CONTROLLED SUBSTANCES

ANALYTICAL METHODS

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Revision History

Revision #	Description of Changes
1	Original issue of Combined method: numbering change to fit new template, updating (clarifying) some procedures, and minor grammatical changes
2	Change to section #1: 4.1.2, 4.1.3.1, 4.1.5, 4.1.6, 4.1.7.2, 4.1.7.3, 4.1.7.4, 4.1.7.10, 4.2.1.1, 4.3, 4.4.1.5, 4.4.2.1, 4.4.3, 4.4.4, 4.5.3, 4.5.4, 4.5.5.5, 4.7.2.1, 4.7.3, 4.8, 4.8.1, 4.8.2 Change section #2: 4.1.1 Change section #3: 4.3.3, 4.5.1.2, 4.5.3.1, 4.6.4, added 4.7 Change section #4: 4.15 Change section #5: 4.1.1, 4.3.1, 4.3.2.1, 4.4.4 Change section #6: 4.1, 4.1.1.3, 4.1.2.5, 4.1.2.6, Change section #7: 1.2, 4.5.5.5 Change section #8: 1.3 Change section #11: 1.2
3	Changed section #1, 4.1.5, 4.1.7.3, 4.1.7.10, 4.2.2.1, 4.6.4, dropped 4.4.4, changed section #3, 4.3.4.3, 4.6.4, changed section #5 4.4.2, changed section #6, 4.1, 4.1.1.3, 4.1.2.2, 4.1.2.3, 4.1.2.5, changed section #7, 4.1.1.1, 4.1,2.4, 4.6.4.1, 4.6.4.2 added 4.6.3.3, changed section #8, 4.2.1.2, 4.2.1.3, deleted 3.6, 3.7 section #9, added 3.7, 3.8, 3.9, deleted 4.4.1, 4.4.2, 4.1.3, changed section #11, 4.1.3.1, 4.1.3.2
4	Changed section #1, 4.1, 4.1.1, 4.1.2, 4.1.7.2.1, 4.1.7.3, 4.2.2, 4.4.1.4, 4.4.3, 4.4.3.1, 4.7.2.1 section #2, 4.1.3, 4.4.1.4, section #3, 4.1.3, 4.5.4.1, 4.5.4.2 section #5, 2.3, 4.4.3, 4.4.4, 4.4.5, 4.4.6, section #7, 3.3, 3.4, 4.3, 4.4, 4.6.3.3 renumbered 4.6.3, section #8, 3.2, 3.5, 3.6, section #9, 4.2.1.2, 4.2.2 Added to section #1, 2.2, 4.6.4.2, section #8 4.2.2.1-6 and renumbered Deleted #1, 4.4.3.4
5	Conversion to PDF
6	Section #1 Changed 4.1.2, 4.1.5, 4.1.7(+renumbered),4.1.8.1, 4.4.2.1, 4.4.3.3, 4.5, 4.6.1, 4.6.2, 4.7.2.1, 4.7.2.2, 4.8.1, 4.8.2, added 4.5.5.6, Section#2 4.1.2, Section #3, 4.1.2, 4.3.3, 4.5.4.2, 4.7.2, dropped 3.6, Section #5, 4.2, Section #6, 1.1, 4.1, 4.1.2.2, 4.1.2.3, 4.1.2.5, 4.1.4 dropped 3.1.5, Section #7, 2.1, 4.6.3.3, 4.2.2, dropped 4.1.2.4, Section #9, 3.9, 4.1

7	Added section #13, section #1 changed, 4.7.2.1, changed section #5, 2.3, 4.2.5, 4.3.1, 4.3.2.1, added 4.4.8, dropped 4.2.6
8	Changed section #1, 4.1.2, 4.1.4, 4.1.5, 4.6.1, added 4.1.3.3, section #3, 4.1.1, 4.1.2, 4.1.3, 4.1.2.3, 4.3.3, 4.1.2.5 section #7 added 4.1.3, changed and renumbered 4.6.3 and 4.6.4, section #8 added 4.1.3.1, section #13 4.3.3.2, 4.3.3.7, 4.3.3.8, 4.3.3.9
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10	Changed section #1-4.1, 4.1.3.3, 4.1.5, 4.1.6, 4.1.7.11, 4.4.1.4, 4.4.1.5, 4.4.2.1, 4.4.2.2, 4.4.3.2, 4.5.4, 4.6.3.2, 4.7.3, 4.8 added 4.1.3.4, 4.1.3.5, 4.6.3.3 changed section #3- 4.1.1, 4.3.3, 4.6.4 section 4- 4.1.4, section #6- 4.1.2 section 7- 4.1.2, 4.1.3, 4.3.1
11	Section #1-4.1.5, 4.1.7.12, 4.4.3.1, 4.4.3.2, 4.4.3.5, 4.8.3 deleted 4.4.3.4 added 4.5.6 changed Section #5 - 4.4.1 Changed section #13- 5.15, 4.4.1, 4.4.1.6 and renumbered all

AM #1: General Quality Measures

1.0 Background/References

1.1 This analytical method contains guidelines describing how suspected controlled substance samples are to be analyzed, laboratory reports worded, what to do about analytical methods that are no longer or rarely used, sample and standards destruction, sampling rules, recipes for color reagents, identification criteria and operations of the GC/MS and the FTIR, and procedures for the analysis of controlled substances that require specific handling.

2.0 Scope

2.1 These methods/procedures are specific for controlled substances but can also be used in identifying other drugs and some chemicals used in the manufacturing process. Analysis may be limited by the scope of ISPFS's accreditation, current instrumentation, and certain environmental conditions. These guidelines are a natural evolution of rules and procedures that have been used by ISPFS for years.

2.2 An infrequent test will be by definition one that has not been performed in a specific laboratory in over six (6) months. With the exception of Lysergic Acid Diethylamide (LSD), only analytes that are specifically listed with separate sections of this manual need to be tracked for frequency, i.e. iodine, phosphorus, psilocin, and GHB. Generally these analytes have distinct extraction schemes. When these tests are performed, the positive controls must be analyzed before case samples. It will be up to each laboratory's management to decide how to monitor the frequency of these tests.

3.0 Equipment/Reagents

3.1 Not applicable

4.0 Procedure

4.1 Documentation in case notes
The electronic signature placed on each page of the examination data in the notes is the identification of the person responsible for the examination associated with the data, unless otherwise noted. When hands of the analyst (HOA) is performed, the notes will clearly indicate who the trainee was and what they did. If HOA is documented for an item it designates the trainee completed all steps of analysis for that item, in cases where they did not, then the analyst will specify which steps were done with HOA. In regards to HOA, analysis is defined as when a sample is touched, i.e. described, weighed, extracted etc. By submitting the case for technical review, the analyst acknowledges that the work done is theirs alone, unless otherwise noted.

All analysis will be performed on the date that the evidence was opened unless otherwise documented.

4.1.1 A description of the evidence, including all packaging and any leakage from internal packaging.

4.1.2 Physical description of evidence. Powder, liquid, plant material, etc. Including color, shape, additional residues, any imprint or writing on pills/tablets/capsules, and when used as part of the analytical scheme any imprint or writing on ampoules or manufacturer's packaging. Documenting the description can be done by words, photos or drawings. If a photo is used it must be annotated.

4.1.3 Original weight, number of pills, etc. of sample. See 4.1.5.

4.1.3.1 "Trace" or "residue" will be defined for solids as anything less than 0.10 grams, and for liquids as anything less than approximately 200 µl.

4.1.3.2 All digits observed from a balance will be reported. For balances that normally read to the hundredths place, there may be instances where the sample weight is reported to the tenths place, in those cases the analyst will explain why in the case notes. This applies to reserve weights as well.

4.1.3.3 In lieu of counting individual pills, or "LSD" dosage units, a total weight of the sample is an acceptable alternative. Reserves need to be noted.

4.1.3.4 In cases where an envelope contains multiple items it is permissible to take a composite weight as long as each item is segregated and analyzed separately. In these situations the items must be kept segregated after analysis as well.

4.1.3.5 Compositing of samples for analysis is discouraged and should only be deployed in limited situations such as where individual items may not contain enough residue to confirm the presence of a controlled substance. It must be clear on the report that the result is from a composited sample. It is never permissible to composite samples if the resultant weight is near a regulatory limit. Exception: Methamphetamine Quantitation. Any questions about compositing on a case should be directed to the controlled substances Discipline Leader before analysis begins.

4.1.4 Amount used for analysis, or reserved weights need to be in the notes but do not need to be reported. If more than half of a pill is analyzed, even if there are more pills available, then the extracts must be returned. Trace amounts of residue used do not need to be noted.

4.1.5 Exceptions: If the charge on a marijuana case is based on the number of plants, then the weight of the sample and the reserve does not need to be recorded. Weights and volumes of liquids and the contents of vape cartridges, are not to be reported. If more than half of the visible residue of a sample is to be consumed in analysis then any extracts must be returned with the evidence. Any extracts or washes from evidence that did not have visible residue will also be returned to evidence. This will be listed on the report. If the nature of the sample precludes an accurate weighing (sticky, sludge, moldy etc.) or the sample cannot be easily removed from packaging, then the sample does not need to be weighed but the reason why must be reported. It is acceptable to list "not opened not analyzed" on additional items in an evidence envelope where the contents are not observed.

4.1.6 If present in a sample a schedule 1 narcotic, LSD, or schedule 2 controlled substance will be confirmed if possible. If two or more CI narcotic, LSD, or CII controlled substances are detected in a sample, the component with the largest response must be reported. The presence of the minor component(s) need to be reported with a "indicates" statement if not confirmed. . Minor components with a response less than 10% relative to the major peak do not need to be reported or noted. Minor components that can reasonably be assumed to be a byproduct of the manufacturing process do not need to be noted either, examples include, but are not limited to, morphine and codeine in a heroin sample or p2p from a suspected methamphetamine clan lab.

4.1.7 Conclusions and Reporting

With a few exceptions there are four possible conclusions for controlled substance cases.

4.1.7.1 Following ASTM E2329-17 analytical guidelines, if the results of testing yield positive results then the presence of the named compound is confirmed/identified. When testifying the analyst will say that the sample contained the listed compound without implying purity. Exception; methamphetamine quant cases.

4.1.7.2 If an analyte is below the level of detection (LOD) the report will state "no controlled substances detected". Unless established through a validated procedure LOD's are determined by each analyst's experience. Exception: On a suspected marijuana sample, if using the marijuana method of analysis yields negative results and the sample will be forwarded on for further testing then report, "Marijuana was not detected, results of further analysis will follow."

4.1.7.3 If a controlled substance is present and could be confirmed/identified but the analyst chooses not to, then the conclusion should read "Preliminary testing indicates the presence of (name of drug), not confirmed." The reason why must also be on the report, i.e. lack of standard, presence of a confirmed controlled substance etc.

4.1.7.4 If following ASTM E2329-17 a controlled substance is unable to be confirmed/identified, the report will read "Results of testing for controlled substances were inconclusive". Exception: On a suspected marijuana sample, if using the marijuana method of analysis yields inconclusive results and the sample will be forwarded on for further testing then report, "Results of testing for marijuana were inconclusive, results of further analysis will follow." In both cases the reason why it was inconclusive must be on the report.

4.1.7.5 In the case of a sample that has a mixture of compounds from different schedules it is up to the analyst to determine if a schedule 1 non-narcotic and/or schedule 3-5 compound warrants confirmation. If it does not then the report should note "Preliminary testing indicates the presence of (name of drug), not confirmed." The reason why must also be on the report, i.e. lack of standard, presence of a confirmed controlled substance etc.

