IV pharmaceutical preparation containing Alprazolam; therefore, the contents were inconsistent with dosage unit labeling."

34.8.4.2.3 "Five dosage units were analyzed separately. Three were found to contain Heroin (Schedule I) and two were found to contain Heroin (Schedule I) and Fentanyl (Schedule II). Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule IV pharmaceutical preparation containing Alprazolam; therefore, the contents were inconsistent with dosage unit labeling."

34.8.4.2.4 "Utilizing a hypergeometric sampling plan, the contents of twenty-eight capsules were analyzed separately. Ten were found to contain Fentanyl (Schedule II), fourteen were found to contain Gabapentin (Schedule V), and four were found to contain no controlled substances. Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule II pharmaceutical preparation containing Amphetamine; therefore, the contents were inconsistent with dosage unit

labeling. No inference can be made as to the contents of the capsules that were not analyzed."

34.8.5 In cases where items have been analyzed for possible tampering or substitution, the results will have an additional statement of either "Meets label specifications" or "Does not meet label specifications". Additional clarifying wording should be used such as "Does not meet label specifications with regard to concentration" at the discretion of the Section Supervisor.

34.8.5.1 Reporting Example: The contents of eight were analyzed separately and each was found to contain Methylone (Schedule I); total net weight of the eight: 1.2345 ± 0.0218 grams of powder. Visual examination of the analyzed capsule determined that the physical characteristics were consistent with a non-prescription pharmaceutical preparation containing Diphenhydramine. Therefore the contents do not meet label specifications.

34.9 Reporting Purity Determinations

34.9.1 The level of confidence statement shall be placed on Certificates of Analysis when reporting purity determinations.

34.9.1.1 For purity determinations with net weights:

Measurement uncertainty of weight and purity measurements is reported at a 95.45% level of confidence.

34.9.1.2 For purity determinations without net weights (i.e., hashish oil):

Measurement uncertainty of purity measurements is reported at a 95.45% level of confidence.

34.9.2 Quantitation results and measurement uncertainty shall be rounded to one (1) decimal place and reported using a 95.45% level of confidence following these examples:

34.9.2.1 24.55 ± 0.07 grams of solid material, found to contain Heroin (Schedule I), 26.7 ± 8.3% pure.

34.9.2.2 12.523 ± 0.011 grams of powder, found to contain Cocaine Hydrochloride (Schedule II), 45.8 ± 9.4% pure.

34.9.2.3 Marijuana, 13.30 ± 0.57 grams (0.469 ± 0.020 ounce) of material found to contain 10.1 ± 1.2% delta-9-Tetrahydrocannabinol by weight.

34.9.2.4 The contents of five were analyzed separately and each was found to contain Methamphetamine Hydrochloride (Schedule II); total net weight of the five: 12.93 ± 0.07 grams of powder. A composite sample of the five was subsequently analyzed and found to be 45.8 ± 9.4% pure.

34.9.2.5 The contents of five were analyzed separately and each was found to contain delta-9-Tetrahydrocannabinol, total net weight of the five: 15.54 ± 0.25 grams (0.548 ± 0.009 ounce) of material. A composite sample of the five was quantitatively analyzed and identified as Marijuana, 10.1 ± 1.2% delta-9-Tetrahydrocannabinol by weight.

34.9.2.6 Utilizing a hypergeometric sampling plan, the contents of eleven were analyzed separately and each was found to contain Methamphetamine Hydrochloride (Schedule II); total net weight of the eleven: 15.77 ± 0.54 grams of powder. Based on these results, there is a 95% level of confidence that at least 90% of the packages contain Methamphetamine Hydrochloride. A composite sample of the eleven was subsequently analyzed and found to be 45.8 ± 9.5% pure. The gross weight of the reminder was 2.02 gram(s) including innermost packaging.

34.9.3 For oils and oily residues with offense dates prior to July 1, 2020:

34.9.3.1 If the mean THC concentration of the quantitation samples, prior to rounding, is 12.0% by weight or greater, report concentration and measurement uncertainty rounded to one (1) decimal place:

- Hashish oil (Schedule I), found to contain 34.6 ± 9.2% delta-9-Tetrahydrocannabinol by weight.

- The contents of five were analyzed separately and each was found to contain delta-9-Tetrahydrocannabinol. A composite sample of the five was subsequently analyzed and found to contain 28.7 ± 10.6% delta-9-Tetrahydrocannabinol by weight, Hashish oil (Schedule I).

34.9.3.2 If the mean THC concentration of the quantitation samples, prior to rounding, is less than 12.0% by weight:

- Marijuana, 13.30 ± 0.57 grams (0.469 ± 0.020 ounce) of material found to contain 10.1 ± 1.2% delta-9-Tetrahydrocannabinol by weight.

- For HPLC quantitative analysis:

  Marijuana, 4.52 ± 0.05 grams (0.159 ± 0.002 ounce) of material. Quantitative analysis of the material determined that it contained 6.3 ± 1.5% Cannabidiol by weight and 8.5 ± 1.7% delta-9-Tetrahydrocannabinol by weight.

- If non-naturally occurring isomers are present and confirmed:

  13.30 ± 0.57 grams (0.469 ± 0.020 ounce) of material found to contain 10.1 ± 1.2% delta-9-Tetrahydrocannabinol by weight and delta-6a(10a)-Tetrahydrocannabinol. The purity of delta-6a(10a)-Tetrahydrocannabinol was not determined.

34.9.3.3 If oily extract contains delta-9-Tetrahydrocannabinol, with or without non-naturally occurring THC isomers present, but unable to report the purity:

- When there is insufficient volume to determine the purity, identify the cannabinoid(s):

  Material found to contain delta-9-Tetrahydrocannabinol and delta-6a(10a)-Tetrahydrocannabinol. The material was insufficient for quantitative analysis.

- When original quantitation failed due to various reasons (e.g., QC fail) and there is not enough sample remaining. Notify customer, document communication on MFR and report:

  Material found to contain delta-9-Tetrahydrocannabinol. Concentration of cannabinoid(s) not determined.

  Material found to contain delta-9-Tetrahydrocannabinol and delta-6a(10a)-Tetrahydrocannabinol. Cannabinoid concentrations not determined.

34.9.3.4 If oily extract contains non-naturally occurring THC isomers only:

Material found to contain delta-6a(10a)-Tetrahydrocannabinol.

34.9.4 For methamphetamine PWID and distribution cases:

34.9.4.1 When no written request for a purity determination has been received from a Commonwealth's Attorney and the net weight falls within an established weight threshold as defined in the Code of Virginia (net weight = 10 – 20 g, 100 – 200 g, or 250 g – 1 kilo):

18.28 ± 0.57 grams of powder, found to contain Methamphetamine (Schedule II). A purity determination was not performed.

34.9.4.2 For resubmissions requesting a purity determination:

- The contents of the five previously examined plastic bag corners were analyzed separately and each was found to contain Methamphetamine Hydrochloride (Schedule II). A composite sample of the five was subsequently analyzed and found to be 45.8 ± 9.4% pure.

- The contents of the previously examined three ziplock bags were analyzed separately and each was found to contain Methamphetamine Hydrochloride (Schedule II). A composite sample of the three was subsequently analyzed and found to be 45.8 ± 9.4% pure.

- The contents of the previously examined plastic bag corner were analyzed and found to contain Methamphetamine Hydrochloride (Schedule II), 95.5 ± 11.0% pure.

34.9.5 For Cannabis Oil determinations per Code of Virginia § 54.1-3408.3:

34.9.5.1 Cannabis oil, found to contain 51.1 ± 11.8% Cannabidiol (CBD) by weight. Per the labeling on the vial, one dose is 10 mL. Therefore, one dose would result in 5.1 ± 1.2 mg CBD.

34.9.5.2 Liquid, found to contain 7.5 ± 1.8% Tetrahydrocannabinolic Acid (THCA) by weight and 0.4 ± 0.2% delta-9-Tetrahydrocannabinol (THC) by weight. Per the labeling on the vial, one dose is 5 mL. Therefore, one dose would result in 0.38 ± 0.09 mg THCA and 0.02 ± 0.01 mg THC.

34.9.5.3 Cannabis oil, found to contain 51.1 ± 11.8% Cannabidiol (CBD) by weight and less than 2% delta-9-Tetrahydrocannabinol (THC) by weight. Per the labeling on the vial, one dose is 10 mL. Therefore, one dose would result in 5.1 ± 1.2 mg CBD and less than 0.008 mg THC.

34.9.5.4 Liquid, found to contain 18.6 ± 4.6% Cannabidiol (CBD) by weight and 2.6 ± 0.8% delta-9-Tetrahydrocannabinol (THC) by weight. No dosage labeling was present on the vial.

34.10 Reporting Cocaine Salt/Base Determination

Resubmissions for cocaine salt/base analysis will be reported as per the following examples:

34.10.1 "The contents of the previously examined five plastic bag corners were analyzed separately and each was found to contain Cocaine base (Schedule II)."

34.10.2 "The contents of the previously examined three ziplock bags were analyzed separately and each was found to contain Cocaine Hydrochloride (Schedule II)."

34.10.3 Solid material, found to contain Cocaine base (Schedule II).

34.10.4 The solid material was found to contain Cocaine base (Schedule II).

34.11 Reporting Precursors

Substances listed as precursors in Code of Virginia § 18.2-248(J) or are defined in § 18.2-248(K) shall be reported as such as per the following examples:

34.11.1 Tablet, found to contain Pseudoephedrine (a listed substance in § 18.2-248(J)).

34.11.2 Liquid, found to contain Ephedrine/Pseudoephedrine (a listed substance in § 18.2-248(J)).

34.12 Reporting Cannabimimetic Agents

Cannabimimetic agents that are specifically listed by both their chemical and common name in the Code of Virginia shall be reported using their common names as generated by LIMS. Cannabimimetic agents will be reported as per the following examples:

34.12.1 When a scheduled cannabimimetic agent is present: "0.254 ± 0.011 gram of powder, found to contain JWH-018 (a Schedule I cannabimimetic agent listed in § 54.1-3446(6)(b))."

34.12.2 When a compound within a defined structural class is present (but not specifically listed): "0.254 ± 0.011 gram of plant material, found to contain JWH-015 (Schedule I). This compound is a cannabimimetic agent as defined in § 54.1-3446(6)(a) and is within the structural class 3-(1-naphthoyl)indole."

34.12.3 When a Non-Listed/Non-Structural Class cannabimimetic agent may be present, but is not identified: "No controlled substances identified."

