

SOUTH CAROLINA LAW ENFORCEMENT DIVISION  
FORENSIC SERVICES LABORATORY

# Toxicology

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## Operations Manual

**V. 5.072517**



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**TOXICOLOGY QUALITY MANUAL**

**I. GENERAL –**

- A. Quality control and quality assurance are integral parts of the forensic activities performed in the SLED Toxicology Department
- B. The following quality guidelines may apply for the analysis and subsequent accepting and reporting of toxicology results, unless otherwise specified in a specific method procedure.
  - Minor deviations and exceptions shall be authorized by the departmental lieutenant or a team/technical leader and be documented in the case record.
  - Major deviations and exceptions shall be authorized by the department lieutenant and documented in the case record.

**II. CASEWORK –**

1. The start date for analysis will be the date of first analysis on the evidence. This is typically volatiles analysis for most cases, but may be drug screen analysis for urine only cases or other appropriate testing. The end date will be the date that the assigned analyst marks the case as complete in LIMS.
2. The following outline will be used as a guide for the typical minimum testing required for an assigned Toxicology case. The assigned analyst may, at his/her discretion, perform limited testing, additional tests, or no testing at all based on the facts surrounding a given case or sample ( ie.. insufficient sample, unsuitable sample, incomplete case history, history of drug abuse, etc.)
3. Universal precautions should be exercised when handling biological specimens in the laboratory.
4. Drug Screen Panels  
The following panel types have been identified for purposes of testing within the Toxicology department. The drug classes that make up the panels and associated thresholds are subject to change in an effort to reflect current trends in substance abuse and availability of assays. Thresholds for each assay are found in the Enzyme Linked Immunosorbent Assay (ELISA) Procedure. Typical Drug Screens are as follows:

| BLOOD 12 Panel: | URINE 12 Panel: |
|-----------------|-----------------|
| Amphetamine     | Amphetamine     |
| Benzodiazepines | Benzodiazepines |
| Buprenorphine   | Buprenorphine   |
| Carisoprodol    | Carisoprodol    |
| Cocaine         | Cocaine         |
| Methadone       | Methadone       |
| Methamphetamine | Methamphetamine |
| Opiates         | Opiates         |
| Oxycodone       | Oxycodone       |

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|              |              |
|--------------|--------------|
| Tramadol     | Tramadol     |
| Zolpidem     | Zolpidem     |
| Cannabinoids | Cannabinoids |

5. Case Type

1. DUI Analysis:

- a. Blood Only- A volatiles analysis will be performed.
  - i) If the ethanol level is less than 0.080% g/dL, a blood drug screen will be performed followed by confirmation/quantitation of the drug(s) indicated. If the drug screen is negative or no sufficient impairment has been established, then a general screen by mass spectrometry will be performed.
  - ii) If the ethanol level is greater than 0.080% g/dL and there is a history of drugs of concern, a blood drug screen and general screen may be performed followed by confirmation/quantitation of the drugs of concern that are indicated.
  - iii) If the ethanol level is greater than 0.080% g/dL and there is no history of drugs, the analysis will be considered complete.
- b. Urine Only- A urine drug screen will be performed followed by qualitative confirmation of the drug(s) indicated. A urine general screen by mass spectrometry may be performed when DS is negative or when there is a history of potentially impairing drugs. Specimens may be analyzed for the presence of alcohol/volatiles or other specific drug(s) based on information received from the submitting official.
- c. Blood and Urine- If a breath test was administered, testing will proceed in the urine only. If no breath test was given, testing will proceed as a blood only case.

2. Felony DUI:

- a. Blood Only- A volatiles analysis will be performed.
  - i. If the ethanol level is less than 0.150% g/dL, a blood drug screen will be performed followed by confirmation/quantitation of the drug(s) indicated. If the drug screen is negative or no sufficient impairment has been established, then a general screen by mass spectrometry will be performed.
  - ii. If the ethanol level is greater than 0.150% g/dL and there is a history of drugs of concern, a blood drug screen and general screen may be performed followed by confirmation/quantitation of the drugs of concern that are indicated.

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- iii. If the ethanol level is greater than 0.150% g/dL and there is no history of drugs, the analysis will be considered complete.
  - b. Urine Only- A urine drug screen will be performed followed by qualitative confirmation of the drug(s) indicated. A urine general screen by mass spectrometry may be performed when DS is negative or when there is a history of potentially impairing drugs. Specimens may be analyzed for the presence of alcohol/volatiles or other specific drug(s) based on information received from the submitting official.
  - c. Blood and Urine- Analysis will be completed in blood only unless there is insufficient sample and the analysis should follow the path of a blood only FDUI case.
- 3. Traffic Fatality:  
A volatiles analysis in blood and ocular will be performed, if available. A blood drug screen will be performed followed by confirmation/quantitation of drug(s) indicated. Specimens may be analyzed for the presence of other specific drug(s) based on information received from the submitting official.
- 4. Homicide, Suicide, and Accidental Death (ie. Electrocution, fall, etc.) where cause of death is obvious:  
A volatiles analysis in blood and ocular will be performed, if available. A blood drug screen will be performed followed by confirmation/quantitation of drug(s) indicated. Specimens may be analyzed for the presence of other specific drug(s) based on information received from the submitting official.
- 5. Criminal Sexual Conduct Victims:  
Analysis will only be performed on cases if there is an indication of drug involvement in the history. The appropriate analysis only for drugs that are specifically indicated in the history may be performed. Typically analysis will proceed in the blood only if the sample was collected within 12 hours of the incident and in urine only if the sample was collected within 36 hours of the incident. Testing will proceed in the appropriate fluid based on time frame. Both fluids should be tested if they were collected within 12 hours of the incident. Deviation may be necessary based on case history. If specimens were collected greater than 36 hours after the incident, no testing will be performed without a specific valid request from the submitting agency. If preliminary drug screen results are negative, and there is indication of drug use, a general should be performed on one or both fluids depending on time frame.
- 6. Explained Deaths (e.g. heart disease, stroke, disease, etc.):

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A volatiles analysis in blood and ocular, if available, will be performed. A blood drug screen and a blood general screen by mass spectrometry analysis will be run followed by confirmation/quantitation of the drug(s) of concern that are indicated.

7. Fire Death

A volatiles analysis in blood and ocular, if available, will be performed. A blood drug screen and a blood general screen by mass spectrometry will be run followed by confirmation/quantitation of the drug(s) of concern that are indicated. A carboxyhemoglobin analysis will be performed.

8. Unexplained Deaths:

A volatiles analysis in blood and ocular, if available, will be performed. A blood drug screen and a general screen by mass spectrometry will be performed on the blood followed by confirmation/quantitation of the drug(s) indicated.

9. Poisoning and/or Drug overdoses (including Lethal Injection):

A volatiles analysis in blood and ocular, if available, will be performed. A blood drug screen and general screen by mass spectrometry in blood followed by confirmation/quantitation of the drug(s) indicated will be performed. Tissue analysis may be required in true overdose cases if sample is available.

10. Child Fatalities:

A volatiles analysis in blood and ocular, if available, will be performed. A drug screen and general screen by mass spectrometry followed by confirmation/quantitation of the drug(s) indicated will be performed in the blood.

**NOTE:** Children involved in fatalities where the cause of death is known (i.e. traffic fatality/homicide by gunshot) may not require a general screen.

11. Moonshine or other non-biological liquids that contain alcohol:

Alcohol/volatiles analysis only.

12. Tox Other

Occasionally cases are submitted that do not meet any of the above categories and these are considered Tox Other. These cases may include food items, materials from syringes, powders, bones, etc.

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Testing of these types of cases may include a combination of analytical techniques to include but not limited to: spot tests, volatiles analysis, pH analysis, mass spectral analysis, etc.

**NOTE:** Coroners are offered an option of a Rapid Panel which would consist of volatiles analyses and drug screening followed by confirmations of positive screens in lieu of the Expanded Panel testing would include this testing plus extensive mass spectrometry screening for cases where a mass spectrometry screen would typically be performed and a faster turnaround time is desired.

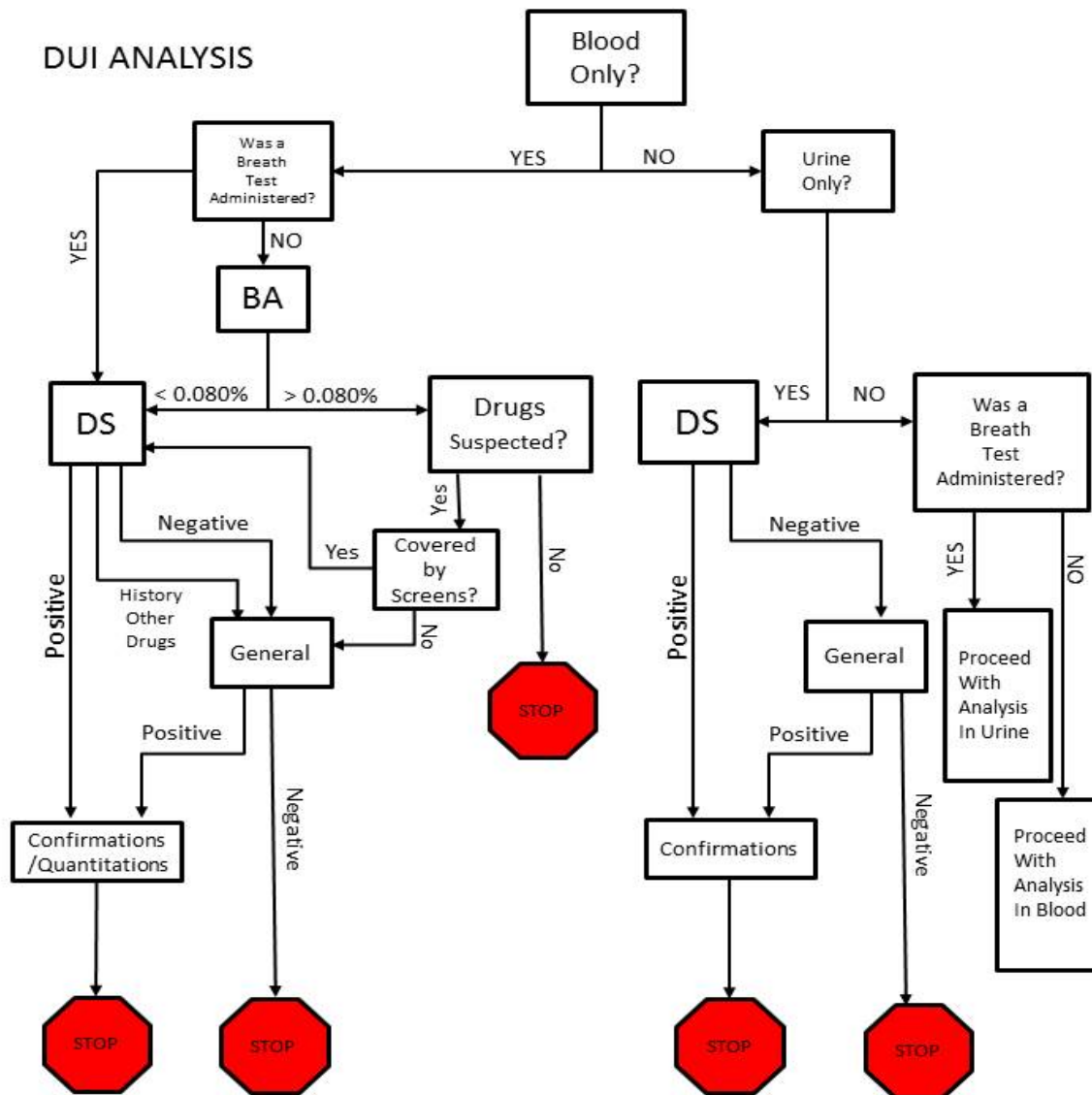
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• Work Flow Chart

**DUI ANALYSIS**

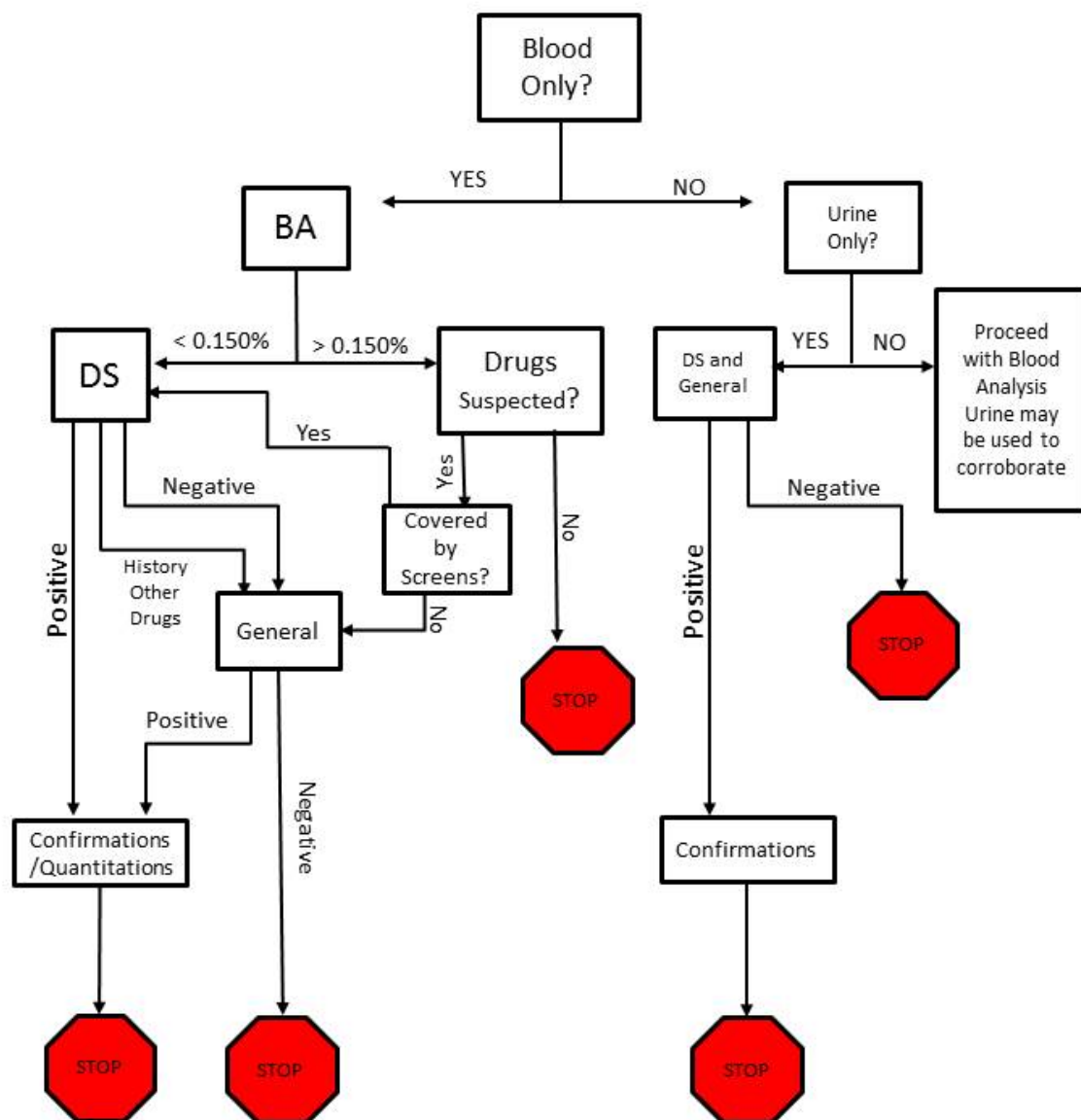


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**FELONY DUI ANALYSIS**

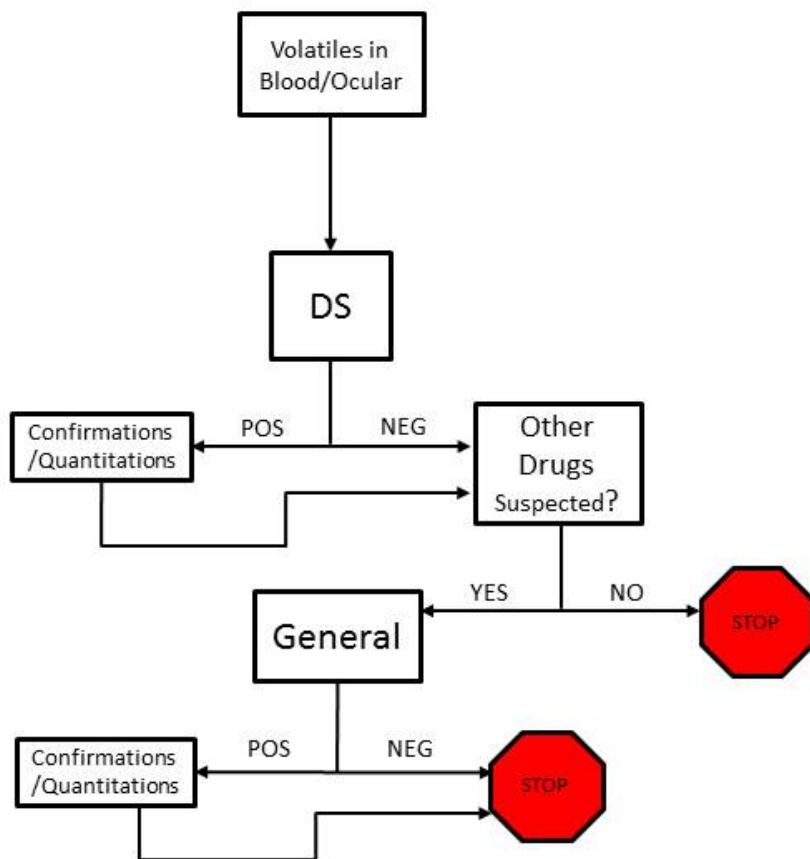


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Traffic Fatality  
Homicide/Suicide (other than  
Alcohol, poison or drug related)



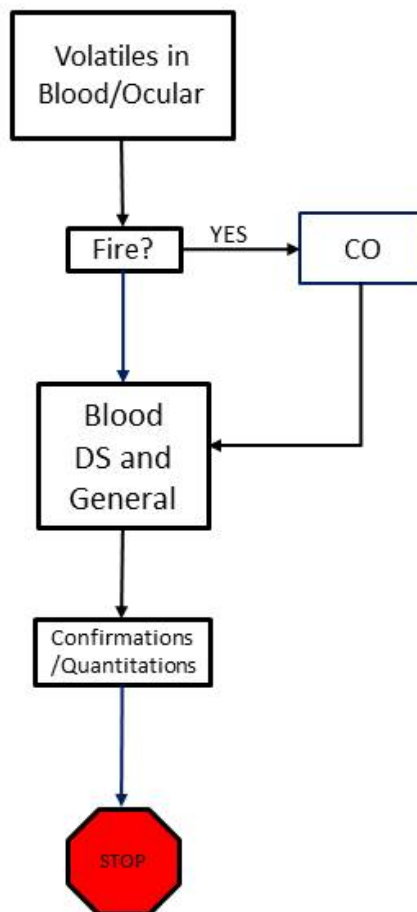
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Child Fatality  
Unexplained Death  
Poisoning/Overdose\*  
Fire Death

\*Tissue or additional  
analysis may be  
necessary



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- Specimens Tested

1. When submitted blood is collected from multiple sites (i.e. Iliac, Aortic, Femoral, Subclavian, Heart blood), the preferred sample for most testing would be peripheral samples.
2. Normally, testing is limited to biological samples that are submitted. However, certain circumstances may necessitate the analysis of non-biological samples that are associated with poisoning or overdoses. Such samples include but are not limited to: syringes, pharmaceuticals, unidentified liquids, or unidentified solids.
3. Multiple fluids or tissues - if more than one specimen type is submitted, analyses should be limited to only those which are likely to provide information which is pertinent to the case. For example, analysis of brain and liver tissue need not be performed except where the levels of drugs in these tissues are needed to confirm results from blood or if no blood was submitted.
4. Drugs that are detected by a screening test in one tissue or fluid may be confirmed in another without the screening test being performed in that fluid or tissue. For example, a positive immunoassay in urine may be confirmed by a GC/MS analysis in the blood.

- Sample Analysis

1. Typically, qualitative and quantitative analyses will be performed only for substances of toxicological significance (i.e. those compounds that cause impairment or can cause significant toxicity).
2. Analytes that are of no probative value will not routinely be identified. Inactive metabolites that have little or no toxicological significance will not normally be quantitated.
3. However, analysis of metabolites such as benzoylecgonine, which are of toxicological significance, will be performed.
4. Additionally, qualitative and quantitative analyses will be limited to those compounds in which suitable standards and methodology are available.
5. As with all cases, these determinations will be made on a case by case basis and documented in the case record.

- Sample Rejection

Under certain circumstances, evidence submitted for analysis may be rejected by the responsible toxicologist prior to completion of requested testing. Each submitted case, based on history, type of case, type of specimen, sample size, or other variables may be rejected for testing. Cases that are called into question regarding specific testing by the toxicologist may be submitted to the departmental Lieutenant for review. Routine case rejection criteria include, but are not limited to, the following:

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1. Where prior toxicological testing has been performed, any testing by the SLED Forensic Toxicology Department may be precluded or limited to specific testing not previously performed.
2. Samples considered to be contaminated or unsuitable for analysis may be rejected for toxicological analysis.
3. Specimens which are thought to contain any microorganism for which normal safety precautions are insufficient, or specimens that pose a high degree of health hazard to the Toxicologist may be rejected for analysis.
4. Any occurrence where the physiological specimen is not labeled with the victim or subject's name, or where the name on the specimen is inconsistent with the Forensic Services Request Form may allow for rejection.
5. Drug Deterrence Program cases from agencies other than SLED will generally not be accepted for analysis without prior authorization.
6. Biological samples or syringes containing traces of biological fluid will not be analyzed for cases involving a drug possession charge, regardless of the presence of any other physical drugs in the individual's possession.

### **III. EVIDENCE HANDLING**

- A. All items of evidence should remain in a secure storage location until authorized Toxicology personnel are ready to accept custody and transfer the evidence to the Toxicology refrigerator (TOX-R) located on the fourth floor. Containers or items which are not properly sealed and/or labeled should be documented and brought to the attention of a departmental supervisor.
- B. After evidence is retrieved from the secure storage area in Evidence Control, it must be inventoried, documented, and routed to the appropriate location.
  - a. Documentation of the evidence and evidence bag is performed via digital photographs. These photographs will be attached to the appropriate case in LIMS or other secure storage area. The outer bag and labels must be photographed so that all labels are documented. Photographs of additional packaging should be made as appropriate.
  - b. Each item of evidence should be marked with the case number and the technician's/analyst's initials and resealed in the evidence bag.
  - c. In LIMS, the analyst/technician will document in the Item description how the item is labeled.
    - i. If an item is not labeled, this should be noted in the description.
    - ii. If it is discovered that an item is labeled with a name that does not match the case, this should be brought to the attention of a departmental supervisor.
  - d. In the Attributes section of the Item Screen in LIMS, the analyst/technician should notate the quantity and type of containers received for each item.