4.1.7.6 All controlled substances should be scheduled. Exception; liquid samples in unmarked bottles or pills that have no observable logo/imprint that contain a controlled substance, where the schedule of the sample is dependent on the concentration of that controlled substance(s) should not be scheduled. If a liquid sample comes in a labeled pharmacy bottle or a pill has a recognizable logo and the results of analytical testing indicate the presence of the ingredients on the label, then any schedule associated with that label should be reported.

4.1.7.7 The reporting of non-controlled substances shall be left up to the discretion of the analyst.

4.1.7.8 In order to assist customers with the conversion between metric and English units of measure on marijuana cases, the following statement can be added to the report: 3oz = 85.05g, 1lb = 453.6g

4.1.7.9 In order to report "No Controlled Substances detected", at a minimum, a sample must be run on the GC/MS using a temperature program and extraction scheme that encompasses a wide range of drugs. Refer to AM #3.

4.1.7.10 In multi-item samples, the report must clearly state what and how many items were tested, i.e. "Three white, oblong, tablets, imprinted M365, <u>analyzed one.</u>"

4.1.7.11 If a controlled substance is detected using an analytical scheme that is restricted to a limited range of compounds, the limitation does not need to be listed on the report. However, if the targeted compounds are not detected then that limitation must be clearly stated on the report along with any qualifiers. Example #1: "no basic controlled substances detected" qualifier: "examples of basic drugs include opiates, amphetamines and cocaine". Example #2: "no psilocin/psilocybin detected".

4.1.7.12 If a wash sample/controls are run on a restricted scheme and the sample tests positive for drug XXX, and nothing is detected in the control, then for the control report "No XXX was detected" or add the qualifier (4.1.7.11).

4.1.7.13 If any drug is detected in the control sample, it must be reported.

4.1.7.14 For synthetic cannabinoids that have ambiguous scheduling report out "XXXX, a synthetic cannabinoid".

4.1.8 Records retention

4.1.8.1 Only the documentation used to reach the conclusion need be kept in the case file. These include chromatograms of sample(s), standard(s), library search results, blank(s), TLC plate photos, IR spectra, pill references etc. If a sample has to be reanalyzed, and the original data is not included, the reason must be in the notes. Results of different instrumental techniques must be included in the case file.

4.1.8.2 Current batch documentation will be stored in an area of the laboratory known and accessible to the controlled substances chemists. An example of batch documentation is GC/MS autotunes and weekly test mixes.

4.1.8.3 GC/MS and FTIR data files will be backed up at least monthly.

4.2 Sample and Standard Destruction

4.2.1 Sample Destruction. For the purpose of this section a sample will be defined as any case work related extract, solution, or solid that is not returned to evidence. Standards of non-controlled substances will also be treated using these procedures.

4.2.1.1 Liquids are stored in waste bottles until disposal.

4.2.1.2 Disposal of aqueous liquids shall consist of neutralization of pH followed by solidification of remaining liquid with absorbent material (kitty litter etc.). The bottle and solid will then be discarded with normal trash.

4.2.1.3 Extracted plant material, test tubes, used vials, and TLC plates are placed in the disposable glass containers. Once these containers are full, they are stored until the next scheduled drug evidence burn, where they will be destroyed.

4.2.1.4 Solid (powder) samples can be either washed down the drain, placed in the disposable glass container, or placed in the liquid (aqueous) waste bottle.

4.2.2 Controlled Substance Standard Destruction. For the purpose of this section, a standard (primary, secondary, bench) is defined as any controlled substance used as a reference for confirmatory analysis.

4.2.2.1 When a controlled substance standard needs to be destroyed, i.e. past the expiration date, contamination, or degradation etc., then the standard will be stored until the next scheduled drug burn and destroyed then. Two lab personnel will witness the removal of the standards from the laboratory and fill out any necessary paperwork required by the agency conducting the drug burn. The laboratory standard log will indicate when the standard was destroyed. Any DEA forms will also be filled out and turned over to the proper authorities.

4.2.2.2 If a controlled substance standard is removed from the laboratory by being totally consumed, accidentally destroyed or spilled, the removal should be witnessed by two lab personnel and both individuals should sign and date the standard log.

4.3 Old Analytical Methods

There are numerous analytical or extraction methods that at one time were used in the Forensic Services laboratory system. These methods are not a part of this analytical method and are not to be used until they have been validated and approved.

4.4 Sample Selection

4.4.1 Sampling guidelines allow for the analysis of key evidence items within a case to maximize the resources of the lab. If during the pretrial process it becomes apparent that items not analyzed will require analysis, then upon resubmission that item may receive rush priority.

4.4.1.1 A felony charge has priority over a misdemeanor. Example: a gram of cocaine found in a suspect's pocket will be tested while a gram of marijuana found in the same pocket may not be.

4.4.1.2 A misdemeanor is treated equally to a felony if it is closer to the suspect or was the probable cause for a subsequent search. Example: A gram of marijuana found in a suspect's pocket would be analyzed in addition to a gram of cocaine found in the suspect's car.

4.4.1.3 Based on the analyst's training and experience, if it is suspected different types of felony drugs are submitted, then one of each type will be analyzed. The analyst may use resources such as: statements of fact, description of items as well as visual inspection of items in making this determination.

4.4.1.4 The analyst will analyze evidence associated with the highest charge, i.e. trafficking, manufacturing, delivery vs. felony possession vs. misdemeanor possession.

4.4.1.5 When only a trace level of sample is present, every effort will be made to use less than one half of the sample. If it is necessary to use more than half of the sample, then any extracts, left over liquids, or residues will be returned with the evidence if the remaining sample is insufficient to repeat testing.

4.4.2 Multiple samples

4.4.2.1 For less than regulatory amounts, only one sample needs to be tested. Additional samples may be analyzed as needed based on location, suspect information, or prosecutor request.

4.4.2.2 At regulatory amounts, **ALL** samples will be analyzed until the weight prescribed by law is reached. Example: Forty balloons come in, each with about 0.1g of suspected heroin. The analyst will weigh out enough to get to the first regulatory level, 2.0 g, and analyze each.

4.4.2.3 ONLY the results of the samples actually tested can be reported and testified to. No representation as to the content of the other samples is to be inferred.

4.4.3 Pharmaceuticals (pills, capsules, ampoules, etc.)

4.4.3.1 Scheduled controlled substances in pharmaceuticals need analytical confirmation if analyzed. Exception, if a controlled substance has been analytically confirmed from another sample in the case, then a pharmaceutical(s) listed to contain the same controlled substance only needs a literature search, (section 4.4.3.2). For the purpose of satisfying the "two test, two sampling" rule, described in 4.7.2.1, a literature search or a label from pharmaceutical packaging (ampoules, vials, blister packs, etc.) will be considered a presumptive test. Therapeutic ingredients other than the controlled substance will be noted in the case record if their presence affects scheduling.

4.4.3.2 Non-analytical identifications of pharmaceuticals will read "*source, including year*, if applicable, (PDR, Logo Index, Drugs.com, etc.) lists as "XXXX, not analytically confirmed."

All literature searches shall be documented in the case notes. Information from poison control centers and non-manufacturers' web sites can be used as a preliminary test when further analytical testing is performed or in conjunction with published books or approved websites to delineate pills with similar imprints and descriptions.

4.4.3.3 If an analyst, through training and experience, can make an educated assumption as to an identity of a partial pills content, that is subsequently analytically confirmed, then the results of a literature search of the partial pill can be used as a presumptive test. Examples of these type of pills are four part bar shaped Xanax, large four part Methadose 40, round Valium heart shaped center holes etc.

4.4.3.4 For counterfeit pharmaceuticals containing a different drug than listed or no drug at all, report any controlled substances detected along with this statement "Contents inconsistent with a published reference."

4.4.3.5 When an analyst decides not to analyze a pharmaceutical a "not analyzed" conclusion is acceptable.

4.5 Color Test Reagents

Unless stated in another section of this analytical method, or below, the recipes for reagents found in "*Clarke's Analysis of Drugs and Poisons, 3rd edition*" are to be used. As needed, recipes can be scaled up or down using the same ratio of ingredients.

4.5.1 The following list of color test reagents are approved for general use. Analyte specific color reagents may be listed in other sections of this method.

Marquis, Cobalt thiocyanate, Liebermann's, Mecke's, Froehde, Fast blue, Duquenois, Simon's (2° amines), Dille-Koppanyi, Sulfuric acid/UV, Van Urk's (p-DMAB).

4.5.2 The following reagents are approved as spray reagents Fast blue, Iodoplatinate, Van Urk (p-DMAB), Fluorescamine, and Dragendorff's.

4.5.3 For each reagent prepared above, a worksheet recording the following will be maintained; reagents name, recipe, lot number and manufacturer of chemicals used if applicable, QC method, date made, name of preparer, and results of QC check. All reagents will be checked against known standards and a blank when they are prepared. Reagents that are prepared for one time use, i.e. Weber test and sulfuric/UV (steroids), the QC results are to be documented in the case notes. If the effectiveness of a reagent is verified with each use and the results are documented in the appropriate case files, then no other documentation is required.

4.5.4 Shelf life. With the exception of Marquis, Cobalt thiocyanate, and Simon's, which are to be tested within 45 days prior to use, , all reagents are to be tested with a positive control and a blank, or negative control as appropriate, with each use. Shelf life is thus considered indefinite.

4.5.5 The following reagents or situations require special attention;

4.5.5.1 Marquis: This reagent will degrade over time especially when not refrigerated. Test with both a positive (methamphetamine) and negative (dimethyl sulfone) control. When testing with methamphetamine, the reaction should flash orange immediately. If the orange reaction is slowed the reagent should be replaced.

The recipe for Marquis: slowly add 100 mL sulfuric acid to 1 mL of approximately 37% (w/w) formaldehyde.

4.5.5.2 Simon's (2° amines). Sodium nitroprusside (Sodium nitroferricyanide) stock solution "A" should be kept in the dark and refrigerated.

4.5.5.3 2% (w/v) cobalt thiocyanate aqueous solution. Mix cobalt thiocyanate with distilled/deionized water and filter if necessary. Solution should be clear and pink. A positive reaction produces a turquoise blue precipitate with cocaine. HCl is added to the test well containing the sample and cobalt thiocyanate if the sample is suspected of containing cocaine base. Test with both a positive (cocaine) and negative (dimethyl sulfone) control.

4.5.5.4 Fast Blue BB salt solution for marijuana and mushrooms. Add enough of the Fast Blue BB salt to distilled/deionized water to change the water to a yellow color. The exact concentration is not relevant as the solution is tested with each use and thus depends on the analyst's personal preference.

4.5.5.5 Duquenois. Add 2.5 mL acetaldehyde and 2 g vanillin to 100 mL of approximately 95% or greater ethanol, may be denatured.

4.5.5.6 Van Urk's (p-DMAB). There are two recipes listed in Clarke's. The one to use is 2 g of p-Dimethylaminobenzaldehyde, add 50 mL of ethanol and 50 mL of hydrochloric acid. This reagent can be used in a test tube or sprayed.

4.5.6 The expected reaction colors can be referenced in Clarke's or from laboratory generated testing.

4.6 Authentication of Standards

Before a standard can be used as a reference for casework, it must be authenticated. This only has to be done once per lot.

4.6.1 Certified reference material may be obtained from ISO Guide 35 (17034 standard) approved providers that are included on the supply service list. These reference materials may be authenticated by a review of the Certificate of Analysis (COA) associated with the reference material. The COA will be centrally filed (in the laboratory or electronically). The review and approval by the analyst will be noted on the first page of the COA. The analyst will ensure the compound was tested and evaluated as positive for containing the anticipated compound by the provider.