34.12.4 When a mixture of compounds is present (where one is within a defined structural class but not specifically listed along with a listed compound): "0.254 ± 0.011 gram of plant material, found to contain a mixture of cannabimimetic agents: JWH-018 (a Schedule I cannabimimetic agent listed in § 54.1-3446(6)(b)) and JWH-015, a Schedule I cannabimimetic agent as defined in § 54.1-3446(6)(a) and is within the structural class 3-(1-naphthoyl)indole."

34.13 Reporting Compounds Scheduled by Board of Pharmacy

Compounds scheduled pursuant to Board of Pharmacy Regulation shall be reported using their common names as generated by LIMS per the following examples:

34.13.1 Cannabimimetic agents:

0.254 ± 0.011 gram of solid material, found to contain NM-2201. This compound was placed into Schedule I of the Drug Control Act as a cannabimimetic agent pursuant to a Board of Pharmacy Regulation.

34.13.2 All other non-cannabimimetic agents:

0.254 ± 0.011 gram of solid material, found to contain 4-bromomethcathinone (4-BMC). This compound was placed into Schedule I of the Drug Control Act pursuant to a Board of Pharmacy Regulation.

34.14 Reporting Schedule I Compound Isomers

34.14.1 The federal definition of "positional isomer" (referred to as "position isomer" in VA State Code) shall be used for interpretation purposes and can be found in the Code of Federal Regulations Title 21, Chapter II, Part 1300, Section § 1300.01 within the definition of the term "isomer":

"As used in §1308.11(d) of this chapter, the term ''positional isomer'' means any substance possessing the same molecular formula and core structure and having the same functional group(s) and/or substituent(s) as those found in the respective Schedule I hallucinogen, attached at any position(s) on the core structure, but in such manner that no new chemical functionalities are created and no existing chemical functionalities are destroyed relative to the respective Schedule I hallucinogen. Rearrangements of alkyl moieties within or between functional group(s) or substituent(s), or divisions or combinations of alkyl moieties, that do not create new chemical functionalities or destroy existing chemical functionalities, are allowed i.e., result in compounds which are positional isomers. For purposes of this definition, the ''core structure'' is the parent molecule that is the common basis for the class; for example, tryptamine, phenethylamine, or ergoline. Examples of rearrangements resulting in creation

and/or destruction of chemical functionalities (and therefore resulting in compounds which are not positional isomers) include, but are not limited to: Ethoxy to alpha-hydroxyethyl, hydroxy and methyl to methoxy, or the repositioning of a phenolic or alcoholic hydroxy group to create a hydroxyamine. Examples of rearrangements resulting in compounds which would be positional isomers include: Tert-butyl to sec-butyl, methoxy and ethyl to isopropoxy, N,N- diethyl to N-methyl-N-propyl, or alpha-methylamino to N-methylamino." [Volume 9, Date 2022-04-01]

34.14.2 Isomers of substances listed in Schedule I will be reported as per the following examples:

34.14.2.1 Option 1 (the isomers can be analytically distinguished): "3-Fluoromethamphetamine (Schedule I), an isomer of 4-Fluoromethamphetamine."

34.14.2.2 Option 2 (the isomers have not been analytically distinguished): "4-Fluoromethamphetamine or one of its isomers as defined in § 54.1-3446(3) (Schedule I)."

34.14.2.3 Option 3 (isomer of a BoP scheduled compound): "Isobutyryl fentanyl (Schedule I), an isomer of Butyryl fentanyl. Butyryl fentanyl was placed into Schedule I of the Drug Control Act pursuant to a Board of Pharmacy Regulation."

34.15 Reporting Substantially Similar Compounds

34.15.1 Compounds which are substantially similar to compounds listed in Schedules I and II shall be reported as per the following examples:

34.15.1.1 If the compound meets requirements as defined in ¶ 17.4.1 or 17.4.2, report as follows:

0.254 ± 0.011 gram of solid material, found to contain (Compound). The chemical structure of (compound) is substantially similar to the chemical structure of (list controlled substance and schedule). A chemical structure is considered substantially similar if there are no more than two minor substituents that have been replaced, added, removed, or extended within the chemical structure.

34.15.1.2 If the compound does not meet requirements as defined in ¶ 17.4.1 or 17.4.2, report the compound as: "No controlled substances found" or "No controlled substances identified".

34.15.2 Compounds which have been evaluated for substantial similarity to a Schedule I or II controlled substance will be reported as per the following examples:

34.15.2.1 If the compound in question is mixed with a Schedule I or II controlled substance, only the controlled substance will be reported.

34.15.2.2 If the compound is the only compound present in an item or is mixed with a Schedule III-VI controlled substance, report the compound. If a standard is not readily available, the following may be reported: "No controlled substances identified. A substance that may be substantially similar to the chemical structure of a controlled substance in Schedule I or II was indicated. If identification of the compound is required as per § 54.1-3401 (controlled substance analog), the evidence should be resubmitted for further analysis along with a letter of request from the Office of the Commonwealth's Attorney".

35 DRUG REVERSALS

35.1 Introduction

The Department will assist law enforcement agencies with preparation of materials to be used in drug reversals, buy/bust scenarios, internal security setup operations, and "show and tell" drugs. In all instances, the requesting agency must assume full responsibility for distribution of these materials.

35.2 Procedure

35.2.1 The agency must make a written request on the Agency's letterhead detailing the scope of the operation, materials needed, and projected timeframes. This request must be approved by the Section Supervisor or Chemistry Program Manager and Laboratory Director.

35.2.2 Any controlled substances must be provided by the requesting agency or a cooperative neighboring jurisdiction.

35.2.2.1 If the material is from a case previously analyzed by the Department, this must be made clear by the agency.

35.2.3 Cutting materials must be supplied by the requesting agency and will be subject to approval by the Section Supervisor.

35.2.3.1 The Department may provide substances added to the material for "marking" purposes. Examples of such substances are benzocaine, lidocaine and diphenhydramine.

35.2.3.2 There may be times when uncut materials are required. However, whenever possible, the controlled substances should be diluted to approximately 1% drug by weight to allow for future analysis while enhancing the safety of the operation. This will be evaluated on a case-by-case basis.

35.2.4 All packaging material must be supplied by the requesting agency.

35.2.4.1 Materials will be packaged at the laboratory in accordance with the agency's request.

35.2.4.2 Packages must allow for future testing of the material when it is re-submitted.

35.2.5 An authentic sample of the final preparation will be kept by the analyst for future comparison. All analytical data such as weights, composition, notes, chromatograms and spectra are to remain with the authentic sample.

35.2.5.1 From the date of preparation, samples and supporting analytical data shall be maintained pursuant to the Library of Virginia Specific Schedule Number 778-001 (Quality Assurance: Equipment, Standards, and Instruments 200540 series).

35.2.6 The requesting agency must submit all cases resulting from a particular operation to the attention of the analyst who prepared the material. These should be clearly marked on the RFLE.

35.2.7 The Department assumes no responsibility or liability for the security of these materials once the law enforcement agency takes possession of them.

35.2.8 The Department assumes no responsibility or liability for any use of these materials by the requesting agency or any other person.

36 QUALITY ASSURANCE

36.1 Introduction

36.1.1 The purpose of this section is to provide a uniform Quality Assurance Program for the Controlled Substances Section of the Virginia Department of Forensic Science. It is to establish a baseline or reference point of reliability and system performance.

36.1.2 It is expected that the analyst will report any unacceptable or anomalous behavior of any of our analytical systems immediately to either their Section Supervisor or the appropriate Instrument Specialist (Primary Operator). It is further expected that appropriate steps to ensure resolution of the issue will follow ASAP and be properly documented.

36.2 Reagents

36.2.1 Chemicals and solvents used in qualitative reagents should be of at least ACS reagent grade.

36.2.2 Solvents used to dissolve samples or standards should be a high quality, low residue solvent (e.g., HPLC grade, OMNISOLV, OPTIMA).

36.2.3 Water used in reagent preparation should be either deionized (DI) or reverse osmosis (RO).

36.2.4 Stock solutions of general color test reagents and TLC sprays will be made up as needed. The *CS Color Test Reagent Preparation Log* or the *CS TLC Bath and Visualization Reagent Preparation Log* shall be used to record reagent preparation. After they are made, they will be checked with the corresponding primary or secondary standard listed below in Table 3 and results, date and initials will be recorded.

36.2.5 Color test reagents and TLC sprays will be verified every three months during the shelf life of the reagent (2 years unless otherwise noted). The *Reagent QA Check Worksheet* shall be used for this purpose.

36.2.6 Individual chemists may have unique reagents other than the ones listed in Table 3 and it will be their responsibility to check them with an appropriate standard and document accordingly. For single use reagents, this documentation may be in the case file.

TABLE 3: Common Reagents and Appropriate Check Compounds

REAGENT	CHECK COMPOUND
Duquenois	Cannabis or Cannabis extract
Marquis	Heroin
Froehdes	Heroin
Meckes	Heroin
Cobalt Thiocyanate	Cocaine
Ehrlich's	LSD
Iodoplatinate	Cocaine
Iodoplatinate/Ceric Sulfate	Caffeine
$KMnO_4$	Acetaminophen
Fast Blue B and Fast Blue BB	Cannabis extract
4-AP	CBD and THC standards

36.3 Standards

36.3.1 Primary and quantitative reference standards should normally be at least of United States Pharmacopeia – National Formulary (USP-NF) quality. This applies to both powders purchased from a manufacturer/supplier and pharmaceutical preparations.

36.3.1.1 Receipt, storage and use of controlled drug standards shall be recorded and records maintained as required by § 54.1-3404.

36.3.1.2 Standards used as reference materials in casework are considered critical supplies and shall be purchased from manufacturers approved by the Chemistry Program Manager.

36.3.1.2.1 The following manufacturers/vendors/suppliers are pre-approved for the purchase of new reference standards:

- USP
- Alltech Associates (Grace – Discovery Sciences)
- Cerilliant
- Sigma-Aldrich and its subsidiaries
- Steraloids, Inc.
- Cayman Chemicals
- Lipomed
- LGC
- Crescent Chemical
- VWR

36.3.1.2.2 Pharmaceutical preparations may be purchased from any licensed pharmacy or the patented drug manufacturer for use as reference materials.

36.3.1.3 Primary reference standards are those purchased from a reputable manufacturer.

36.3.1.4 Secondary standards are those that are obtained or synthesized within the laboratory. These may be from previously analyzed case samples.