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- e. All biological specimens of the same type will be considered one item unless it is from multiple collection sites or differentiated by collection time or other means. When multiple types are present, the original item will be sub itemized and the proper differentiation noted in the item description.
    - i. If samples are differentiated based on time, the order will go by the earliest collection.
    - ii. If samples are differentiated based on collection site, the order is typically peripheral followed by central sites.
  - f. Upon inventory, if the items are found to be incorrect in LIMS based on the “Said to contain inventory”, the following steps will be taken:
    - i. If an item is not found on inventory: In LIMS, scan to location “Not submitted”
    - ii. If an item is found that was not on receipt: In LIMS, create the found item and make a note in the LIMS narrative that the item was discovered during inventory.
    - iii. If an item is found in a container upon inventory that is required by another department, it will be removed from the container and packed for transfer to the appropriate department.  
NOTE: If any deletions, additions or significant changes are made to the items of evidence upon inventory, the submitting agency must be contacted to notify them of the changes made and this notification must be captured in the case narrative.
  - g. Upon completion, the analyst/technician should reseal the evidence and their initial and date should be placed on the seal.
- C. Once the evidence is properly documented, the evidence bag/container and its contents will be transferred to the appropriate analyst or secure refrigerator via LIMS.
- D. Upon initial retrieval of evidence from the Toxicology refrigerator or freezer, the responsible analyst shall transfer custody of the evidence from the Toxicology refrigerator to themselves via LIMS.
- E. Subsequent access into the evidence bag for any purpose will be documented through the use of the examiner's initials and date placed at the site of entry/resealing of the bag. The evidence bag must be properly resealed prior to storage. Analysts may keep evidence in their custody, stored in their assigned secured area while analysis is ongoing.
- F. Only one evidence bag will be open at one time by an analyst when aliquotting samples for analysis.
- G. All biological evidence must be stored in a secure refrigerator or freezer when not being examined, assessed, or processed.

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- H. Evidence that is in process may remain in an analyst's area for short term storage during the work day. It may be stored refrigerated in the analyst's bench refrigerator.
- I. Evidence items may be containerized, re-containerized, separated, or combined as necessary so long as the activities are properly recorded in LIMS.
- J. After final toxicological analysis has been performed, evidence bags containing biological sample(s) from a completed case that requires no further analysis should be placed into temporary hold bins as soon as practical. The transfer to temporary hold bins should be recorded in LIMS.
- K. Containers in temporary hold bins can be returned to the submitting agency after 90 days from the date the report was completed. Earlier return can be done at the submitter's request. The return should be documented in the LIMS and typically occurs via transfer to the evidence room.

#### **IV. SPECIMEN STORAGE**

- A. In order to provide meaningful results from specimens received by this department, it is necessary to insure that storage conditions be adequate for maintenance of specimen integrity.
- B. Biological fluids will be refrigerated upon arrival and during storage when not being tested at 2-8°C.
- C. Tissue specimen(s) should be frozen upon receipt and remain frozen during storage when not being tested. Frozen specimens should be stored at temperatures not exceeding -10°C.
- D. In some instances evidence may be submitted such as clothing, bed linens, hair/nail samples, etc. that do not require refrigeration. These specimens may be stored within the access limited Toxicology Department Laboratory as long as they are secured in a proper container and sealed in a tamper evident manner.

#### **V. REAGENT AND STANDARDS PREPARATION/VALIDATION**

- A. REAGENT PREPARATION
  - 1. Preparation/validation of reagents that are disseminated throughout the laboratory should be documented on a Reagent Preparation/Verification Log Form.
  - 2. Pertinent information includes:

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- a) Reagent name
- b) Recipe
- c) Batch number (date of preparation formatted YYMMDD)
- d) Date prepared
- e) Description of chemical(s) used
- f) Lot number of chemical(s) used
- g) Date verified
- h) Initials of verifying individual
- i) Date in service (if different from date of verification)

NOTE: Formula weight of the hydrate salts of buffers along with the calculations used should be documented on the Reagent Preparation/Verification Log Form.

3. Storage containers should be labeled with the following information:

- a. Name of Reagent
- b. Initials of Preparer
- c. Date Prepared
- d. Date of Expiration (if applicable)
- e. Applicable Safety Codes or Warnings
- f. Batch Number (if applicable)

NOTE: Organic Solvents that are recontainerized from the bulk container will be labeled with the above information (a-e) and in addition, marked with the Manufacturer Name and Contact information.

**B. CRITICAL SUPPLY VERIFICATION:**

Due to the diversity of supplies used within the toxicology laboratory, no single procedure will suffice for the validation of all items. All critical supplies must be verified before use in analyses. All critical supplies will be maintained by the department on the Critical Supplies List which includes the name of the supply, approved vendor, catalog number (if available), evaluation criteria and acceptance criteria. Approved methods for verification of critical supplies include, but are not limited to the following:

Aqueous buffer solutions –

- 1. Aqueous buffers should be prepared in accordance with recipes found in the formulary.
- 2. The pH of these solutions should be checked prior to use. (Refer to pH Meter operations in Instrumental Section of Toxicology Manual.) When necessary, the pH should be adjusted with an appropriate acid or base to achieve the desired value.
- 3. Additionally, these buffers are often prepared from crystalline or powdered form and can exist in various states of hydration. Some stocks

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may be anhydrous, while others may exist as hydrate salts of the compound. Care must be taken when preparing these buffers to account for the varying formula weights encountered in these situations.

**Organic solvents –**

1. Solvents may be verified concurrent with analysis but will be purchased according to a specified quality criteria (ex. ACS grade, HPLC grade). Acceptability will be evaluated using quality control criteria outlined in the specific analytical procedure or quality manual.
2. When questions arise as to the purity of a reagent, that reagent should be removed from use until verification is performed. Verification may involve analysis by GC, GC/MS, HPLC, or UV/Vis spectrometry.

**Blank Blood/Urine Matrix-**

1. Blank Blood/Urine will be shown to contain no analytes of interest prior to use in analytical assays.
2. This will be accomplished by screening the matrix with all available immunoassays and analyzing via Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry and/or Liquid Chromatography/Time of Flight.

**Certified Reference Material-**

1. Standards and Controls purchased from a qualified vendor that have a certificate of analysis are considered acceptable and do not need to be evaluated prior to use.
2. Certified Reference Materials for quantitation should be used within 6 months from the date the standard was opened unless validated by UV-Vis. Subsequent validation is valid for two weeks from date of validation.
3. Qualified Vendors Include but are not limited to:
  - a. Cerilliant
  - b. Toronto Research Chemicals
  - c. Cayman Chemical
  - d. Sigma Aldrich
  - e. Lipomed
  - f. UTAK
4. Standards should be labeled when opened with the:
  - a. Drug Name
  - b. Manufacturer
  - c. Concentration
  - d. Lot Number
  - e. Date Opened

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f. Initials of Analyst who opened

Controls for Immunoassay-

Controls for the ELISA are prepared as needed and are evaluated against the current controls prior to use.

**C. DRUG STANDARDS PREPARED INTERNALLY**

1. Internally prepared drug standards must be validated within two weeks prior to their use in a drug quantitation. No validation is required for use as a purely qualitative standard.
2. A Drug Standard Recording Sheet (SLED Forensics Form #TOX 006) should be filled out on each drug and should be on file in the drug validation notebook. A copy of the UV-Vis data used for validation should be placed with this form. Information recorded on this log should include:
  - a. Drug name and molecular weight
  - b. Calculations used to determine amount of drug used
  - c. Source and lot # of drug standard
  - d. Date Prepared and initials of preparer
  - e. Date of UV-Vis measurement
  - f. Wavelength used for measurement
  - g. Absorbance reading and copy of Absorbance spectrum
  - h. A<sub>i</sub> information found in *Clarke's Analysis of Drugs and Poisons* or appropriate UV-Vis information from an external certification source
  - i. Acceptable absorbance range.

**3. PREPARING THE STANDARD:**

First determine the molecular weight of the free drug and the molecular weight of the salt. Calculate the ratio of the salt to free drug as follows:

$$\text{Ratio} = \frac{\text{Drug salt molecular weight}}{\text{Free drug molecular weight}}$$

The amount of drug salt required to make 10 mLs of a 1 mg/mL solution can be calculated by multiplying this ratio by 10 mg.

$$\text{Ratio} \times 10 \text{ mg} = \# \text{ mg} \times \frac{1 \text{ g}}{1000 \text{ mg}} = \# \text{ g drug salt}$$

Weigh the appropriate grams of drug using an analytical balance and place in a 10

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mL volumetric flask. Dissolve the salt using an appropriate solvent and filling to the mark on the volumetric flask. (Refer to Clarke's Analysis of Drugs and Poisons for solvent, typically Methanol is used if not found in this reference.)

Standards should be placed in an appropriate screw top vial and labeled with the following information:

- Name of Drug and Solvent in which dissolved. (ex.: Cocaine in EtOH)
- Concentration of Standard
- Initials of Preparer
- Date Prepared and/or Validated

4. UV-Vis VALIDATION:

**BACKGROUND:** UV-Vis validation refers to the use of UV-Vis spectrophotometry to validate standards. The UV-Vis spectrum recorded of a standard can be compared to a reference spectrum to ensure the identity of the standard as well as its concentration. The identity is validated by comparing the UV-Vis spectrum qualitatively with the reference spectrum, and the concentration is verified by using Beer's Law:  $A = abc$ , where  $A$  is the measured absorbance,  $a$  is the absorptivity of the compound at a given wavelength (this is a constant value for the compound),  $b$  is the path length, and  $c$  is the concentration of the sample. The  $A_1^1$  values of many drugs are provided in Clarke's Analysis of Drugs and Poisons, and this value is defined as the absorbance of a 1% solution (with units of g/100mL) using a 1 cm length cell. The absorptivity ( $a$ ) is equal to  $A_1^1/10$  and has units of L/(g cm). Hence, the concentration of a standard can be calculated from Beer's Law:  $c = A/(a b)$ .

Generally the standard will have to be diluted before the absorption spectrum is recorded. It is desirable to record the absorbance at the wavelength of interest to be between 0.1 and 3.0. The dilution factor that is necessary to achieve this absorbance can be easily calculated from the reference. For example, a 1 mg/mL (or 1 g/L) diazepam standard is to be measured, and the  $A_1^1$  is referenced to be 1020 at 242 nm in aqueous acid. Therefore,  $a = 102.0$  L/(g cm) and the absorbance ( $A$ ) would be expected to be 102 from Beer's Law since  $b$  is 1 cm and  $c$  is 1 g/L. Therefore, a 1/100 dilution of the diazepam standard must be made.

A rule for calculating this dilution is to look up the  $A_1^1$  value and appropriate wavelength in Clarke's. (ex. 257 nm  $A_1^1 = 177a$ )

If  $A_1^1 < 300$ , a 1/10 dilution will be used

If  $A_1^1 > 300$  a 1/100 dilution will be used.

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Clarke's will list the solvent to be used for dilution. Most often it is a 0.25M Sulfuric Acid solution. (0.25M Sodium Hydroxide solution and stock methanol are also used.)

Make the dilution in a 10 mL volumetric flask using 100 uL drug solution for a 1/100 dilution and 1 mL drug solution for a 1/10 dilution (a 5 mL volumetric flask may also be used if there is limited sample volume provided proper volume adjustments are performed). Add dilution solvent to the mark on the flask and mix the dilution well. Record the absorbance reading at the appropriate wavelength. (The  $A_{\lambda}$  wavelength)

Refer to protocol on operation of the UV-Vis for further information on instrument operation procedures.

**NOTE:** It is essential that the solvent being used to dilute the standard is the same as the solvent used for the blank.

**CALCULATIONS:**

$$\% \text{ error} = [ |( \text{expected} - \text{calculated} )| / (\text{expected}) ] (100\%).$$

**QUALITY CONTROL:**

a. Compare the recorded spectrum with the referenced spectrum qualitatively by ensuring that the spectra have similar band shapes and the absorption maxima are at the correct wavelengths.

b. Calculate the concentration of standard and compare to the expected concentration. The % error between the calculated concentration and the expected concentration should be within 10 %. As an example, suppose the diazepam sample from the example above (diluted 1/100) gave an absorbance of 1.00 at 242 nm. The calculated concentration is determined by:

$$c = A / (a \cdot b) = (1.00) / [(102.0 \text{ L}/(\text{g cm})) (1 \text{ cm})] = 0.00980 \text{ g/L}.$$

Since the standard was diluted 1/100, the concentration of the standard is calculated to be 0.980 g/L. The % error is therefore calculated to be:

$$\% \text{ error} = [ |(1.00 - 0.980 \text{ g/L})| / (1.00 \text{ g/L}) ] (100\%) = 2.00 \%.$$

Therefore, the standard is verified to be acceptable for use.

**NOTE:** It is realized that the UV-Vis procedure by itself does not provide ample information as to the identity of a substance since many compounds have

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similar UV-Vis spectra. The use of this procedure as a validation method also relies on the quality control and quality assurance of the manufacturer, and the identity of the standards are further indirectly validated by GC/MS data when using the standards as positive controls for casework.

**5. REVALIDATING A STANDARD:**

When revalidating standards:

The standards should be removed from the freezer or refrigerator and allowed to warm to room temperature before any manipulation is done.

A visual inspection should be performed to insure the drug has not fallen out of solution. If this is the case, the standard should be sonicated to try to assist redissolution.

Once the standard is at or near room temperature, the appropriate dilutions can be performed on the sample and absorbance measurements are made as with a new standard.

The date of revalidation and initials of analyst performing the revalidation should be placed on the tube as well as on the Drug Standard Recording Sheet on file. A copy of the UV-Vis Data should also be initialed and included with this record in the drug validation notebook.

**NOTE:** When revalidating a standard, the solution may be out of the acceptable absorbance range and thus out of concentration range. The analyst may perform limited dilutions or partially dry down the sample to get the standard within the acceptable absorbance range. These manipulations should be recorded on the Drug Standard Recording Sheet and any samples which require more than minor manipulation should be reprepared or referred to the analyst in charge of standards.

**VI. SAMPLING PROCEDURE**

- A.** Sampling of evidence is critical in toxicological analyses. One must be sure that what is sampled is representative of the total sample submitted. The analyst should take into consideration the homogeneity of the submitted sample. Inverting the tube or vortexing may be required to ensure homogeneity. A representative sample should be removed from the biological specimen.
- B.** For blood samples, when clotting is observed, it may be necessary to centrifuge samples and identify the sample as serum, particularly for volatiles analysis.

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- C. For gastric samples, if quantitation is to be performed, the entire gastric contents must be available. A total volume or mass will be measured (using graduated cylinders of appropriate size or a balance). The sample should be homogenized prior to analysis. Weigh or pipette a portion of the gastric contents (1-2 g or mL) and dilute to desired dilution factor with water (typically 1/10).
- D. In cases where only a portion of the gastric contents is submitted, gastric may be analyzed for qualitative purposes only. Pill fragments from gastric may also be analyzed independently but reported appropriately as such.
- E. Tissue samples are generally considered to be homogenous throughout the tissue and a portion of the tissue may be sampled and analyzed. Refer to Analysis of Tissue Specimens procedure for detailed instructions.
- F. Sample aliquots are not considered subitems. They are representative of the parent item. The location of the aliquot is tracked through the case analysis QC pack and the entirety of the aliquot is consumed each time.

**VII. QUANTITATIVE/QUALITATIVE MASS SPECTRAL BATCH ANALYSES**

**A. Sample Volume**

1. The appropriate sample volume for most standard mass spectral analyses is 1.0 mL of biological fluid (blood, tissue homogenate, etc.) unless otherwise notated in the procedure.
2. Some samples require less volume due to the presence of higher concentrations of the drugs. Dilutions of the sample may be required in order to keep the case sample within the linear dynamic range of a particular calibration curve.
3. Dilutions can be achieved by using a smaller volume of sample and then adding DI water to the sample to bring the final volume to 1.0 mL.
4. Dilutions should be documented and for quantitations, appropriate calculations performed to account for the dilution when determining the quantitative value.

**B. Internal Standards**

Appropriate internal standard will be used. Internal standard identity and concentration are determined based on the concentration and identity of the analyte thought to exist in the sample.

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1. Internal standards should be of similar chemical characteristics and structure to the analyte of interest to ensure that the extraction efficiency of both internal standard and analyte are similar.
2. The internal standard should be able to be chromatographically resolved from the analyte and/or produce ions that are readily distinguishable from those of the analyte.
3. Where appropriate, deuterated internal standards should be used. Due to fluctuations in availability of standards, the analyst should refer to previously analyzed quantitations/qualitative analyses for current compounds available as internal standards and concentrations used.
4. Hexobarbital is an appropriate internal standard for acidic and neutral compounds and, similarly, SKF-525A is an appropriate internal standard for basic compounds for qualitative analysis.

**C. Interpretation of Results**

1. A compound is identified by comparing the mass spectrum and retention time of the unknown compound with the mass spectrum and retention time of a known compound.
2. The source of the mass spectrum of the known compound can be derived from purchased data libraries, data libraries from reputable sources or in-house libraries. The mass spectrum may also be determined by analyzing a neat standard of the compound.
3. The relative retention time should be within  $\pm 2\%$  of the comparison control for GC-MS and  $\pm 3\%$  for LC-MS/MS.
4. A positive confirmation of a compound can only be reported when the unknown compound has been identified, in the toxicologist's opinion, as a match. The toxicologist's opinion should be based on the following criteria: presence of appropriate characteristic fragments, relative abundances, base peak, molecular weight, and retention time.
5. The signal to noise (S/N) for qualitative analyses should be a minimum of approximately 3/1 for a compound to be identified.
6. Additional criteria may include results from immunoassay or other approved analytical techniques.

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**NOTE:** The autotune on the HP 5973 is a maximum sensitivity autotune that optimizes the instrument for greater sensitivity of higher molecular weight ions. As a result, the abundance of ion 219 is much greater than with other tunes and the base peaks may be either 69 or 219. This increased sensitivity of higher molecular weight ions may result in ion ratios that differ significantly from previously published library results. This should be accounted for when identifying analytes by GC-MS.

**D. Selected Ion Monitoring (SIM) and Multiple Reaction Monitoring (MRM)**

**Quantitations:**

1. When quantitations/qualitative analyses are performed that use SIM/MRM, guidelines are needed to establish the acceptable ion ranges for the qualifier ions. The following ranges should be used for the corresponding relative abundances:

| % Abundance of qualifier ion relative to the Target Ion | Acceptable relative % ion abundance ranges |
|---------------------------------------------------------|--------------------------------------------|
| > 50%                                                   | ±20%                                       |
| 25-50%                                                  | ±25%                                       |
| < 25%                                                   | ±30%                                       |

2. When performing quantitations in SIM/MRM, it is preferred to have one target ion and two analyte qualifying ions. For analytes with limited qualifying ions, it is minimally acceptable to use one target ion and one qualifying ion.
3. When quantitations are run in SIM/MRM mode, compounds at levels below the lowest calibrator will not be reported. Due to the increased sensitivity of the SIM/MRM mode, methods are designed to analyze down to the low therapeutic range for most compounds and the lowest calibrators are at or near the limit of quantitation. Reporting values below these levels reduces the confidence of the identification to a level that is unacceptable on a routine basis.
4. Also in SIM/MRM mode, if the qualifier ions are out of range, the compound will not be identified.

**E. Calibration Curves**

1. At least 4 calibrators must be included in the calibration curve.
2. The response curve is prepared comparing integrated area under the curve of an analyte to integrated area under the curve of internal standard ratio vs calibrator

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concentration. It is acknowledged that some assays are inherently non-linear (e.g. LC-MS/MS) and the use of quadratic models may be necessary and appropriate. The response curve and determined unknown specimen concentration(s) are generated by the instrument software.

3. The correlation coefficient (R) of the linear least squares fit of the data should be greater than 0.990 or an  $R^2$  of 0.980 in order for the quantitative results to be considered reliable.
4. A weighted curve fit ( $1/x$  or  $1/x^2$ ) should be used at all times regardless of whether linear or quadratic curve model is selected.

**F. Quality Control for Batch Analyses-**

1. Batch analyses should each have appropriate calibrators, a negative control and appropriate number of positive controls.
2. For batch analyses using autosamplers, the identity of each vial and vial placement must be verified by an independent analyst with the instrument autosampler sequence list. Additional batch verifications may be documented throughout the process as needed.
3. For quantitations, a positive control should bracket unknown samples with a maximum of ten samples in between each positive control. For qualitative analyses, the batch will contain at a minimum a positive and negative control.
4. For quantitations, the value of the positive control should fall within 30% of the actual value if a generic internal standard is used or 20% of the actual value if a matched deuterated internal standard is used.
5. If a positive control is outside of the acceptable range, values from unknown samples bracketed by that positive control cannot be reported for the compound that was out of range.

NOTE: If a trend of unacceptable positive control results is identified, the source of the nonconformity needs to be identified before continuing with nonconforming work. Possible routes of investigation may include pipette re-verification, standard evaluation (ie. UV-Vis or GC-MS), evaluation of replicate neat controls for repeatability/instrument performance, etc..

6. For quantitations, the positive controls should be made from differing sources than calibrators. Alternatively, if differing sources are not available, positive

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controls must be made by a secondary analyst and documented on the Quantitation Worksheet.

7. The different sources for standards can be from two vendors, from different lot numbers from the same vendor or from two vials from the same vendor and lot that can be uniquely identified. If standard vials from the same vendor and lot are opened on the same date, vials will be marked with a letter to be able to distinguish them uniquely.
8. It is recommended that the positive control be at a concentration that is near the midpoint of the calibration curve. Deviations from this procedure must be approved by the departmental Lieutenant.
9. For a quantitative value to be reported, it should not fall above the linear range of the curve. Dilutions and reextraction may be necessary to bring the concentration into the linear range of analysis. In some instances, it may be appropriate to report a compound as greater than the highest calibrator but an absolute numerical value will not be reported that is greater than the highest calibrator. Reporting compounds as less than the lowest calibrator may be appropriate with certain compounds that are analyzed in SCAN mode.
10. Manual integrations should be avoided if possible. In case where a manual integration may be necessary, (ie. Overabundance or low abundance of analyte, coeluting peaks of similar mass to charge, broad or tailing peaks) the specific reason for the manual integration must be documented in the case record. For SIM/MRM analyses, this documentation must be approved by the departmental lieutenant or a team/technical leader.
11. For quality assurance purposes, each batch analysis must be reviewed by an authorized independent analyst and this review is documented with a signature and date on the batch worksheet.
12. The quality control packet will be maintained electronically within the Toxicology Department Files on a secure network folder and will contain at a minimum the following:  
QUANTITATION PACKET:
  - Quantitation Worksheet(s)
  - Autotune/Tune Evaluation of Instrument prior to Neat Standard Analysis
  - Neat Standard Mix Analysis
  - Autotune/Tune Evaluation prior to batch sequence (if repeated prior to batch)

QUALITY MANUAL

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- Extraction Log
- Instrument Sequence Log and/or Worklist
- Data Analysis Method Parameters
- Calibration Curves
- Detailed Quantitation Report for Negative Control mass spectra for internal standards identified in analysis and Summary Quantitation Report for Blank prior to Negative Control, if applicable.
- Detailed Quantitation Report for one Positive Control from Batch with mass spectra for all compounds identified in analysis and Summary Quantitation Report for Blank prior to Positive Control, if applicable
- Summary Quantitation Report for subsequent Positive Controls from Batch with Summary Quantitation Report for Blank prior to Positive Controls, if applicable.