4.6.2 Qualitative standards that are not obtained by an approved ISO Guide 35 provider will be authenticated by an instrument that provides structural information (such as GCMS or FTIR) and has been validated and approved for use in the lab.

A standard will be considered authenticated (after 12/30/2019) when the match factor from the NIST library is greater than 850, (Agilent Q of 85%) as compared to a library search. If the match is less than 850 (Q 85%) then two analysts must concur on the validity of the match. Initials of each analyst will be kept on the printout in the standards logbook or file. Reference libraries can come from any reliable source including but not limited to instrument libraries, scientific journals, or publications. When comparison to a journal, compendium or other document is not an option, mass spectral interpretation may be used in conjunction with the COA (certificate of analysis). This would apply in cases where instrumental data for a drug metabolite is not yet published, but a structurally similar compound is available to assist with interpretation. A second trained analyst must also review and initial the printout verifying the interpretation.

4.6.3 Authentication documentation will be kept for each standard.

4.6.3.1 Standards will be obtained from commercial or governmental sources i.e. Sigma, Cerilliant, Cayman, and DEA, etc. Standards may also be obtained from previously analyzed casework.

4.6.3.2 For qualitative analysis only, manufacturer's expiration dates and storage temperature requirements may be ignored if the standard produces the expected mass/infrared spectra.

4.6.3.3 For quantitative analysis, reference materials can be kept colder than manufacturer's recommendations as long as any solvents do not freeze.

4.6.4 Unauthenticated reference material must be stored in a designated area or clearly marked that authentication is needed.

4.6.5 It is the responsibility of each analyst to verify that each standard or control used has been properly authenticated.

4.7 Identification Criteria

4.7.1 General Guidelines. The following identification criteria will be applied to both controlled and non-controlled substances unless different criteria are listed in another section of this Analytical Method.

4.7.2 Testing Rules

4.7.2.1 For each controlled substance, two positive tests from two different sampling events must be employed for identification. For confirmation, one of the tests must provide structural information, i.e. either MS or FTIR. A positive test is defined as one that gives a reaction or result that indicates the presence of the analyte in question. Non-confirmatory tests include but are not limited to color tests, mass spectral data including incomplete spectra, retention time data, and a FTIR match to an external library. If mass spectral data is used as a test, then the reference used for comparison must be in the notes. A negative reaction to a color test cannot be used for a positive test even if a negative reaction was expected. Example: a negative reaction of methamphetamine and cobalt thiocyanate even though no color change is expected.

4.7.2.2 For non-controlled substances i.e. inorganics, cutting agents and non-scheduled prescription drugs, the second sampling event does not have to be used.

4.7.3 If a sample's MS or FTIR spectra matches the spectra of a standard, the GC has a retention time within the acceptable time window, and the second test is positive, then the compound is confirmed.

4.8 Uncertainty of Measurement on Qualitative Samples

Of the many possible variables that contribute to the uncertainty of measurement using this Analytical Method, only one is accurately measurable, the use of a balance. For weights near regulatory limits, the contribution of that variable is what will be reported. It must be clear that that uncertainty number applies only to the act of weighing and not to the sample as a whole. All analysts must be aware of other possible variables and to be able to explain their potential impact on the reported weight.

Examples of other variables include but are not limited to, moisture/solvent content, static, and the ability to remove all of a sample from packaging. A set of sample pillows has been provided to each lab. These need to be weighed and the results recorded at the same time that the class 2 weight check is performed. Once a year the UM for the balances will be recalculated and any change will be published.

4.8.1 The uncertainty of measurement (UM) is; for less than 100g it is (+/-) 0.06g, for greater than 100g it is (+/-) 0.18g. For results on Meridian balance #11, the uncertainty of measurement will be (+/-) 2.0. For results on Coeur d'Alene balance Ranger, the uncertainty of measurement will be (+/-) 0.9g.

4.8.2 When the total weight of a sample(s) falls within the window of uncertainty at regulatory limits, then the uncertainty associated with each weighing event must be listed on the report. Current windows:

For Cocaine and Methamphetamine:

27.40 to 28.60 g

198.20 to 201.80 g

398.20 to 401.80 g

For Heroin:

1.40 to 2.60 g

6.40 to 7.60 g

27.40 to 28.60 g

For Marijuana:

84.45 to 85.65 g (3 ounces)

451.80 to 455.40g (1 pound)

2266.20 to 2269.80 g (5 pounds)

11338.20 to 11341.80 g (25 pounds)

4.8.3 The measurement result shall include the measured quantity value (X) along with the associated expanded uncertainty (U), and this measurement shall be reported as X (+/-) U where U is consistent with the measurand of X, i.e. 28.05 (+/-) 0.06g. On the report reference that the uncertainty was calculated at the 95% confidence level.

4.8.4 The total U is a product of the calculated uncertainty and the number of weighing events (samples weighed). For example:

0.30=0.06(5) for less than 100grams with five weighing events.

AM #2: **FTIR**

1.0 Background/References

1.1 The Fourier Transform Infrared Spectrometer (FTIR) is an analytical instrument that is used to identify compounds based on their infrared absorption properties.

2.0 Scope

2.1 The FTIR is generally used with samples of higher purity. Samples may need cleanup prior to analysis.

2.2 The FTIR is primarily used to analyze organic compounds, compounds containing carbon atoms.

3.0 Equipment/Reagents

3.1 A FTIR and corresponding analytical software.

3.2 IR grade potassium bromide (KBr). Should be kept in a desiccator.

3.3 ACS grade solvents.

3.4 Hydraulic or other press for making KBr windows.

3.5 Any other sample introduction equipment, such as an ATR.

4.0 Procedure

4.1 Routine Maintenance

4.1.1 The desiccant in the instrument should be checked prior to running the monthly performance verification (4.1.2). If the desiccant is replaced it will be noted in the maintenance log.

4.1.2 Monthly (45 days before analyzing samples) performance verifications of the instrument with and without the ATR attachment. Using the manufacture's procedures, performance verification of the instrument is done using polystyrene film. These procedures will be performed monthly and after any maintenance. The "System Validation Report" or "Valpro Qualification Report" printouts are to be initialed by the analyst and kept in the maintenance logbook. If the verification does not pass and/or there is any other symptom of system failure, perform a bench alignment and repeat the verification and or consult the manufacturer. Any maintenance is recorded in the logbook. Each analyst who uses the instrument must verify and then initial the monthly printouts before analyzing casework.

4.1.3 The polystyrene "lollypop" ATR standard should be stored in a clean dry place. To preserve the film, a different section of the window should be used with each monthly check.

4.2 Background spectra will be collected immediately before every sample but do not need to be retained.

4.3 Standard Library Preparation

4.3.1 Production of valid standard library.

A pure sample of a standard is analyzed using the same procedures that will be used with an unknown (ATR vs. KBR etc.). Once a scan has been produced it can then be stored in an internal library. These standard scans can be produced and entered into the library as they are encountered in casework.

4.4 Analysis and Identification Criteria

4.4.1 Analysis

4.4.1.1 Run background

4.4.1.2 Obtain a spectra

4.4.1.3 Perform a library search of resulting spectra.

4.4.1.4 In order to confirm the presence of an analyte in a sample, the scan of the sample must match that of a known standard from the laboratory produced library. It is not acceptable to confirm on the basis of a match from a commercially produced library (Georgia State etc.). A match with an external library can be used as a preliminary (secondary) test.

4.4.2 Identification

4.4.2.1 FTIR spectra are considered matched if the peaks of the standard are present in the sample, in location, shape, and relative intensities. Any extra major peaks in the sample must be explainable.

4.4.2.2 If a sample's FTIR spectra matches a spectra of a standard and the second test is positive, then the compound is confirmed.

4.4.2.3 If FTIR spectral subtraction is done then what was subtracted needs to be in case notes.

4.4.2.4 If a FTIR spectra is inconclusive or negative for a controlled substance then the sample will be analyzed on a GC/MS.

AM #3: GC/MS

1.0 Background/References

1.1 The gas chromatograph mass spectrometer (GC/MS) is an analytical instrument that separates and identifies a wide variety of organic compounds based on their mass spectra and retention time data.

2.0 Scope

2.1 The purpose of this section is to layout the basic daily tune, scheduled periodic maintenance, sample preparation, and data interpretation necessary to perform quality analysis using a GC/MS. This method is limited to those compounds that produce adequate spectra and chromatography using the instruments owned and operated by the ISPFS.

3.0 Equipment/Reagents

3.1 A GC/MS and corresponding analytical software.

3.2 Capillary column and data acquisition methods sufficient to separate the analytes of interest.

3.3 ACS grade, or better, organic solvents.

3.4 Standards of the analytes of interest. Standard solutions may be prepared in-house or purchased from a commercial source. They can contain a single analyte or a mixture but all must be authenticated before use in casework.

3.5 Sodium carbonate and bicarbonate.

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4.0 Procedure

4.1 Mass Spectrometer Tune

4.1.1 Frequency

Using Hewlett-Packard/Agilent software and instrumentation an AUTOTUNE (either atune or etune) will be run after every major maintenance procedure, i.e. source cleaning or column change.

4.1.2 Using Hewlett-Packard/Agilent software and instrumentation, a successful MS AUTOTUNE, will be run at least once a week in which the instrument will be used. A tune evaluation will be run each day that the instrument is used. A day is defined as a calendar day. Each analyst who uses the instrument that day must verify and initial the tune evaluation and the weekly tune before their samples are analyzed. The exception to this is if the sequence of a methamphetamine quantitation run lasts longer than 24 hours.

4.1.3 Definition of a Successful Tune (using PFTBA)

Using Chemstation/Masshunter software the following parameters should be met.

4.1.3.1 Mass assignments within +/- 0.2 AMU of 69, 219, and 502

4.1.3.2 Peak widths (PW) should be between 0.55 and 0.65 AMU

4.1.3.3 The relative abundances should show 69 as the base peak, although it might switch with the 219 peak. Under no circumstances should the base peak be anything other than 69 or 219. The relative abundances should be anything greater than 40% for 219, anything higher than 2.4% for 502.

4.1.3.4 The Isotope mass assignments should be approximately 1 AMU greater than the parent peak and the ratios should be 0.5-1.6% for mass 70, 3.2-5.4% for mass 220, and 7.9-12.3% for mass 503.

4.1.3.5 The presence of mass 18 (water), 28 (nitrogen), and or 32 (oxygen) may indicate an air leak into the system. If any of these masses are above 10% relative abundance, 20% for water, then maintenance to the instrumental system might be required. Although elevated levels indicate an issue, by themselves, they do not affect analysis other than possibly an elevated background noise level. Prolonged exposure to oxygen or water can lead to a decrease in the life of a column and thus should be addressed through maintenance. An indication of an air leak on the Auto Tune and/or tune evaluation should be evaluated based on the normal operating parameters for that particular instrument, and thus does not require that the instrument be taken out of service.

4.1.4 The Tune Evaluation and AUTOTUNE printouts shall be centrally stored.

4.2 GC/MS Quality Assurance

4.2.1 For each GC/MS, a standard containing at least one controlled substance will be analyzed within 24 hours of samples being run. If for any reason this standard fails, change of retention time, MS scan, weak or no response etc., then a determination of the cause of the failure and corrective actions must be undertaken. Samples may need to be reanalyzed. Consultation with the discipline leader may be necessary. The failure of the standard due to instrument failure should be noted in the logbook, along with whatever maintenance that was performed to remedy the situation.