36.3.1.5 Quantitative standards have a known purity, known accuracy and are purchased from a reputable manufacturer including those listed in ¶ 36.3.1.2. Certified reference materials shall be used, where available, for all quantitations and include the supplier's Certificate of Analysis. For obtaining reference materials, see QM ¶ 8.3.3.

- Reference materials used for quantitative analyses shall not be used past the manufacturer's expiration date or retest date, but may be used for qualitative analyses. The reference material can continue to be used for quantitative analyses beyond the retest date if an updated CoA is obtained following the manufacturer's retest.

36.3.2 For all standards obtained for drug lab use, a qualified examiner will be responsible for obtaining a mass spectrum, IR or other suitable definitive instrumental data (data provided by the manufacturer is not sufficient, although it should be stored with lab generated data). The hard copy data will be filed. The documentation should include the following information:

- Lot# or logbook code
- Standard name
- Concentration, as appropriate
- Amount injected (for MS only)
- Date and analyst's handwritten initials
- Certificate of Analysis from manufacturer (if available)

36.3.3 Qualitative reference materials:

36.3.3.1 A manufacturer's retest date stated for a reference material is the date when a material should be re-examined to ensure that it is still suitable for use. While manufacturers will retest reference materials according to the retest date for a particular lot and update the Certificate of

Analysis for that lot accordingly, the manufacturer may not always have that lot available for retesting.

- To maintain confidence in their continued suitability for use beyond the manufacturer's retest date and/or expiration date, reference materials shall be evaluated concurrently with each use. It is acceptable for substances that are associated with the breakdown of the reference material to be present in minor abundances.

36.3.4 After the examiner gathers the data and ensures that it agrees with known published spectra or that the data is consistent with the unique compound represented from both a chemical and data interpretation perspective, label the standard bottle with "MS", "IR", etc., the date and initials. Include the source of the standard and record all pertinent information in the Standards File.

36.3.5 If an examiner needs a standard from a new lot that has not been documented in this fashion, the examiner must perform the above procedure prior to using it for drug casework.

36.3.6 When positive results are achieved in casework, the corresponding standard(s) must be properly documented in the case file. Standards used for TLC should be documented in the case notes, whereas standards used for instrumental methods may be documented either in the case notes or on the data.

36.4 Balances

36.4.1 All analytical and toploading balances will be checked **weekly** for accuracy using ASTM Class 2 weights (previously referred to as Class S) or better.

36.4.2 All high capacity balances will be checked at least **monthly** for accuracy using ASTM Class 3 weights (previously referred to as Class S-1) or NIST Class F weights or better.

36.4.3 Place the appropriate vessel on the balance, tare and add weight (dynamic weighing).

- Plastic weigh boats shall not be used for check weights on 4-place balances.
- Plastic beakers and weigh boats shall not be used for check weights on 5-place balances.
- Dynamic weighing shall be used for 5-place balance check weights.

36.4.4 Record all performance check weight values in the balance QA log with the date, initials, and appropriate notation(s) for values that are outside acceptance criteria.

36.4.4.1 Weight values may be recorded using the electronic worksheet or hand-written worksheet.

36.4.4.2 If a result from the performance check is outside of the acceptable range:

36.4.4.2.1 First, ensure that the balance is level and clean prior to rechecking.

36.4.4.2.2 If applicable, use the internal calibration function of the balance prior to rechecking.

36.4.4.2.3 If a result remains outside of the acceptable range after performing the above actions, the balance shall be immediately taken out of service until maintenance and/or calibration are performed by an approved vendor.

36.4.4.3 As an ongoing component of measurement assurance, all weekly performance check values within acceptance criteria are compiled electronically and should be uploaded to the shared drive within 60 days of collection.

36.4.5 The balances listed below in Table 4 represent examples of a balance class or type along with the corresponding check weights. If a balance does not fit into these categories, use three weights within its range as approved by the Chemistry Program Manager.

TABLE 4: Balances and Appropriate Check Weights

BALANCE TYPE	BALANCE EXAMPLES	CHECK WEIGHTS
Analytical (dual range)	Mettler XS 105	0.01000* (± 0.00005) gram 20.00000* (± 0.00005) grams 50.0000 (± 0.0005) grams 100.0000 (± 0.0005) grams
Analytical	Mettler AE 160 Sartorius Basic	0.0500 (± 0.0002) gram 1.0000 (± 0.0003) gram 20.0000 (± 0.0005) grams
Toploading (± 0.01) gram	Mettler PE 2000 Mettler PE 1600 Mettler PB302 Ohaus Port-O-Gram Sartorius 2100	5.00 (± 0.02) gram, 10.00 (± 0.03) grams, 100.00 (± 0.03) grams
Toploading (± 0.001) gram	Ohaus Explorer Mettler PB303	0.500 (± 0.002) gram 1.000 (± 0.002) gram 100.000 (± 0.005) grams
High Capacity (g)	A.N.D. Electronic (1000, 10,000, 30,000 g) Ohaus CQ10R33 (100, 1000, 10,000 g)	100 (± 1) grams 1000 (± 1) grams 10,000 (± 5) grams 30,000 (± 10) grams
High Capacity (kg)	Ohaus DS5-M (1 or 2, 10, 20kg) Ohaus DS10-L (1 or 2, 10, 30 kg) Ohaus ES50L (1 or 2, 10, 30 kg)	1.00 or 2.00 (± 0.02) kilograms 10.00 (± 0.02) kilograms 20.00 (± 0.02) kilograms 30.00 (± 0.02) kilograms

*Use the Conventional Mass Calibration Value as reported on the most recent calibration certificate, rounded to the fifth decimal place.

36.4.6 As an ongoing component of measurement assurance and for uncertainty of measurement calculations, surrogate weights shall be weighed **weekly** on all balances. Record the weights in the *Surrogate Weight* folder on the shared drive. Occasional lapses in weekly measurements for personal balances (e.g., due to absences from the laboratory) are anticipated and do not require compensatory measurements. The surrogate weights shall be weighed similarly to net weights in casework.

36.4.6.1 There may be times when more than one surrogate weight is measured weekly (e.g., following the preparation of new surrogate weights); however, annual uncertainty of measurement calculations will incorporate data from only a single surrogate weight per balance type, per laboratory.

36.4.6.2 In the event of a surrogate weight breaking or requiring a replacement prior to the routine repreparation of surrogate weights, more frequent intervals of weighing may be necessary to ensure adequate collection of data for uncertainty of measurement calculations.

36.4.7 Maintenance:

36.4.7.1 Balances shall be calibrated annually by an external vendor that is accredited to ISO/IEC 17025 and whose scope of accreditation covers the calibration performed. Following annual calibration, balances may be placed back into service after a successful performance check. Once received, calibration certificates shall be reviewed for accuracy by the Section Supervisor, Group Supervisor, or designee. Guidance on *Calibration Certificate Review* is available in the Resources/Pipettes folder in Qualtrax.

36.4.7.2 Weights used to check balance accuracy shall be re-calibrated every three years by an ISO/IEC 17025 accredited vendor whose scope of accreditation covers the calibration performed.

36.4.7.2.1 If more than one calibrated weight or set is available, the balance QA log will denote which weight and/or weight set was used for the respective performance check values.

36.4.7.3 Accuracy and precision must be established after a balance has been repaired. The *Balance Accuracy and Precision Worksheet* should be used for this purpose.

36.4.7.3.1 The check weights listed in Table 4 are weighed and recorded five times.

36.4.7.3.2 The mean and % relative standard deviation (%RSD) are calculated for each weight.

%RSD = 100 * (standard deviation / mean)

36.4.7.3.3 Acceptance Criteria:

- The accuracy of each weight should meet the criteria in Table 4.
- %RSD shall be less than or equal to 5%.
- The balance will be immediately taken out of service if these criteria are not met.

36.4.7.4 New surrogate weights will be prepared every 5 years using the following procedure:

36.4.7.4.1 Dry play sand in an oven for approximately 1 week.

36.4.7.4.2 Prepare packets such that each plastic packet of sand is heat sealed within another labeled plastic packet. Double heat seals are used to prevent leaking and premature deterioration.

36.4.7.4.3 Packets are prepared for each balance type with the following target weights:

Balance Type	Target Weights
2-place	100 g
3-place	11.5 g
4-place	2.5 g
5-place	0.5 g
Large (g)	2012 g
High capacity (kg)	2 kg

36.4.7.4.4 Patience and care must be taken to ensure as much consistency of packet weights as possible within the same balance type.

36.4.7.4.5 Record preparation weights as they will be helpful in the event a surrogate weight breaks or requires a replacement prior to the routine repreparation of packets.

36.4.7.4.6 Surrogate weights will be prepared at one laboratory and disseminated to the other laboratories following preparation.

36.4.8 New balances: Prior to being placed into service, new balances shall be calibrated and evaluated to ensure that the balance meets the requirements for accuracy and precision as well as being within the current estimated uncertainty.

36.4.8.1 Calibration certificates shall be reviewed for accuracy by the Section Supervisor, Group Supervisor, or designee. Guidance on *Calibration Certificate Review* is available in the Resources/Pipettes folder in Qualtrax.

36.4.8.2 The *Balance Accuracy and Precision Worksheet* should be used to ensure accuracy and precision criteria are met. Further, the balance must meet check weight performance check acceptance criteria.

36.4.8.3 The balance's uncertainty on the certificate shall be no greater than the line item "value" for the "balance calibration uncertainty" found on the appropriate UoM budget in Qualtrax. If the uncertainty from the certificate is greater than the line item "value", notify the Chemistry Program Manager to assess the impact in the reported UoM, further assess the calibration certificate, evaluate any associated risk, or republish the UoM budget, as needed.

36.5 Thin Layer Chromatography

36.5.1 TLC bath solutions will be made up as needed. After they are made, they will be checked with the compound(s) listed below in Table 5 and results, date and initials will be recorded in the logbook. Limited use baths not listed in Table 5 will be checked by running appropriate standards along with the sample(s).

TABLE 5

REAGENT	CHECK COMPOUNDS
TLC1 (9:1)	Cocaine/Heroin
TLC2 (18:1)	Cocaine/Heroin
TLC3 (T-1)	Cocaine/Heroin
TLC4 / TLC5	Cannabis extract

36.5.2 The baths should be refreshed daily.

36.5.3 Day-to-day performance shall be checked by running a standard along with the sample(s).

36.6 Gas Chromatographs

36.6.1 Record any maintenance performed in the logbook, date and initial.

36.6.2 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

36.6.3 Following inlet maintenance, a 2.5 mg/mL CBD in internal standard solution shall be injected on those instruments used for the semi-quantitative analysis of Cannabis. To demonstrate acceptable conditions, no THC shall be detectable. Acceptable results must be documented either in the logbook or on the data.