**QUALITATIVE PACKET:**

- Qualitative Worksheet(s)
- Autotune/Tune Evaluation of Instrument prior to Neat Standard Analysis
- Neat Standard Mix Analysis
- Autotune/Tune Evaluation prior to batch sequence (if repeated prior to batch)
- Extraction Log
- Instrument Sequence Log and/or Worklist
- Data Analysis Method Parameters
- Detailed Report for one Positive Control from Batch with mass spectra for all compounds identified in analysis and Summary Quantitation Report for Blank prior to Positive Control
- Detailed Report for Negative Control with library matches for internal standards identified in analysis and Summary Report for Blank prior to Negative Control, if applicable.
- Summary Report for subsequent Positive Control(s) from Batch with Summary Quantitation Report for Blank prior to Positive Control(s), if applicable.

13. Once the Quality Control Packet has been reviewed by the technical reviewing scientist or other qualified approver, the case data can be released to the case files.

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14. The signing analyst will document the review of all Quality Control Data that was relied upon to support the reported conclusions on the case Table of Contents.

**G. Multiple Data Sets for Quantitations**

1. When multiple quantitations exist for the same analyte, the results having demonstrably better QC data should be reported. (For example, QC data may be characterized by the degree of fit of the curve, the extraction efficiency or by a calibration curve which more closely reflects the range of interest.)
2. When multiple dilutions are analyzed, report the least dilute sample that falls within the quantitation range of the assay.
3. Alternately, if the QC data is similar and results are within 20% of the mean, the mean should be reported. (Due to the lack of significant figures for quantitative values on the lower end of the curve, reporting the average is acceptable for cases that fall within Calibrators 1-3 regardless of the percent difference.) If the two results are outside of 20% of the mean then an additional quantitation should be performed.
4. If after this repeated testing, no two values meet this criteria, the sample may be reported as “unsuitable for quantitation” for this compound.
5. The explanation for why one data set was used over another or why the sample was deemed unsuitable for quantitation should be documented in the case record.

**VIII. DATA RETENTION**

**A.** Case files will contain the following:

1. SLED Toxicology Case Jacket Table of Contents/Data Acceptance Form.
2. All data specific to the case that was utilized to formulate the final report.
3. All pages should contain the case lab number and initials of assigned analyst or electronic equivalent.

**B.** Digital analytical data will be stored on instrument computers for a sufficient time to allow processing and review. Data will then be transferred to a secure server storage. If necessary, data will be written to CD for archival storage. The CD's will be indexed and made searchable for retrieval if needed.

**IX. REFERENCE COLLECTIONS**

- A.** Reference collections of data or materials used for identification, comparison or interpretation shall be fully documented, uniquely identified and properly controlled.

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- B.** Purchased data libraries (ie. NIST) are fully documented and uniquely identified. No changes may be made to these libraries. Removal of these libraries must be approved by a departmental supervisor.
- C.** Data libraries obtained from reputable forensic sources (ie. AAFS, SWGDRUG, Cayman Chemicals) are fully documented and uniquely identified. These libraries will be treated the same as purchased libraries.
- D.** In-house libraries may be created and utilized. Each entry should be identified with a unique tracking number generated by the instrument software. These libraries should only be edited by personnel as approved by the department Lieutenant. Entries in in-house libraries shall have listed at a minimum the following information for each compound entered:
  - 1. Compound name.
  - 2. Standard lot number and manufacturer.
  - 3. Relative retention times for DB-1(or equivalent) and XTI-5(or equivalent) column types.
  - 4. Initial of person making the entry and the date entry made.
  - 5. Additional information may be entered at the discretion of the assigned analyst or departmental supervisor.
- E.** A reference collection of drug standards is available for use as reference standards. These are uniquely identified by lot number and manufacturer and an inventory is maintained.
- F.** Controlled substances will be tracked utilizing the SLED Toxicology Drug Standard Register (Form TOX 043). The form tracks the drug name, manufacturer, lot number, date received and method of receipt. All controlled substances will be weighed to include their container upon receipt. At each subsequent entry of the standard a weight will be taken before and after a portion of the standard is removed for analysis. Any discrepancy in these weights should be reported to the Toxicology Supervisor.

**X. TECHNICAL REVIEW OF TOXICOLOGY BATCH DATA**

- A.** Review of quality control measures performed in the Toxicology Department will be done by a senior analyst within the Toxicology department as specified by the departmental Lieutenant.
- B.** Multiple individuals may be assigned to review the different types of data produced within the department.
- C.** The technical review of quality control data will be documented in the case record prior to batch case data being released to analysts.
- D.** The technical review of Quality Control Data will include the following as applicable:
  - i) Compare QC results to target and acceptable ranges
  - ii) Review QC for typographical or handwriting errors
  - iii) Ensure standards used are valid and appropriate

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- iv) Review linearity of quantitation curves
  - v) Review peak shapes and area counts
  - vi) Review QC for consistency with written procedures
  - vii) Ensure that each page of analytical data bears the initials of the analyst responsible for the analyses or electronic equivalent.
- E. The analysts assigned to technical review of QC data are expected to:
- i.) Maintain awareness of processes and procedures used in the department and identify departures from written procedures or good laboratory practice.
  - ii.) Recommend changes to procedures, policies and processes to ensure and improve laboratory quality
  - iii.) Advise supervisory staff to initiate action when QC is out of tolerance or other quality issues are identified.
- F. All Toxicology staff are expected to:
- i.) Actively participate in quality processes of the department
  - ii.) Run appropriate QC as noted in analytical procedures
  - iii.) Properly document procedures as followed and make note of any quality related issues within QC documentation.
  - iv.) Place QC documentation in the designated area for review by the QC reviewer in a timely manner
  - v.) Participate to actively resolve noted issues identified in any laboratory processes and to further develop the laboratory quality processes.

## **XI. METHOD VALIDATION**

### **A. Introduction**

1. Method validation is an integral step in the measurement assurance process as it ensures new and updated methods are appropriate, accurate and reliable.
2. The Supervisor, Technical Leader or responsible analyst will design a specific testing protocol to validate the accuracy of a new method or to verify the accuracy of a method transferred from one instrument to another.
3. An appropriate validation plan is created.
4. Upon successful completion of the validation, the validation form is approved and signed by the Supervisor, Technical Leader, Captain and Quality Manager; the report and a summary of the data are retained by the Quality Manager. Full Validation Data will be maintained in the Toxicology Department.
5. Reference materials utilized in method validations will be purchased from an approved vendor and will be fit for purpose.

### **B. Transfer of a Method to New Instrumentation**

1. Proper calibration and operation of new instrumentation is verified and documented using the applicable instrument calibration process.
2. Analytical results are compared between the new and old instrumentation.

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3. Where possible, side by side operation of old and new instrumentation is compared using the same samples or extracts.
4. Standards, internal standards, and quality control samples should meet their respective criteria described in the analytical procedure.
5. Test specimens should also be run and should meet acceptability criteria established for quality control samples or similar proficiency test results.
6. As applicable, replicate analytical runs should be made on a single day and also compared between multiple days; criteria should meet those established for standards, internal standards, and quality controls; test specimens should meet acceptability criteria established for quality control samples or similar proficiency test results.
7. As applicable, a new procedure for instrument operation is written.
8. As applicable, measurement uncertainty may be calculated (quantitative methods only).
9. As applicable, analysts are trained in operation of new instrumentation.

**C. Development of a New Analytical Method**

1. The departmental lieutenant or a team/technical leader outlines proposed steps for a new analytical method based upon literature, other established procedures, or knowledge and experience of the materials to be analyzed.
2. The target sensitivity and linear range is established based upon expected sample concentrations and instrument performance.
3. Standard curves are run to assess the applicability of the proposed analytical technique in comparison to the targeted analytical needs.
4. Once it is determined that sensitivity and range are appropriate, the method is evaluated for interference from expected matrices or co-occurring substances.
5. The assay is evaluated for stability by repeating analysis of single prepared samples and duplicate preparation of samples both within day and between days.
6. Variability of standards, internal standards, quality control samples, and specimens are evaluated with respect to literature values and/or similar established procedures.
7. Criteria for acceptable standard curves, internal standards, and/or quality control samples are established.
8. A formal procedure is written.
9. As applicable, analysts are trained.
10. Results are reviewed for stability between analysts.
11. As applicable, measurement uncertainty may be calculated if the method will result in the reporting of a quantitative result.
12. Method results may also be evaluated against external standards or specimen results from reference laboratories; criteria for acceptability will be similar to that established for quality control samples.

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**D. Components of Method Validation**

1. Method validation is the process of testing an analytical method, technique, or instrument to determine its suitability for meeting its intended purpose and to document its reliability under expected conditions of use.
2. A validation plan is developed to outline steps necessary to evaluate the reliability of the new technique, procedure, instrument, or significant modification thereof.
3. Generally the validation process is expected to:
  - a. Evaluate whether a new testing method meets identified analytical needs and current scientific practices.
  - b. Compare the new test method's performance with existing laboratory methodology.
  - c. Describe the conditions under which a testing method will produce valid results.
  - d. Predict possible sources of error.
  - e. Determine limitations of a testing method.
  - f. Establish baseline characteristics of the testing method (linearity, accuracy, etc.) which serve as benchmarks to evaluate future method. These characteristics may include:
    - i. Linearity/linear range: Linear range is a defined concentration range across which the method is valid. Criteria evaluated, as applicable, to determine the linear range include: accuracy of quantitations, completeness of mass spectra, peak shape, retention time, ion ratios, and signal to noise ratio. Weighting schemes such as  $1/x$  or a quadratic curve may be utilized. Linearity is expressed by the correlation coefficient ( $r$ ) or coefficient of determination ( $r^2$ ).
    - ii. Accuracy/precision: Accuracy is the ability to quantitate the true value of a control, standard, or sample. Precision is the closeness of agreement between many quantitations. Both accuracy and precision will be calculated using known controls and/or standards at several concentrations. The relative standard deviation and/or coefficient of variation will be used to determine precision and accuracy.
    - iii. Specificity/selectivity: The selectivity of a method is the extent to which the method can determine a particular analyte of interest accurately in the presence of other components that may be expected to be present in the sample matrix. This parameter will be evaluated using actual samples and comparing the results to known methodologies.
    - iv. Ruggedness/robustness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. These would include different analysts, reagents, extraction days, etc. These parameters are similar to precision and accuracy and will be evaluated using the results obtained from repeat analysis from several analysts. Robustness is the capacity of a method to remain unaffected by small deliberate variations in the method parameters. These changes/variations will be selected based on possible deviations from

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the analytical procedure such as extraction time, dry down time, solvent volume, changes in LC buffers, etc.

v. Recovery/process efficiency/matrix effects:

1. The recovery of a method is calculated by comparing the detector response of an extracted sample to that of a sample spiked with the same concentration of drug and internal standard (IS) after the dry down step.
2. The process efficiency of a method is calculated by comparing the detector response of an extracted sample to that of a pure standard of the same concentration.
3. The matrix effect of the method is determined by comparing area of neat standard to area of neat standard combined with extracted matrix.

vi. Limit of detection/quantitation: The limit of detection is the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitated. The limit of quantitation is the lowest concentration of an analyte that can be determined with acceptable precision and accuracy or is the amount equal to the lowest point on the calibration curve that can be determined with acceptable precision and accuracy.

vii. Carryover: Carryover will be evaluated using high control samples, where possible, followed by blanks or negative controls. The high control samples will begin at the high calibration curve point and move upward through concentrations that would far exceed those expected in casework. If high concentration control samples are unavailable, a number of casework samples will be evaluated to ensure that routine concentrations encountered in casework will not carry over into the next sample.

## **XII. REPORTING GUIDELINES**

The following formats will be regarded as acceptable verbiage to be used in the generation of formal reports as issued by the Toxicology Department of the South Carolina Law Enforcement Division. Inclusion of additional information (ie. drug information, synonyms, therapeutic, toxic, or lethal ranges) may be included at the discretion of the responsible Toxicologist, with prior approval by the departmental Lieutenant.

The analytical method used will be designated as a section header in the Results portion of the Toxicology report.

- A. **ETHANOL**: All positive ethanol levels from biologicals will be reported after truncating to three decimal places. All positive ethanol levels from non-biologicals will be reported after truncating to one decimal place. Proof will be reported to the nearest whole number. The average of all valid values from replicate/duplicate samples will be reported. The uncertainty of measurement range for blood/serum/plasma levels will be reported for all case types involving living subjects (ie. DUI and CSC).

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**1. Blood (DUI or CSC):**

| Analyte | Result           | Units    | Threshold |
|---------|------------------|----------|-----------|
| Ethanol | 0.###<br>± 0.### | % (g/dL) | 0.010     |

The above range is calculated at 99.7% confidence.

|         |          |          |       |
|---------|----------|----------|-------|
| Ethanol | Negative | % (g/dL) | 0.010 |
|---------|----------|----------|-------|

**2. Serum or Plasma (DUI or CSC):**

| Analyte | Result          | Units    | Threshold |
|---------|-----------------|----------|-----------|
| Ethanol | 0.###<br>±0.### | % (g/dL) | 0.010     |

The above range is calculated at 99.7% confidence.

(Calculated from serum/plasma Ethanol levels.)

|         |          |          |       |
|---------|----------|----------|-------|
| Ethanol | Negative | % (g/dL) | 0.010 |
|---------|----------|----------|-------|

**3. Blood (Postmortem),Urine or Ocular Fluid:**

| Analyte | Result | Units    | Threshold |
|---------|--------|----------|-----------|
| Ethanol | 0.###  | % (g/dL) | 0.010     |

|         |          |          |       |
|---------|----------|----------|-------|
| Ethanol | Negative | % (g/dL) | 0.010 |
|---------|----------|----------|-------|

**4. Serum or Plasma (Postmortem):**

| Analyte | Result | Units    | Threshold |
|---------|--------|----------|-----------|
| Ethanol | 0.###  | % (g/dL) | 0.010     |

(Calculated from serum/plasma Ethanol levels.)

|         |          |          |       |
|---------|----------|----------|-------|
| Ethanol | Negative | % (g/dL) | 0.010 |
|---------|----------|----------|-------|

**5. Tissue Specimens:**

| Analyte | Result | Units  | Threshold |
|---------|--------|--------|-----------|
| Ethanol | 0.###  | g/100g | 0.010     |

|         |          |        |       |
|---------|----------|--------|-------|
| Ethanol | Negative | g/100g | 0.010 |
|---------|----------|--------|-------|

**6. Non Biologicals:**

| Analyte | Result | Units | Threshold |
|---------|--------|-------|-----------|
| Ethanol | ##.#   | % v/v | 1.00      |

This is equivalent to ## proof.

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- B. VOLATILES (excluding ethanol).** The accepted reporting format for volatiles includes the level of the analyte present (truncated to three decimal places), the analyte identified, and the body fluid or tissue tested. No corrections will be made for volatiles found in serum, plasma, ocular, or urine specimens based strictly on water content. Volatiles excluding ethanol in non-biological samples will be reported in w/v (g/dL) percent. The average of all valid values from replicate/duplicate samples will be reported.
- C. ETHYLENE GLYCOL:** When quantitated, the accepted units for reporting Ethylene Glycol are mg/mL. Quantitative values are not typically reported for ethylene glycol due to the fact that the presence alone of ethylene glycol provides adequate interpretive value.
- D. DRUGS and/or POISONS:** Quantitative drug or poison results should be reported to two significant figures as ng/mL or µg/mL, or ng/g or µg/g for tissue. Qualitative results should only report "compound" and/or metabolites found. Thresholds may be reported when known. Interpretive notes may be included if verified by a departmental supervisor. There must be two valid tests in order to report a drug either qualitatively or quantitatively (i.e. Positive drug screen and a valid confirmation, or valid match on GC/MS general and valid confirmation). The analyst must submit two independent aliquots for analysis.

**NOTE:** Tissue samples only need to be homogenized one time. The analyst may utilize two aliquots from the same homogenate for the two independent tests.

**E.**

For blood, ocular fluid, urine (qualitative only):

| Analyte    | Result   | Units | Threshold |
|------------|----------|-------|-----------|
| "Compound" | 0.##     | ng/mL | 0.##      |
| "Compound" | Positive | ng/mL | 0.##      |

For tissue samples:

| Analyte    | Result   | Units | Threshold |
|------------|----------|-------|-----------|
| "Compound" | 0.##     | ng/g  | 0.##      |
| "Compound" | Positive | ng/g  | 0.##      |

**NOTE:** Quantitative values are not reported in body fluids other than blood, ocular or tissue unless extenuating circumstances exist which warrant such measures. All such circumstances should be reviewed by a departmental supervisor prior to performance of quantitation and documented in the case record.

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**NOTE:** Compounds which have little or no toxicological significance, either in general or for a specific case, should be documented only in the appropriate place in the case record. These compounds will not normally be included in the final report, unless warranted by extenuating circumstances.

**F. IMMUNOASSAY:**

Results from immunoassays can be listed as follows:

| Analyte | Result   | Units | Threshold |
|---------|----------|-------|-----------|
| "Assay" | Positive | ng/mL | ###       |
| "Assay" | Negative | ng/mL | ###       |

**NOTE:** See other acceptable write-ups for additional information.

**G. CARBON MONOXIDE:**

Positive results should contain two significant figures.

| Analyte           | Result   | Units | Threshold |
|-------------------|----------|-------|-----------|
| Carboxyhemoglobin | ##       | %     | 10        |
| Carboxyhemoglobin | Negative | %     | 10        |
| Carboxyhemoglobin | > 75     | %     | 10        |

**NOTE:** Carboxyhemoglobin levels found in excess of 75% will be reported as seen above.

**CARBON MONOXIDE ALTERNATE:** If samples are found to be unsuitable for quantitative analysis utilizing the AVOXimeter 4000 an alternate Ammonia/UV-VIS semi-quantitative procedure is available. Results from the Ammonia Method should be reported as follows under the UV-Vis Result Section:

| Analyte           | Result   | Units | Threshold |
|-------------------|----------|-------|-----------|
| Carboxyhemoglobin | Negative | %     | 20        |
| Carboxyhemoglobin | 20-50    | %     | 20        |
| Carboxyhemoglobin | > 50     | %     | 20        |

**G. SPOT TESTS:**

Spot tests may be performed as qualitative screening tests. These tests typically provide additional evidence to corroborate other analyses or may be indicative that additional testing may be required. Spot test results may be reported at the analyst's discretion.

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- H. OUTSIDE TESTING:** Results of tests performed by independent laboratories will be properly annotated as follows:  
(Analysis performed by "Name of Laboratory")  
Alternate units as reported by the external laboratory may be used for reporting outside laboratory analysis as approved by a departmental supervisor.
- I. OTHER ACCEPTABLE WRITEUPS MAY INCLUDE:**
- A. Sample unsuitable for analysis due to specimen condition.
  - B. Sample unsuitable for analysis due to contamination with embalming fluid.
  - C. Insufficient sample for further analysis.
  - D. No analysis performed due to insufficient sample.
  - E. Insufficient sample for quantitation/confirmation.
  - F. Requested analysis not performed due to submission of inappropriate specimen.
  - G. Determination of "analyte" in "specimen type" not available from this laboratory at this time.
  - H. Immunoassay indicates the presence of "analyte". Insufficient sample for confirmation/quantitation by "method".
  - I. Immunoassay indicates the presence of "analyte" and/or "analyte" metabolites. Insufficient sample for confirmation by "method"
  - J. No analysis performed.
  - K. If the concentration of an analyte in an unknown specimen is less than the concentration of the analyte in the lowest calibration specimen then the report may reflect the concentration of the analyte as "less than" the concentration of the lowest calibrator. This is only pertinent to GCMS analysis performed in SCAN mode where an identification of the compound meets S/N requirements.
  - L. In some situations it may be appropriate if the concentration of an analyte in an unknown specimen is higher than the concentration of the analyte in the highest calibration specimen then the report may reflect the concentration of the analyte as "greater than" the concentration of the highest calibrator
  - M. Analysis performed by "external laboratory".
  - N. Any notation as approved by the departmental Lieutenant.

**SIGNING OF REPORTS:**

The reporting analyst marking the report complete in LIMS indicates that they have reviewed all case data and corresponding quality data, agree with the results included on the report and are issuing the findings based on their opinions and interpretations.

In the event that the reporting analyst is not available for courtroom testimony the supervisor or another analyst can perform a secondary case review for testimony. The secondary reviewer must sign and date the SLED Toxicology Case Jacket Table of Contents/Data Acceptance form (TOX012) after reviewing the case jacket contents, report, and all associated Quality Control data.

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### **XIII. ESTIMATION OF THE UNCERTAINTY OF MEASUREMENT**

- A. An estimation of the uncertainty of measurement shall be determined for all analytical procedures in the toxicology section in which a quantitative measurement is reported.
- B. Estimating the Uncertainty of Measurement
1. Estimations of the uncertainty of measurement shall be conducted and documented using an uncertainty budget.
  2. The uncertainty budget for a given procedure shall include both random (Type A) uncertainties and systematic (Type B) uncertainties.
  3. Since the uncertainty of measurement is only an estimate, generally uncertainties shall not exceed two significant figures.
  4. To be conservative, calculations used to estimate the uncertainty and the final combined uncertainty should be rounded up.
  5. In order to combine the uncertainty, the units of uncertainty values should be measured in the same units. Typically, it is beneficial to express all uncertainty values in % to eliminate the necessity to convert measurements to the same units.
- C. Random (Type A) Uncertainty
1. Random (Type A) uncertainty results from measurement values being scattered in a random fashion due to laws of chance and thus has a normal or Gaussian shaped distribution.
  2. Random (Type A) uncertainty is best determined by historical data of a large number of repeated measurements.
  3. Where appropriate, the toxicology section relies on the use of control charts to establish the historical standard deviation for common quantitative procedures.
  4. The standard deviation used for uncertainty calculations shall be updated annually.
  5. For new methods that lack historical control data, the toxicology section may use repeatability data available from the test method validation data.
  6. For less frequently used quantitative analyses which do not have a historical record of many measurements to rely upon, multiple measurements shall be taken to determine the standard deviation of the mean.
    - a. The standard deviation of the mean for multiple measurements is divided by the square root of the number of measurements (n),  $\sigma_{\text{mean}} = \sigma/\sqrt{n}$ .

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- b. When using the standard deviation of the mean for measurements that lack repeatability data, a corrective coverage factor (kcorr) must be calculated from the Student t table (see table below)
  - c. Alternatively, due to the very large calculated uncertainty associated with this type of statistical analysis, the analytical result may be reported as qualitative only.
7. When multiple measurements are performed on case specimens (e.g. BAC analysis performed in duplicate) the measurements shall be averaged.
- a. In these instances, multiple measurements reduce the uncertainty of measurement.
  - b. The improvement in the uncertainty arises from the number of measurements that went into the generation of the mean result.
  - c. When multiple measurements are performed and the results are averaged, uncertainty is calculated using the standard deviation of the mean ( $\sigma_{\text{mean}}$ ) which is calculated by dividing the historical standard deviation ( $\sigma$ ) by the square root of the number of measurements (n) using the equation  $\sigma_{\text{mean}} = \sigma/\sqrt{n}$ .