4.2.2 To confirm any substance, there must be a valid standard of that substance analyzed within twenty-four hours of the sample run, regardless of when the tune(s) were run.

4.2.3 If a sample is analyzed using a GC/MS acquisition method that is of limited scope and the results are negative or if there are indications of a second unrelated compound then the sample will be analyzed on the "DRUG" general screening method or a variant of the "DRUG" method.

4.3 General Scheduled Maintenance

All non-consumable items, that are repaired or replaced, must be entered into the maintenance logbook. Entrees into the logbook should include any symptoms of problems along with the status of the system after the repair has been completed.

4.3.1 Daily (consumables, i.e. items used once and then discarded). These items are needed to operate the GC/MS system but their replacement, or repair, does not need to be entered into the maintenance logbook.

4.3.2 Check and fill solvent rinse vials on autosampler, empty waste solvent vials as needed

4.3.3 At the start of the week before samples are run, and after maintenance, run a standard mix containing 200 µg/mL each of methamphetamine, caffeine, cocaine, morphine, and alprazolam. The stock of this mix should be kept in a freezer and a few drops of it added to a clean insert equipped vial just before analysis. The response of each compound in the mix must have a signal to noise ratio of greater than five (5) before samples can be analyzed. The noise is measured (response difference between peak to valley) just before each analyte and ratioed to the corresponding peak's response. Peak response is measured from the base to the apex. Peaks do not have to be measured if it is obvious that their signal to noise ratio is greater than five. For instruments that use hydrogen as a carrier gas then the chromatography (peak shape and retention time) of methamphetamine does not need to be considered as long as the ions are detected. By comparing a test run to previous runs can help identify potential problems, i.e. loss of response, and the need for maintenance. A printout of the TIC (Total Ion Chromatograph) is kept in the maintenance logbook. If a new column is installed, or the "DRUG" general screening temperature program is altered then a standard or set of standards will be analyzed consisting of at a minimum alprazolam, phentermine and methamphetamine. Alprazolam must be identified and phentermine and methamphetamine must be able to be separated by greater than 0.100 minutes, preferably to baseline, and identified. The TIC of these run(s) will be initialed by all analysts prior to them running samples and kept in the maintenance logbook.

4.3.4 Annual

4.3.4.1 Replace solvent trap, and pump oil, if so equipped. Should be done when other maintenance is performed, approximately once a year.

4.3.4.2 Vacuum dust from electronics fans.

4.3.4.3 If possible, inspect and clean the autosampler guides.

4.4 Non-scheduled Maintenance

All non-scheduled maintenance is to be performed on an "as needed" basis as indicated from failure of the autotune, poor chromatography, and or other indications of a system failure. All of these types of repairs will be noted in the maintenance log.

4.4.1 Replace or trim column.

4.4.2 Clean MSD, replace filaments, gold seal, and injection liner, when needed. Consult with manufacturer's manual or software for cleaning procedure.

4.4.3 Replace electron multiplier if, after repeated cleaning of the source, the EM volt readings remain at or above 2800.

4.4.4 Replace any part, or system of parts, as necessary.

4.5 Data Interpretation and Conclusions

4.5.1 Retention time

4.5.1.1 A sample's retention time will be considered acceptable if a mass spectral scan of the analyte is within +/- 0.040 min of a matching scan from a known standard. Retention time window was determined using the method described in "EPA SW846, method 8000B, section 7.6, Revision 2, December 1996 ".

4.5.1.2 The instrumentation and data acquisition parameters must be sufficient to maintain a 0.100 minute retention time difference between analytes of interest that produce similar mass spectra. Positional, geometric and other isomers may be exempt from this requirement.

4.5.2 The analyte of interest's peak shape must be acceptable, i.e. limited tailing or fronting. *Note* Some compounds do not chromatograph well, i.e. stanozolol.

4.5.3 Mass spectral interpretation.

4.5.3.1 For the purpose of drug identification, analysis of mass spectra is one of pattern recognition. A great deal of the interpretation is dependent on each analyst's opinion as to what constitutes a match. All comparisons for the purpose of confirmation are made between analytical standards, not library searches, and the sample spectra. The following are the minimum requirements to determine a match.

4.5.3.2 Identification of the molecular (parent) ion, if normally present. *Note* Some compounds do not have detectable molecular ions in their mass spectra.

4.5.3.3 Presence of the correct base ion. Exception, some compounds have several ions that depending on spectral shifting may change base ions, cocaine is an example of this. In these cases the base ion of the sample does not have to match that of the standard but does have to be present in significant abundance.

4.5.3.4 The ratios of the relative abundances of the major ions, from the sample, should be similar to those of the standard.

4.5.4 Conclusions

4.5.4.1 Confirmation. The retention time must be within (+/-) 0.040 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied and, if possible, the analysis of a second sampling event, then the GC/MS data may be used for confirming the presence of a compound.

4.5.4.2 Non-confirmation. If a drug is present but is unable to be confirmed, the report will read "Results of testing for controlled substances were inconclusive". The reason why the drug was not confirmed must be on the report

4.5.4.3 If the RT or MS do not match, or there is no peak at all, then report, "No controlled substances detected".

4.5.4.4 As with all cases it is up to the analyst to decide whether or not to report non-controlled substances.

4.6 Blanks

4.6.1 The purpose of a blank is to check for carry-over between samples, and to verify the lack of contamination of the solvents.

4.6.2 Frequency. A blank will be run preceding each sample. If two extracts of the same sample are run consecutively then the sample blank only has to be run before the first extract.

4.6.3 Interpretation. A blank run is considered blank if a analyte(s) of interest would not be identified using the above criteria from 4.5.3.

4.6.4 If a blank has an identifiable analyte of interest, then the blank will be rerun to rule out contamination of the blank vs carryover. If it is determined that the blank is contaminated, then the sample(s) immediately following the suspect blank(s) will be reanalyzed after an acceptable blank has been generated. If the samples contain the same analyte found in the contaminated blank, then the samples must be re-sampled/re-extracted and reanalyzed.

4.7 "DRUG" method parameters:

Initial temp 80°C

Hold time 2 min

Ramp 10-30°C/min

Final temp 280-310°C

Final hold time 0-5 min

Carrier gas flow 1 mL/min

Split ratio 100:1

Inlet temp 280+ °C

MSD transfer line 280-310°C

4.7.1 The analyst may vary GC/MS instrument parameters without performance verification other than a positive control. This includes changing the inlet temperature, temperature ramp and/or hold time, pressure program, injection volume and split ratio, and ion scan range. Acquisition parameters are stored with the data files.

4.7.2 When using GC/MS methods that are of a limited scope, i.e. LSD, GHB, Mushrooms etc. then a positive control must be run with the analysis of case samples to ensure that the analyte of interest can be detected.

AM #4 Balance Calibration Verification

1.0 Background/References

1.1 In order to ensure the integrity of the reported weights of controlled substances each laboratory within the ISPFS system maintains a set of weights that are used to verify the calibration of all balances and scales located in each laboratory

2.0 Scope

2.1 This section intentionally left blank

3.0 Equipment/Reagents

3.1 One set of ASTM Class 2, or better, weights. These weights must be NIST traceable and certified at the time of purchase. The documentation for the certification/calibration of the weights will be retained.

3.2 Top loading balances

4.0 Procedure

4.1 Verification

4.1.1 Within 45 days prior to use, each balance is to have its calibration checked (intermediate check) against a set of certified NIST traceable weights. Results are to be recorded in a log for future reference.

4.1.2 Each balance is checked using a set of ASTM weights as reference. This set should span the expected weights of samples that will be measured on each balance. An example: for the typical top loader 1 g, 100 g, and 2000 g weights would be sufficient. The allowable deviation from the standard weights will be 0.01 g or 0.1%, whichever is greater.

4.1.3 Each laboratory will keep a log sheet for each balance in use. The log sheet will list the balance identification, the weights used, their indicated weight, whether or not the observed weight is within the tolerance of the balance, the analyst and the date on which the check was performed.

4.1.4 Once a year an independent vendor will calibrate each balance. Acceptance criteria is the same as 4.1.2.

4.1.5 An independent vendor will calibrate each weight set once every five years. Upon return to the laboratory and before the weights are placed back into service the calibration will be verified. Calibration Certificates will be checked for compliance with ISO/IEC 17025, and initialed.

4.1.6 The weights will be handled with gloves or tweezers to keep them clean. They will be transported and stored in their case.

4.2 Consequences

If a balance fails a calibration check, the check is repeated. If the balance still fails then it will be taken out of service until it can be recalibrated or repaired. The balance shall be tagged indicating that it is out of service.

AM #5 Methamphetamine Quantification

1.0 Background/References

1.1 Under normal circumstances quantification of a methamphetamine sample's purity is not part of the analytical scheme used by the Idaho State Police Forensic laboratories. By special request this analysis can be performed. This analysis will only be performed on casework in which a federal court has a stated interest. This analytical method was derived from the principles and methods detailed in EPA publication "SW-846" and the states of Oregon and Utah's quantitation analytical methods.

2.0 Scope

2.1 The following procedures have only been approved for the analysis of samples containing methamphetamine in a solid matrix. The Idaho State Police Laboratory reserves the right to reject any sample for quantitative analysis based on sample size or circumstance.

2.2 In order to minimize the largest potential source of error, samples that have a high moisture or solvent content may need special consideration.

2.3 At the laboratory's discretion all samples may be analyzed as a composite unless specifically requested by the prosecutor.

3.0 Equipment/Reagents

3.1 Gas Chromatograph/ Mass Spectrometer (GC/MS) and corresponding software.

3.2 Injector should have a split liner with a glass wool plug.

3.3 Solid methamphetamine hydrochloride. The purity is to be documented with a certificate of analysis from the vendor.

3.4 ACS grade chloroform stabilized with either ethanol or pentene.

3.5 Class A volumetric flasks.

3.6 1.0 mL Gastight® type syringes. Syringes that are used to generate the standard calibration curve will have their accuracy checked before each use via section 4.4.7 of this method. The verification must encompass the expected working range of the syringe, 200 µL and 800 µL. Syringes that fail to meet the acceptance value of (+/-) 3% will be evaluated for accuracy and if necessary replaced. A syringe check is good for two weeks.

3.7 Internal standard: With a ratio of 1.3 mL of (98% or greater) n-tridecane per 1 liter of chloroform, prepare at least four liters per batch. Each sequence of samples and standards must be made with the same internal standard.

3.8 0.5 N sodium carbonate solution.

3.8.1 Add 2.7 g of sodium carbonate to 100 mL of water.

4.0 Procedure

4.1 Generation of Standard Curve

A six point calibration curve will be generated.

4.1.1 Prepare a standard stock solution of 1,200 to 2,000 µg/mL. With an analytical balance weigh 30-50 mg of methamphetamine HCl salt, add to a 25 mL volumetric flask and dissolve and bring to volume with the internal standard. Calculate the concentration.

4.1.2 Using the syringe, auto-sampler vials, and stock standard prepare an additional five 1.0 mL standards. Into five autosampler vials place 0.1, 0.2, 0.4, 0.6, 0.8 ml of stock standard and then dilute to 1.0 mL using the internal standard. The undiluted stock standard must be one of the points on the curve. If the stock standard point does not fall within the linear range of the instrument then a more dilute stock standard is prepared and a new curve is run or the acquisition parameters of the instrument can be altered, i.e. split ratio, and the original curve rerun.

4.1.3 Add approximately 100 µL (3 drops) of a 0.5 N sodium carbonate solution to each vial and mix.

4.1.4 Using the GC/MS software set up the calibration acquisition parameters and tables. The curve is to be generated using linear regression with the points weighted using the inverse square. For Agilent Chemstation/MassHunter software, the parameters and tables are found in the data analysis/calibration section.