36.6.4 Weekly

36.6.4.1 Change all septa (unless a Merlin Microseal is installed).

36.6.4.2 The column performance is checked by injecting a standard with the sample(s).

36.6.4.3 A 2.5 mg/mL CBD in internal standard solution shall be injected weekly on those instruments used for the semi-quantitative analysis of Cannabis. To demonstrate acceptable conditions, no THC shall be detectable. Acceptable results must be documented either in the logbook or on the data.

36.6.5 Monthly

36.6.5.1 Run a mixture of DFTPP, Methamphetamine, Cocaine, Tetracaine and Heroin standards. The concentration of these standards should be 2 mg/mL or less. Method conditions should mimic those used in a general screen run by examiners.

- Record in logbook, date and initial. The chromatogram should demonstrate good chromatographic performance. Acceptable results must be documented either in the logbook or on the data.
- Any performance discrepancies or degradation must be reported immediately to a supervisor.
- Store hardcopy of data for approximately one year.

36.6.5.2 Back up data files, sequence files, and sequence log files by copying them to the Department's intranet. Data files will be retained for a period of no more than two years. Sequence files and sequence log files will be retained for a period of five years.

36.6.5.3 Change injection port liners, as needed.

36.6.6 Semi-annually

Macros and methods will be backed up by copying them to the Department's intranet and retained there for a period of five years.

36.6.7 Yearly

36.6.7.1 Remove columns. Reinstall or replace columns as needed.

36.6.7.2 Clean injection ports and FID detectors annually, or more as needed.

36.6.7.3 Replace the split vent traps for Agilent 6890 and higher series gas chromatographs.

36.6.8 Placement of Instrument into Service

36.6.8.1 After significant maintenance has been performed, run the standard mixture as outlined in ¶ 36.6.5.1.

36.6.8.2 New instrument installation

- Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer's specifications.
- Load/modify appropriate macros and test functionality.
- After methods are created, run a standard on a representative sample of the methods (e.g., low temp., mid temp., high temp., screen methods) to demonstrate efficacy.
- Run the standard mixture as outlined in ¶ 36.6.4.1 ten times to demonstrate chromatographic reproducibility.
- Archive methods and data analysis macros to suitable long-term storage media.
- A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.
- Retain instrument verification documentation.

36.6.9 Hydrogen Generator Maintenance

36.6.9.1 Fill with deionized water as needed.

36.6.9.2 Perform, at a minimum, annual preventive maintenance which may include changing filters, electrolytes, cartridges, or other parts as necessary. Semiannual preventive maintenance may be performed to troubleshoot or if it provides enhanced performance. When performing annual or semi-annual preventive maintenance, follow instructions from the manufacturer.

36.6.9.3 The *Hydrogen Generator Maintenance Log* shall be used to record any maintenance performed (excludes water refilling).

36.7 Liquid Chromatographs

36.7.1 Record any maintenance performed in the logbook, date and initial.

36.7.2 Day of Use

36.7.2.1 Check mobile phase volumes and dates of preparation. Mobile phase shelf-life is one month unless otherwise stated. Prepare fresh mobile phase if necessary.

36.7.2.2 The column performance is checked by injecting a standard(s) with the sample(s).

36.7.2.3 A record of all samples will be kept in a logbook that includes the date, file/sequence name, and the initials of the user.

36.7.3 Monthly

36.7.3.1 Data files will be backed up by copying them to the Department's intranet and retained there for a period of no more than two years. Sequence files will be backed up by copying them to the Department's intranet and retained there for a period of five years.

36.7.4 Semi-Annually

36.7.4.1 Important, non-data files (i.e., methods and libraries) may be backed up by copying them to the Department's intranet and retained there for a period of five years.

36.7.5 Yearly

36.7.5.1 Schedule preventative maintenance as required.

36.7.6 As Needed

36.7.6.1 Instrument components shall be maintained to manufacturer's specification, using repair/replacement guidelines set forth by the manufacturer, or to maintain optimum operating conditions.

36.7.6.2 Columns and other components should be replaced as necessary.

36.7.6.3 During long periods of dormancy, it may be necessary to shut down the LC for long-term storage.

- Flush the channels used for buffered mobile phases with pure solvents of the same type.
- Flush the column according to manufacturer's recommendations.

- Remove the column from the instrument leaving it in the manufacturer's recommended solvent storage conditions and cap it tightly to store for future use.
- In place of the column in the instrument, place a zero dead volume fitting so the flow cell and other lines are flushed.
- Flush all channels with methanol. Flush all channels with isopropanol. Leave the system in isopropanol.

36.7.7 Placement of Instrument into Service

36.7.7.1 After significant maintenance has been performed, run an appropriate standard to check instrument performance.

36.7.7.2 New instrument installation

- Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer's specifications.
- After methods are created, run a representative standard sample to demonstrate efficacy.
- Run a standard mixture ten times to demonstrate chromatographic reproducibility.
- Archive methods to suitable long-term storage media.
- A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.
- Retain instrument verification documentation.

36.8 Mass Spectrometers

36.8.1 Record any maintenance performed in the logbook, date and initial.

36.8.2 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

36.8.3 Daily

- Autotune - Use "Reset to Defaults", if available, and "Autotune" for GC/MS systems for routine "seized" drug analysis. Quicktune or its equivalent may be used as determined by the primary operator. For applicable instruments, "Etune" may be substituted for "Autotune". Specialized tunes may be used on a case–by-case basis as determined by the primary operator and the examiner in concert with the Chemistry Program Manager.
- An acceptable tune will be noted in the logbook with the date and initials of the approving examiner.

Table 6: Autotune/Etune Acceptance Criteria

TUNE PARAMETER	SPECIFIC PARAMETER	ACCEPTANCE RANGE
Peak widths	0.50 Da	± 0.05 Da (spread between values ≤ 0.05)
Mass assignment	69.00, 219.00, 502.00 Da	± 0.10 Da
Isotope Ratios	Ratio of mass 70 to 69	0.5 – 1.6%

Isotope Ratios	Ratio of mass 220 to 219	3.2 – 5.4%
Isotope Ratios	Ratio of mass 503 to 502	7.9– 12.3%

- Run Background and note in logbook with the date and initials.
- Retain a copy of the daily Autotune/Etune report and background for five years.
- Change septum or perform maintenance on “Merlin Microseal”, if needed.

36.8.4 Weekly

- Run a mixture of Methamphetamine, DFTPP, Cocaine, Tetracaine and Heroin reference standards and store representative peaks and spectra in file. Concentration should be 2 mg/mL or less. Method conditions should mimic those used in a general screen run by examiners. The chromatogram should demonstrate good chromatographic performance. Acceptable results must be documented in the logbook. Retain a copy for two years.
- Check Helium and other gas supplies, replace as needed.
- Replace injection port liner as needed.

36.8.5 Semi-monthly

- Data files will be backed up by copying them to the Department’s intranet and retained there for a period of no more than two years. Sequence files and sequence log files will be backed up by copying them to the Department’s intranet and retained there for a period of five years.

36.8.6 Monthly

- Check PFTBA level and do full source clean, if needed.
- Check mechanical pumps oil level.
- Clean injection port if needed.

36.8.7 Yearly

- Replace GC column as needed.
- Macros, methods, and libraries will be backed up by copying them to the Department’s intranet and retained there for a period of five years.
- Schedule preventive maintenance as required.

36.8.8 Placement of Instrument into Service

36.8.8.1 After significant maintenance has been performed

- Tune and run background, as necessary, as outlined in section 36.8.3.
- Run the standard mixture as outlined in section 36.6.4.

36.8.8.2 New instrument installation

- Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer’s specifications.
- Run blanks with the threshold set to various values, beginning with zero, to determine the proper mass detect threshold setting for the instrument.
- Load/modify appropriate macros and test functionality.

- After methods are created, run a standard on a representative sample of the methods (e.g., low temp., mid temp., high temp., screen methods) to demonstrate efficacy.
- Run either the QA mixture or a cocaine standard ten times to demonstrate chromatographic reproducibility.
- Load applicable user libraries.
- Archive methods and data analysis macros to suitable long-term storage media.
- A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.
- Retain instrument verification documentation.

36.9 FTIR

36.9.1 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

36.9.2 Record all maintenance performed in a logbook, date and initial.

36.9.3 Day of Use

36.9.3.1 For the bench, perform the VAL-Pro iS50 System Qualification Test. This test checks a number of different performance characteristics including collecting polystyrene and NG11 glass spectra as well as checking the peak-to-peak noise using samples mounted in an internal validation wheel. The validation wheel shall be replaced prior to either the expiration of its calibration certificate or five years from the certification date if no expiration date is provided. If any test fails, troubleshoot and re-run VAL-Pro. Do not proceed until this issue is resolved. All copies of the VAL-Pro report, including those with notations of “Fail”, will be generated and retained for 5 years. Record VAL-Pro results in the logbook, date, and initial.

36.9.3.2 For the ATR, perform the Val-Pro Qualification Test for the integrated ATR. This test checks performance characteristics such as wavenumber accuracy and optical resolution using a certified polystyrene standard. In lieu of the polystyrene standard, an uncorrected spectrum of procaine hydrochloride shall be run and searched against a library containing uncorrected spectra with a match of 95% or greater. Record in logbook, date, and initial. A hardcopy of the ValPro Report or standard spectrum and library match will be generated and stored in the laboratory for five years.

36.9.3.3 A record of all samples will be kept in a logbook that includes the date, FS Lab# and/or file name, and the initials of the user.

36.9.4 Weekly: The bench will be aligned weekly using the "Align Bench..." function.

36.9.5 Monthly:

Data files will be backed up by copying them to the Department’s intranet and retained there for a period of no more than two years.

36.9.6 Placement of Instrument into Service

36.9.6.1 After significant maintenance has been performed, align and run the daily QA as outlined above.

36.9.6.2 New instrument installation

- Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer's specifications.
- After experiments are created, run a cocaine base and a cocaine hydrochloride standard on each to demonstrate efficacy. An additional standard of procaine hydrochloride must be run on methods utilizing the ATR attachment.
- Run a cocaine standard ten times to demonstrate reproducibility.
- Archive experiments and macros to suitable long-term storage media.
- A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.
- Retain instrument verification documentation.