**D. Systematic (Type B) Uncertainties**

1. Systematic uncertainty results from the inherent biases in measuring systems and quantitative analytical methods. These uncertainties may be reduced by optimizing the method or measuring system, but can never be completely eliminated.
2. Examples of some of the systematic uncertainties within the Toxicology Section include the following:
  - Using an analytical balance to weigh a powdered standard in the preparation of a calibrator, control or internal standard. This uncertainty includes digital balance resolution, corner loading (shift test) and uncertainty associated with the calibration weight.
  - Preparation of calibrator, internal standard or control solution using 10 mL volumetric flask.
  - Using an analytical pipette to prepare calibrator or controls.
  - Using a pipette to sample 1 mL of specimen.
  - Using a Hamilton diluter to prepare calibrators, controls and specimens for BAC analysis by headspace GC.
  - Using a repeater pipette to dispense internal standard into all calibrators, controls and case specimens.
  - Uncertainty associated with Certificates of Analysis on analytical standards (e.g., Cerilliant Certificate of Analysis on Ethanol-50 standard indicating ethanol concentration of  $50 \pm 1.55$  mg/dL at 95% confidence). Note: A 95% confidence level is equivalent to  $2\sigma$  and should be divided by 2 for use in uncertainty calculations (which calculates uncertainty based on  $1\sigma$ ).
3. Within the toxicology department, the scope of the control data used in the estimation of Type A uncertainty already encompasses the errors associated with Type B uncertainty including

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instruments, maintenance, analysts, pipettes and calibrators. Nonetheless, a comprehensive statistical analysis should be performed to verify that the contribution of Type B uncertainty is insignificant relative to the Type A uncertainty.

4. The use of an internal standard for quantitative analysis minimizes other sources of uncertainty including instrumental factors such as the injector, GC column and detector. These miscellaneous uncertainty factors have no significant impact on the overall estimation of uncertainty and are therefore not included in the calculation.
5. Systematic (Type B) uncertainties resulting from measurement bias typically have an equal chance of falling within a range and therefore follow a rectangular or random distribution. With rectangular distribution, the range ( $\pm a$ ) of the outer limits is used to estimate the standard deviation ( $\sigma$ ) using the equation  $\sigma = a/\sqrt{3}$ .

For example, a 10 mL volumetric flask has a tolerance of  $\pm 0.2$  mL. The calculated uncertainty associated with this measurement is  $0.2/\sqrt{3}$  or 1.15. To maintain only 2 significant figures, the uncertainty for this measurement used in the uncertainty budget is 1.2 (after rounding up).

E. Combination of Uncertainties

Uncertainties are combined using the Root Sum Squares technique

$$U_{\text{combined}} = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 \dots)}$$

F. Determination of Confidence

1. In order to determine the expanded uncertainty, the combined uncertainty is multiplied by the coverage factor ( $k$ ) using the equation  $U_{\text{expanded}} = U_{\text{combined}} \times k$  for routine measurements with a large amount of historical data ( $n > 30$ )
  - The coverage factor for 95% confidence is  $k = 2$
  - The coverage factor for 99.7% confidence is  $k = 3$
2. For analysis with reduced confidence due to lack of historical data, a corrected coverage factor ( $k_{\text{corr}}$ ) is used based on the Student's  $t$  Distribution.

For example, for an analysis with no historical control data, a control is analyzed 15 times (degrees of freedom or  $d_f = n - 1$ , or 14 in this example). Using the Student's  $t$  table below,  $k_{\text{corr}}$  value of 3.32 would be used to calculate the expanded uncertainty at 99.7% confidence limit. The expanded uncertainty would be:  $U_{\text{expanded}} = U_{\text{combined}} \times 3.32$

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The following table contains the Student-t Distribution for  $k_{corr}$  at a 99.7% confidence level ( $d_F$ = degrees of freedom):

| $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| 1     | 127.3      | 8     | 3.83       | 15    | 3.28       | 22    | 3.11       | 29    | 3.03       |
| 2     | 14.09      | 9     | 3.69       | 16    | 3.25       | 23    | 3.10       | 30    | 3.03       |
| 3     | 7.45       | 10    | 3.58       | 17    | 3.22       | 24    | 3.09       | 40    | 2.97       |
| 4     | 5.59       | 11    | 3.49       | 18    | 3.19       | 25    | 3.07       | 50    | 2.93       |
| 5     | 4.77       | 12    | 3.42       | 19    | 3.17       | 26    | 3.06       | 60    | 2.91       |
| 6     | 4.31       | 13    | 3.37       | 20    | 3.15       | 27    | 3.05       | 80    | 2.88       |
| 7     | 4.02       | 14    | 3.32       | 21    | 3.13       | 28    | 3.04       | 100   | 2.87       |

The following table contains the Student-t distribution for  $k_{corr}$  at 95 % confidence level ( $d_F$ = degrees of freedom)

| $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ | $d_F$ | $k_{corr}$ |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| 1     | 12.71      | 8     | 2.31       | 15    | 2.13       | 22    | 2.07       | 29    | 2.05       |
| 2     | 4.30       | 9     | 2.26       | 16    | 2.12       | 23    | 2.07       | 30    | 2.04       |
| 3     | 3.18       | 10    | 2.23       | 17    | 2.11       | 24    | 2.06       | 40    | 2.02       |
| 4     | 2.78       | 11    | 2.20       | 18    | 2.10       | 25    | 2.06       | 50    | 2.01       |
| 5     | 2.57       | 12    | 2.18       | 19    | 2.09       | 26    | 2.06       | 60    | 2.00       |
| 6     | 2.45       | 13    | 2.16       | 20    | 2.09       | 27    | 2.05       | 80    | 1.99       |
| 7     | 2.37       | 14    | 2.15       | 21    | 2.08       | 28    | 2.05       | 100   | 1.98       |

**G. Reporting the Estimated Uncertainty of Measurement**

1. Measurement uncertainty will be calculated for all quantitative measurements.
2. The measurement uncertainty will only be reported when it is relevant to the validity of or application of the test results, when a customer requires it be reported or when it affects compliance to a specification limit.
3. The measurement uncertainty will be routinely reported for ethanol quantitations in blood. This is due to the statutory specifications within the state code of laws.
4. Other measurement uncertainties will be maintained internally and will be provided as information as needed but will not be reflected on the report without specific request.

**XIV. CASE REVIEW – SCOPE**

**A. Administrative Review –**

1. Definition – The administrative review is a review of the report and case file to check for consistency with laboratory policy and for editorial correctness
2. Scope – The administrative review will include the following as applicable:
  - The case report is free of typographical/spelling errors

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- The case number is on each page of the case file
- The analyst's initials are on each non-administrative page in the case file.
- The analyst performing the work is clearly identified.
- The Chain of Custody is complete
- The meaning of all notes is clear
- The case file is free of any obliterations
- All strike-throughs are initialed.
- The report is addressed to the appropriate agency/individual
- All CCs are included as requested
- All suspect/victim names are listed appropriately on the report
- Evidence labeling is listed correctly
- The Table of Contents is complete
- The total number of pages in each pack is indicated
- All quality packs relied on for conclusions are listed
- Testing performed by external laboratory has been appropriately identified
- If applicable, report headers are correct

**B. Technical Review -**

1. Definition – The technical review is a review of the report, notes, data, and other documents which form the basis for a scientific conclusion.
2. Scope – the technical review will include the following as applicable:
  - The appropriate specimens were analyzed
  - Testing is appropriate based on case history
  - Volatiles – For positives, the replicates are within 8% agreement,
    - The average is correct
    - The U of M is correct
    - All positives have been confirmed appropriately
  - Screens – Positive screens are reported appropriately
    - All positive screens are appropriately confirmed
    - All negative screens are reported
  - Appropriate units are used.
  - When multiple data sets are present, it is clear how they were evaluated and why one set was use over another or an average was used.
  - Appropriate internal standards were used
  - Extraction efficiency is appropriate based on internal standards
  - Qualitative :
    - Compounds are at the correct retention time
    - Compounds have the correct ions and ion ratios
    - Compound does not exhibit as carryover from a previous sample. (blanks have been evaluated)

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- Compounds have appropriate S/N response
- Quantitative:
  - Compounds are within the linear range of the assay
  - If reported out of the range, compounds are reported as < or > appropriately.
  - For SIM/MRM – all qualifiers are within appropriate ranges
  - Dilutions are appropriately accounted for
  - Integration of peaks of interest is correct
  - Appropriate ions were used in methods
- All compounds are reported appropriately
- Correct analytical methods are specified
- Results identified in two or more assays are in general agreement
- Metabolites and parent drugs are present in reasonable proportion
- Inconsistent or unexpected findings have been evaluated

**NOTE:** All case reviews are to be performed by a senior analyst designated by the departmental Lieutenant. No cases shall be reviewed by an analyst that performed analysis on the case to be reviewed.

**NOTE:** Preliminary results may be issued when necessary (ie. Child fatalities, officer involved, etc.). The analyst at the time the data is generated may communicate the results. All positive results must first be technically reviewed before the results can be communicated to the customer. The review must be documented in the case record and the communication must be captured in the case narrative.

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**BALANCE CALIBRATION/VERIFICATION**

**PURPOSE:**

The purpose of the following procedure is to provide guidelines for the calibration and verification of balances used in the Toxicology Department.

**SUPPLIES REQUIRED:**

Certified Reference Weights:  
10 mg, 200 mg, 1 g, 2 g, 100 g

**APPARATUS REQUIRED:**

Analytical or Top Loading Balance

**CALIBRATION REQUIREMENTS:**

Balance calibration, cleaning and preventive maintenance will be performed yearly by an ISO 17025 Vendor. If a balance does not meet performance verification and is removed from service requiring maintenance, a calibration will be required prior to returning the balance to service. Calibration of balances is typically done on-site and shipping/handling is not required. Calibration records will be maintained within the department.

All weights will be certified every two (2) years by an ISO 17025 certified Metrology Laboratory or other equivalent certification laboratory. Check weights will be stored in appropriate cases and located near the balances. When weights are sent for calibration, a reputable courier service with tracking will be used (i.e. Fed-Ex or UPS). These calibration records are archived by the department.

Weighing should only occur when humidity in the laboratory is 20% or higher. Humidity must be checked and notated on the balance verification form before any weighing is performed.

**PROCEDURE:**

**PERFORMANCE VERIFICATION:**

1. The performance of analytical balances will be verified prior to use. The verification will be performed using NIST traceable certified weights. The weights utilized will be 10 mg, 200 mg, 1 g, 2 g or 100 g where appropriate. Weights may be combined to attain a desired verification level. The verification at a specific weight is not required more than once daily.
2. The results of this verification will be recorded in the Balance Verification Logbook assigned to each balance.
3. The accuracy of the weight measurement must be within 3% of the target value.
4. The balances listed in Table 1 are intended as examples of a balance class or type with appropriate check weights and acceptable ranges.

BALANCE VERIFICATION

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TABLE 1

| Balance Type | Balance Example        | Readability | Check Weights          |
|--------------|------------------------|-------------|------------------------|
| Analytical   | Mettler Toledo XS-64   | 0.0001 g    | 0.010 g $\pm$ 0.0003 g |
|              |                        |             | 0.200 g $\pm$ 0.0060 g |
|              |                        |             | 1 g $\pm$ 0.03 g       |
|              |                        |             | 2 g $\pm$ 0.06 g       |
|              |                        |             | 3 g $\pm$ 0.09 g       |
|              | Mettler Toledo PG-203S | 0.01g       | 1 g $\pm$ 0.03 g       |
|              |                        |             | 2 g $\pm$ 0.06 g       |
|              |                        |             | 3 g $\pm$ 0.09 g       |
| Top Loading  | Fisher XL-5000         | 0.1 g       | 100 g $\pm$ 3 g        |

5. If the accuracy of a weight is outside the acceptable range, ensure that the balance is level and clean prior to rechecking.
6. Perform the balance check again. If the accuracy fails again, use the internal calibration function to recalibrate the balance. Record all pre and post calibration measurements on the log sheet.
7. If after internal calibration, the balance sill is outside of the acceptable range, the balance should be taken out of service and labeled as such. Maintenance and/or calibration should be performed by a qualified and approved vendor.

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**OPERATION OF BECKMAN DU<sup>®</sup> 800 UV-VIS SPECTROPHOTOMETER**

**PRINCIPAL OF PROCEDURE:**

The following procedure will provide the analyst with a method for the operation of the Beckman Coulter DU<sup>®</sup> 800 UV-Vis Spectrophotometer. Unknown compounds may be qualitatively identified and known compounds may be quantitated.

**SPECIAL HANDLING:**

None

**SPECIMEN REQUIRED:**

Specimens include extracts from biological specimens or neat standards.

**SUPPLIES REQUIRED:**

Reference solution will vary dependent upon analyte.

**APPARATUS REQUIRED:**

Adjustable Micropipette (0.1 - 1.0 mL range)

Micro pipette (1.0, 2.0, and 5.0 mL)

Quartz Sample Cuvette or Quartz Flow Sample Cell apparatus

Beckman DU<sup>®</sup> 800 UV-Visible Spectrophotometer

**CALIBRATION REQUIREMENTS:**

Calibration is performed by the factory representative as part of the routine preventative maintenance and should not be attempted by the analyst unless otherwise directed. A self-test should be run daily before instrument use.

**PROCEDURE:**

**INSTRUMENT START-UP:**

1. Open the DU<sup>®</sup> 800 Spectrophotometer program. The system should automatically run its diagnostics program test. Ensure that the test was successful and that all appropriate parameters are within acceptable limits (according to the manufacturer's recommendations). Print the results, initial, date, and save in DU<sup>®</sup> 800 notebook. If the instrument does not pass the diagnostics test, inform the toxicologist in charge of the instrument and terminate testing.

2. Turn on lamps (both UV and Visible) by selecting the UV and Visible icons at the bottom left hand corner of the screen. The source lamps must warm up for a minimum 30 seconds before a scan is acquired (30 minutes is recommended). The instrument is ready when the icon stops flashing and the Status & Control Frame reads "UV Lamp On for x time".

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**SCANNING:**

1. Select “RediScan”. The screen will flash “blank required”.
2. Change the scan range on the x-axis from 200-800 to 190-800 by selecting “Set scan Limits” under the “AXIS” icon.
3. Place the cuvette containing the blank solution into the sample compartment. Select “Blank (BLK)” icon.
4. After the instrument has performed the blank scan, remove the blank cuvette from the sample compartment and place a cuvette containing the sample into the sample compartment.
5. Select the “Scan” icon.
6. After the scan is complete, select “Print” to print a hard copy of the scan. Additional scans can be performed at this time by replacing the cuvette in the sample compartment with additional samples. These scans can be printed individually or overlaid to compare the scans and printed as multiple scans.
7. The data can be saved by selecting the “Save File” icon. Alternatively, the data can be deleted by selecting the “Clear” icon. At this point, a new scan can be initiated, another application may be started, or the instrument can be shut down.

NOTE: The sample compartment lid must be closed in order to acquire data and correctly blank the instrument.

**SINGLE WAVELENGTH READINGS:**

1. Select “Fixed Wavelength” from the dropdown menu.
2. Select “Create/Edit Method” from the “Methods” menu. Select “absorbance” under the “read mode” menu and “none” under the “calculation mode” menu.
3. Change the Number of Wavelengths to “1” and enter the appropriate wavelength for the compound being measured. This wavelength will vary depending on the compound being measured. At this point, the method can be saved by selecting “Save As” and naming the method according to the compound being measured. In this case, the saved method can be utilized by selecting the method name from the drop down menu.
4. Place a cuvette containing blank solution into the sample compartment and close the lid. Select the “Blank (BLK)” icon.
5. After the instrument has performed the blank reading, remove the blank cuvette from the sample compartment and place a cuvette containing the sample into the sample compartment. Close the lid to the sample compartment.
6. Type the name of the sample in the appropriate block in the Sample I.D. table. The instrument will automatically label the samples as “Sample 1.1” etc.
7. Select the “read” or “GO” icon. Repeat this step for the required number of readings.
8. Save the results as “drugdate” (i.e. risperidone 05222008).
9. Print the results and file the data accordingly.

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**INSTRUMENT SHUT DOWN:**

1. Turn off lamps by selecting the lamp icons in the lower left-hand corner of the screen.

**QUANTITATIVE DATA:**

Quantitative analytical results can be obtained from UV-Vis Spectral data for a known compound. A known compound is considered to be one which originated from a reference standard solution or has been confirmed by GC/MS. Refer to QUANTITATIVE RESULTS for method of calculation.

**INTERPRETATION:**

**QUALITATIVE RESULTS:**

The UV-VIS spectrum can serve as evidence of identity when supported by other analytical data, however it should not be used as the sole basis for identification of an unknown compound. When a spectrum is acquired from a solution containing an unknown compound, the wave lengths of the principal peaks and the corresponding Absorptivity values should be noted. Comparison with data from a reliable reference (ie. *Clarke's Analysis of Drugs and Poisons*) or from a known reference standard will assist the analyst in identifying the unknown compound of interest.

**NOTE:** *Clarke's* contains listings of both UV-Vis scans from reference standards as well as an appendix listing principal peaks in ascending numerical order

**QUANTITATIVE RESULTS:**

The Absorptivity is a fundamental property of a molecule at a specified monochromatic wavelength. When concentration is expressed as 10 mg/mL (or gm/100 mL), absorptivity is described as the specific absorbance and represented by the symbol  $A_1^1$  or  $A(1\%, 1\text{ cm})$ , defined as 'the absorbance of a 1% w/v solution in a cell of 1 cm path length'. If the  $A_1^1$  value is known for a respective compound at a specific wavelength, then the concentration of compound in the sample may be calculated using the following equation:

$$\frac{A_1^1}{10\text{ mg/mL}} = \frac{\text{Absorbance @ Maxima}}{\text{conc. of unknown}(x)}$$

Rearrangement of the equation yields:

$$x = \frac{(10\text{ mg/mL}) (\text{Absorbance @ Maxima})}{A_1^1}$$

$x(\text{mg/mL of solution}) = \text{conc. of unknown in 1.0 mL of solution.}$

Therefore, y (conc. of drug (mg) per 1.0 mL of extract) is determined by:

$$y(\text{mg/mL}) = x * (5\text{ mL of solution/volume of extract(mL)})$$

UV-VIS SPECTROPHOTOMETER

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**NOTE:** On occasion, it will be necessary to make a dilution of the sample due to the high concentration of drug present in the sample. This dilution factor must be taken into account in the calculation as follows.

$$y(\text{mg/mL}) = x * (\text{dilution factor}) * (5 \text{ mL/vol of extract})$$

To calculate the concentration of drug present in the total sample:

$$\text{conc. of drug(mg)} = y(\text{mg/mL}) * \text{tot vol of sample(mL)}$$

**DOCUMENTATION:**

Scans should be filed with analytical data in case file. The following information will be recorded and kept with the spectrum: total sample volume, extract volume, solution volume, dilution factor, wavelength of principal peak and corresponding absorbance.

Scans of reference standards will be filed in the UV-Vis Spectral notebook for Standards.

**REFERENCES:**

Clarke's Analysis of Drugs and Poisons.

Beckman Coulter DU<sup>®</sup> 800 Spectrophotometer Installation and Operating Instructions, Beckman Coulter, Inc., July 2002.

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**OPERATION OF FISHER SCIENTIFIC accumet AE150 PH METER**

**PRINCIPAL OF PROCEDURE:**

The following procedure will provide the analyst with a method for the operation of the Fisher Scientific accumet AE150 pH Meter. This instrument is used to measure the pH of solutions.

**SPECIMEN REQUIRED:**

Specimens include liquid samples and buffered solutions.

**SUPPLIES REQUIRED:**

Buffer solution, pH 4

Buffer solution, pH 7

Buffer solution, pH 10

**APPARATUS REQUIRED:**

Fisher Scientific accumet AE150

**CALIBRATION REQUIREMENTS:**

Calibration is performed as part of the following procedure.

**PROCEDURE:**

1. To power the meter on, press the ON/OFF key. All the display segments will be displayed briefly and then the pH measurement mode will be displayed.
  - a. If necessary press the MODE key to select the pH measurement mode.
2. The display will indicate the following:
  - a. "MEAS" icon indicates the meter is in the measurement mode
  - b. "pH" icon indicates the pH measurement mode is selected
  - c. "°C" icon indicates the measured temperature reading
  - d. "ATC" icon indicates automatic temperature compensation is active when a temperature probe is connected
3. Rinse the pH electrode with deionized water or rinse solution.
  - a. Do not wipe the pH electrode, as this may cause a build-up of electrostatic charge that may cause measurement instability.
4. Select the first pH buffer (pH 7) and pour the buffer into a clean container.
5. Immerse the pH electrode and temperature probe into the first pH buffer.
  - a. The tip of the pH electrode must be completely immersed in the buffer. Stir the electrode gently to create a homogenous solution.

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6. Press the CAL/MEAS key to enter the calibration mode.
  - a. The “CAL” icon will be shown on top of the display to indicate the meter is in pH calibration mode.
  - b. The upper display will show the measured reading and lower display will indicate the pH buffer standard value, based on the selected buffer set.
7. Wait for the measured pH value to stabilize and the “READY” icon to be shown on the display.
8. Press the ENTER key to confirm the calibration point.
  - a. The upper display will flash the calibration value briefly before storing the calibration point in the meter.
  - b. The lower display will toggle between the next calibration buffer values, based on the selected buffer set.
9. Rinse the pH electrode with deionized water or rinse solution.
10. Select the second pH buffer (pH 4 or 10) and pour the buffer into a clean container.
11. Immerse the pH electrode and temperature probe into the second pH buffer.
  - a. The lower display will update to the pH buffer standard value, based on the selected buffer set.
12. Wait for the measured pH value to stabilize and the “READY” icon to be shown on the display.
13. Press the ENTER key to confirm the calibration point
  - a. The upper display will flash the calibration value briefly before storing the calibration point in the meter.
  - b. The lower display will switch to the next calibration buffer value, based on the selected buffer set.
14. Save the calibration. Track the pH calibrators used (Level and Lot number), pH measurement of the calibrators, slope of calibration and intended use of the pH meter on the Laboratory pH Meter Log. Press the CAL/MEAS key to return to the measurement mode and start taking pH readings.
15. Immerse the electrode and temperature probe into the sample.
  - a. Make sure that the glass bulb of the pH electrode is completely immersed in the sample.
  - b. Gently stir the electrode to create a homogeneous sample.
16. Wait for the reading to stabilize. When the reading stabilizes, the “READY” icon will be shown. Record all applicable readings.
  - a. The “READY” icon is shown when pH readings are stable within a range of  $\pm 0.01$  pH.

**NOTE:** As a general rule when measuring pH, the liquid being measured should be gently swirled or stirred to insure constant contact with a "fresh" portion of the solution. To adjust the pH of the solution, add appropriate acid or base drop-wise with stirring until desire pH is reached. Allow sufficient time for equilibration of the solution before reading final pH.

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**NOTE:** During the pH calibration, if the upper measured pH reading is not within the acceptable buffer value range for automatic buffer recognition, the “ERR” error icon and electrode icon will flash when the ENTER key is pressed. Press the CAL/MEAS key to exit the error calibration condition and return to the measurement mode. Refer to the Troubleshooting Guide section of the User Manual to verify the pH electrode performance and repeat the calibration. There is a chart on page 16 of the User Manual showing the range of acceptable Buffer Value Ranges.

**DOCUMENTATION:**

Record of this calibration and meter use will be recorded in a pH log.

**REFERENCES:**

Fisher Scientific accumet AE150 pH Meter instruction manual.

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**MAINTENANCE OF EXTRACTION MANIFOLDS AND EVAPORATION STATIONS**

**PRINCIPLE:**

The purpose of the following procedure is to provide information concerning the recommended method for cleaning and routine maintenance of extraction manifolds and evaporation stations.