4.2 Sample Preparation and Analysis

One of the basic requirements in determining an accurate quantification is that the sample must be homogenous. The sample must also be prepared using the same extraction procedure that was used in generating the standard curve. If a sample has been previously qualitatively analyzed and been resubmitted for quantitative analysis the sample must be reweighed before proceeding to 4.2.1. This new weight will be used in the final calculations. It will be documented which samples exhibit an abnormally high level of moisture or solvent.

4.2.1 Initially rough grind the sample with a mortar and pestle until the entire sample will pass through a US No. 4 sieve. Roll and quarter the sample until a representative sub sample of about 10 grams is obtained. Grind the sub sample until a fine powder is formed. ***Note*:** If the sample is less than 10 grams then grind the entire sample into a fine powder.

4.2.2 Using an analytical balance that is accurate to at least 0.1 milligram, accurately weigh out an amount of sample that is equal to, or less than, what was used for the stock standard, and place into a 25 mL volumetric flask. Add internal standard, dissolve, and bring to volume with internal standard. See 4.3.1.

4.2.3 Into an auto sampler vial aliquot approximately one milliliter of sample extract, add approximately 100 µL (3 drops) of 0.5 N Na_2CO_3, mix and analyze.

4.2.4 Samples are to be run in duplicate (two separate weighing's and extractions). The results are averaged before being used for calculating the final result. The duplicate results must have a Relative Percent Difference (RPD) (labeled "differential" in the BEAST LIMS) of less than (+/-) 10%, if they are not then either first rerun the extracts or proceed to extracting a new pair of samples and analyze.

$$RPD = \frac{(R1-R2) * 100}{A}$$

Where R1 = Result of first run in percent

R2 = Result of second run in percent

A = Average of R1 and R2 in percent

4.2.5 If a sample(s) is to be forwarded to another laboratory for quantitative analysis, the originating laboratory has the option of either sending the entire sample or preparing the sample(s) as per 4.2.1 above and then send a maximum of 1g per sample to the laboratory doing the quantitative analysis. If the original sample is less than 5 grams then the original sample can be sent without preparation.

4.3 *Calculation and Reporting of Final Results*

4.3.1 Calculation

Using Agilent software calculate the concentration in the vial (the computer software should do this). Use the following equation to calculate the concentration of the analyte in the original sample. All calculations may be done by hand or by using computer software:

$$\frac{(\mathbf{A}\ \mu g/mL) \times (\mathbf{Milliliters\ of\ solvent})}{(10) \times (\mathbf{B}\ mg)} = \mathbf{C}\ \%\ \text{analyte}$$

A = Concentration given by curve, rounded to the hundredths.

B = Weight of sample used, in milligrams

If C is less than 20% then the sample is re-extracted and reanalyzed using a larger sample size. The calculated concentration of the re-extraction must be 100< C <20%.

4.3.2 Reporting

4.3.2.1 Using the formula:

$$\frac{C \times D}{100} = X$$

- Where **C=** average of the two duplicate results from the equation in 4.3.1
- (If the average is greater than 100% the results will be calculated using C= 100%)
- **D=** total weight of sample in grams

The uncertainty range of each sample will be reported out by weight using:

X (+/-0.07) = Range

The original value of C will be used to calculate the uncertainty range even if C is greater than 100%

Report the result that "All samples calculated as the hydrochloride salt." Each report will have the statement, "The expanded uncertainty value was calculated at the 95% confidence level".

4.3.2.2 If a sample is less than 0.5 grams the sample will not be quantitated and the report shall state "sample is unsuitable for quantitative analysis, insufficient amount". These samples will be qualitatively analyzed.

4.3.2.3 If the concentration of a sample is below 10% then the report shall state, "Sample is unsuitable for quantitative analysis, concentration is below the limit of quantitation".

4.3.2.4 All calculated results will be reported to the same degree of significance.

4.3.2.5 All results will be rounded using standard rounding rules, i.e. 1-4 down, 6-9 up and 5 to the nearest even number.

4.4 Notes and QA/QC

4.4.1 The curve must be linear as defined by a R^2 correlation coefficient of 0.998 or better. The correlation coefficient is generated by the Agilent Chemstation/MassHunter software.

4.4.2 The area counts of the internal standard should be consistent from the beginning to the end of the run (+/- 10% of the mean). If a sample's internal standard falls outside of the range then the sample can be added to the end of the sequence and rerun. If the drift is noticed after the sequence has finished then the sample needs to be reanalyzed with the next batch.

4.4.3 A new curve will be generated before each quantitation sequence. A sequence is defined as a batch(s) run consecutively without the introduction of non-quantitation samples. A batch is defined as up to twenty injections. At the end of each batch a positive control will be run, the results of which must be (+/-) 4% of the stated value. The Relative Percent Difference (RPD) will be calculated for each batch of positive controls:

$$RPD = \frac{|R1-R2|}{E} * 100$$

- Where R1 = calculated result of the first positive control run after the generation of the curve.

- Where R2 = calculated result of positive control run at the end of the batch, or sequence if two or more batches are run together.
- Where E = Expected value

The RPD will be less than 8%.

4.4.4 A positive control will be analyzed each time a curve is generated. The positive control will come from a source other than what was used to generate the curve. Another in-house standard from a different lot, if available, and prepared by a different analyst is to be used as the positive control. To a 25 mL volumetric flask, add approximately 0.025 g of methamphetamine hydrochloride salt, that has been accurately weighed using an analytical balance, then dissolve and bring to volume with internal standard. The positive control is made with the same batch of internal standard as the rest of the run. Aliquot one milliliter into an auto-sampler vial and add sodium carbonate solution.

4.4.5 The accuracy of the curve is validated when the value of the positive control is within (+/-) 4% of the stated value.

4.4.6 The calibration curve, chromatograms and quantitation reports of the positive controls, excel spreadsheets containing RPD (positive controls) calculation results and syringe verification results for each run, and sequence logs will be centrally located on the common forensics drive. Chromatogram(s) and quantitation report(s) of all samples, and chromatograms of all applicable blanks are to be kept in the case notes. Chromatograms of standards used to generate the curve do not need to be kept.

4.4.7 For the 1.0 mL syringe weigh 10 replicate aliquots of water at 200 µL and 800 µL. For the purpose of the calculations, the density of water is 0.998 g/ml. The acceptance criteria are (+/-) 3% of 0.1996 (for 200 µL) and 0.7984 (for 800 µL) for all measurements.

4.4.8 If a blank, or sample, is missing the target or any of the qualifier ions then it is considered blank.

4.5 Uncertainty of Measurement

The (+/-) 7% value was derived from a validation study that took into account this method, different instruments and analysts. As long as the constraints described in this method are followed, the stated UM value is valid. Before a new analyst or instrument is used on casework it must be demonstrated that they/ it can meet the original target goals of the validation study. The UM value will be reviewed annually and adjusted if necessary.

AM #6 Analysis of Solid and Liquid Unknowns

1.0 Background/References

1.1 These procedures are designed to analyze most solid and liquid samples. Two different tests, and two different sampling events will be employed in confirming the presence of controlled substances. One of the tests must provide structural information, i.e. either MS or FTIR

2.0 Scope

2.1 Generally samples submitted to the laboratory fall into two categories, the first and most common, through information provided with the case or through training and experience the analyst has some idea of what the sample might contain. The second are complete unknowns. Analysis of some special/unique samples are covered under different sections of this manual.

3.0 Equipment/Reagents

3.1 The following pieces of equipment and reagents can be used in any combination to identify the analytes of interest.

3.1.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.1.2 FTIR and appropriate analytical software. Reference AM #2.

3.1.3 ACS grade, or better, solvents.

3.1.4 0-14 pH paper.

4.0 Procedure

4.1 Sample Preparation and Analysis

This section details the minimum requirements for analysis, any additional testing if necessary may be performed at the analyst's discretion. As per AM#1 section 4.8.2.1 a positive color test can always be used in lieu of a second GC/MS run or in conjunction with the FTIR in order to satisfy the second sampling event rule. This applies to both solid and liquid samples. While performing testing using two GC/MS sampling events, if the first GC/MS run is negative the second sampling event does not need to be analyzed. Liquid samples approximately as viscous as honey, or thicker, may be treated as a solid. Trace amounts of liquids may be treated as a solid or as a liquid at the analysts' discretion.

4.1.1 Solids

4.1.1.1 Weight. If possible obtain the weight of the sample. Packaging is never included in the reported weight unless the sample cannot be separated. In that case the report will clearly describe that packaging was included in the reported weight. On very rare occasions the submitting agency can request that a weight of the packaging, separate from the substance, be reported.

4.1.1.2 Perform screening tests.

4.1.1.3 Extractions. Acidic, basic, neutral, and dry extractions, in any combination, can be employed in order to separate diluents or other interferences, or improve chromatography. If screening tests are negative a dry extraction using methanol or a mixture of methanol and chloroform should be employed. Consideration of solubility's of the different analytes into various solvents must always be considered.

4.1.1.4 Analysis. Selection of appropriate instrumentation for confirmation is at the analyst's discretion, see methods 2 and 3.

4.1.2 Liquids

Acidic, basic, neutral extractions, in any combination, can be employed in order to separate diluents or other interferences, or improve chromatography.

4.1.2.1 Option A: If possible, determine if the sample is aqueous, this may be done through immiscibility or flame testing (preferred) or from information submitted with the sample. If the sample is organic go to 4.1.2.5. If it is not possible to determine if the sample is aqueous then treat the sample as if it is. If submitted, a control can be used to determine if the sample is aqueous, they are assumed to be of the same matrix.

4.1.2.2 If possible, split the sample and perform a basic extraction using approximately 0.5 N sodium carbonate or 0.5 N sodium bicarbonate and an immiscible solvent, chloroform preferred.

4.1.2.3 Option B: an aliquot may be evaporated with air/nitrogen and the resultant residue can be treated as a solid.

4.1.2.4 Refer to AM #1 section 4.1.7 for reporting conclusions.

4.1.2.5 Organic solvents may be analyzed directly with the GC/MS. Split the sample if possible. In order to produce adequate chromatography the sample may be diluted with appropriate solvent and/or base may be added before or after initial analysis.

4.1.2.6 Meth lab samples may be cleaned up using back extractions and solvent exchange.

4.1.2.7 Oil or glycerin based samples (steroids, e-cig etc.) may be diluted with an appropriate solvent or another clean-up method may be used.

4.1.2.8 If a sample is suspected of containing a controlled substance that is covered by a separate section, i.e. GHB, then that method should be used.

4.1.3 Analysis

4.1.3.1 Run samples using an appropriate data acquisition method, like "DRUG".

4.1.3.2 If a peak appears, and is not recognized, perform a library search.

4.1.3.3 If a controlled substance is recognized from a library search or other means, then a standard is run if identity is to be confirmed. Library search reports do not need to be retained in the case file.

4.1.4 Conclusions: see AM #1 section 4.1.7.

4.2 FTIR Sample Preparation and Analysis

4.2.1 Direct.

Solid samples or dried liquid samples may be analyzed directly with the ATR. Samples may also be mixed with KBr, pressed into a pellet/window and then analyzed.

4.2.2 Extractions

4.2.2.1 The organic layer from either a basic or acidic extraction may be mixed with ground KBr, evaporated and analyzed.4.2.2.2 Samples undergoing a basic extraction may require bubbling with HCl gas and filtering before HCl salt can be isolated and analyzed.