36.10 DART-TOF

36.10.1 Record any maintenance performed in the logbook, date and initial.

36.10.2 Daily

36.10.2.1 Start DART and Mass Center or msAxel software, and turn on DART gases and power supply.

36.10.2.2 Turn on DART gas temperature control, load the appropriate tune or method file, and put AccuTOF in "operate" mode.

36.10.2.3 Using the appropriate positive mode switching method, perform calibration and acquire a calibration check spectrum.

- Mass Calibration: generate and save a PEG600 internal mass calibration file.
- Calibration Check: run a positive control mix containing methamphetamine, cocaine, and nefazodone. These three drugs exhibit masses that span the spectral mass range. Acceptance criteria for methamphetamine, cocaine, and nefazodone positive controls: the $[M+H]^+$ peaks shall be within ± 5 mDa of the calculated protonated molecules at 150.1283 Da, 304.1549 Da, and 470.2323 Da, respectively.
- For internal mass drift compensation (when applicable), generate and save a mass drift compensation file on the protonated molecule of cocaine (304.1549 Da). Methamphetamine and nefazodone peaks should also be checked and meet the above acceptance criteria. After saving the mass calibration file and the mass drift compensation file, produce an averaged background subtracted, centroided spectrum of methyl stearate (30V function). Acceptance criterion for methyl stearate: the $[M+H]^+$ peak must be within ± 3 mDa of the calculated protonated molecule at 299.2950 Da.

36.10.2.4 Store copy of daily calibration for at least five years.

36.10.3 Weekly

36.10.3.1 Check rough pump oil level and siphon oil back into pump from mist filter, as needed.

36.10.3.2 Check helium and other gas supplies and replace as needed.

36.10.4 Monthly

36.10.4.1 Clean ion guide components of TOF, as needed, to maintain performance.

36.10.4.2 If ion guide is cleaned using the isolation valve, meaning only the ion optics region is vented while the analyzer remains under vacuum, the daily calibration procedure is performed before placing the instrument back into service. If the ion guide is cleaned and the whole system is vented, after pumping down the system and conditioning the MCP detector, attach electrospray ionization source and infuse 100 ppb reserpine in methanol to check resolution and intensity of reserpine $[M+H]^+$.

- Adjust TOF settings as needed, and save tune or method file. Print screen shot of "Spectrum Monitor" showing resolution and intensity achieved.
- Reset the PEG+H global calibration file, as needed.
- Resave tune or method files with appropriate Orifice1 voltages, as needed.

36.10.4.3 Data files will be backed up by copying them to the Department's intranet and retained there for a period of no more than two years. Spectral libraries and QA data will be backed up by copying them to the Department's intranet and retained there for a period of five years.

36.10.5 Annually

36.10.5.1 Schedule preventative maintenance for DART-TOF system, as required.

36.10.5.2 Perform maintenance on nitrogen generator, as required.

36.10.6 Placement of Instrument into Service

36.10.6.1 After significant maintenance has been performed, run the daily and weekly QA as outlined above.

36.10.6.2 New instrument installation

36.10.6.2.1 Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer's specifications.

36.10.6.2.2 Run QC check mix (methamphetamine, cocaine, nefazodone) 10 times within one data file (using PEG600 and cocaine drift lock calibrations, as needed) using the function switching method (20, 30, 60 and 90 volts). Using the 30V data, determine that all protonated molecules are within ± 5 mDa of their calculated mass.

36.10.6.2.3 Run QC check mix 5 times in individual data files with function switching. Using the 30V data, determine that all protonated molecules are within ± 5 mDa of their calculated mass.

36.10.6.2.4 LLOD studies: Using cocaine, heroin, methamphetamine and alprazolam standards, prepare dilutions in methanol of 0.5, 0.1, 0.05, 0.03 and 0.01 mg/mL. Using function switching, run each dilution 5 times (five data files that contain all four drugs at each dilution level). Determine the dilution level where protonated molecules in the 30V data begin to stray beyond ± 5 mDa.

36.10.6.2.5 Load all applicable libraries for Mass Mountaineer. Using the data from #4, ensure operation of Mass Mountaineer by searching lists with 30V data and matching spectra with 90V data. Ensure that all searches correctly identify the peaks of interest.

36.10.6.2.6 Selectivity check: Run, using function switching, methamphetamine/phentermine, hydromorphone/morphine and cocaine/scopolamine standards. Check to ensure differences can be seen between the pairs of spectra at higher voltages.

36.10.6.2.7 A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.

36.10.6.2.8 Retain instrument verification documentation.

36.11 Refrigerators/Freezers

36.11.1 The temperature of refrigerators and freezers that store reagents, standards or evidentiary material should be checked and recorded on a weekly basis. The *CS Refrigerator Temperature Log* and the *CS Freezer Temperature Log* should be used for this purpose.

36.11.2 For refrigerators, the temperature shall be between 2 – 8ºC.

36.11.3 For freezers, the temperature shall be below -5ºC.

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

36.11.4.1 Critical reagents and standards should be re-verified if the temperature in the refrigerator exceeds 15ºC or the freezer exceeds 0ºC prior to use in casework.

36.11.4.2 Refrigerator and/or freezer temperature records will be retained pursuant to the Library of Virginia Specific Schedule Number 778-001 (Quality Assurance: Equipment, Standards, and Instruments 200540 series).

36.12 DiscovIR GC-FTIR

36.12.1 Record any maintenance performed in the logbook, date and initial.

36.12.2 Day of Use.

36.12.2.1 Refer to the DiscovIR Instructions for Daily Start Up.

36.12.2.2 Run “IR signal” function and record the Maximum Interferometer Voltage after adding liquid nitrogen to the detector. Range: 4 – 7.5 volts.

36.12.2.3 Run “Noise” function and record the average of five scans. Range: 0 – 2.0 mAbs.

36.12.2.4 Run “WN Accuracy” function. The 1601 cm^{-1} peak must be 1601 ± 2 cm^{-1} and have a height of at least 400. If the peak height is less than 400, adjust the polystyrene film and repeat.

36.12.2.5 Fill liquid nitrogen Dewar and allow all temperatures to reach set points (see ¶ 14.3.4).

36.12.2.6 Run a blank and a mixture of AB-CHMINACA, Methamphetamine, Cocaine, and Pseudoephedrine standards on an appropriate method. The concentration of these standards should be approximately 1-2 mg/mL.

36.12.3 Monthly

36.12.3.1 Run a blank and a mixture of AB-CHMINACA, Methamphetamine, Cocaine and Pseudoephedrine standards on an appropriate method. The concentration of these standards should be approximately 1-2 mg/mL.

- Record in logbook, date and initial. The chromatogram should demonstrate good chromatographic performance. Acceptable results must be documented either in the logbook or on the data.
- Any performance discrepancies or degradation must be reported immediately to a supervisor.
- Store hardcopy of data for approximately one year.

36.12.3.2 Set the instrument to standby conditions and perform a system reboot by power cycling the computer.

36.12.3.3 Change septum or Merlin Microseal, as needed.

36.12.3.4 Change injection port liner as needed.

36.12.3.5 Clean disk as needed.

36.12.3.6 Data files will be backed up by copying them to the Department's intranet and retained there for a period of no more than two years. Sequence files (.csv and .squ) will be backed up by copying them to the Department's intranet and retained there for a period of five years.

36.12.4 Placement of Instrument into Service

36.12.4.1 After significant maintenance has been performed

- Perform daily QA as outlined in ¶ 36.12.2.
- Run the blank and standard mixture as outlined in ¶ 36.12.3.1.

36.12.4.2 New instrument installation

36.12.4.2.1 Obtain documentation from the instrument service representative that demonstrates that the instrument performs to manufacturer's specifications.

36.12.4.2.2 Create methods listed in ¶ 14.3.4.3. After methods are created, run a standard on each of the methods to demonstrate efficacy.

- Low: Amphetamine
- Mid: MDPV
- High: a cannabimimetic agent
- Screen: QA mixture

36.12.4.2.3 Run the QA mixture ten times to demonstrate chromatographic reproducibility.

36.12.4.2.4 Load applicable user libraries.

36.12.4.2.5 Archive methods to suitable long-term storage media.

36.12.4.2.6 A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.

36.12.4.2.7 Retain instrument verification documentation.

36.13 Glassware

Glassware used to prepare calibrators and check standards shall be calibrated by an external vendor that is accredited to ISO/IEC 17025 and whose scope of accreditation covers the calibration performed. New volumetric flasks, requiring measurement traceability, shall be calibrated prior to being placed into service. Calibration certificates shall be reviewed for accuracy by the Section Supervisor, Group Supervisor, or designee prior to placing the flask into service. Guidance on *Calibration Certificate Review* is available in the Resources/Pipettes folder in Qualtrax. Volumetric glassware shall be visually inspected prior to each use.

36.14 Mechanical Pipettes

36.14.1 Pipettes shall be calibrated annually by an external vendor that is accredited to ISO/IEC 17025 and whose scope of accreditation covers the calibration performed. New pipettes shall be calibrated prior to being placed into service. Calibration certificates shall be reviewed for accuracy by the Section Supervisor, Group Supervisor, or designee prior to placing the pipette into service. Guidance on *Calibration Certificate Review* is available in the Resources/Pipettes folder in Qualtrax.

36.14.2 Repeater and adjustable volume pipettes shall have their performance checked on a monthly basis, as well as following a repair or after purchase and placement of pipette into service. Check and record the performance and calibration by weighing volumes of water with an analytical balance using one of the following two methods:

36.14.2.1 Use of the *Adjustable Pipet Calibration Check Worksheet*: Ten measurements will be taken at two different volumes (e.g., 20% and 80% of maximum volume). Record the QC data on the worksheet, which is available in the Resources/Pipettes folder in Qualtrax. Acceptance criteria:

36.14.2.1.1 The pipette precision must be within ± 5% coefficient of variation (CV) and inaccuracy (accuracy error) must be within ± 5% for both volumes checked to be considered passing.

36.14.2.1.2 For small volume pipettes (20 µL or less), pipettes must be within ± 10% CV and ± 10% accuracy error for both volumes checked to be considered passing.

36.14.2.1.3 If the pipette fails these checks, the pipette will be sent to the manufacturer for repair or the external calibration vendor per ¶ 36.14.1. Repair and/or maintenance of pipettes will be recorded in a log maintained for pipettes.

36.14.2.2 Use of the Rainin SmartCheck: Four aliquots at two different volumes will be used to check pipette performance. The QC data should be recorded with the use of the *CS SmartCheck and Pipette QA Log (Electronic)*. Acceptance criteria:

36.14.2.2.1 Both volumes must pass, indicating the pipette is dispensing within the ± 5% tolerance. Guidance for volume selection:

- There are six fixed volume options available on the SmartCheck, which range from 20 µL to 1000 µL.
- In general, use the following volumes to check the pipette performance.