**SUPPLIES REQUIRED:**

DI Water  
10% Bleach Solution (Prepared fresh prior to usage)  
Disposable Towels  
Methanol or other suitable solvent

**APPARATUS REQUIRED:**

None.

**PROCEDURE:**

**Extraction Manifolds**

**CLEANING:**

1. After use, extraction manifolds should be disinfected with a 10% bleach solution (freshly prepared) or other suitable disinfectant. Specifically, the entire apparatus, both interior and exterior should be cleaned with bleach solution or other suitable disinfectant. Special care should be taken to ensure that the valves and cannulas have been flushed with appropriate solvents and disinfected.
2. Rinse thoroughly with DI water and dry.

**MAINTENANCE:**

Prior to use, examine the apparatus to ensure it is functioning properly. Replace any defective parts such as valves and gaskets as soon as practical.

**Evaporation Stations**

**CLEANING:**

Prior to and after each use, clean the cannulas by wiping thoroughly with a disposable towel which has been moistened with methanol or some other appropriate solvent.

**MAINTENANCE:**

When necessary, the cannula assembly can be thoroughly cleaned by sonication in an appropriate solvent such as methanol.

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**FREEDOM EVO ROUTINE MAINTENANCE**

**PRINCIPLE:**

To insure the proper operation of the Tecan Freedom EVO instrument certain routine preventative maintenance procedures should be performed on a regular basis.

**SPECIAL HANDLING:**

The Tecan Freedom EVO 75 system is designed to process biological specimens. Universal precautions should be exercised during maintenance procedures.

**SUPPLIES REQUIRED:**

DI water  
Isopropyl alcohol  
1 M HCl solution  
1 M NaOH solution

**APPARATUS REQUIRED:**

Tecan Freedom EVO instrument including the Columbus Washer and Sunrise reader  
2-100 mL beakers  
400 mL beaker

**PROCEDURE:**

**DAILY:**

1. Flush the system with distilled or DI water using at least 50 mls over washer and 1 ml over waste.
2. Remove racks from the instrument surface and clean work surface with appropriate disinfectant and lint-free cloth.
3. Empty waste containers.
4. Clean the Teflon sample tip by gently wiping it with a moist lint-free cloth.
5. Check the level of DI water in fluid container. Refill as needed.

**WEEKLY:**

1. Perform daily maintenance.
2. Check the syringes for leaks, bubbles or internal contamination. If required, clean the syringes taking care in removing syringes. If the syringes are leaking, replace the caps on the syringe plungers.
3. Check around the valves for signs of moisture.
4. Check the green Teflon coating of the stainless steel pipette tip for any damage.
5. Check that there are no air bubbles or contamination in the pipetting tubing. Tighten the tubing connections or replace the tubing as required.

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**MONTHLY:**

Monthly Maintenance should be performed by a designated Toxicologist or Technician.

Monthly Maintenance includes, but is not limited to, the following procedures:

1. Perform an acid/base wash to the Freedom EVO.

Acid Wash: Prepare a solution of 1 M HCl solution. Put 100 mL into a beaker and place the system intake tubing into the acid. Follow instrument directions

Base Wash: Prepare a solution of 1 M NaOH solution. Put 100 mL into a beaker and place the system intake tubing into the base. Follow instrument directions.

2. Remove and clean the manifold on the Hydroflex washer. Refer to Hydroflex Washer Operating Manual, for procedures on removal and cleaning of manifold.

**PERIODIC (As needed):**

1. Change pipette tubing.
2. Change syringe or syringe cap.
3. Change valves.
4. Clean filter on reader.
5. Archive data.

**DOCUMENTATION:**

All maintenance should be recorded in the appropriate Freedom Maintenance Log (Freedom Daily Checklist – TOX 036, Freedom Weekly and Monthly Maintenance Checklist – TOX 037).

**REFERENCES:**

Freedom EVO 75 Workstation Operating Procedures.

Columbus Washer Operating Manual.

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**PREVENTATIVE MAINTENANCE AGILENT GCMS SYSTEMS**

**PURPOSE:**

To ensure the reliability of GC/MS systems, it is necessary that certain preventive maintenance (PM) steps or procedures be performed by each primary operator. This list of recommended preventive maintenance is based on manufacturer recommendations and should be performed in accordance with guidelines as set forth in the Mass Spectrometer System Manual.

**SPECIAL HANDLING:**

Specific procedures may involve the removal or inspection of hardware heated to temperatures in excess of 200°C. Proper care should be exercised when handling or removing these parts.

**SPECIMEN REQUIRED:**

None.

**SUPPLIES REQUIRED:**

Injection Port Liner (Agilent No. 5062-3587 or equivalent)  
Viton O-ring (Agilent No. 5180-4182 or equivalent)  
Inlet Seal (Agilent No. 18740-20880 or equivalent)  
Ferrule, short, 0.4mm id 15% graphite, 85% Vespel (Agilent No. 5062-3323 or equivalent)  
Ferrule, long, 0.4mm id 15% graphite, 85% Vespel (Agilent No. 5062-3508 or equivalent)  
Vacuum Pump Oil (Agilent No. 6040-0517 or equivalent)  
Perfluorotributylamine, PFTBA (Agilent No. 5800-0656 or equivalent)  
10 µL or 5 µL Syringe  
Septum (Merlin Microseal or Thermogreen LB-2 11.5 mm or equivalent)

**CALIBRATION:**

None.

**PROCEDURE:**

**ROUTINE MAINTENANCE:**

Prior to the analysis of routine case work, the following preventive maintenance/instrument checks should be performed:

1. The following items should be considered for replacement before starting any auto injection sequence (batch) or at any time deemed necessary by the instrument operator based on characteristics of instrument operation:
  - Injection port liner
  - Viton o-ring
  - Septum (if standard septum is used)

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**NOTE:** The gas chromatograph may be equipped with a Merlin Microseal septum. This septum is a long life replacement for the standard septum found on most capillary inlet systems. Microseal septa should be replaced if an excessive septum leak rate ( $> 2$  mL/minute) is detected or excessive amounts of atmospheric contaminants are observed during tuning.

2. Prior to beginning an auto injection sequence (batch), wash bottles should be checked and if necessary rinsed and/or filled with appropriate solvents.
3. Prior to beginning an auto injection sequence (batch), the syringe should be flushed with solvent to ensure the syringe is functioning properly and the plunger moves with ease.
4. Perform check tune or auto tune. See tuning procedure for guidelines on tune acceptance criteria.

**NOTE:** If a problem occurs during tuning, please see primary operator prior to analysis of any samples

5. Prior to analysis of samples, it is recommended that an appropriate check sample be injected, analyzed, and reviewed. Check samples provide valuable information about the condition of GC columns, column positioning, and GC inlets.

**PERIODIC MAINTENANCE:**

The following procedures are to be performed on an as needed basis:

1. Reboot work station.
2. Check level of rough pump oil. The rough pump is provided with a sight glass which allows for the visual inspection of pump oil level and condition. Oil should be visible in window with meniscus between upper and lower lines. Oil level should be inspected at least quarterly.
3. Replace GC inlet seal. Inlet seals should be replaced or cleaned as necessary to maintain acceptable GC performance.
4. Mass spectrometer sources should be removed and cleaned when MS tuning begins to reflect an unusual trend or tune parameters are outside of acceptable limits. Consult primary operator prior to installation or removal of source.

**PREVENTIVE MAINTENANCE PROVIDED BY MANUFACTURER/TRAINED ANALYST:**

Existing Agilent GC/MS systems may be covered under manufacturer's maintenance contracts. Preventive maintenance performed by Agilent service personnel includes the following:

1. Change oil in rough pump a minimum of once a year and diffusion pump as needed.
2. Replace foreline oil trap as needed.

**NOTE:** Should maintenance contracts not be renewed, these steps may be performed by a trained analyst.

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**DOCUMENTATION:**

GC/MS Maintenance Checklist -TOX 010 are provided for use as documentation of routine and periodic maintenance. Operators should complete checklist after performing the various required maintenance steps or checks. At the end of each month, this checklist should be archived. Maintenance performed, but not notated on checklist, should be hand written in maintenance log and include a brief description of work performed, date performed, analyst's initials, and a brief justification for performing task. In addition, maintenance logs should include references to specific problems encountered and a description of action taken to rectify problem.

**REFERENCES:**

HP 5973 Mass Selective Detector Reference Collection, Hewlett-Packard Company, 1998.

Merlin Microseal Septum Kit for HP Split/Splitless Capillary and Purged Packed Inlet Systems Installation Instructions and Product Information, Merlin Instrument Company, 1993.

Agilent 5975 Series Mass Selective Detector Operation Manual, Agilent Technologies, 2010.

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**TUNING SPECIFICATIONS FOR AGILENT GCMS SYSTEMS**

**PURPOSE:**

To ensure accurate results with maximum sensitivity, it is necessary to periodically adjust various parameters (tuning parameters) associated with the mass selective detector (MSD). The auto tune program allows the computer to adjust various parameters in an attempt to achieve predefined performance criteria.

Tuning should be done prior to the operation of this instrument to ensure that all MSD parameters are within acceptable values. However, it is generally not necessary to tune more than once per day.

**SUPPLIES REQUIRED:**

Perfluorotributylamine (PFTBA)

**APPARATUS REQUIRED:**

AGILENT GCMS SYSTEM (5973, 5975 or equivalent)

**PROCEDURE:**

**PERFORMING AUTOTUNE:**

1. From the instrument control menu, select perform MS autotune.
2. When complete, evaluate tune report to determine if additional adjustments are indicated.
3. From the Top menu select Checkout then select Evaluate Tune.
4. If tune is not acceptable, repeat steps one and two. Subsequent failure may require additional maintenance.

**TUNE EVALUATION:**

The following conditions should be met before continuing with operation of the Mass Spectrometer:

1. Peak width of mass ions 69, 219, and 502 should be 0.5 +/- 0.1 at half height.
2. The ratio of relative abundance for ion 219 to 69 should be > 40%.
3. The ratio of relative abundance for ion 502 to 69 should be greater than 2.4%.
4. The peak shape of ions 69, 219, 502, and their respective isotopes should be acceptable with minimal or no splitting.
5. The mass assignments shown in the upper "profile" part of the display should be within +/- 0.2 amu of 69, 219, and 502.
6. The mass assignments shown in the lower "scan" part of the display should be within +/- 0.1 amu of 69, 219, and 502.
7. The isotope (Iso) mass assignments should each be 1 amu greater than the mass assignments of the parent peaks.

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8. The isotope (Iso) ratio figures (indicating the relative abundances of the naturally occurring isotopes to their parent ion) should be close to the theoretical values of 1.08 for  $m/z$  69, 4.32 for  $m/z$  219, and 10.09  $m/z$  502. (The tune evaluation will reflect if these values are out of the acceptable range.)
9. Check to insure that the abundance of mass 18 is no greater than 20% and the abundance of mass 28 no greater than 10% of the abundance of ion 69. The presence of large amounts of any of these ions may indicate a leak and necessitate contacting the primary operator of this instrument before continuing.

If the above specifications are met and the tune evaluation (system verification-tune) passes, the instrument is ready for normal operation. Instrument maintenance checklist (GCMS Maintenance Checklist – TOX 010) should reflect that an Autotune has been completed for the appropriate day of the month.

**NOTE:** The autotune on the Agilent instruments is a maximum sensitivity autotune which optimizes the instrument for greater sensitivity of higher molecular weight ions. As a result, the abundance of ion 219 is much greater than with other tunes and the base peaks may be either 69 or 219. This increased sensitivity of higher molecular weight ions may result in ion ratios which differ significantly from previously published library results. This should be accounted for when identifying analytes by GC/MS.

Any changes made to tune parameters during tuning procedure should be justified and documented on the tune.

**DOCUMENTATION:**

All acceptable Tune Reports should be maintained and placed in appropriate log. The GCMS Maintenance Checklist – TOX 010 should reflect that a Check or Autotune has been completed for the appropriate day of the month.

**REFERENCES:**

HP5972A Mass Selective Detector Hardware Manual, Hewlett-Packard Company, 1994.

HP G1034C MS ChemStation Software, MS ChemStation User's Guide, Hewlett-Packard Company, 1993.

HP 5973 Mass Selective Detector Reference Collection, Hewlett-Packard Company, 1998.

Agilent 5975 Series Mass Selective Detector Operation Manual, Agilent Technologies, 2010.

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**PROCEDURE FOR VERIFICATION OF HEAT BLOCKS/EVAPORATION  
UNITS**

**PURPOSE:**

In the Toxicology Department, heat blocks are typically used for evaporation or derivatization of samples. At least once a month, the temperature of the heat block should be checked with a thermometer to ensure the temperature falls within the appropriate range for the procedure. Additional checks may be performed as needed.

**PROCEDURE:**

- A. Heat blocks are typically maintained at temperatures not exceed 45 °C for evaporation and approximately 80 °C for derivatization. Temperatures should be verified within the range of 40-45 °C for evaporation and 75-85 °C for derivatization.
- B. Adjust the thermostat as necessary to achieve the desired temperature.
- C. If the correct temperature range cannot be achieved, remove the heat block from service and label it as such until a repair can be made. Document the removal from service and repair on the heat block instrument log book.
- D. Successful performance of controls and calibrators within an analytical batch further substantiates performance of the heat blocks used.

**DOCUMENTATION:**

A Heat Block Instrument Log should be maintained for each unit that is used for evaporation or derivatization. Logs should include the unique identifier of the unit, date temperature taken, and temperature, initials of the individual recording the measurement and the thermometer used for verification. Comments should be used to indicate temperatures that are out of the control range and should be accompanied by a description of action taken.

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**OPERATION OF AGILENT 1200 SERIES LIQUID CHROMATOGRAPH/6400  
SERIES TANDEM MASS SPECTROMETER**

**PURPOSE:**

The following procedure outlines the basic operating sequence for the Agilent 1200 Series Liquid Chromatograph/6400 Series Tandem Mass Spectrometer.

**SPECIAL HANDLING:**

None.

**SPECIMEN REQUIRED:**

The following procedure is designed for use with extracts derived from biological or non biological specimens.

**SUPPLIES REQUIRED:**

DI Water  
HPLC Grade Organic Solvents  
HPLC Grade Buffers  
Source Cleaning Solvent (1:1 Water:MeOH)  
Lint Free Wipes  
Needle Rinse Solution (1:1:1 Water:MeOH:Isopropanol)

**APPARATUS REQUIRED:**

Agilent 1200 Series Liquid Chromatograph/6400 Series Tandem Mass Spectrometer  
Appropriate glassware

**CALIBRATION:**

None.

**PROCEDURE:**

**INSTRUMENT PREPARATION:**

1. With the source below 100 °C (view this temperature in the ACQUISITION METHOD pane, MS tab, SOURCE tab, or in the INSTRUMENT ACTUALS of the STATUS pane), rinse the stainless steel face plate and the black surfaces of the source chamber and door with source cleaning solvent from a non-plasticized wash bottle. Catch the drainage in a beaker.
2. Using a lint free wipe moistened with source cleaning solvent, wipe any visible residue from beneath the nebulizer and on the spray shield.
3. Ensure solvent bottles are filled for the mobile phase and for needle rinse solution.
4. Ensure proper analytical column is installed for the assay.
5. Perform Tuning as required in TUNING SPECIFICATIONS FOR AGILENT 6400 SERIES LC-MSMS section.

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6. Load the appropriate worklist template for the batch (FILE>LOAD>WORKLIST) or create a new worklist (FILE>NEW>WORKLIST).
7. Open WORKLIST RUN PARAMETERS (Right-click WORKLIST header or use menu WORKLIST>WORKLIST RUN PARAMETERS)  
Enter the following:
  - Data File Path
  - Choose any Post Acquisition Cleanup steps (Standby or PumpsAllOff)
  - Ensure the CLEAR SELECTION AFTER RUN box is checked, to be able to keep track of what samples have been completed.Click OK to close WORKLIST RUN PARAMETERS
8. Edit the Template Worksheet to represent your batch or create a new worklist with ADD MULTIPLE SAMPLES from a right-click.
9. Save the Worklist.
10. Verify the Correct position of samples in the tray and worklist.
11. Verify that all sample lines have a checkmark ✓ in the second column so that each line will run. These can all be turned on or off at once by clicking the topmost checkbox in that column.
12. Verify the WORKLIST RUN PARAMETERS to ensure that any desired Post Acquisition cleanup step is selected.
13. Run the Worklist  
Verify:
  - Correct method for the batch is loaded
  - All module icons are ready (green)
  - LC flow is steady (pressure is stable and/or ripple <1%)
14. Click the RUN WORKLIST icon (three vials) in the ACQUISITION toolbar.

**QUALITY CONTROL:**

The appropriate control samples should be processed with each batch of unknown samples to include a known test mix with a suitable internal standard.

**DOCUMENTATION:**

Documentation will be kept as instructed in Quantitative/Qualitative Mass Spectral Analysis of the Toxicology Quality Manual.

**REFERENCES:**

Agilent 6400 Series QQQ/Liquid Chromatograph Techniques and Operation, Agilent Technologies, May, 2010.

Agilent 6400 Series QQQ LC/MS Techniques and Operation; Course R1893A, Student Manual, Agilent Technologies, 2010.

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**TUNING SPECIFICATIONS FOR AGILENT 6400 SERIES LC-MSMS**

**PURPOSE:**

To ensure accurate results with maximum sensitivity, it is necessary to periodically adjust various parameters (tuning parameters) associated with the mass selective detector (MSD). Tuning or a check tune should be done prior to the operation of this instrument to insure that all MSD parameters are within acceptable values. However, it is not necessary to tune more than once per day.

**SPECIAL HANDLING:**

None.

**SUPPLIES REQUIRED:**

Polypropylene glycol (calibrant)

**APPARATUS REQUIRED:**

Agilent 6400 Series Triple Quad LC-MS/MS with Agilent 1200 Series Infinity Liquid Chromatograph with ESI (Electrospray Ionization) interface

**PROCEDURE:**

Tuning should be performed in order to obtain settings for the LC-MS/MS that will maximize signal, minimize noise and give the appropriate peak widths and mass assignments in the range of the masses to be analyzed. A check tune will be performed on the day of each run. If the specifications are met and the check tune passes, the instrument is ready for operation. A failed check tune will require maintenance to bring the instrument back within acceptable parameters. An Autotune will be performed monthly, after any maintenance to the instrument or as needed to keep the instrument functioning properly within acceptable parameters.

Prior to tuning, run a MS2 scan of calibrant to check for contaminants.

This is performed by:

1. Go to Tune Context, Manual Tune Tab, select the Calibrant On button.
2. Select the Acquire button.
3. Under SCAN TYPE drop down menu, select MS2 SCAN.
4. Compare to spectrum of calibrant found in tune manual for extraneous peaks.

DAILY (or whenever running samples):

Run a CHECKTUNE

- Go to Tune context, Autotune Tab, select the Checktune button.
- This Generates a Tune Report
- From the Tune Report, identify any resolution mode for both quads which does not meet "passing" criteria. Masses which do not pass can be adjusted with the "Adjust Width and Gain" button in Manual Tune.

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**NOTE: (for adjustments)**

Increasing the Width Gain will:

- Decrease Peak Width
- Decrease Peak Amplitude
- Large effect at high mass/small effect at low mass

Increasing the Width Offset will:

- Decrease Peak Width
- Decrease Peak Amplitude
- Equal effect at low and high mass

**MONTHLY (or as needed)**

Run Autotune (takes 5-10 minutes per polarity)

- This step optimizes ion optics, verifies and corrects resolution and mass axis calibration for both quadrupoles, in all three resolution settings.
- Checks and adjusts the EM gain to maintain constant response.

All parameters for masses below 600 amu must pass for a tune to be considered valid.

EM Volts should be monitored. The sum of Autotune EMV and  $\Delta$ EMV from the method should be less than 2500 volts.

**DOCUMENTATION:**

Records of Check Tunes and Tunes will be notated on the LC-MSMS Maintenance Checklist - TOX 037 and a copy of the Check Tune or Tune will be stored in the Instrument Log as well as with the batch data for the analysis.

Tunes are stored electronically on the instrument in D:/Tune/QQQ/

**REFERENCES:**

Agilent 6400 Series QQQ LC/MS Techniques and Operation; Course R1893A, Student Manual, Agilent Technologies, 2010.

Agilent 6400 Series QQQ/Liquid Chromatograph Techniques and Operation, Agilent Technologies, May, 2010.

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**PIPETTE ROUTINE MAINTENANCE**

**PURPOSE:**

The purpose of the following procedure is to provide information concerning the recommended method for cleaning and lubrication of MLA and Eppendorf pipettes. Regular cleaning and maintenance will help to ensure the continuing performance of pipettes of all types. If liquid has been accidentally aspirated into the pipette, the pipette should always be disassembled and cleaned immediately. Reagent deposits on the piston and seals will damage the pipette and impair its performance.

**SUPPLIES REQUIRED:**

DI Water  
10% Bleach Solution  
Disposable Towels

**APPARATUS REQUIRED:**

None.

**CALIBRATION REQUIREMENTS:**

Pipettes should be checked for accuracy after cleaning or lubrication. For instruction concerning calibration see Pipette Calibration procedure in this manual.

**PROCEDURE (MLA):**

**CLEANING:**

1. Hold pipette in vertical position with the nose down.
2. Grasp the nose with one hand and unscrew the body in a counterclockwise direction with the other hand.
3. Remove metering seal housing and wave spring washer from the protruding end of the piston or from the housing recess in the nose.
4. Remove metering seal from the housing, using fingers. Do not use sharp instruments.
5. Using a soft tissue, remove old grease from the metering housing, seal, and the housing recess in the nose.
6. With a gentle stream of DI water flush the inside of the nose, metering seal and housing, and wave spring washer. For stubborn deposits, substitute 10% bleach solution for DI water.
7. Holding pipette vertically, piston down, flush piston with a gentle stream of DI water.
8. Wipe piston, nose, seal, housing, and washer to remove excess water with a soft tissue.
9. Allow all components to air dry prior to reassembly.
10. Using a finger tip, apply a small amount of grease from the plastic tube supplied with the pipette to the inner surface of the seal and housing.
11. Replace metering seal in its housing and install into nose recess. Make sure the metering seal housing is installed properly.

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12. Install wave spring washer on top of the metering seal housing.
13. Carefully insert the body into the nose and screw in a clockwise direction to a firm stop.
14. Depress and release plunger several times before using pipette.
15. Wipe exterior of pipette with a cloth dampened with dilute bleach solution.

**LUBRICATION:**

1. Disassemble pipette as described in steps 1-5 above.
2. Lubricate piston.
3. Reassemble as indicated in steps 10-14 above.

**PROCEDURE(Eppendorf)**

**ROUTINE CLEANING AND MAINTENANCE OF THE EXTERNAL COMPONENTS:**

Eppendorf pipettes can be cleaned with most common household or laboratory cleaning agents, soap solutions or isopropyl alcohol.

- Simply spraying or wiping the cleaning agent over their entire surface can clean the outside of the pipettes.
- Let the cleaner sit on the pipette for 10 minutes then wipe off or rinse with distilled water.