4.2.3 Analysis

See AM #2, section 4.4.1

4.2.4 Identification

See AM #2, section 4.4.2

AM #7 Marijuana

1.0 Background/References

1.1 Marijuana (Cannabis Sativa) has been used for its sedative, euphoriant and hallucinogenic properties for over 3000 years. Written references to it date back to 2700 BC. It is primarily smoked but can be taken orally. The active compound, delta-9-tetrahydrocannabinol (THC) is most concentrated in the resin that is obtained from the flowers of the female plant. It is imperative that the analyst be familiar with the current Idaho code as it pertains to the legal definition of marijuana.

1.2 References

Identification of Marijuana, *by J.I. Thornton and G.R. Nakamura* Journal Forensic Science (1972), 12, 461

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of marijuana in plant material, residues, and samples containing extracted resins. The procedure is composed of a series of tests, none of which by themselves are specific for marijuana or THC, but taken in combination are considered specific for the presence of marijuana or its resins. GC/MS is not routinely applied to marijuana analysis but may be used. If a plant material sample is suspected of containing a substance other than marijuana then the sample must be extracted and analyzed using a GC/MS.

3.0 Equipment/Reagents

3.1 Stereo microscope

3.2 Thin layer chromatography tank and plates.

3.3 Aqueous Fast Blue BB solution.

3.4 ACS grade Petroleum ether, hexane, diethyl ether, methanol, toluene, acetonitrile, and chloroform. Solvents may be used past any expiration dates listed by the manufacturer, since they are checked with a positive and negative control with each batch/use to demonstrate their reliability.

3.5 GC/MS and analytical software, see AM #3.

4.0 Procedure

4.1 *Solvent Extraction*

4.1.1 Plant material

4.1.1.1 Place some plant material in a test tube.

4.1.1.2 Cover with appropriate solvent.

4.1.1.3 Use extract for thin layer and/or modified Duquenois-Levine, see 4.3 and 4.4

4.1.1.4 Retain small amount of unused solvent as blank.

4.1.2 Resins (including samples from Vape cartridges and edibles)

4.1.2.1 Flush/swab/extract item(s) containing suspected resins with appropriate solvent and collect solvent in test tube.

4.1.2.2 Use extract for analysis.

4.1.2.3 Retain small amount of unused solvent as blank.

4.1.3 Oils

4.1.3.1 In a test tube extract 1.0g of oil with 1.0mL of methanol, mix well. The amounts used may be scaled up or down as long as an accurate 1:1 ratio is maintained.

4.1.3.2 Analyze methanol layer via GC/MS (4.6.2)

4.1.3.3 Analyze sample via TLC and duquenois

4.2 Microscopic Examination

4.2.1 Plant material is examined using a stereo microscope for the following characteristics:

4.2.1.1 Cystoliths and/or Cystolithic hairs – Small "bear claw" shaped hairs with bases of calcium carbonate. The cystoliths and hairs are located on the topside of the leaf or leaf fragment.

4.2.1.2 Unicellular hairs – Fine hairs located on the underside of the leaf or leaf fragment. **Note** Unicellular hairs are not always observed on the leaves from the budding parts of the marijuana plant.

4.2.2 Seeds are examined using a stereo microscope for the following characteristics:

4.2.2.1 Veined shell.

4.2.2.2 Ridged edges.

4.2.2.3 Point on one end and dint on the end of plant attachment

4.3 Thin Layer Chromatography

The extract used for the TLC should not be heated.

4.3.1 Spot a small amount of solvent extract onto a thin layer plate alongside of a standard and a solvent blank.

4.3.2 Develop the plate using one or more of the following mobile phases:

Hexane/diethyl ether 4:1 (petroleum ether may be substituted for hexane).

Chloroform or Toluene.

4.2.3 Visualize by spraying the plate with Fast Blue BB salt solution.

4.2.4 Compare results of unknown to those of standard, see 4.5.2. Photograph the plate for the case file.

4.4 Modified Duquenois-Levine

Unless otherwise noted the Duquenois-Levine test will be performed on the same day noted on the TLC plate.

4.4.1 In a test tube containing a portion of the evaporated solvent extract, mix 2-10 drops of Duquenois reagent and an equal amount of concentrated HCl.

4.4.2 Let stand ½ to 3 minutes and observe color change.

4.4.3 Add chloroform.

4.4.4 Observe if the purple/blue color transfers into chloroform layer. * **Note**: Transferring the solution from step 4.4.1 into a clean test tube before the addition of chloroform will decrease the color interference from chlorophyll.

4.4.5 A blank and a standard need to be run with each batch and the results recorded in the case notes.

4.5 Results and Reporting

A positive test shall be defined as the following:

4.5.1 Microscopic

Observation of cystolithic hairs on the leaf and/or the presence of characteristic seeds.

4.5.2 Thin Layer

Presence of a red spot with migration distance consistent with the red THC spot of the standard. The line of spots across the TLC plate should form a continuum, be it linear (straight line) or parabolic (slight curve with the ends being higher than the middle).

Negative blank.

4.5.3 Modified Duquenois-Levine

A purple* color developing after the addition of the HCl (*color may vary from blue to reddish purple depending on the sample).

Transfer of the color into the organic layer after the addition of chloroform.

4.5.4 A positive result shall be defined as the following:

4.5.4.1 Positive microscopic, single TLC system, and modified Duquenois-Levine. Report using the words “marijuana or the resins thereof, (CI)”.

4.5.4.2 Negative microscopic. Positive modified Duquenois-Levine and two positive TLC systems. The conclusion should contain the words “marijuana or the resins thereof, (CI).”

For positive GC/MS see 4.6.3.

4.5.5 Germination

Marijuana seeds without THC are only controlled if they are fertile. The germination test should only be performed if it has been determined that the seeds do not contain THC. ***Note*** In determining the presence of THC, soaking the seeds for up to thirty minutes in petroleum ether/hexane, does not affect germination rates.

4.5.5.1 Wrap a minimum of 10, to a maximum of 100 seeds, in a moist paper towel and place in a covered container. The container is then placed in a safe dark place for 14 days.

4.5.5.2 Check seeds daily making sure they do not dry out. Also watch out for mold.

4.5.5.3 Report how many fertile marijuana seeds sprouted as a percentage of the original total. Conclusion should be, “The sample contains XX % viable seeds with the botanical characteristics of marijuana seeds”.

4.6 GC/MS Confirmation

4.6.1 Extract sample with appropriate solvent.

4.6.2 Run extract according to AM #3 along with a THC standard.

4.6.3 Compare retention time and ion chromatograph of sample with THC standard.

4.6.3.1 On MCT oil based samples, on some instruments, it may be necessary to use spectral subtraction and/or extracted ion profiles to separate co-eluting compounds in order to identify THC

4.6.3.2 Extra blanks and/or a hotter/longer temperature program may need to be run when analyzing MCT oil samples to reduce carryover.

4.6.4 Reporting results

4.6.4.1 Report positive results as “marijuana or the resins thereof, (CI)” if a positive microscopic result was also observed.

4.6.4.2 Report “tetrahydrocannabinol, (CI)” if a positive GC/MS and positive TLC are observed.

4.6.4.3 Report ”tetrahydrocannabinol (CI) and/or tetrahydrocannabinolic acid” where there are two positive GC/MS analyses or a positive GC/MS, positive duquenois, and an inconclusive TLC.

4.6.4.4 Report “inconclusive for tetrahydrocannabinol(CI)/tetrahydrocannabinolic acid” where there is a positive GC/MS, and negative TLC .

AM #8 Psilocyn/Psilocybin Mushrooms

1.0 Background/References

1.1 Psilocyn and psilocybin are related tryptamines that are found in many species of mushrooms. The mushrooms have been used in religious ceremonies for at least 3000 years by the native peoples of Mexico and Central America. Psilocyn and psilocybin are Schedule I hallucinogens.

1.2 More information is available through the "Drug Identification Bible".

1.3 The Weber Test for the Presence of Psilocyn in Mushrooms", Garrette, Siemens, and Gaskill, NEAFS vol. XVIII, No.1, 1993.

2.0 Scope

2.1 The following procedures are used to identify psilocyn and/or psilocybin from mushrooms.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.2 ACS grade, or better, solvents: methanol, acetone, chloroform.

3.3 Fast Blue BB, or B salt.

3.4 Deionized/distilled water.

3.5 Hydrochloric and acetic acid.

3.6 Sodium bicarbonate.

4.0 Procedure

4.1 Color Spot Test

"Weber test".

4 .1.1 Add sample to well of spot plate after the addition of a Fast blue BB, or B, solution. Should turn orange-red within a couple of minutes if psilocin/psilocybin is present.

4.1.2 Remove some of the liquid to another well and then add a drop of concentrated HCl. A positive test is one that turns a blue-green color.

4.1.3 Negative and positive controls need to be run with each batch, and the results documented in the case notes.

4.1.3.1 The positive control can be either a mushroom secondary standard or a commercial psilocin/psilocybin standard.

4.2 GC/MS Sample Preparation and Analysis

4.2.1 Extraction.

4.2.1.1 Extract with just enough methanol to cover sample. ***Note*** At this stage the methanol extract may be injected into the GC/MS.

4.2.1.2 Centrifuge and decant solution into clean test tube. Cap and place into freezer until cold.

4.2.1.3 Remove from freezer and immediately add equal volume of cold acetone and mix.

4.2.1.4 Centrifuge, decant, and if necessary concentrate the supernatant.

4.2.2 Acid base extraction

4.2.2.1 Grind sample, add 10% acetic acid, grind, add water, grind to a slurry.

4.2.2.2 Add chloroform, mix and centrifuge/separate layers. Discard chloroform layer.

4.2.2.3 Slowly add solid Sodium Bicarbonate to pH 8-8.5.

4.2.2.4 Extract with chloroform, discard aqueous layer.- Extract chloroform layer with a 2% sodium bicarbonate solution.

4.2.2.5 Analyze chloroform layer on a GC/MS.

4.2.3 Analysis.

4.2.3.1 Run samples on GC/MS using a split or splitless data acquisition method depending on the sensitivity of the instrument.

4.2.3.2 Compare with a commercial standard containing either psilocyn or psilocybin. ***NOTE** psilocybin breaks down into psilocyn in the hot injection port of a GC.

4.2.4 Conclusions and Reporting.

4.2.4.1 Confirmation. The retention time must be within (+/-) 0.040 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied then confirmation can be reported as "psilocyn and/or psilocybin (CI)".

4.2.4.2 If THC is detected in the GC/MS run then an analysis by TLC must be performed in order to confirm the THC.

AM #9 Lysergic Acid Diethylamide (LSD)

1.0 Background/References

1.1 LSD was originally synthesized from lysergic acid found in the fungus *claviceps purpurea.* Street LSD is found most often on blotter paper. It is also found on sugar cubes, candies like “Sweet Tarts”, gelatin squares called windowpanes, and on small pills called microdots. It breaks down in the presence of light and heat, because of this the samples are often found wrapped in metal foil.

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of lysergic acid diethylamide (LSD).

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3

3.2 Ultraviolet light source

3.3 Thin Layer Chromatography (TLC) plates and tank.

3.4 ACS grade, or better, solvents

3.5 Distilled or deionized water

3.6 $NaHCO_3$ or Na_2CO_3

3.7 The recipe for T1 is 7drops of ammonium hydroxide per 10ml of methanol.

3.8 The ratio of chloroform to methanol is 9/1.

3.9 p-DMAB is 2 gram of p’dimethylaminobenzaldehyde in50 mL of ethanol and 50 mL of conc. hydrochloric acid.