Pipette's Calibrated Volume Range	SmartCheck Available Volumes w/in Range	Recommended Volume(s) to Check
100 – 1000 µL	100, 200, 300, 1000 µL	200 and 1000 µL
50 – 250 µL	50, 100, 200 µL	50 and 200 µL

10 – 100 µL	20, 50, 100 µL	20 and 100 µL

36.14.2.2.2 This method cannot be used for checking pipettes in which two volumes are not available for evaluation within the pipette range.

36.14.2.2.3 If the pipette fails either of these checks, the pipette will be re-checked using the *Adjustable Pipet Calibration Check Worksheet* method above.

36.14.3 The SmartCheck device performance shall be verified at least monthly, prior to use in pipette performance checks. The verification is performed with the use of the weigh pan adapter, draft shield, and a traceable 1 g weight and recorded on the *CS SmartCheck and Pipette QA Log (Electronic)*. The 1 g weight shall be weighed with 10 repetitions.

36.14.3.1 Maintenance:

36.14.3.1.1 The housing shall be cleaned as needed with alcohol, isopropanol, or with a soapy water solution. Chemicals such as acetone will damage the surface and shall not be used.

36.14.3.1.2 At a minimum, empty the SmartCheck vessel when the device indicates it is full. More frequent emptying, such as after the monthly checks, is recommended.

36.14.3.1.3 SmartCheck devices used to check the performance of mechanical pipettes shall be serviced annually and calibrated as needed by an external vendor that is accredited to ISO/IEC 17025 and whose scope of accreditation covers the calibration performed.

36.15 Heat Blocks and Ovens

The temperature of ovens and heat blocks used during the SIM quantitative analysis of Cannabis plant material will be checked prior to use with casework and the value recorded on a log sheet kept with the equipment.

36.15.1 The thermometers used for the temperature checks will be NIST traceable. Thermometers shall be replaced prior to their calibration due date (typically every two years).

36.15.2 If the NIST traceable thermometer temperature is not consistent with the temperature display on the equipment, then the thermostat will be adjusted to obtain the correct temperature. Following adjustment, the temperature will be measured and this value recorded. The correct temperature shall be achieved prior to utilization with casework.

36.15.3 Documentation shall be maintained with the respective temperature log that associates the serial number of the NIST traceable thermometer with the corresponding oven or heat block.

36.15.4 Any maintenance or repair will be documented in the respective temperature log.

36.15.5 Temperature log(s) and thermometer certificates will be retained pursuant to the Library of Virginia Specific Schedule Number 778-001 (Quality Assurance: Equipment, Standards, and Instruments 200540 series).

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See Training Manual for additional references

38 SCHEDULING

38.1 Schedule I

38.1.1 High potential for abuse with no legal medical use in the U.S.

38.1.2 Examples include: Heroin, MDA, LSD, mescaline, peyote, psilocybin, and psilocyn

38.2 Schedule II

38.2.1 High potential for abuse, have some medical use, use may lead to severe dependence

38.2.2 Examples include: Cocaine, opium, morphine, codeine, oxycodone, PCP, methamphetamine, amphetamine, amobarbital, secobarbital, and pentobarbital

38.3 Schedule III

38.3.1 Lower potential for abuse than Schedule II substances, have a medical use, cause some dependence

38.3.2 Examples include: Most barbiturates, some codeine preparations, ketamine, phendimetrazine, and anabolic steroids

38.4 Schedule IV

38.4.1 Low potential for abuse, have a medical use, can cause low dependence

38.4.2 Examples include: Barbital, diazepam, meprobamate, phenobarbital, chlordiazepoxide, pentazocine, and benzodiazepines

38.5 Schedule V

38.5.1 Similar to Schedule IV but less potential for abuse and less dependence

38.5.2 Examples include: Low concentrations of codeine, ethylmorphine or opium, and numerous cough syrups

38.6 Schedule VI

38.6.1 All other drugs requiring a prescription

38.6.2 Examples include: Antibiotics, antihistamines, tricyclic antidepressants

38.7 If there is any question as to the scheduling of a particular substance, refer to the current Code of Virginia. If any question still remains, report only the identity of the material and leave the scheduling to the court system.

39 LEGAL THRESHOLDS FOR WEIGHT AND COUNT

39.1 Any Schedule I or II Controlled Substance

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth

39.2 Coca Leaves

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

39.3 Cocaine

1 ounce (28.35 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture) § 18.2-248.01: transportation of controlled substances into Commonwealth
½ pound (226.8 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture)
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

39.4 Cocaine Base

1 ounce (28.35 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture) § 18.2-248.01: transportation of controlled substances into Commonwealth
½ pound (226.8 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture)
250 grams	§ 18.2-248: distribution or possession with intent to distribute
2.5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute

39.5 Ecgonine

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

39.6 Heroin

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
100 grams	§ 18.2-248: distribution or possession with intent to distribute
1 kilogram	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute

39.7 Marijuana

½ ounce	§ 18.2-255.2: sale of drugs on or near certain properties
1 ounce	§ 4.1-1100: possession by persons 21 years of age or older § 18.2-248.1: marijuana distribution § 18.2-255: distribution to persons under 18
4 ounces	§ 4.1-1100: possession by persons 21 years of age or older
1 pound	§ 4.1-1100: possession by persons 21 years of age or older § 18.2-308.4: possession of firearms and certain Controlled Substances
5 pounds	§ 18.2-248.01: transportation of controlled substances into Commonwealth § 18.2-248.1 marijuana distribution
100 kilograms	§ 18.2-248: distribution or possession with intent to distribute
250 kilograms	§ 18.2-248: distribution or possession with intent to distribute
5 plants	§ 4.1-1101 home cultivation of marijuana for personal use
11 plants	§ 4.1-1101 home cultivation of marijuana for personal use
50 plants	§ 4.1-1101 home cultivation of marijuana for personal use
101 plants	§ 4.1-1101 home cultivation of marijuana for personal use

39.8 Methamphetamine (methamphetamine, its salts, isomers, or salts of its isomers)*

10 grams	§ 18.2-248: distribution or possession with intent to distribute
1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
100 grams	§ 18.2-248: distribution or possession with intent to distribute
250 grams	§ 18.2-248: distribution or possession with intent to distribute

(*quantitation will only be performed at the request of a Commonwealth's Attorney)

39.9 Methamphetamine (a mixture or substance containing a detectable amount)

20 grams	§ 18.2-248: distribution or possession with intent to distribute
28 grams	§ 18.2-248.03: Manufacturing, distribution or intent to manufacture or distribute
1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
200 grams	§ 18.2-248: distribution or possession with intent to distribute
227 grams	§ 18.2-248.03: Manufacturing, distribution or intent to manufacture or distribute
1 kilogram	§ 18.2-248: distribution or possession with intent to distribute

40 HYPERGEOMETRIC TABLE

Population (N) Nmax=1000	Proportion of Positives = 90% Confidence Level=95%	Population (N) Nmax=1000	Proportion of Positives = 90% Confidence Level=95%
1 – 10	ALL	40	18
11	9	41	18
12	9	42	18
13	10	43	19
14	11	44	19
15	12	45	20
16	12	46	20
17	13	47	21
18	14	48	21
19	15	49	22
20	12	50-59	23
21	13	60-69	23
22	14	70-79	24
23	14	80-89	25
24	15	90-99	25
25	16	100-199	27
26	16	200-1000	28
27	17		
28	18		
29	18		
30	15		
31	16		
32	17		
33	17		
34	18		
35	18		
36	19		
37	19		
38	20		
39	20		

Note: For sample populations greater than 1000 please refer to “Calculator for Qualitative Sampling of Seized Drugs”, “Hypg_Proportion” tab, (version July 2017). The value for Nmax may need to be changed for populations over 1000.

Reference

Table derived from European Network of Forensic Science Institutes Drug Working Group (ENFSI DWG) “Calculator for Qualitative Sampling of Seized Drugs” (version July 2017).

European Network of Forensic Science Institutes Drug Working Group (ENFSI DWG) “Calculator for Qualitative Sampling of Seized Drugs” (version July 2017)

http://enfsi.eu/wp-content/uploads/2017/06/ENFSI-DWG-Qualitative-Sampling-Calculator-Revision-July-2017xls2007-20....xlsm

41 COMMONLY USED ABBREVIATIONS

The following is a list of abbreviations commonly used by examiners in the Controlled Substances Section. This list has been generated to assist in the interpretation of case file notes and is not a standardized list of required abbreviations. The abbreviations are appropriate written in either lower or upper case and they are appropriate with or without punctuation such as periods or hyphens. Common chemical formulas, chemical, mathematical and shorthand abbreviations are equally acceptable and will not be listed here.

Definitions	Abbreviations
Acetic Anhydride	AA
Alexandria	Alex
Alternate Non-Aqueous Organic Ratio Extraction	ANOR
Ammonia saturated chloroform	ASC
Ammonia saturated hexane	ASH
Arlington County	ARL
autosampler vial	ASV
Background	Bkg
Bag	b, bg
Because	Bc
Black	Bl
Blank	Blk
Bleed	Bld
blotter paper	blot pap, blp
Board of Pharmacy	BoP
Bottle	btl, bot
brought sample back up in solvent	↑
Brown	Brn
Burnt residue	Br
Canister	Cn
Capsule	cap, cp, cps
Cardboard box	Cdbx
Cartridge	ctg
Cellophane	cel, cell, cello
Charred	chr, char
Chunky	Chy
Cigar	cgr
cigarette	cig
Clear	Clr
Column	Col
color test(s)	Ct
commercial	com
compressed	cmps
Concentrated	Conc
Concentration of cannabinoid(s) not determined	CCND
Container	Cont
Containing	c/, =>, c (with a line over it), →

Continued	Cont'd
Corner	C
cross contamination	x-cont, x-contam
Crystalline	cryst, xtalline
Controlled substance	CS
crystalline material	CM
cystolithic hairs	Cyst, ch, cyst hair
Currency	cur
Dark	Dk
Data not used	DNU
Development	devel
Device	dev
Difference	dif
Diluted	dil
Dimethyl sulfone	DMSO2
dimethyl terephthalate	DMTP
dried down sample	↓
Drug Identification Bible	DIB
Duquenois-Levine	D-L
Each	ea
Effervescence	eff, effer, efferv
Electronic cigarette	e-cig
Electronic smoking device	ESD
Electronic smoking device cartridge	ESDC
Empty	MT
Envelope	Env
Evidence	ev, evid, evd, e
Extract	ext
Extraction	xt
Extraction blank	EB
Fairfax County	FFX
Filtered	Fil
Fingerprints	LX
Fluorescence	flu
fragment(s)	frag
General	Gen or G
Glass tube	gt
Glass	gl
Glassine	glas, gla
Green	gr, grn
Gross weight	GW
hand made	hdm
hand rolled	hr
Heat	Δ, H
Heat-sealed	HS or H-S