**ROUTINE CLEANING AND MAINTENANCE OF THE INTERNAL COMPONENTS:**

1. Remove tip ejector (where applicable, as with Eppendorf Research pipettes).
2. Disconnect bottom part from upper part.
3. Remove seal/O-ring assembly and replace with new one if necessary. *Refer to the instruction manual for information regarding placement of the internal parts such as seals and o-rings. It varies for different sizes of pipettes*
4. Clean air passages in the bottom part with cotton swab (long stem) dipped in cleaning solution
5. Rinse with distilled water and allow to air dry.
6. Inspect nose cone and free air passage, if clogged.
7. Remove piston assembly and inspect.
8. Pistons—clean and rinse well with DI water.
9. Grease piston lightly with the lubricant that was provided with the pipette at time of purchase and wipe off any excess grease. Too much grease will make the pipette “sluggish”.
10. Reassemble pipette. Check all its functions for proper smooth operation.

**NOTE:** After any cleaning or lubrication, the pipette should be verified by following the Pipette Verification Protocol.

**DOCUMENTATION:**

Verification following cleaning should be documented on the appropriate pipette log.

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**REFERENCES:**

The MLA Macro Pipette System Operating Instructions, Medical Laboratory Automation, Inc., 1990.

*CARE AND MAINTENANCE OF EPPENDORF PIPETTES*

([http://www.eppendorf.com/img/au/lhsc/care\\_maintenance\\_pipettes\\_eag.pdf](http://www.eppendorf.com/img/au/lhsc/care_maintenance_pipettes_eag.pdf)): Web. 05 May 2014

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**PIPETTE VERIFICATION**

**PURPOSE:**

In order to provide accurate, meaningful results on tests performed within this laboratory, it is necessary to periodically check the accuracy and precision of pipettes and other liquid dispensing devices. Pipette accuracy checks are to be performed twice yearly. Calibration will be performed by an ISO 17025 compliant vendor once per year and calibration verification will be performed in-house once per year. The following procedure describes the use of the gravimetric methods as well as spectrophotometric methods using the ARTEL PCS Pipette Calibration System for use in verifying the calibration of pipettes internally.

**SPECIAL HANDLING:**

Pipettes should be stored clean and without a tip attached.

**SUPPLIES REQUIRED:**

DI Water, Room Temperature  
Pipette Tip

**REAGENTS AND STANDARDS:**

ARTEL PCS Instrument Calibrator Kit  
ARTEL PCS Standard Reagent Kit (SRK)

**APPARATUS REQUIRED:**

Analytical Balance or  
Suitable Pipette Accuracy Test Kit  
ARTEL PCS3 Instrument

**CALIBRATION REQUIREMENTS:**

Calibration of pipettes/diluters is scheduled to occur once per year by an external calibration service with a calibration verification to occur at approximately 6 months following calibration. Verification may be performed more frequently, if necessary, due to cleaning or lubrication and at any time deemed necessary by the analyst/owner.

Upon internal verification, pipettes/diluters should be labeled with the date of service, analyst's initials, and expiration date of the calibration/verification.

Adjustable pipettes/diluters should be verified at volumes equal to or approximating those volumes commonly dispensed by that pipette. It is recommended to test adjustable pipettes at 20% of capacity and at 80% of capacity. For example, a 10-100  $\mu$ L pipette will be tested at 20  $\mu$ L and at 80  $\mu$ L. Pipettes utilizing 10  $\mu$ L or less will only be verified at 80%.

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Pipette calibration by an outside vendor is typically performed in-house. When it is necessary to ship pipettes for calibration, a reputable courier will be used that allows for insurance and tracking (i.e. Fed-Ex, UPS).

Pipettes/diluters should be clean and in good working order prior to calibration/verification.

**PROCEDURE:**

If a pipette/diluter appears to be out of calibration between normally scheduled performance/calibration verifications, the pipette will be sent to an authorized vendor for repair.

Any maintenance will be documented in the pipette log along with the calibration certificate generated by the vendor.

**FOR GRAVIMETRIC VERIFICATION:**

1. Attach the appropriate tip to the pipette. (For Hamilton diluters, adjust to the appropriate volume)
2. Depress push button to first positive stop. Immerse into distilled water. Draw up water. Wait a moment. Withdraw the tip from the water.
3. Dispense into a previously tared container.
4. Calculate the volume dispensed based upon the weight of the aliquot and density of water.
5. Record volumes, and repeat a minimum of 5 times.
6. Volumes should be within +/- 3 % of the expected volume.
7. Replicates should be within +/- 3% of each other.

**FOR SPECTROPHOTOMETRIC VERIFICATION WITH ARTEL PIPETTE  
VERIFICATION SYSTEM:**

**A. INSTRUMENT CALIBRATION:**

Calibration of the ARTEL PCS Pipette Calibration System is to be performed before the start of individual pipette verifications.

1. Turn instrument on
2. Press NO on main menu pipette calibration screen.
3. Press YES to perform instrument calibration.

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4. Press YES to instrument cal menu. Perform instrument cal.
5. Enter operator ID
6. Enter calibrator lot from ARTEL PCS Instrument Calibrator Kit
7. Insert CAL A vial (continue with CAL B, C and D vials, following directions)
8. When completed the system will print calibration results indicating pass or fail.
9. Keep for records
10. In the event of calibration failure, external service of the artel device may be required.

**B. PIPETTE VERIFICATION:**

1. Press YES for main menu pipette calibration
2. Enter reagent lot code (Standard Reagent Kit)
3. Insert CAL A vial from SRK.
4. Take a blank from kit and insert it
5. Enter operator ID
6. Enter pipette ID (serial number) to be verified
7. Enter pipette volume (10, 20, or 200 µL)
8. Pipette amount out using appropriate RANGE reagent solution indicated.
9. Repeat step 8 two times for a total of three.
10. Press end of run
11. Press NO for reprint results
12. Depending on how many tests are left, press YES or NO for proceeding with same blank
13. Relative Inaccuracy and Imprecision must fall within +/- 3%
14. Record in PIPETTE CALIBRATION LOG

**EXTERNAL CALIBRATION:**

If service or cleaning is performed on pipettes by an external laboratory or company and calibration is performed by acceptable methods, this calibration may serve as one of the two semi-annual checks. Calibration certificates will be archived for each of the pipettes.

The pipette precision and inaccuracy must be within 3%. For small volume pipettes (less than 20 µL), the precision and inaccuracy must be within 5%.

**INTERPRETATION:**

Acceptable calibration is achieved when a percentage error of less than or equal to 3% is found for multiple dispensing of a predetermined aliquot.

**CALCULATIONS:**

Percent error is defined by the following calculation:

$$\text{Percent Error} = \frac{|(\text{measured value}) - (\text{expected value})|}{(\text{expected value})} \times 100$$

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**DOCUMENTATION:**

Accuracy and precision calibration verification should be recorded on the appropriate calibration log (Adjustable Volume Pipette Calibration Log – TOX 005, Fixed Volume Pipette Calibration Log – TOX 008).

Bulk records may be stored for calibration services from outside vendors.

Pipettes which have been verified will be marked accordingly.

**REFERENCES:**

The MLA Macro Pipette System, Operating Instructions, Medical Laboratory Automation, Inc., Pleasantville, NY, 1993.

Eppendorf Varipipette 4810, Instruction Manual, Brinkman Instruments, Inc., Westbury, N.Y

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**PROCEDURE FOR CHECK OF REFRIGERATOR/FREEZER**

**PURPOSE:**

In the Toxicology Department, refrigerators and freezers that are used to store biological evidence or critical reagents should be monitored to ensure the appropriate storage temperature. This includes walk-in refrigerators and those at the analyst's bench used for temporary storage of evidence.

**PROCEDURE:**

- A. Each Refrigerator/Freezer will have a Refrigerator/Freezer Log – TOX 002.
- B. Each Refrigerator/Freezer will be equipped with a thermometer. All thermometers should be NIST traceable or verified annually against a NIST traceable thermometer. (Refer to Thermometer Verification Procedure)
- C. Refrigerator and Freezer temperatures are to be checked weekly to insure proper operation.
- D. Record temperatures on the log along with the initials of the staff conducting the check.
  - 1.) Refrigerators should fall between 2-8 °C.
  - 2.) Freezers should fall below -10 °C.
- E. In the event that a refrigerator does not fall within the acceptable range, the responsible analyst should notate this on the appropriate refrigerator log and make an adjustment to refrigerator temperature setting.
- F. This refrigerator should be rechecked after an acceptable period of time has elapsed to allow stabilization of temperature (approximately 2-3 hours). After adjustment, should the temperature still fall out of range, all contents will be moved to a valid refrigerator until the refrigerator in question is brought within range. Actions taken will be notated on the refrigerator log.

**DOCUMENTATION:**

A refrigerator/freezer log should be maintained for each unit that may be used to house samples from active or completed cases and also reagents that require refrigeration. Logs should include refrigerator/freezer ID, date temperature taken, and temperature. Comments should be used to indicate temperatures that are out of the control range and should be accompanied by a description of action taken.

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**PROCEDURE FOR ROBOTIC EXTRACTION**

**PURPOSE:**

Compounds of interest may be extracted from urine using the Zymark robotics system in conjunction with solid phase extraction columns. The robot is capable of processing a maximum of fifty (50) samples per batch. Completion of a fifty sample run takes place approximately 20 hours after initialization of the robot.

**SPECIAL HANDLING:**

See specific extraction procedure for special handling procedures.

**NOTE:** Before beginning any robotic procedure, the individual operator must determine if auxiliary ventilation is in operation. If auxiliary fans are non-operational, the analyst should attempt to restart them before continuing. Contact primary operator for further assistance.

**SPECIMEN REQUIRED:**

The following extraction protocol is intended for use with 1 mL of blood, serum or urine. Other sample volumes may be substituted, if necessary, due to insufficient sample volume or as needed for better analytical results (i.e. sample needs to be within the linear quantitative range or to prevent overloading the column with high levels of drugs in urine). If amount of sample deviates from suggested volume, amount of sample should be documented in the case notes and retained for future reference. This procedure may also be used to prepare samples of bile, ocular fluid, gastric contents, tissue homogenates, or other specimens for confirmation/quantitation. Tissue homogenates and gastric samples should be centrifuged and decanted into a clean tube before beginning the extraction procedure.

**SUPPLIES REQUIRED:**

See specific extraction method for expendable supplies required.

**NOTE:** Directions for the preparation of solutions and buffers can be found in the formulary section of this manual.

**NOTE:** All solvents should be ACS reagent grade or better unless otherwise noted.

**APPARATUS REQUIRED:**

Zymark Robotic System

**CALIBRATION:**

None

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**PROCEDURE:**

**ROBOT PREPARATION:**

Complete the Robot Daily Maintenance Checklist-TOX 025:

1. Check to ensure the test tube dispenser is full of tubes.
2. Refill column dispenser with columns of like batch number.
3. Inspect all solvent reservoirs and refill as necessary.
4. Remove any foreign objects from the robot's work area.
5. Check to ensure the temperature of the evaporation rack is less than 45°C.
6. Check to ensure the temperature of the incubation station is between 60 and 65°C.
7. Check the air pressure as indicated on the robot manifold. This should be between 30 and 35 psi.
8. Check the level of waste in each of the waste containers. If full, replace with empty container.
9. Check weekly the nitrogen pressure as indicated on the robot manifold.  
Pressure should be between 25 and 30 psi.
10. Ensure exhaust hoods are operational.
11. Wipe the O-ring on the extraction station with 10% bleach solution.
12. Perform sample preparation steps according to specific extraction procedure.
13. Place samples into rack 1 of robot.
14. Prepare QC worksheet and/or extraction log.
15. Have a second analyst/technician verify vial placement and document the verification on the Extraction Log – TOX 004.
16. Start robot.
17. Watch the liquid lines purge.

**EXTRACTION SEQUENCE:**

See specific extraction method for a detailed description of the extraction sequence.

**AFTER THE EXTRACTION:**

1. Clean the robot as necessary.
2. Flush the waste lines with 10% bleach solution.

**DOCUMENTATION:**

The appropriate worksheet (Qualitative worksheet – TOX 030, Quantitation Worksheet – TOX 028) and/or Extraction Log – TOX 004 should document the extraction protocol followed, specimen type, and the date procedure was performed. In addition, deviations from approved method should be notated and accompanied by a justification for changes made.

If the robot stops mid-assay, notes should be made as to where the stop occurred and that the robot was restarted.

Consult the primary operator if a basic restart is not possible or if additional assistance is required.

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Daily Maintenance will be documented on the appropriate log form (Robot Daily Maintenance Checklist – TOX 025).

**REFERENCES:**

Clean Screen DAU Forensic Applications, World Wide Monitoring Inc., Horsham,  
Pennsylvania 19044, Revised 9/10/89.

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**PREVENTATIVE MAINTENANCE FOR ZYMARK ROBOTIC SYSTEM**

**PURPOSE:**

To ensure the reliability of robotic systems within the Toxicology Department, it is recommended that certain preventative maintenance checks be performed. These procedures should be carried out in accordance with guidelines set forth in the Zymark PySystems Service Manual, Volume 1, section entitled Maintaining the Zymate II System.

**SPECIAL HANDLING:**

Before beginning any work on the Zymark robotics systems, it is recommended that the exterior of the robot and the table top or PySections be cleaned with a diluted bleach solution (1:10, made fresh daily) or other approved disinfectant.

**SPECIMEN REQUIRED:**

None.

**SUPPLIES REQUIRED:**

Cable Tie, 4", Zymark P/N 36740 or equivalent  
Masking Tape  
Finger Pad, Replacement Zymark P/N 42868 or equivalent  
Appropriate Adhesive  
Disinfectant – (Dilute bleach 1:10, made fresh daily or other appropriate)

**APPARATUS REQUIRED:**

Screwdriver, blade, 1/8, 3/16, 1/4  
Screwdriver, holding  
Screwdriver, phillips, #1, #2  
Wrench, hex (Allen), .050, 3/32, 9/64, 3/16  
Rule, steel, 12" or 18" (with 0.10 " increments)  
Digital Voltmeter (DVM)  
Hex driver, ball-end, 9/64  
Wrench, open box-end, 5/16  
Robot post clamp, Zymark P/N 38236 or equivalent  
Wire cutters  
Snap wring pliers

**CALIBRATION REQUIREMENTS:**

Robot calibration (Py Calibration) will be performed as needed to maintain proper robot operation. Calibration should be performed by the primary operator or their designee.

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**PROCEDURE:**

**DAILY (BEFORE EACH RUN):**

Each operator is responsible for checking robot waste containers and replacing them if full. In the event of a spill, the individual operator should notify the primary operator. Clean-up should be performed in accordance with SLED procedures for the removal of biological/organic spills. Operator is also responsible for wiping down O-ring on extraction station, checking to ensure there is an empty rack in the output station, and watching the solvent lines purge to check for leaks and proper delivery.

**DAILY (AFTER EACH RUN):**

As soon as is practical after completion of a batch of samples, the exterior surfaces of the robot and surrounding stations should be cleaned and disinfected with an approved disinfectant. Disinfectant bleach solution (1:10 dilution, made fresh daily) should be poured down waste lines on the extraction station to prevent clogging of tubes.

Additional preventative maintenance will be performed as needed and may include but is not limited to the following:

Arm/post lubrication

Documentation of unusual sounds during operation

Check cable tension (reach, rotary, rotary feedback, vertical)

Reach bearing adjustment

Wrist self-restoring play and zero-position

Rotary axis self-restoring play and soft key test

Hand wear and parking smoothness

Clean centrifuge air filter

Check MLS mounting screws, chatter, and leakage

Clean air filter and screen for Zymate controller

**DOCUMENTATION:**

Individuals performing robot maintenance should record the maintenance in the maintenance log provided for each robotic system.

Routine maintenance will be documented on the appropriate form (Robot Daily Maintenance Checklist – TOX 025, Zymate Maintenance Check Schedule – TOX 016)

**REFERENCE:**

Zymate System Service Manual Volume 1 and 2, Zymark Corporation, Hopkinton MA, 1988.

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**PROCEDURE FOR VERIFICATION OF THERMOMETERS**

**PURPOSE:**

In the Toxicology Department, thermometers that are used to monitor temperatures on refrigerators and freezers as well as heat blocks/evaporators should be certified as NIST traceable or they may be checked for accuracy against a NIST traceable thermometer annually.

**PROCEDURE:**

- A. NIST or NIST traceable thermometers are good for 2 years from their original date of calibration. Purchase a new NIST traceable thermometer or borrow a NIST traceable thermometer from the DNA department when the units need verification. Traceability paperwork will be maintained by the responsible department.
- B. Each Thermometer should be given a unique identifying number.
- C. Thermometers should be checked for accuracy at temperatures similar to those of their intended use.
- D. To verify one thermometer at a time, place the NIST traceable thermometer in the same conditions as the thermometer being verified. Allow approximately 5 minutes for the temperature to equilibrate. Record the temperature of the Thermometer in question and of the NIST traceable thermometer.
- E. To verify the accuracy of multiple thermometers at one time follow the following procedures:
  1. For thermometers used in the range of 2-8°C (refrigerator), prepare an ice/water slurry in a beaker. Place a NIST traceable thermometer and all other thermometers in the beaker. Record the temperature of each thermometer.
  2. For thermometers used below 0°C, prepare an/ice water slurry in a beaker and add salt to lower the temperature below 0°C. Place a NIST traceable thermometer and all other thermometers in the beaker. Record the temperature of each thermometer.
  3. For thermometers used in the high temperature range (heat blocks and evaporators), partially fill the wells of a heat block with sand or fill a test tube with glycerol. Turn on the heat block to be in range of 50-80°C. Place a NIST traceable thermometer and all other thermometers in the heat block or glycerol. Record the temperature of each thermometer.
- F. Additionally, SLED has an electronic temperature monitoring system which electronically monitors critical refrigerator/freezers used for long term storage. This system alerts departmental supervisors or designated personnel when the unit is out of range via text

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message. Should units fail on weekends or at night, notifications are delivered and action can be taken to correct the issue.

**DOCUMENTATION:**

A refrigerator/freezer log should be maintained for each unit that may be used to house samples from active or completed cases and also reagents that require refrigeration. Logs should include refrigerator/freezer ID, date temperature taken, and temperature. Comments should be used to indicate temperatures that are out of the control range and should be accompanied by a description of action taken. This log is the primary log for the units.

As a secondary log, electronic records can be generated from the electronic system to document temperature ranges for a specific unit over a given time period.

THERMOMETERS

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**ANALYSIS OF ETHANOL AND OTHER VOLATILES**

**PRINCIPLE:**

The following procedure is designed to be used in conjunction with approved headspace gas chromatograph systems in the quantitation of ethanol and the other volatiles including: methanol, isopropanol, acetaldehyde, and acetone. This procedure makes use of a head space gas chromatograph equipped with a capillary column to quantitate ethanol as well as other volatiles. Results are reported as a percent volatile found in body fluid. This is a weight/volume measurement also accurately expressed in units of grams per deciliter (g/dL). For tissues, results are reported in g/100g. For liquids, results are reported as % v/v and will also be reported in proof.

**SPECIAL HANDLING:**

Universal precautions should be exercised when handling any biological specimens.

**SPECIMEN REQUIRED:**

Specimens suitable for analysis by the following method include, but are not limited to the following: whole blood, serum, urine, ocular fluid, gastric contents, bile, and tissue or tissue homogenate, liquids and/or beverages. The recommended minimum sample is 0.5 mL of liquid or 0.5 g of tissue.

**SUPPLIES REQUIRED:**

BA Vials  
Septa  
Aluminum Seals  
DI Water  
0.01% Tertiary Butanol Internal Standard  
NIST traceable Cerilliant Volatiles Mix for use as calibrators/controls (0.050%, 0.100%, and 0.400% w/v) containing acetone, methanol, ethanol and isopropanol.  
NIST traceable Cerilliant Ethanol standards for use as calibrators/controls (0.010%, 0.025%, 0.080%, 0.150%, 0.200%, 0.300%, 0.400%)  
0.05%(0.025%) Mixed Volatiles Check Mix containing Ethanol, Isopropanol, Methanol, Acetone and (Acetaldehyde) – Made internally  
UTAK Whole Blood Volatiles Mix  
0.08 UTAK Whole Blood Volatiles Mix

**NOTE:** Ethanol standards may also be prepared in house from USP 100% Ethanol. Standards of volatile substances other than Ethanol are prepared from commercially available high purity reagents.

**NOTE:** All reagents should be ACS reagent grade or better unless otherwise noted.

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**APPARATUS REQUIRED:**

Headspace Gas Chromatograph/Flame Ionization Detection  
Headspace Gas Chromatograph/Mass Spectrometer  
Chromatographic Data Station  
Hamilton pipetter/diluter or suitable dispenser  
Adjustable pipette  
Analytical balance  
Vial Crimper

**CALIBRATION:**

Instrument calibration should be performed at least once every six months, or when:

1. A complete change of reagents or controls is introduced.
2. There is major preventive maintenance performed or a critical part is replaced.
3. Controls begin to reflect an unusual trend or are outside of acceptable limits (see quality control section of this procedure for definition of limits).

**CALIBRATION PROCEDURE:**

1. The Instrument Method will be saved using an alternate name to prevent overwriting without backup.  
**NOTE:** For example, on HS Instrument #4, the Method for volatiles analysis is BA4. Prior to calibration, this method may be saved as BA4OLD and the calibration will be performed using method BA4, overwriting the previously stored values. If the current internal standard lot is still valid, this allows all quantities of internal standard to be used while the instrument is being prepared for the next lot of internal standard.
2. Complete a Headspace Calibration Log – TOX 034.
3. Add 2.0 mL of 0.01% tertiary butanol internal standard to the appropriate number of vials
4. Calibration is typically performed using the following calibrators run in 4 replicates at a volume of 0.20 mL each:
  - a. 0.025% Acetaldehyde
  - b. 0.01% Volatiles Mix (If this standard is unavailable, use the Hamilton Pipetter to pipette 40 µL of the 0.05% Volatiles mix into the vial and then adding 160 µL of DI water by manual pipette)
  - c. 0.025% Volatiles Mix
  - d. 0.05% Volatiles Mix
  - e. 0.10% Volatiles Mix
  - f. 0.20% Volatiles Mix
  - g. 0.40% Volatiles Mix

**NOTE:** All standards should be allowed to warm to room temperature before use.

**NOTE:** When a Hamilton pipetter/diluter is used steps 3 and 4 are performed simultaneously.

**NOTE:** Based on availability, calibrators at similar levels may be substituted if needed.

5. A Negative Control should follow the last 0.400% Calibrator to ensure there is no carryover.

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6. After the calibration curve is analyzed, the curve is validated using a series of positive controls.

Typical controls are:

- a. Duplicate 0.010% Ethanol
- b. Duplicate 0.025% Ethanol
- c. Duplicate 0.080% Ethanol
- d. Duplicate 0.150% Ethanol
- e. Duplicate 0.300% Ethanol
- f. Duplicate 0.400% Ethanol
- g. Negative Control
- h. Duplicate 0.05% Mixed Volatiles Check Mix
- i. Duplicate UTAK Whole Blood Control

**NOTE:** Based on availability, positive controls at similar levels may be substituted if needed.

**LINEARITY OF CALIBRATION:**

The determination of linearity for a given calibration is shown through the graphical representation of a calibration curve as produced from representative points of interest. This is performed by plotting response (integrated area under the curve) verses concentration of calibrators and performing a linear least squares regression analysis. This analysis is performed by the instrumentation software. The acceptable correlation coefficient range for volatiles should be 0.99 or greater.

**DOCUMENTATION:**

Following Calibration, a Quality Pack will be prepared which contains at a minimum:

1. The Headspace Calibration Log – TOX 034
2. The Instrument Sequence
3. The Whole Blood Control Verified and Expected Range as published from Manufacturer
4. All Chromatograms from Batch
5. The Calibration Method and Calibration Curves

The Calibration Quality Pack will be reviewed by a Senior Toxicologist and approved prior to using the method for casework. This review will be documented on the Headspace Calibration Log.