4.0 Procedure

4.1 Ultraviolet (UV)

UV can be useful in identifying which side of a sugar cube, or candy, has been spiked with LSD.

4.2 GC/MS Sample Preparation and Analysis

4.2.1 Sample preparation.

4.2.1.1 As with all GC analyses it may be necessary to concentrate the extracts from either of the following methods; this is done by blowing a stream of air, or other suitable gas, over the top of the solvent. Do not heat!

4.2.1.2 "Window panes", blotter paper, and pulverized microdots can be extracted using a basic extraction or directly with methanol. Place sample in a test tube and add just enough methanol or basic solution to cover sample. Shake and then let soak for at least an hour. Microdots should soak overnight if possible. Centrifuge if necessary and analyze.

4.2.1.3 Sugar cubes, "Sweet Tarts" or other candy. Check under UV to find the side that is suspected of being spiked. Scrape off upper layer until up to one half of the sample, has been used. Dissolve in water and make basic. Extract with a minimal amount of chloroform. Analyze on GC/MS. Using the extraction procedure in 4.3.2, without the derivatizing agent, also works well.

4.2.1.4 Aqueous samples should be extracted using base and a minimal amount of chloroform.

4.2.2 Analysis

4.2.2.1 Due to the typically dilute nature of LSD samples, the GC may need to be set to splitless mode. The injector liner may have to be changed to a splitless model depending on the sensitivity of the particular MS being used. The retention time for LSD is concentration dependent. A series of standards of varying concentrations may have to be run in order to achieve the standard (+/-) 0.040 minute retention time window.

4.3 TMS Derivative

At times, it may be necessary to derivatize weak LSD samples. The following is a summary of one possible method.

4.3.1 Reagents

4.3.1.1 Ammonium hydroxide (NH_4OH)

4.3.1.2 Methylene chloride, chloroform, or ethyl ether as solvents

4.3.1.3 N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)

4.3.1.4 bis(trimethylsilyl)trifluoroacetamide (BSTFA)

4.3.2 Procedure

Place sample in concentrated NH_4OH and let soak for at least ten minutes. Add 200 µL of solvent and extract. Separate and evaporate the solvent. Add 30-200 µL of either MSTFA or BSTFA. Analyze on the GC/MS looking for the TMS derivative and comparing it to a derivatized standard.

4.4 TLC Analysis

A T1 system followed by p-DMAB color development works well for LSD. Other appropriate solvent systems, such as chloroform/methanol and acetone, may also be used. After the plate has been spotted with the sample extract, blank, and a standard, and the solvent has risen at least three quarters of the way up, remove the plate, dry, and then develop with p-DMAB. A purple color should develop with LSD.

4.4 Color Spot Tests

4.4.1 Marquis, grey color

4.4.2 p-DMAB, purple violet color

AM #10 GHB, GBL, and 1,4,BD

1.0 Background/References

GHB (gamma-hydroxybutyrate) is a controlled substance in Idaho while its precursors GBL (gamma- butyrolactone) and 1,4 Butanediol (1,4 BD) are not. This is problematic in that the interconversion of GBL to GHB is simply pH dependent and 1,4 BD is converted to GHB in the body. In aqueous solutions GHB and GBL will exist in equilibrium, the relative concentrations of each are also pH dependent.

The following analytical scheme was developed to separate and identify GHB, GBL, and 1,4 BD while ensuring that GHB is not produced during the process.

This method was based on a procedure found in "Microgram, Vol XXXV, No.1 January 2002".

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of GHB and its related analogs in samples.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM# 3.

3.2 FTIR and appropriate analytical software. Reference AM# 2.

3.3 pH paper

3.4 ACS grade, or better, chloroform, ethyl acetate, methanol, and ethanol.

3.5 H_2SO_4, BSTFA (with 1% TMCS) a TMS derivatizing reagent. ***Note*** the BSTFA is available premixed from Cerilliant Corporation.

3.6 Distilled or deionized water.

3.7 Bromocresol green, methyl orange, dextrose, aniline hydrochloride, sodium hydroxide. ***Note*, aniline is acutely toxic handle with care.**

4.0 Procedure

4.1 Screening Tests

4.1.1 Color Spot Test

A mixture of Bromocresol green, Methyl orange, and Schweppes reagents are tested with samples. A positive reaction for the presence of GHB is one that turns green.

4.1.1.1 Bromocresol green

Mix 0.03 g bromocresol green in 100 mL of 4:1 methanol: water. Adjust to pH 7 with NaOH.

4.1.1.2 Methyl Orange

Mix 0.01 g of methyl orange in 100 mL of methanol. Adjust pH to 7.

4.1.1.3 Modified Schweppes

4.1.1.3.1 Solution A: mix 2.0 g dextrose in 20 mL of water.

4.1.1.3.2 Solution B: mix 2.4 g aniline hydrochloride in 20 mL of ethanol.

Mix solution A & B and dilute to 80 mL with methanol.

4.1.1.4 For the final color reagent mix Bromocresol green solution with the Methyl Orange solution in a 1:1 ratio. Add 3 parts of this combined solution to one part of the Schweppes reagent.

4.1.2 Physical tests

4.1.2.1 Pure GBL and 1,4 BD are viscous liquids at room temperature. 1,4-BD will solidify when placed in a refrigerator (approximately 4°C) while GBL will not.

4.1.2.2 GBL is soluble in chloroform and 1,4 BD is not.

4.1.3 GC/MS

Add concentrated Sulfuric acid to aqueous sample, extract with chloroform and analyze (GC/MS). If GBL is detected then proceed with confirmational GC/MS.

4.2 GC/MS Sample Preparation and Analysis

GHB cannot be analyzed directly on a GC/MS as it will convert to GBL in the heated injector port. GHB must be derivatized with BSTFA before injection.

4.2.1 Butanediol

4.2.1.1 If pure 1,4 BD is suspected then dilute with methanol and inject into GC/MS.

4.2.1.2 In aqueous samples, if the concentrations of 1,4 BD are high enough, then the 1,4 BD may be observed in a chloroform extract.

4.2.1.3 Dry down sample, add methanol, and analyze.

4.2.1.4 1,4 BD will derivatize with BSTFA as per 4.2.2.3.

4.2.2 GHB

4.2.2.1 Extract aqueous samples with chloroform, discard chloroform layer.

4.2.2.2 Dry down aqueous layer with nitrogen or dry air. Sample can be warmed to expedite drying as long as the temperature remains below 60°C.

4.2.2.3 Once sample is completely dry then add 100-200 ul of BSTFA. Cap sample and heat at 60-70°C for 15-20 minutes.

4.2.2.4 Add ethyl acetate and analyze on GC/MS.

4.3 FTIR

Aqueous samples appropriate for FTIR are defined as clear, colorless liquids that appear to be water. This doesn't include sodas, sport drinks, etc.

4.3.1 1,4-BD.

If suspected to be pure, run as a liquid sample, i.e. liquid cell, salt windows Gemini, ATR etc.

4.3.2 GBL

4.3.2.1 If pure then analyze as a liquid.

4.3.2.2 If aqueous, extract with chloroform. Discard aqueous layer. Evaporate off chloroform and run as a liquid.

4.3.3 GHB

4.3.3.1 If solid, analyze via the ATR or as a KBr pellet.

4.3.3.2 If aqueous, extract with chloroform. Discard chloroform layer. Evaporate to dryness and run via the ATR or as a KBr pellet.

4.4 Scheme

4.4.1 Solids

4.4.1.1 Run color test per section 4.1.1 of this method.

4.4.1.2 If color test is negative, dissolve in methanol and analyze on GC/MS.

4.4.1.3 If color test is positive, skip to 4.4.1.5.

4.4.1.4 If GC/MS is negative then analysis is complete.

4.4.1.5 If GC/MS has GBL then derivatize original sample with BSTFA and analyze on GC/MS as per sections 4.2.2.3 and 4.2.2.4. Or run sample on FTIR.

4.4.2 Clear, thick liquids

4.4.2.1 Place 1-5 mL of the sample in the freezer for fifteen minutes. If it solidifies, extract with methanol and analyze with GC/MS.

4.4.2.2 If results indicate the presence of GBL proceed to section 4.4.3.4 and 4.4.3.5.

4.4.2.3 If sample remains a liquid go to section 4.4.3.

4.4.3 Aqueous samples

4.4.3.1 Perform color test per 4.1.1.

4.4.3.2 Acidify a portion of the sample with concentrated H_2SO_4 and extract with chloroform. Analyze the chloroform layer with GC/MS. If results are negative for GBL then proceed with section 4.4.3.3. If GBL is present then skip to section 4.4.3.4. If 1,4 BD is present then report.

4.4.3.3 If results from 4.4.3.2 indicate the presence of 1,4 BD then report. If results were negative then take a portion of original sample and dry down with nitrogen/air and heat (approximately 60C). Extract with methanol and analyze with GC/MS.

4.4.3.4 Take a portion of original sample extract with chloroform. Analyze chloroform layer with GC/MS. Report GBL if found.

4.4.3.5 Take aqueous layer from 4.4.3.4 and analyze using sections 4.2.2.2 through 4.2.2.4.

AM #11 Iodine

1.0 Background/References

1.1 Iodine is one of the essential ingredients in the production of methamphetamine using the ephedrine/pseudoephedrine/HI reduction method. The following methods, when used in combination, can be used to confirm the presence of iodine in samples typically found at clandestine laboratories.

1.2 A full copy of the Leuco Crystal Violet method can be found in Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, Method 4500-I B.

2.0 Scope

2.1 The primary test is the Leuco Crystal Violet method. By itself this test cannot be used to confirm the presence of iodine due to the possible false positive reaction to oxides of manganese (MnO_2 and MnO_4). By using any of the additional tests the presence of the interferences is eliminated thus confirming iodine.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Flasks and Stoppers

3.1.2 Pipets

3.1.3 pH test strips

3.1.4 Starch paper

3.2 Reagents. All reagent solutions can be scaled up or down from the following recipes. All water is deionized or distilled.

3.2.1 Stock iodine standard. Dissolve approximately 1.3 g of KI into 1 L of water.

3.2.2 Citric Buffer

3.2.2.1 Citric acid. Dissolve 182.2 g $C_6H_8O_7$ or 210.2g $C_6H_8O_7.H_{19}$ into 1 liter of water.

3.2.2.2 Ammonium hydroxide 2 N. Dilute 131 mL of conc. NH_4OH to 1 L with water.

3.2.2.3 Final Buffer. Mix 350 mL 2N NH_4OH to 670 mL citric acid. Add 80 g ammonium dihydrogen phosphate ($NH_4H_2PO_4$) stir to dissolve.

3.2.3 Leuco crystal violet indicator

3.2.3.1 Add 200 mL water, 3.2 mL H_2SO_4, and 1.5 g 4,4',4'=-methylidynetris (N,N-dimethylaniline) ** to a 1 L brown glass bottle. Mix upon each addition. ** **NOTE**** AKA Leuco crystal violet.

3.2.3.2 Dissolve 2.5 g mercuric chloride ($HgCl_2$) into 800 mL water. ***Note*** Mercuric chloride is very toxic, avoid skin contact and inhalation.

3.2.3.3 Add $HgCl_2$ solution to Leuco crystal violet. Adjust pH to less than 1.5 with H_2SO_4 if necessary. Store, away from light.

3.2.4 Oxone. Potassium peroxymonosulfate ($KHSO_5$). Dissolve 1.5 g into 1 L of water.

4.0 Procedure

4.1 *Procedure for Leuco Crystal Violet*

The goal of this test is the identification of iodine and not quantification; the following procedure has been condensed from the original. The following recipe is based on a final volume of 100 mL for both standard and sample solutions. Using the same proportion of reagents the volume can be successfully reduced. A test is considered positive if a violet color is developed. As with all procedures a blank and a standard are run with every batch.