Hexane	C6 or Hex
Imprinted	imp
Including innermost packaging	iip
Individual	ind
Insert (for weak samples in ASV)	INS
Instrumental Data for Drug Analysis by Mills *et al.*	IDDA
Insufficient	IF
Integrate	int
Internal Standard	IS
Juvenile	juv
Knotted	k, kn
Large	lge, lg
leafy material	lm
Light	lt
Laboratory Information Management System	LIMS
Listed to contain	ltc
Liquid	liq
Lottery paper packet	lpp
Manila	man
Material	mat, mat'l
Metal	met
Methanol	MeOH
microscopic examination	micro
multi-colored	mc, multi
Mushrooms	mush
negative Result for a test	-, circled -, neg
Net weight	NW
nitroprusside color test	np
no change	nc
no color reaction	ncr
no reaction	nr
no significant reaction	nsr
not analyzed	na, not anal., nap, nt, DIRP
not determined	ND
not examined	ne
not opened	n/o, no
off-white	off-wh, ow
One plastic container which contained one glass container which contained	clp
Orange rectangular sublingual film	ORS
Pack	pk
Package	pkg
Packet	pkt
Paper	pap
Pentane	pent
physical identification code for brand name product	PIB, PIBr

physical identification code for generic product	PIG
physically identified with references	PID
Physicians' Desk Reference	PDR
placed in zip(s) in lab	PIZIL
plant material (only)	pm
Plastic	p, plas, pls, plst
Plastic Safety Tube	PST
Powder	pwd, pW, pwdr
Precipitate	ppt
Prescription	Rx
Prince William County	Pr W
Probable	prob
Purple	prpl, pur, pl, ppl
quantity insufficient	qns
Reaction	rxn
Rectangular	rec
Remaining	rem
reopened to return	rtr
repackage(d)	repkged
Residue	res
Returned	ret
Rock-like	rl
rolling papers	roll pap
round	rnd
Sample	Sam
schedule	Sch, sched
Schedule I	CI
Schedule II	CII
Schedule III	CIII
Schedule IV	CIV
Schedule V	CV
Schedule VI	CVI
Sealed	"circled" word or acronym, s (in front of another code)
Septum	Sep
smoking device	sd, smok dev, s-d
Solid	sol
solid material	sm
Solvent	solv
Strong	str
Sublingual film	SF
Substance	subst
Syringe	syr
tablet(s)	tab(s)
Threshold	thr
twist-tied	tt

Typical	typ
Vacuum	vac
Vacuum sealed	v-s, vac-s
Very	v
Vial	vl
Violet	vio
Visual Identification	VIS-ID
Volume	vol
Weak	wk
Which contained(s)	w/c
White	wh, wht
Wrapper	wrap
Yellow	y, yell, yl
Ziplock	zip, z

Drug Name	**Abbreviation**
3,4-Methylenedioxyamphetamine	MDA
3,4-Methylenedioxyethcathinone	MDEC
3.4-Methylenedioxymethamphetamine	MDM, MDMA
6-Monoacetylmorphine	MAM, 6-MAM
Acetaminophen	AC, APAP
Acetylcodeine	ACC
Acetyl fentanyl	AceF
Acryl fentanyl	AFEN
Alprazolam	AL, ALP
Amphetamine	A, AMP
Aspirin	ASP, ASA
Benzocaine	BNZ, BENZO
Bufotenine	BUF
Buprenorphine	BUP
Benzylpiperazine	BZP
Bromazolam	BZL
Caffeine	CAF
Cannabidiol	CBD
Cannabinoid(s)	cann
Cannabinol	CBN
Cannabimimetic agent	CMA, CA
Cannabis	CBS
Carisoprodol	CAR
Clonazolam	CZL
Clonazepam	CZM
Cocaine	CO, COC
Cocaine base	CB, CFB
Cocaine Hydrochloride	COH
Codeine	COD

Cyclobenzaprine	CBP
1,4-cyclohexadienyl-2-methaminopropane	CMP
Dextromethorphan	DXM
Diazepam	DZM
Dibutylone	DBU
Diltiazem	DLT
Dimethyltryptamine	DMT
Diphenhydramine	DPH
Ephedrine	EP
Etizolam	ETZ
Eutylone	EUT
Fentanyl	FENT, FEN
Flualprazolam	FALP
Flubromazepam	FBZP
Flubromazolam	FBZL
Fluoroisobutyryl fentanyl	FIBF
Furanyl fentanyl	FUF
Gabapentin	GAB
Gamma-butyrolactone	GBL
Gamma-hydroxybutyric acid	GHB
Guaifenesin	GUI
Heroin	HER
Hydrocodone	HC, HYD
Hydromorphone	HM
Ibuprofen	IB
Insufficient for analysis	IFA
Insufficient for identification	IFI
Ketamine	KET
Levamisole	LEV
Lidocaine	LIDO
Lisdexamfetamine	LIS
Lorazepam	LO
Lysergic acid diethylamide	LSD
Marijuana	MJ
Medetomidine	MDT
meta-fluorofentanyl	m-FF
Methadone	MN, MDN
Methamphetamine	M, METH
3,4-Methylenedioxymethcathinone	MDMC
Methylphenidate	MPH
Miscellaneous	MIS
Morphine	MO
Naloxone	NAL
N-Ethylpentylone	NEP
Nicotine	NIC

N,N-Dimethylpentylone	DMP
No controlled substance identified	NCSI, NCI
No controlled substances found	NCS, NCSF
Noscapine	NOS
ortho-fluorofentanyl	o-FF
Oxycodone	OC, OXY
Oxymorphone	OX, OM
Papaverine	PAV
para-fluorobutyryl fentanyl	p-FBF
para-fluorofentanyl	p-FF
para-fluoroisobutyryl fentanyl	p-FIBF
Pentylone	PENT
Pethidine (Meperidine)	PE
Phenacetin	PHN
Phencyclidine, liquid	PCL
Phencyclidine	PCP
Phenylpropanolamine	PPA
Previously analyzed	PRE
Procaine	PR, PRO
Promethazine	PRT
Propoxyphene	PRP
Pseudoephedrine	PSE, PSU
Psilocybin	PSB
Psilocyn	PS
Quinine	QUI
Tetrahydrocannabinol	THC
Tetrahydrocannabinolic Acid	THCA
Tetramisole	TET
Tramadol	TRA
Tropacocaine	TRO
Trifluoromethylphenylpiperazine	TFMPP
Xylazine	XYL
Zolpidem	ZOL

Any drug abbreviations listed in the Virginia Code need not be listed to be used as an abbreviation.

Appendix A: Evaluation and Estimation of Weight Measurement Uncertainty Records

This summarizes the records required for the Estimation of Uncertainty of Measurement as it applies to drug weights in the Controlled Substances Section. The annual Uncertainty of Measurement update shall be completed and the estimations shall reflect the current measurement assurance data and calibration information.

1.) Measurand: The weight of evidentiary samples submitted for controlled substances analysis
2.) Traceability of measurement:
    a. Balance calibration: The traceability is established by the annual, external calibration of the balances by an ISO/IEC 17025 accredited calibration provider. The certificate and scope of the provider is kept by Quality Assurance. Certificates of calibration for each balance are kept in the laboratory.
    b. Steel weight calibration: Additional traceability is established by the use of steel calibration weights to check the balance calibration. The weights are calibrated every three years by an external, ISO/IEC 17025 accredited calibration provider. The certificate and scope of the provider is kept by Quality Assurance. Certificates of calibration for each weight and weight set are kept in the laboratory.
3.) Equipment used:
    a. Calibrated balances (5-Place Analytical, 4-Place Analytical, 3-Place Top Loading, 2-Place Top Loading, and High Capacity)
    b. Calibrated, traceable steel weights
    c. Bags of sand for measurement assurance
    d. Log worksheet for calibration checks
    e. Spreadsheet for measurement assurance
4.) Uncertainty components considered and evaluated:
    a. Type A Evaluation: Measurement process - standard deviation from measurement assurance values
        i. Multiple analysts differing in years of experience
        ii. Multiple balances of same readability specification
        iii. Measurements taken in multiple laboratories
        iv. Balances placed in different locations within laboratories
        v. Differences in static vs. dynamic measurement processes
        vi. Differences in weigh vessels
        vii. Static electricity
        viii. Drafts
        ix. Vibration
        x. Humidity
        xi. Temperature
        xii. Display resolution – For balances with at least 100 values of measurement assurance data, this is covered by the Type A Evaluation of process reproducibility data
        xiii. Eccentricity (location of placement of object on pan)
    b. Type B Evaluation: Digital Display Resolution – for balance types with less than 100 measurement assurance values
    c. Type B Evaluation: Buoyancy – Buoyancy is not evaluated. (See AL-PD3063 Ver 1.0, page 13)
    d. Type B Evaluation: Uncertainty of Measurement for balance calibration
        i. Balances were grouped by readability and included balances from all four laboratories. The highest balance calibration uncertainty for each group was used in the calculations for the most conservative estimation.
    e. Type B Evaluation: Uncertainty of Measurement of steel weight calibration
        i. Steel weight calibration uncertainties were grouped for each balance readability type. The highest steel weight calibration uncertainty for each weight used in each balance group was utilized in the calculations for the most conservative estimation.
    f. Type B Evaluation: Balance Linearity (maximum deviation allowed for calibration checks)
        i. The largest weight tolerance was used for the most conservative estimation.