The Calibration Packet will be archived in Tox\QC Data Folder on the secure server.

A note will be made on the front of the instrument, typically on an index card, indicating the lot of internal standard the instrument is currently calibrated to use.

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**PROCEDURE:**

**FOR LIQUID/BEVERAGES:** The responsible analyst must dilute the sample so that the concentration of the analyte falls within the linear range of the assay and reporting limits for the item type.

For a 1/100 dilution, the following procedure will be followed:

1. Pipette 100  $\mu$ L of the liquid into a 10 mL volumetric flask
2. Fill volumetric to 10 mL with DI water.
3. Each sample is to be prepared and analyzed in replicate
4. For replicates, a separate dilution will be prepared

**NOTE:** All samples should be allowed to warm to room temperature before dilutions are performed.

**SAMPLE PREPARATION:**

1. Complete a Headspace Volatile Log – TOX 003.
2. Add 2.0 mL of 0.01% tertiary butanol internal standard to the appropriate number of vials.
3. Transfer 0.200 mL of the appropriate body fluid, liquid or approximately 0.200 g of tissue to an appropriate headspace vial (for Tissues record actual weight on Headspace Volatile Log).

**NOTE:** When a Hamilton pipetter/diluter is used, steps 2 and 3 are performed simultaneously.

**NOTE:** When necessary, the responsible analyst may be required to use less than the amount of specimen indicated in this procedure. If less than 0.200 mL of biological specimen is aliquoted, sufficient DI water should be added to bring the total sample volume to 0.200 mL. For example, when 0.100 mL of a biological specimen is prepared for analysis, an additional 0.100 mL of DI water should be added to the vial. The final result should be corrected to account for the dilution.

**NOTE:** Sample preparation steps may be reversed at the analyst's discretion.

4. Insert septum after each sample is delivered.
5. Once batch is pipetted, seal vials with aluminum seal and crimper.
6. Program the appropriate sequence file to reflect standards, controls, and cases for the current batch and save.
7. Print the sequence from the instrument.
8. From the printed sequence, place the sealed vials in appropriate position on the headspace sampler.
9. Have an independent analyst/technician verify the placement of the vials on the instrument autosampler and document this review on the Headspace Volatile Log – TOX 003.
10. Start the sequence on the data station.

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**QUALITY CONTROL:**

For quantitation, the following control samples will be necessary:

- a. 0.08% ethanol standard in position 1 and position 2, one 0.08% ethanol standard after no more than each and every fifteenth sample within the batch, and one after the final case sample
- b. one ethanol control measuring 0.30% or greater
- c. one vial containing 2.0 mL internal standard and 0.200 mL of DI water or other appropriate sample to be used as a negative control placed immediately following the high control of 0.30% or greater
- d. one Mixed Volatiles Check Mix
- e. one ethanol control measuring 0.15%

**NOTE:** The exact positioning and order of the final 0.08% standard, high control and negative control is not defined due to variations in the number of samples routinely analyzed. It is recommended that the high control and negative control samples be placed after the last case sample, but actual position may vary.

**ACCEPTANCE CRITERIA:**

1. The 0.08% control samples must have a percent error of no more than 5% as compared with the expected value. For example, 0.08% standards must fall within the range of 0.076-0.084% to be considered as valid.
2. If the 0.08% controls have a difference outside the 0.076-0.084% range, then no positive ethanol determinations will be reported for case samples preceding or succeeding that control until an acceptable control is analyzed. An exception is at the beginning of the run where instrument equilibration may take place and where two controls are run. In this instance, the first control may be out of range while the instrument equilibrates but the second standard must be in range.
3. With the exception of the 0.08% controls, controls with a value below 0.100% must be within 0.005% absolute difference from the target value.
4. For positive ethanol levels to be reported, the following criteria must be met:
  - a. The high control will also have a percent error of no more than 5% as compared to the expected value. For example, 0.400% standards should fall within the range of 0.380-0.420% to be considered valid.

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- b. The 0.150% control must have a percent error of no more than 5% as compared with the expected value. For example, 0.150% standards must fall within the range of 0.142-0.157% to be considered as valid.
  - c. The negative control must be less than the reporting threshold of 0.010%.
5. Samples which have values greater than the highest control should be either:
- a. re-prepared with less biological fluid (e.g. 100  $\mu$ L of whole blood with the addition of 100  $\mu$ L of DI water) and the reported values corrected to account for the dilution.
  - b. re-prepared and analyzed with a control of sufficient concentration as to ensure curve stability, and linearity.
6. Samples which test positive for Methanol, Isopropanol or Acetone must be analyzed with a suitable control containing the analyte of interest before it can be reported.
- a. The batch will be reprepared as per Sample Preparation above and bracketed with appropriate controls containing the analyte of interest.
  - b. Case samples will be bracketed with 0.05% positive controls with a maximum of 15 case samples between brackets.
  - c. A High Control of 0.30% or higher will be analyzed followed by a negative control.
  - d. All controls >0.100% must be within 5% of the expected value for positive results to be reported.
- NOTE:** Cases with a history of embalming do not need to be quantitated. Non-ethanol volatiles may be reported as positive due to contamination with embalming fluid.
7. If there are any significant peaks which cannot be identified as ethanol, methanol, isopropanol, acetone or acetaldehyde the sample may be run using Headspace Gas Chromatography/Mass Spectrometry to determine the unknown peak.

In order to show separation as well as identify other volatiles, a quality control standard will be run which includes at a minimum methanol, isopropanol, and acetone. The recipe for a suitable standard can be found in protocol formulary – Mixed Volatiles Check Mix.

Positive cases will be confirmed with Headspace Gas Chromatography/Mass Spectrometry.

**REPLICATE SAMPLE AGREEMENT:**

Quantitated samples are to be prepared and analyzed in replicate with the average of all valid values being reported. A replicate is defined as an analysis of a separate aliquot of the specimen on a separate run.

The replicate results must have a percent difference less than or equal to 8% for ethanol values equal to or greater than 0.100% w/v, or must have an absolute difference of no more than 0.008% w/v for ethanol values less than 0.100% w/v. Percent difference is defined as:

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$$\frac{(\text{high value} - \text{low value})}{((\text{high value} + \text{low value}) / 2)} \times 100$$

All samples will be run in singlet on the first run and any positive samples will be re-analyzed in replicate to confirm the quantitated value. In addition, samples that are positive for any volatile substance will also be confirmed via Headspace Gas Chromatography/Mass Spectrometry.

**NOTE:** For some samples such as RUSH cases or other extenuating circumstances, it may be necessary to run samples in duplicate within the same run. Duplicate analysis would be defined as two aliquots, pipetted from the specimen, at the same time and analyzed sequentially. Use of duplicates must be approved by the departmental Lieutenant.

#### MULTIPLE BODY FLUIDS:

When submitted for analysis, ocular fluid will be analyzed for the presence of ethanol in an attempt to determine if post-mortem ethanol production has occurred. In addition, this information may assist in determining which phase of the ADME cycle an individual was experiencing at the time of sample collection. The acronym ADME refers to the cycle beginning with **a**bsorption, and continuing through **d**istribution, **m**etabolism, and **e**limination. Additional body fluids may be analyzed at the discretion of the responsible analyst.

#### NON-BIOLOGICAL SAMPLES (LIQUIDS)

Volatiles quantitated by this method result in a weight/volume percent measurement that is accurately expressed in grams per deciliter (g/dL). Although these units are appropriate for the quantitation of ethanol in biological fluids, ethanol content in non-biological fluids is usually expressed as percent ethanol content by volume or proof. For ethanol, the percent weight/volume should be converted to percent volume/volume (v/v). This can be accomplished by dividing the weight measurement by the density of ethanol (0.79 g/mL) as follows:

$$\text{###\% (w/v)} = \text{###g/100mL}$$

$$\text{###g/100mL} \times 1 \text{ mL}/0.79\text{g} \times 100\% = \text{X \% (v/v)}$$

or

$$31.7\text{g} / 100\text{mL} \times 1 \text{ mL}/0.79 \text{ g} \times 100\% = 40.1 \% \text{ (v/v)}$$

The resulting percent volume/volume should be rounded to one decimal place.

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Proof can be calculated by multiplying the percent volume/volume by two. For example, a liquid was analyzed and found to contain 40.1% ethanol (v/v). The proof content can be calculated as follows:

$$40.1 \times 2 = 80.2$$

Proof will be reported to the nearest whole number therefore the above sample is 80 proof. The proof can be rounded using the rules for rounding.

**LIMITATIONS OF METHOD:**

Under normal casework situations, acetaldehyde will not be reported.

Ethanol, methanol, isopropanol, and acetone levels less than 0.010% will be reported as negative except for investigatory purposes.

For non-biological liquids, volatile levels less than 1.0% v/v will be reported as negative except for investigatory purposes.

**CALCULATIONS:**

1. Interpretation of results

For the purposes of consistency in report writing, the following correlations will be used between the concentration of ethanol in whole blood and the concentration in various other body fluids or samples:

|                      |              |
|----------------------|--------------|
| Whole Blood          | Actual Value |
| Hemolyzed Blood      | Actual Value |
| Serum : Whole Blood  | 1.18 : 1     |
| Plasma : Whole Blood | 1.18 : 1     |

**NOTE:** Samples will be considered serum if a clot activator, serum separation, or a sterile (no additive) tube has been used regardless of centrifugation or case type. Samples are generally considered to be whole blood when the tube used for collection contains one or more anti-coagulant(s), unless the sample has an obvious clot caused by improper mixing after sample draw. Examples of anti-coagulants include, but are not limited to, preparations containing heparin, EDTA, potassium oxalate or sodium citrate.

Conversion of an ethanol value in body fluids, other than whole blood, to the corresponding whole blood value may be accomplished as follows:

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$$C_{\text{Reported}} = \frac{C_{\text{Actual}}}{\text{Ratio of sample to Whole blood}}$$

Ratio of sample to Whole blood

where  $C_{\text{Actual}}$  is the value or concentration of ethanol which was indicated on original chromatogram (without correction), and  $C_{\text{Reported}}$  is the value reported on the final report.

For example, conversion from a serum ethanol of 0.100% to the corresponding whole blood ethanol requires that the actual value be divided by 1.18. Thus, the corresponding whole blood ethanol value would be 0.085%.

## 2. TISSUE SAMPLES:

The approved unit for the reporting of ethanol found in a tissue sample is grams per 100 g of tissue (g/100 g). If 200 mg of tissue are used in the determination of tissue ethanol concentration, the uncorrected value as reported by the work station is correctly expressed as grams ethanol per 100 grams of tissue. Deviations from 200 mg of sample should be corrected to reflect the concentration based on 200 mg. For example, two liver samples, each weighing 221 mg, were analyzed with the following results:

|          |                 |
|----------|-----------------|
| Sample 1 | 0.0427% Ethanol |
| Sample 2 | 0.0438% Ethanol |

The report should reflect 0.039 g/100 g ethanol found in liver tissue. This is based on the following correction equation:

$$\frac{\text{Result from instrument}}{\text{Weight of tissue (mg)}} \times 200 \text{ mg} = \text{Corrected value}$$

## REPORTING:

Refer Guidelines for Generation of Formal Toxicology Reports in the Toxicology Quality Manual for reporting of volatiles.

## DOCUMENTATION:

Documents for case files should include copies of all relevant GC chromatograms from processed specimens.

A quality control packet will be created for each batch of case samples that are analyzed. The QC Pack should include at a minimum:

1. The Headspace Volatile Log – TOX 003
2. The Instrument Sequence Log
3. Chromatograms from all QC Samples

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The Quality Control Pack will be reviewed by a Senior Toxicologist and approved prior to the data being used for casework. Upon completion of their review, the reviewing analyst will initial or sign the appropriate space on the log.

The Quality Control Pack will be archived in the Tox\QC Data Folder on the secure server.

**REFERENCES:**

Winek, Charles L., and Mark Carfagna, "Comparison of Plasma, Serum, and Whole Blood Ethanol Concentrations", Journal of Analytical Toxicology, 11 (1987): 267-268.

Blood Alcohols Analysis by Packed Column GC, (Application Note 72), 1995 Supelco, Inc., Bellefonte, PA.

Solanky, Anil A. and Phillip L. Wylie, "Analysis of Blood- Alcohol Concentrations using the HP 7694 Headspace Sampler, (Hewlett Packard Application Note 228-250)", Gas Chromatography, September 1993.

Hewlett Packard Company, HP 5890 Series II and Series II Plus Reference Manual, 1994.

Hewlett Packard Company, Understanding Your Chemstation, 1994.

Hewlett Packard Company, HP 5890 Series II and Series II Plus Operating Manual, 1994.

Hewlett Packard Company, HP 7694 Headspace Sampler Operating and Service Manual, 1993.

SOFT/AAFS Forensic Toxicology Laboratory Guidelines, 2006 Version.

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**CONFIRMATION OF VOLATILE COMPOUNDS BY HEADSPACE GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PURPOSE:**

The following procedure provides a general method for the qualitative confirmation of various volatile compounds including, but not limited to the following: ethanol, methanol, isopropanol, acetaldehyde, and acetone. This procedure makes use of a headspace gas chromatograph/mass spectrometer (HSGC/MS).

**SPECIAL HANDLING:**

Universal precautions should be exercised when handling any biological specimens.

**SPECIMEN REQUIRED:**

Specimens suitable for analysis by the following method include, but are not limited to the following: whole blood, serum, urine, ocular fluid, gastric contents, bile, and tissue or tissue homogenate. The recommended minimum sample is 0.5 ml of liquid or 0.5 g of tissue.

**SUPPLIES REQUIRED:**

BA Vials  
Septa  
Aluminum Seals  
2 mL 0.01% Tertiary Butanol Internal Standard/sample  
0.30% g/dL or greater Ethanol Standard (Certified Stock Solution)  
Volatile control containing but not limited to the following: acetaldehyde, acetone, methanol, ethanol, and isopropanol.

**NOTE:** Ethanol standards may also be prepared in house from USP 100% Ethanol. Standards of volatile substances other than ethanol are prepared from commercially available high purity reagents.

**NOTE:** All reagents should be ACS reagent grade or better unless otherwise noted.

**APPARATUS REQUIRED:**

Headspace gas chromatograph/mass spectrometer  
Hamilton pipetter/diluter or suitable dispenser  
Adjustable pipette  
Analytical balance  
Vial crimper

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**PROCEDURE:**

**TUNE:**

Before acquiring any data, it is necessary to tune the mass spectrometer. An acceptable tune indicates that the mass spectrometer is working satisfactorily. The methods employed to tune the HP GC/MS Systems are outlined in the user's guide provided with each instrument. In addition, the various criteria for determining if a tune is acceptable are outlined in Tuning Specifications for Agilent GCMS Systems.

**SAMPLE PREPARATION:**

1. Complete a sequence log- Headspace Volatile Log- TOX 003.
2. Add 2 mL 0.01% tertiary butanol internal standard (when appropriate) to the appropriate vials.
3. Transfer 0.200 mL of the appropriate body-fluid or approximately 0.200 g of tissue to an appropriate headspace vial.

**NOTE:** When necessary, the responsible analyst may be required to use less than the amount of specimen indicated in this procedure.

**NOTE:** When a Hamilton pipetter/diluter is used, steps 2 and 3 are performed simultaneously.

4. Insert septum and seal with aluminum seal.
5. Place the sealed vials in appropriate position on the headspace sampler.
6. Load the appropriate method on the headspace sampler.
7. Program the appropriate sequence file to reflect standards, controls, and cases for the current run and save.
8. Have a second analyst/technician verify vial placement on the HS autosampler and document this verification on the Headspace Volatile Log –TOX 003.
9. Begin sequence acquisition on the data station.
10. Initiate headspace run.

**NOTE:** DO NOT initiate the headspace run until the data station is ready and in the pre-run mode.

**QUALITY CONTROL:**

Within any batch of cases, the following control samples will be necessary:

- a. One control containing, but not limited to the following: acetaldehyde, acetone, methanol, ethanol, and isopropanol.
- b. One ethanol control measuring 0.30% g/dL or greater.
- c. One vial containing 2.0 ml internal standard and 0.200 mL of DI water or other appropriate sample to be used as a negative control placed immediately following the high control of 0.30% g/dL or greater.

**INTERPRETATION OF RESULTS:**

Refer to Toxicology Quality Manual for criteria on qualitative identification of compounds.

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**OPERATING CONDITIONS:**

HSGC/MS operating conditions which provide adequate separation of analytes with minimum upper oven temperatures are recommended. To properly analyze volatiles it is important to start at a low oven temperature, at or near room temperature. The maximum ramp temperature typically is significantly lower than a standard general run.

The method most commonly used for volatiles confirmation is: BA2SCR

Method parameters can be found in the methods binder next to the instrument.

**DOCUMENTATION:**

A quality control pack shall be prepared which contains the Headspace Volatiles Log-TOX 003, appropriate tune and tune evaluation, instrument sequence, the Total Ion Chromatograms (TIC) from the controls, and mass spectra of all compounds positively identified.

The Quality Control Pack will be reviewed by a Senior Toxicologist and approved prior to the data being used for casework. Upon completion of their review, the reviewing analyst will initial or sign the appropriate space on the log.

The Quality Control Pack will be archived in the Tox\ QC Data Folder on the secure server.

**REFERENCES:**

Screening and Confirmation of Drugs of Abuse with Toxi-Lab TLC and Hewlett-Packard GC/MS Systems, Analytical Systems, Division of Marion Laboratories, 1988.

HP G1701BA Enhanced ChemStation Software, MS Chemstation User's Guide, Hewlett-Packard Company, 1989-1998.

HP 5973 Mass Selective Detector Hardware Manual, Hewlett-Packard Company, 1998.

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**EXTENDED VOLATILES ANALYSIS**

**PRINCIPLE:**

The following procedure is designed for the qualitative confirmation of hydrocarbons and other volatile substances other than those routinely screened by headspace gas chromatography/mass spectrometry (Ethanol, Methanol, Isopropanol, Acetone, Acetaldehyde). This procedure utilizes a head space gas chromatograph with mass spectrometer detector to separate and identify volatile compounds of interest in the samples.

**SPECIAL HANDLING:**

Universal precautions should be exercised when handling any biological specimens.

**SPECIMEN REQUIRED:**

Specimens suitable for analysis by the following method include, but are not limited to, the following: whole blood, serum, urine, ocular fluid, gastric contents, bile, and tissue or tissue homogenate. The recommended minimum sample is 0.5 mL of liquid or 0.05 g of tissue.

**SUPPLIES REQUIRED:**

BA Vial  
Septum  
Aluminum Seal  
2 mL 0.01% tertiary butanol Internal Standard  
Standard of Volatile of Interest  
Gas Tight Syringe

**NOTE:** Standards of volatiles are prepared from commercially available high purity reagents. All reagents should be ACS reagent grade or better unless otherwise noted.

**APPARATUS REQUIRED:**

Headspace Gas Chromatograph/Mass Spectrometer  
Adjustable Pipette  
Analytical balance  
Vial Crimper

**PROCEDURE:**

**TUNE:**

Before acquiring any data, it is necessary to tune the mass spectrometer. An acceptable tune indicates that the mass spectrometer is working satisfactorily. The methods employed to tune the HP GC/MS Systems are outlined in the user's guide provided with each instrument. In addition, the various criteria for determining if a tune is acceptable are outlined in Tuning Specifications for Agilent GCMS Systems.

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**SAMPLE PREPARATION:**

1. Complete a sequence log.
2. Add 0.01% tertiary butanol internal standard (when appropriate) to the appropriate vials.
3. Transfer 0.200 mL of the appropriate body-fluid or approximately 0.200 g of tissue to an appropriate headspace vial.

**NOTE:** When necessary, the responsible analyst may be required to use less or more than the amount of specimen indicated in this procedure.

**NOTE:** When a Hamilton pipetter/diluter is used, steps 2 and 3 are performed simultaneously.

4. Insert septum and seal with aluminum seal.
5. Place the sealed vials in appropriate position on the headspace sampler.
6. Load the appropriate method on the headspace sampler.
7. Program the appropriate sequence file to reflect standards, controls, and cases for the current run and save.
8. Begin sequence acquisition on the data station.
9. Initiate headspace run.

**NOTE:** DO NOT initiate the headspace run until the data station is ready and in the pre-run mode.

**NOTE:** It may be necessary to use an unopened vial of blood, especially if an extremely volatile substance is being analyzed. In addition, depending on the volatile of interest, it may be necessary to analyze lung tissue (preferably submitted to the lab by having the whole lung sealed in a paint can and frozen).

**QUALITY CONTROL:**

Within any batch of cases, the following control samples will be run:

- a. One vial containing internal standard and DI water or other appropriate sample in a similar amount to the sample being analyzed to be used as a negative control.
- b. One control containing, but not limited to the following: acetaldehyde, acetone, methanol, ethanol, and isopropanol.

**NOTE:** Gaseous samples may be used as positive controls. For this method, fill the vial with the gas of interest and seal after addition of internal standard. Analyze by the same method as the sample.

**INTERPRETATION OF RESULTS:**

Refer to Toxicology Quality Manual for criteria on qualitative identification of compounds.

**OPERATING CONDITIONS:**

**EXTENDED VOLATILES**

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HSGC/MS operating conditions which provide adequate separation of analytes with minimum upper oven temperatures are recommended. To properly analyze volatiles it is important to start at a low oven temperature, at or near room temperature. The maximum ramp temperature typically is significantly lower than a standard general run.

The method most commonly used for Extended Volatiles confirmation is: EXT\_VOL2  
Method parameters can be found in the methods binder next to the instrument.

**LIMITATIONS OF METHOD:**

Numerous volatile substances are known to exist. It is therefore difficult to have one method or set of standards that permit the quantitative analyses of all volatiles.

**DOCUMENTATION:**

A quality control pack shall be prepared which contains the Headspace Volatiles Log-TOX 003, appropriate tune and tune evaluation, instrument sequence, the Total Ion Chromatograms (TIC) from the controls, and mass spectra of all compounds positively identified.

The Quality Control Pack will be reviewed by a Senior Toxicologist and approved prior to the data being used for casework. Upon completion of their review, the reviewing analyst will initial or sign the appropriate space on the log.

The Quality Control Pack will be archived in the Tox\ QC Data Folder on the secure server.

Refer to the Toxicology Quality Manual Guidelines for Generation of Formal Toxicology Reports for reporting of extended volatiles.

**REFERENCES:**

Solanky, Anil A. and Phillip L. Wylie, "Analysis of Blood-Alcohol Concentrations using the HP 7694 Headspace Sampler, (Hewlett Packard Application Note 228-250)", Gas Chromatography, September 1993.

Hewlett Packard Company, HP 5890 Series II and Series II Plus Reference Manual, 1994.

Hewlett Packard Company, Understanding Your Chemstation, 1994.

Hewlett Packard Company, HP 5890 Series II and Series II Plus Operating Manual, 1994.

Hewlett Packard Company, HP 7694 Headspace Sampler Operating and Service Manual, 1993.