4.1.1 Standard preparation.

4.1.1.1 Add 0.25 mL of iodine standard to a 100 mL flask. Dilute with 50 to 75 mL of water.

4.1.1.2 Add 1 mL citric buffer and 0.5 mL $KHSO_5$ solution. Mix and let stand one minute.

4.1.1.3 Add 1 mL Leuco violet indicator, mix, and QS to 100 mL.

4.1.1.4 Color will often develop immediately. If not wait up to five minutes.

4.1.2 Blank preparation. Substitute deionized water for the standard and proceed with 4.1.1

4.1.3 Sample Preparation. The most difficult part of this analysis is judging how much sample to use. It is easy to use too much sample. If this occurs, a light blue-green- yellow color will develop instead of the expected violet.

4.1.3.1 Solid samples. Place a small piece of sample in a flask. Dissolve with 50 mL of water. Wait approximately one minute. Sample does not need to be completely dissolved. Proceed with 4.1.1 substituting the standard with the dissolved sample.

4.1.3.2 Liquids. Place a small amount of sample into a flask. Dilute with 50 to 75 mL of water. The color of the sample solution at this point should be a very light yellow. Proceed with 4.1.1 substituting the standard with the diluted sample.

4.2 Complementary Methods for the Detection of Iodine

4.2.1 Heat. When a capped test tube containing solid iodine is subjected to moderate heating, violet fumes are created. Condensation of the fumes into shiny grey crystals at the cool top of the test tube will often be observed. The oxides of manganese that interfere with the Leuco crystal violet method do not produce this effect. As with all experiments involving heat this test should be done in a hood.

4.2.2 Starch Test. A liquid iodine solution when added to starch paper produces a blue-black stain, solutions made from the oxides of manganese do not.

4.2.3 Hexane Color Test. Hexane turns to a violet color when added to an iodine solution. Solutions containing oxides of manganese do not produce this effect.

4.2.4 pH Shift. Add sample to water and check pH, it should be neutral. Add red phosphorus and let stand. Check for a drop in pH (<2).

AM #12 Phosphorus

1.0 Background/References

The following method is used to identify elemental phosphorus. Phosphorus is typically found at clandestine methamphetamine laboratories. Although white phosphorus may be found, red phosphorus is most often encountered due in large part to its greater accessibility.

Method published in *"Clandestine Laboratory Investigating Chemists Journal, Vol. 10, #3".*

2.0 Scope

2.1 The following GC/MS analytical procedure is used to identify the presence of phosphorus.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.2 Test tube and holder

3.3 Bunsen burner or hand held propane torch

3.4 Chloroform ACS grade.

4.0 Procedure

4.1 This test is based on the fact that white phosphorus is soluble in chloroform while red phosphorus is not. Being soluble allows white phosphorus to be injected into a GC/MS.

4.1.1 Conversion of red to white phosphorus.

4.1.1.1 Take approximately 0.1 g of red phosphorus into a test tube and heat with a Bunsen burner until the red phosphorus starts to emit yellow-white fumes signaling the conversion.

4.1.1.2 Remove from heat and immediately add chloroform. If white phosphorus is present then the above step is omitted.

***NOTE** Caution, the chloroform will boil and spit when added to test tube. The white phosphorus may ignite upon exposure to air. This part of the procedure **MUST** be done in a hood.

4.1.2 Analyze the extract on the GC/MS. Confirmation is achieved when the presence of the P2, P3, P4 ions are detected (MW 62, 93, 124 respectively) and compared to a standard.

4.2 Conclusions

The GC/MS procedure by itself can give a positive confirmation.

AM #13 Total THC by HPLC

1.0 Background/References

The HPLC (High Performance Liquid Chromatograph) equipped with a Diode Array Detector (DAD) will be used to determine the total delta 9-THC (tetrahydrocannabinol) concentration in plant material. For the purposes of this analysis total delta 9-THC consists of delta 9-THC and delta 9-THC-A (tetrahydrocannabinolic acid) using the formula

Total 9-THC = 9-THC + (0.877* 9-THC-A).

2.0 Scope

2.1 This method will be used to quantitate the concentration of total THC in plant material on cases submitted as suspected "Hemp" only.

3.0 Equipment/Reagents

3.1 Agilent 1220 HPLC with DAD, associated software, and method parameters supplied by Agilent

3.2 2mL autosampler vials

3.3 HPLC grade methanol for extraction

3.4 HPLC grade methanol with formic acid, 0.05% (v/v)

3.5 HPLC grade water with formic acid, 0.1% (v/v). Water from a functioning Nanopure system is acceptable substitute

3.6 Spex 1600 Mini G Homogenizer

3.7 Spex steel balls or equivalent

3.8 50mL plastic centrifuge tubes, with caps

3.9 25mL graduated cylinder

3.10 Glass test tubes

3.11 Centrifuge

3.12 1.5 mm screen

3.13 Gas tight syringes capable of measuring 50, 200, 500, 800, 900 and 950µl

3.14 2-3mL disposable syringes with 4mm RC syringe filters 0.45µm

3.15 delta 9-THC and delta 9-THC-A standards, 1mg/mL.

3.16 CRM Hemp as a positive control

3.17 THC free dried plant material as a negative control/extraction blank

4.0 Procedure

4.1 Sample Drying

4.1.1 Place approximately 2 grams of plant material, which has been broken into pencil eraser size pieces into a weigh boat.

4.1.2 Place into an oven set to approximately 70°C for 2 hours

4.1.3 Remove from oven and place in desiccator for 15 minutes to cool, then weigh. If constant weight has been achieved proceed to 4.2, if not proceed to 4.1.4.

4.1.4 Place back into oven for 30 minutes

4.1.5 Remove from oven and place in desiccator for 15 minutes to cool, then weigh again.

4.1.6 Repeat the heating and cooling process until a constant weight is observed

4.1.7 Constant weight is defined as (+/-) 5%. Once dried, the plant material should easily crumble.

4.2 Homogenization

4.2.1 Place approximately 1-2 grams of dried plant material into a 50mL plastic centrifuge tube, add three steel balls, and cap.

4.2.2 Homogenize for 5 minutes at 1000 RPM

4.2.3 Pass homogenized plant material through a 1.5 mm screen and collect.

4.3 Extraction

Samples and a positive control are to be run in duplicate (two separate weighing's and extractions). Only one extraction blank of THC free plant material needs to be run per batch of samples.

4.3.1 Place 0.20g of homogenized plant material into a 50mL centrifuge tube, add one steel ball, add 20mL of methanol, and cap.

4.3.2 Shake at 500RPM for 2 minutes

4.3.3 Transfer 1-2mL of methanol into a glass test tube and centrifuge for 5 minutes. Run at approximately 2000RPM if a speed setting is available.

4.3.4 Dilute 50µL of the supernatant into 950µL of methanol.

4.3.5 Filter through the 0.45 filter into an autosampler vial.

4.4 HPLC

4.4.1 Calibration Curve

A five point calibration curve is made from 1.0 mg/ml delta 9-THC and delta 9-THC-A standards via serial dilutions. Standards are good for up to 3 months after initial opening.

4.4.1.1 Add 50µL from the two 1mg/mL standards to 900µl of methanol (level 5), 50µg/mL.

4.4.1.2 Add 200µL of level 5 to 800µL of methanol (level 4), 10µg/mL.

4.4.1.3 Add 500µL of level 4 to 500µL of methanol (level 3), 5µg/mL.

4.4.1.4 Add 200µL of level 3 to 800µL of methanol (level 2), 1µg/mL.

4.4.1.5 Add 500µL of level 2 to 500µL of methanol (level 1), 0.5µg/mL.

4.4.1.6 Analyze standards, generate a curve with a (R^2) linearity of greater than 0.9990.

4.4.2 Instrument Parameters

The following parameters were originally provided by the manufacturer and may be modified if necessary.

4.4.2.1 Formic acid in water and methanol may be purchased or made in house. Add 1mL formic acid to 1 L of water for Channel A, and 0.5mL formic acid to 1 L of methanol for Channel B.

4.4.2.2 Mobile phase gradient program with flow rate of 1mL/min

4.4.2.2.1 At time 0 is 60% channel B, 40% channel A

4.4.2.2.2 At time 7.0 minutes 77% channel B, 23% channel A

4.4.2.2.3 At time 8.2 minutes 95% channel B, 5% channel A

Time (minutes)	Channel A (%)	Channel B (%)
0	40	60
7.0	23	77
8.2	5	95

4.4.2.3 Column temperature isothermal at 50°C

4.4.2.4 DAD settings

4.3.2.4.1 Signal Wave length: 230nm, band width 4

4.3.2.4.2 Peak width: >0.0063 min (0.13 seconds response time)(40Hz)

4.3.2.4.3 Range 190 to 400nm

4.3.2.4.4 Analog Output: Zero offset: 5% attenuation: 1000 mAU

4.3.2.4.5 Margin for Negative Absorbance: 100 mAU

4.3.2.4.6 Slit: 4nm

4.4.3 Sample Analysis and QA/QC

4.4.3.1 Samples are to be run in duplicate (two separate weighing's and extractions). The results are averaged for the final result. If the final result falls within 0.3% and 1 % total THC, then the duplicate results must have a Relative Percent Difference (RPD) (labeled "Calc. conc. differential" in the BEAST LIMS) of less than (+/-) 25%. If they are not, then either rerun the extracts or proceed to extracting a new pair of samples and analyze.

$$RPD = \frac{(R1-R2)*100}{A}$$

Where R1 = Result of first run in percent

R2 = Result of second run in percent

A = Average of R1 and R2 in percent

4.4.3.2 Extraction blank (negative control) must not detect any THC or THC-A above 0.02%. If the extraction blank fails then the entire batch needs to be re-extracted with new, clean solvent.

4.4.3.3 The CRM Hemp positive control value (AVG) must be within (+/-) 20% Of the Total THC value calculated from the Certificate of Analysis (COA)

4.4.3.4 Controls are to be analyzed with every sample run.

4.4.3.5 Two of the three tests for marijuana must also yield positive results before the measured concentration of Total THC can be reported.

4.4.3.6 Central data will be kept on the ISP common drive and will consist of the sequence file, THC and THC-A calibration curves, positive and negative controls final results, and lot numbers for all standards and controls

4.4.3.7 A methanol blank will be run before every sample.

4.3.3.8 A blank will be considered blank if it does not contain any THC or THC-A that is above 0.02%.

4.4.3.9 If the blank has THC or THC-A above 0.02% then the blank will be rerun to determine contamination vs. carryover. If it is determined that the blank is contaminated, then the sample(s) immediately following the suspect blank(s) will be reanalyzed after an acceptable blank has been generated.

4.4.4 Conclusions

The total delta 9-THC concentration will be reported as:

4.4.4.1 If greater than 1% report as "Cannabis with a total delta 9-THC greater than 1%"

4.4.4.2 If averaged result is between 1% and 0.3% report as the calculated number (+/-) the UM, example: "Cannabis with total delta 9-THC of 0.45 (+/-) 0.09%"

4.4.4.2 If the result is less than 0.30% report as "Cannabis with total measured delta 9-THC of less than 0.3%"

4.4.4.3 If neither THC or THC-A is detected on the HPLC then the sample must be analyzed on the GC/MS.

4.4.4.4 The Uncertainty of Measurement (UM) is (+/-) 20 % at the 95% confidence level.