5.) Data used to estimate repeatability/reproducibility

a. While Measurement Assurance data has been collected over a longer period of time, only data collected during a two-year time span was used, as indicated on the uncertainty budgets. Bags containing sand were heat sealed to minimize loss and change in the contents. Each laboratory was assigned one set of sand bags of varying weights for use on each type of balance. Each examiner weighed the sand bags weekly using the weighing method that they most often used in casework, as appropriate for net weights. The weights and method of weighing were entered into a spreadsheet for analysis. Available methods are:

- Weigh paper – dynamic
- Weigh boat – dynamic
- Plastic beaker – dynamic
- Weigh paper – static
- Weigh boat – static
- Plastic beaker – static

b. Analysis of Measurement Assurance data

i. For 4- and 5-place analytical balances, the method of weighing with vessels other than weigh paper was investigated. The Standard Operating Procedure currently specifies plastic weigh boats may not be used on either the 4- or 5-place balances and that plastic beakers may not be used on 5-place balances. Therefore, only appropriate vessels are used in uncertainty estimation calculations.

ii. Static vs. dynamic weighing: SWGDRUG SD-3 states that for static weighing processes, if the correlation coefficient is not known between the two separate weighing events (i.e., the taring of the weigh container and the weighing of the material), then the most conservative approach would be to assume that the two weighing events are completely negatively correlated and assign r_1 as a value of -1.

$$u_{total} = \sqrt{2 - 2r_1} * u_{combined}$$

This would therefore require the combined uncertainty to be multiplied by 2 prior to expansion.

Prior to analysis of the measurement assurance data from the Central Laboratory and Eastern Laboratory indicated that the values obtained from both the dynamic and static weighing processes on the 4-place analytical balance were similar enough to assume them uncorrelated.

Central Laboratory 4-Place Analytical Balance Data

Tared Weigh Paper (Static)		Tared Weigh Paper (Dynamic)	
Minimum	2.6378	Minimum	2.6379
Maximum	2.6393	Maximum	2.6419
Average	2.6389	Average	2.6390
Standard Dev	0.000208051	Standard Dev	0.000251516
Count	107	Count	662

Tared Plastic Beaker (Static)		Tared Plastic Beaker (Dynamic)	
Minimum	2.5583	Minimum	2.5589
Maximum	2.5594	Maximum	2.5595
Average	2.5591	Average	2.5592
Standard Dev	0.000179117	Standard Dev	0.000205653
Count	53	Count	53

Tared Microcentrifuge Tube (Static)		Tared Microcentrifuge Tube (Dynamic)	
Minimum	2.5586	Minimum	2.5587
Maximum	2.5597	Maximum	2.5596
Average	2.5591	Average	2.5592
Standard Dev	0.000228836	Standard Dev	0.000181331
Count	53	Count	53

Tared Glass Test Tube (Static)		Tared Glass Test Tube (Dynamic)	
Minimum	2.5584	Minimum	2.5589
Maximum	2.5594	Maximum	2.5589
Average	2.5591	Average	2.5592
Standard Dev	0.000241578	Standard Dev	0.000151097
Count	53	Count	53

Eastern Laboratory 4-Place Analytical Balance Data

Tared Weigh Paper (Static)		Tared Weigh Paper (Dynamic)	
Minimum	2.6169	Minimum	2.6156
Maximum	2.6181	Maximum	2.6183
Average	2.6174	Average	2.6175
Standard Dev	0.000192875	Standard Dev	0.000236728
Count	166	Count	394

Therefore, DFS assigns r_1 as a value of +1, which makes the formula the same for both dynamic and static processes.

Measurement assurance data from the Central Laboratory indicated that values obtained from the dynamic and static weighing processes on the 5-place analytical balance were not similar enough to assume them uncorrelated, and therefore, only dynamic weighing will be used on the 5-place balance.

c. Calculations: See *UoM DX* folder in *Quality System Documents* for Budgets and Calculations for each type of balance

d. For each balance, calculations are performed to combine individual uncertainties and then expanded using a coverage factor of k=2 which results in a 95.45% coverage probability.

6.) Uncertainty of Measurement Estimation Reviews:

a. Estimated uncertainties will be reviewed on an annual basis using updated measurement assurance data (Type A evaluation). In addition, sources that are evaluated as Type B will also be reviewed to ensure that no changes have occurred.

b. Balances purchased prior to the annual review will be evaluated before being put into service for casework to ensure that the balance meets the requirements for accuracy and precision as well as being within the current estimated uncertainty.

Appendix B: Evaluation and Estimation of Uncertainty of Measurement: Quantitative Analysis of Total THC in Plant Material using GC/MS (SIM)

This summarizes the records required for the Estimation of Uncertainty of Measurement as it applies to the quantitative analysis results of total THC in plant material using GC/MS (SIM) in the Controlled Substances Section. The annual Uncertainty of Measurement update shall be completed and the estimations shall reflect the current measurement assurance data and calibration information.

1.) Measurand: The quantitative analysis of total THC using GC/MS (SIM) in evidentiary plant material samples submitted for controlled substances analysis.
2.) Traceability of Measurement:
    a. Delta-9-Tetrahydrocannabinol and Hemp certified reference materials: Traceability is established by the procurement of certified reference materials for use as calibration and check standards from an appropriately accredited reference material provider. The certificate and scope of the provider is kept by Quality Assurance. Certificates of Analysis for each certified reference material are kept in the laboratory.
    b. Calibration of pipettes: Traceability is maintained by the utilization of mechanical pipettes that are externally calibrated annually by an appropriately accredited calibration provider and their performance is checked monthly. The calibration certificate and scope of the provider is kept by Quality Assurance. Certificates of calibration and the performance checks of each mechanical pipette are kept in the laboratory.
    c. Balance Traceability:
        i. Balance Calibration: The traceability is established by the annual, external calibration of the balances by an appropriately accredited calibration provider. The certificate and scope of the provider is kept by Quality Assurance. Certificates of Calibration for each balance are kept in the laboratory.
        ii. Steel weight calibration: Additional traceability is maintained by the use of steel calibration weights to check the balance calibration weekly. The weights are calibrated every three years by an external, appropriately accredited calibration provider. The certificate and scope of the provider is kept by Quality Assurance. Certificates of calibration for each weight and weight set are kept in the laboratory.
3.) Equipment used:
    a. Silanized glassware
    b. Vortex mixer
    c. Sonicator
    d. Centrifuge
    e. Mill grinder and disposable grinder cups
    f. GC autosampler vials (with or without inserts)
    g. Vial seal crimper
    h. Gas chromatograph with data system, mass spectrometer and autosamplers
    i. Capillary columns. DB-5MS (or an equivalent), 15 meters long x 0.25 mm i.d. x 0.25 µm film thickness.
    j. Calibrated balances
    k. Calibrated steel weights
    l. Calibrated mechanical pipettes
    m. Class A volumetric flasks (for Internal Standard preparation)
    n. Certified reference materials
4.) Uncertainty components considered and evaluated:
    a. Type A Evaluation: Measurement process – relative standard deviation from measurement assurance values. The √2 was used as the divisor for the Type A evaluation since case samples are analyzed in duplicate but measurement assurance controls are analyzed with a single analysis.
        i. Multiple analysts differing in years of experience

ii. Multiple mechanical pipettes in multiple laboratories
iii. Multiple gas chromatograph systems with mass spectrometer detectors and autosamplers
iv. Multiple grinder mills in multiple laboratories
v. Multiple ovens and heat blocks in multiple laboratories
vi. Multiple vortex mixers in multiple laboratories
vii. Multiple centrifuges and sonicators in multiple laboratories
viii. Measurements taken in multiple laboratories
ix. Multiple glass autosampler vials
x. Multiple lot numbers of THC and Hemp certified reference materials
xi. Humidity
xii. Temperature

b. Type B Evaluation: Certificates of Analysis for THC reference materials
    i. The certificates of analysis from all four laboratories for the certified reference materials used for the calibration standard were considered. From the certificates of analysis, the highest uncertainty was used in the estimation.

c. Type B Evaluation: Pipette standard for preparation of THC working standard
    i. For preparation of the THC working standard, 100 µL of the THC standard is transferred into an autosampler vial. All pipettes from all four laboratories capable of dispensing 100 µL were considered, with the exclusion of Eppendorf Repeater pipettes as these pipettes are not used for the preparation of this standard. The pipette uncertainty with the highest relative uncertainty at 100 µL was used in the estimation.

d. Type B Evaluation: Pipette solvent for preparation of THC working standard
    i. The THC working standard preparation also requires that the THC standard above be diluted with 900 µL of 80:20 acetonitrile:methanol in an autosampler vial. As such, all pipettes from all four laboratories capable of dispensing 900 µL were considered, but the pipette uncertainty with the highest relative uncertainty nearest to 900 µL was used in the estimation.
e. Type B Evaluation: Pipette for preparation of calibration curve
    i. To prepare the calibration curve, volumes ranging from 15 – 63 µL of THC standard are dispensed. As such, all pipettes from all four laboratories capable of dispensing each necessary volume were considered, but the pipette uncertainty with the highest relative uncertainty nearest to the dispensed volumes of 15, 20, 30, 40, or 63 µL was used in the estimation.
f. Type B Evaluation: Pipette internal standard for plant material and calibrators
    i. For the preparation of the calibration curve, 2500 µL of the internal standard is used for each of the calibrators and controls. As such, all pipettes from all four laboratories capable of dispensing 2500 µL were considered, as well as some pipettes which allow a 2500 µL volume to be dispensed with certain pipette tips. The pipette uncertainty with the highest relative uncertainty nearest to 2500 µL was used in the estimation.
g. Type B Evaluation: Balance uncertainty budget for 4-place analytical balance for weighing samples (See *UoM DX* folder in *Qualtrax* for a Budget and Calculation for balance uncertainty)
    i. The combined, unexpanded uncertainty from the 4-place balance uncertainty of measurement budget was used in the estimation.

5.) Data used to estimate repeatability/reproducibility:
    a. Measurement Assurance data was gathered over the validation period and alongside casework. The minimum normalized concentration, maximum normalized concentration, mean, median, standard deviation and relative standard deviation were calculated. A histogram was created to assess the normality of the historical control data.
    b. Total THC demonstrated a normal distribution when normalized by taking the ratio of the calculated THC content and the calculated total THC content from the certificate of analysis.

c. The percent relative standard deviation for the normalized values was utilized for the historical Type A data.

6.) Calculations: See *UoM DX* folder in *Quality System Documents* for Budgets and Calculations for THC quantitation. Calculations are performed to combine individual uncertainties using root sum and then expanded using a coverage of k=2 which results in a 95.45 % level of confidence.

7.) Uncertainty of Measurement Estimation Reviews:
   a. Estimated uncertainties will be reviewed on an annual basis using updated measurement assurance data (Type A evaluation) as well as updated calibration information for Type B evaluations. In addition, sources that are evaluated as Type B will also be reviewed to ensure that no changes have occurred.
   b. Equipment purchased prior to the annual review will be evaluated before being put into service for casework to ensure it meets the requirements for accuracy and precision as well as being within the current estimated uncertainty.