EXTENDED VOLATILES

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**ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) PROCEDURE**

**PRINCIPLE:**

The Enzyme Linked Immunosorbent Assay (ELISA) system is an automated system which performs drug screening assays on a variety of samples. The procedure described below is for competition immunoassays in which typically horseradish peroxidase (HRP) labeled drug competes with the unlabeled drug found in the subject sample for binding sites on antibodies immobilized onto the assay reaction well. After incubation for a fixed time, separation of bound from free drug is achieved by washing the plate. Finally, a substrate is added which produces a color change based on the amount of labeled drug present. Absorbance is determined by a plate reader and the absorbance level is inversely proportional to the amount of drug present in the sample or calibrator/control.

**TERMINOLOGY:**

1. ELISA – Enzyme-linked Immunosorbent Assay
2. Plate - 96-well polystyrene microplate, approx. 300 µL well volume.
3. Antibody Coated Plate - Plate with typically rabbit anti-drug antibodies immobilized on the well walls.
4. Enzyme Conjugate – Horseradish peroxidase (HRP) labeled with the drug of analysis and diluted in a protein matrix with stabilizers. It is this conjugate which competes with any drug present in the subject sample.
5. Substrate Reagent – Containing 3,3',5,5'-tetramethylbenzidine (TMB) and urea peroxide in buffer or other appropriate substrate for HRP.
6. Stop Solution – Containing 1 M hydrochloric acid.
7. Positive control – Either a whole blood or urine matrix containing dissolved drugs. This is to be diluted to the established cutoff.
8. Negative control – A drug free whole blood or urine matrix.

**SPECIAL HANDLING:**

Handle all components and all biological samples as recommended for any potentially infectious human specimen. Discard substrate if obvious blue color develops. Do **NOT** add sodium azide to samples as a preservative. Do **NOT** freeze kits unless instructed. Store kits at 2-8°C until expiration date on label.

**SPECIMEN REQUIRED:**

Biological specimens which have been refrigerated or frozen. The recommended minimum sample is 100 µL for blood and 50 µL for urine.

**SUPPLIES REQUIRED:**

Appropriate ELISA kit for drug to be analyzed.  
12x75mm Test Tubes  
ELISA Buffer (0.1M Phosphate Buffered Saline or other appropriate buffer)

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**APPARATUS REQUIRED:**

TECAN Automated ELISA comprised of:  
TECAN Freedom EVO 75 sample processor  
Microplate reader  
Columbus microplate strip washer  
Microplate incubator  
Micropipettes: 10-100 µL and 100-1000 µL with tips

**CALIBRATION:**

Routine positioning calibration of the TECAN Freedom EVO 75 is not required as the instrument goes through a self check calibration procedure before each test to ensure the instrument is working properly. Calibration is needed after major failures or replacement of parts. Calibration of individual components is performed as described in the operation manual.

**PREPARATION OF BLOOD CONTROLS:**

Blood should first be treated with sodium bisulfite at a concentration of 0.01g/50 mL. To prepare controls (High, Medium/Cut-Off, and Low), 10 mLs of High Control Stock is prepared in the proper matrix. For the Cut-off control, a ½ dilution of the High Control is performed. For the Low control, a ½ dilution of the Cut-off control is performed.

**URINE CONTROLS:** High Control Stock is prepared by adding the following amounts to 10 mLs of blank urine matrix. The table provided shows dilutions and final concentrations.

**High Control 1:**

60 µL of 0.10 mg/mL Benzoylcegonine  
20 µL of 1.0 mg/mL Methamphetamine  
20 µL of 0.10 mg/mL Morphine  
20 µL of 0.10 mg/mL Methadone  
20 µL of 0.01 mg/mL THC-COOH  
20 µL of 0.10 mg/mL Tramadol

**High Control 2:**

20 µL of 1.0 mg/mL Amphetamine  
20 µL of 0.01 mg/mL Buprenorphine  
20 µL of 1.0 mg/mL Carisoprodol  
40 µL of 0.10 mg/mL Oxazepam  
20 µL of 0.10 mg/mL Oxycodone  
10 µL of 0.10 mg/mL Zolpidem

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|                 | Stock conc. | 1:10 dilution | 1:100 dilution | Low (ng/mL) | Med (ng/mL) | High (ng/mL) | 10 mLs (ng)   |
|-----------------|-------------|---------------|----------------|-------------|-------------|--------------|---------------|
| PC1             |             |               |                |             |             |              |               |
| Benzoylcegonine | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 150         | 300         | 600          | 6000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 60 μL 1:10    |
| Methamphetamine | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 500         | 1000        | 2000         | 20000         |
|                 | 1000 ng/μL  | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL stock   |
| Morphine        | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 50          | 100         | 200          | 2000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL 1:10    |
| Methadone       | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 50          | 100         | 200          | 2000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL 1:10    |
| THC-COOH        | 0.100 mg/mL | 0.01 mg/mL    | 0.001 mg/mL    | 50          | 100         | 200          | 2000          |
|                 |             | 10 ng/μL      | 1 ng/μL        |             |             |              | 20 μL of 1:10 |
| Tramadol        | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 50          | 100         | 200          | 2000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL 1:10    |
|                 |             |               |                |             |             |              |               |
| PC2             |             |               |                |             |             |              |               |
| Amphetamine     | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 500         | 1000        | 2000         | 20000         |
|                 | 1000 ng/μL  | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL stock   |
| Buprenorphine   | 0.10 mg/mL  | 0.01 mg/mL    | 0.001 mg/mL    | 5           | 10          | 20           | 200           |
|                 | 100 ng/μL   | 10 ng/μL      | 1 ng/μL        |             |             |              | 20 μL 1:10    |
| Carisoprodol    | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 500         | 1000        | 2000         | 20000         |
|                 | 1000 ng/μL  | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL stock   |
| Oxazepam        | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 100         | 200         | 400          | 4000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 40 μL 1:10    |
| Oxycodone       | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 50          | 100         | 200          | 2000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 20 μL 1:10    |
| Zolpidem        | 1.0 mg/mL   | 0.1 mg/mL     | 0.01 mg/mL     | 25          | 50          | 100          | 1000          |
|                 |             | 100 ng/μL     | 10 ng/μL       |             |             |              | 10 μL 1:10    |

**BLOOD CONTROLS:** High Control Stock is prepared by adding the following amounts to 10 mLs of blank blood matrix. The table provided shows dilutions and final concentrations.

**High Control 1:**

40 μL of 0.10 mg/mL Benzoylcegonine  
 20 μL of 0.10 mg/mL Methamphetamine  
 15 μL of 0.10 mg/mL Morphine  
 10 μL of 0.10 mg/mL Methadone  
 60 μL of 0.01 mg/mL THC-COOH  
 10 μL of 0.10 mg/mL Tramadol

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**High Control 2:**

20 µL of 0.10 mg/mL Amphetamine  
20 µL of 0.001 mg/mL Buprenorphine  
10 µL of 1.0 mg/mL Carisoprodol  
10 µL of 0.10 mg/mL Oxazepam  
15 µL of 0.10 mg/mL Oxycodone  
40 µL of 0.01 mg/mL Zolpidem

|                  | Stock concentration | 1:10       | 1:100       | Low (ng/mL) | Med (ng/mL) | High (ng/mL) | 10 mLs (ng)   |
|------------------|---------------------|------------|-------------|-------------|-------------|--------------|---------------|
| PC1              |                     |            |             |             |             |              |               |
| Benzoyllecgonine | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 100         | 200         | 400          | 4000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 40 µL 1:10    |
| Methamphetamine  | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 50          | 100         | 200          | 2000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 20 µL 1:10    |
| Morphine         | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 37.5        | 75          | 150          | 1500          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 15 µL 1:10    |
| Methadone        | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 25          | 50          | 100          | 1000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 10 µL 1:10    |
| THC-COOH         | 0.100 mg/mL         | 0.01 mg/mL | 0.001 mg/mL | 15          | 30          | 60           | 600           |
|                  |                     | 10 ng/µL   | 1 ng/µL     |             |             |              | 60 µL of 1:10 |
| Tramadol         | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 25          | 50          | 100          | 1000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 10 µL 1:10    |
| PC2              |                     |            |             |             |             |              |               |
| Amphetamine      | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 50          | 100         | 200          | 2000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 20 µL 1:10    |
| Buprenorphine    | 0.10 mg/mL          | 0.01 mg/mL | 0.001 mg/mL | 0.5         | 1           | 2            | 20            |
|                  |                     | 10 ng/µL   | 1 ng/µL     |             |             |              | 20 µL 1:100   |
| Carisoprodol     | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 250         | 500         | 1000         | 10000         |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 10 µL stock   |
| Oxazepam         | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 25          | 50          | 100          | 1000          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 10 µL 1:10    |
| Oxycodone        | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 37.5        | 75          | 150          | 1500          |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 15 µL 1:10    |
| Zolpidem         | 1.0 mg/mL           | 0.1 mg/mL  | 0.01 mg/mL  | 10          | 20          | 40           | 400           |
|                  |                     | 100 ng/µL  | 10 ng/µL    |             |             |              | 40 µL 1:100   |

**PROCEDURE:**

**NOTE:** All components must be at room temperature before use.

1. Prepare a sequence table to include lab number and assays

ELISA

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requested.

2. Pipette samples in duplicate into 12x75 mm tubes, minimum volume 0.1 mL.  
Dilute blood samples 1/10 with ELISA buffer. Dilute Urine Samples 1/20 with ELISA buffer. (Example blood dilution: 100 µL sample, 900 µL buffer) (Example urine dilution: 50 µL sample, 950 µL buffer). Minimum required volume for EVO operation is 500 µL.
3. Perform daily maintenance on Freedom EVO 75.
4. Dilute all controls and calibrators to appropriate dilution with ELISA buffer.
5. Using the plate map generated by the instrument, place correct number of antibody coated well strips into an empty frame. Fill incomplete strips with blank wells. Place plate into correct position on the instrument.
6. Place reagents into correct positions on instrument worktable and check that appropriate volume is in each container. Also verify that wash bottles are filled and waste bottles are emptied.
7. Select Navitrak icon and select start. Follow on screen instructions.
8. Alternatively, the ELISA may be performed manually utilizing the plate washer and reader in manual mode. Instructions for operation in the manual mode are located within the Magellan software.

**DATA ANALYSIS:**

Results generated by the plate reader must be reviewed by the technician or analyst and then compared to the controls for each assay to determine which samples are positive or negative. A report reflecting those results will then be generated.

**QUALITY CONTROL:**

An assay is considered positive at or above the following levels:

Assay Screening Thresholds:

| Assay           | Matrix | Cut-Off (ng/mL) |
|-----------------|--------|-----------------|
| Amphetamine     | Blood  | 100             |
|                 | Urine  | 1000            |
| Benzodiazepines | Blood  | 50              |
|                 | Urine  | 200             |
| Buprenorphine   | Blood  | 1.0             |
|                 | Urine  | 10              |
| Carisoprodol    | Blood  | 500             |
|                 | Urine  | 1000            |
| Cocaine         | Blood  | 200             |
|                 | Urine  | 300             |
| Methadone       | Blood  | 50              |
|                 | Urine  | 100             |

ELISA

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|                 |       |      |
|-----------------|-------|------|
| Methamphetamine | Blood | 100  |
|                 | Urine | 1000 |
| Opiates         | Blood | 75   |
|                 | Urine | 200  |
| Oxycodone       | Blood | 75   |
|                 | Urine | 200  |
| Tramadol        | Blood | 50   |
|                 | Urine | 100  |
| Zolpidem        | Blood | 20   |
|                 | Urine | 50   |
| Cannabinoids    | Blood | 30   |
|                 | Urine | 100  |

In DUI cases, screen threshold levels will be adhered to unless there is a case history of a substance which is known to have a low cross-reactivity with a particular assay. In Death Investigation, the screen is used as a guide and discretion is given to the analyst to pursue analytes which may provide additional information to the case.

1. Interpretation

- a. If the absorbance of the sample is equal to or less than the absorbance of the Cut-off Control, the sample is presumptive positive for that class of drugs.
- b. If the sample absorbance is greater than the absorbance of the Cut-off Control, the screen is considered negative.
- c. If the sample absorbance falls between the Cut-off Control and the Low Control, the sample could contain low concentrations of the drug of interest. In death investigation cases, it is the analyst's discretion to pursue drugs in this range based on case history.
- d. Samples will be run in duplicate to prevent issues generated by robotic sampling. If one screen is positive and one is negative, the sample will move forward with confirmation.

2. Quality Control Criteria

- a) The Negative Control must be negative relative to the Cut-off Control.
- b) The Low Controls must be negative relative to the Cut-off Control.
- c) The High Controls must be positive relative to the Cut-off Control.
- d) The % CV between the duplicate Cut-off Controls should be less than 20%.
- e) The % CV between the duplicate Negative Controls should be less than 20%.

3. Corrective Action for Failed Quality Control

- a. If the Negative Control is positive, repeat all samples.
- b. If the low control is positive or the high control is negative, repeat or send to confirmation all case samples with absorbance within 20% above the Cut-off

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Control. All case samples higher than 20% of the Cut-off Control should be considered negative.

- c. Exceptions to these guidelines must be authorized by the departmental Lieutenant.

4. Additional guidelines:

- a. All positives are presumptive and must be confirmed by a more specific, selective procedure.
- b. A toxicologist may, at their discretion, order confirmation testing even if the ELISA result is negative. For example, some benzodiazepines differ in their cross-reactivity with the benzodiazepine assay.
- c. All negative results with an absorbance greater than 1.5 times that of the negative control absorbance will be considered invalid and should be repeated.

**DOCUMENTATION:**

1. Vial placement on the ELISA system will be verified from the instrumental sequence log and documented on the ELISA worksheets.
2. For each batch of samples run on the ELISA system, a quality packet will be prepared which contains:
  - The Toxicology Drug Screen Log
  - The instrumental sequence list
  - List of reagents and lot numbers
  - Drug Screen reports for the Low Controls, High Controls
  - Raw data from the batch
3. A technical review of the ELISA quality Packet will be conducted by a Senior Analyst.
4. ELISA quality packets will be archived in the Toxicology department electronic files.
5. All maintenance records are maintained in the instrument log.

**REFERENCES:**

OraSure Technologies Cannabinoids Micro-Plate EIA Kit Package Insert, OTI, 2000.

Immunalysis Corporation Package Inserts for ELISA Kits

TECAN Freedom EVO 75 Workstation Operating Procedures

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**DETERMINATION OF CARBON MONOXIDE IN BIOLOGICAL SAMPLES**  
**VIA AVOXimeter 4000**

**PURPOSE:**

The following procedure describes the use of the AVOXimeter 4000 CO-Oximeter for the determination of carboxyhemoglobin levels in whole blood. The procedure may be used with liquid specimens from tissues if there is sufficient (>4 g/dL) total hemoglobin present.

**SPECIAL HANDLING:**

Due to the potential for aerosol formation or spatter of biological fluids, sample preparation should be performed in an appropriate biohazard hood or behind proper splash guards.

**SPECIMEN REQUIRED:**

The following procedure requires approximately 1 mL of whole blood. Smaller sample volumes may be used in cases of insufficient sample.

**SUPPLIES REQUIRED:**

1 ml (approximate) DI Water  
10 mg (approximate) Sodium Hydrosulfite (for samples with high metHb)  
Glass cuvettes or micro glass culture tubes  
Syringe (with needle optional) for injecting sample into cuvette  
AVOX sample cuvettes  
Cleaning Solution (approximately 10% v/v bleach solution in water, made fresh daily)  
Multi-Level CO-Oximeter Controls (I-III)  
Yellow and Orange AVOX optical filters  
**NOTE:** All reagents should be ACS reagent grade or better unless otherwise noted.

**APPARATUS REQUIRED:**

AVOXimeter 4000 CO-Oximeter

**CALIBRATION REQUIREMENTS:**

Calibration of the AVOXimeter 4000 CO-Oximeter is to be performed by the primary operator and is scheduled to occur:

- 1.) Following major preventative maintenance or a when a critical part is replaced
- 2.) When controls begin to reflect an unusual trend or are outside of manufacturer specified limits.

To perform calibration, Technical Support should be contacted for instructions on calibration. Contact information is located on the instrument and in the Operations Manual.

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**PROCEDURE:**

Before performing this procedure, it is recommended that unknown samples be screened for the presence of carboxyhemoglobin via Kunkel's Test. For a complete description of this procedure, see Kunkel's Test.

**CARBOXYHEMOGLOBIN DETERMINATION (AVOXimeter 4000):**

**TO FILL A SAMPLE CUVETTE WITH A LIQUID SAMPLE:**

1. Using a syringe, draw up approximately 0.5 mL undiluted blood.
2. Connect the syringe to an unused cuvette. Hold the cuvette by means of the finger grip on the black cap.
3. Firmly, holding the syringe and cuvette at approximately a 45 degree angle, fill the cuvette by gently depressing the syringe plunger.  
**NOTE:** Never force sample into the cuvette. If a cuvette does not fill easily, discard it and use a new one.
4. Stop filling the cuvette when the sample reaches the vent patch. Do not continue to fill the cuvette and cause the vent patch to bulge.
5. Verify that the light path (the first cm immediately above the vent path) is free of bubbles.
6. With the syringe still attached to the cuvette, remove any blood from the exterior of the cuvette before placing it in the test chamber.

**INSTRUMENT OPERATION:**

1. Press Enter/On. The instrument will start and performs a series of self-tests.
2. The display will say "READY" and "Insert Cuvette" when a test can be run.
3. The Cal Code will display at the bottom of the display screen. Confirm that the Cal Code is the same as the one marked on the package of cuvettes, if not, see CHANGE CAL CODE below.
4. Run the Quality Control tests for the day.
  - a. Optical Filter Quality Control (to be run each day of instrument operation)
    - i. Verify the instrument is ready to run and that the "READY--- Insert Cuvette" screen is displayed.
    - ii. Insert the yellow optical filter into the test chamber. The instrument will begin to analyze the filter.
    - iii. The "Select Sample Type" screen will appear. Choose "2 – QC" followed by "Enter/On".
    - iv. It will ask what type of QC, choose "2. Optical" followed by "Enter/On".
    - v. The "Select Filter" screen will appear. Choose "1- Yellow" followed by "Enter/On".
    - vi. A Confirmation screen will appear titled "Yellow Optical Filter" with choices: "1. OK" or "2. Re-enter". Choose "1. OK" followed by "Enter/On".

AVOX COOXIMETER

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- vii. Results will print.
- viii. Verify that the results for each filter are within the expected ranges (see table below).
- ix. Remove the cuvette and press “Enter/On” to return to the main screen.
- x. Repeat this process for the orange filter.

| Optical Filter | tHb (g/dL)   | %O <sub>2</sub> Hb | %COHb        | %MetHb      |
|----------------|--------------|--------------------|--------------|-------------|
| Yellow         | 7.8 to 8.2   | 93.7 to 96.3       | 0.6 to 3.4   | -0.4 to 2.4 |
| Orange         | 16.7 to 17.3 | 37.8 to 40.2       | 20.0 to 23.0 | 0.2 to 1.8  |

- b. Liquid CO Quality Controls (Run three levels of Multi-level liquid controls I-III once per week of instrument operation)
  - i. Verify the instrument is ready to run indicated by the “READY—Insert Cuvette” message displayed on the screen.
  - ii. Following instructions above for filling a cuvette, fill cuvette with a liquid control.
  - iii. Insert the cuvette into the test chamber. Analysis will begin automatically.
  - iv. The screen specifying the sample type will be displayed – Choose “2. QC” followed by “Enter/On”.
  - v. The screen specifying the QC type is displayed- Choose “1.-Liquid” followed by “Enter/On”.
  - vi. The screen specifying the control level is displayed. Choose the appropriate control level. (i.e. Level 1, 2, or 3)
  - vii. A menu for selection of the liquid control lot number will be displayed. Select the previously entered lot number and press “Enter/On”.
  - viii. A menu to confirm the cuvette lot number will be displayed. Choose “1.OK” or “2. Enter New Value”. The cuvette lot number should match the lot number on the cuvette packaging.
  - ix. Confirm the liquid level lot number and cuvette lot number by selecting “1. OK” then “Enter/On” or “2. Re-enter” followed by “Enter/On” if changes need to be made.
  - x. Results from the analysis will automatically print.
  - xi. Remove the cuvette and press “Enter/On” to return to the main screen.
  - xii. Repeat the previous steps for each additional liquid control level.
  - xiii. Verify that the results for each level of liquid controls are within the expected ranges. See table for the appropriate lot # of liquid controls in the AVOX notebook located next to the instrument.
- c. Running the Sample.
  - i. Verify the instrument is ready to run. The “READY—Insert Cuvette” screen should be displayed.

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- ii. Following previously stated instructions for filling a cuvette, fill cuvette with the sample.
  - iii. Insert the cuvette into the test chamber. Analysis will begin automatically.
  - iv. The screen specifying the sample type will displayed – Choose “1. Patient” followed by “Enter/On”.
  - v. The screen will display the option to enter the sample ID or to use a previous ID. If this is a new sample, choose “1. Enter Patient ID” followed by “Enter/On”.
  - vi. Enter the numeric Sample ID in the appropriate space followed by “Enter/On”.
  - vii. A confirmation screen for the sample ID will appear with the options “1. OK” or “2. Re-enter”. Choose the appropriate response followed by “Enter/On”.
  - viii. Results from the analysis will print automatically.
- d. Shutdown.
- i. Press “Main Menu”
  - ii. Select “Shutdown” followed by “Enter/On”.

**NOTE:** Typically sample dilution is not required but may be performed with particularly viscous samples using DI Water. In addition, samples with High MetHb may be treated with approximately 10 mg Sodium Hydrosulfite to reduce the MetHb prior to analysis. If Sodium Hydrosulfite is used, centrifuge samples prior to analysis.

**NOTE:** A 10% v/v solution of bleach in water is used as the cleaning solution, made fresh daily.

**ALTERNATE PROCEDURES:**

See procedures for KUNKEL'S TEST or Carboxyhemoglobin Determinations by UV Spectrophotometry (AMMONIA METHOD CONFIRMATION FOR THE CONFIRMATION OF CARBOXYHEMOGLOBIN)

**LIMITATIONS OF METHOD:**

Methemoglobin levels above 25% may cause absorbance variations at the wavelengths measured by the AVOXimeter resulting in inaccurate results. Therefore, only those samples which contain less than or equal to 25% Methemoglobin will be considered for reporting purposes. Samples which contain in excess of 25% methemoglobin should be reanalyzed after treatment with sodium hydrosulfite (sodium dithionite).

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**DOCUMENTATION:**

CO-Oximeter Results worksheets should reflect the date analysis was performed, lab number, subject's name and the final CO value as reported by the AVOXimeter 4000. In addition, the Met Hb results and the results of the Kunkel's Test should be reported as either positive or negative (qualitative test).

Weekly and Daily QC Data should be recorded on the CO-Oximeter Checklist.

Optical controls must be evaluated each day the instrument is utilized and all controls must be within the acceptable range.

Liquid controls must be evaluated once a week and all controls must be within the acceptable range.

Results should be reported as directed in the Guidelines for Generation of Formal Reports portion of the Toxicology Quality Manual.

A maintenance and QC log will be maintained at or near the instrument.

**REFERENCES:**

Operator's Manual AVOXimeter<sup>®</sup> 4000 CO-Oximeter, International Technidyne Corp, Edison, NJ, 2007.

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**AMMONIA METHOD FOR THE CONFIRMATION OF CARBOXYHEMOGLOBIN**

**PRINCIPLE:**

When dissolved in dilute aqueous ammonia solution, oxyhemoglobin and carboxyhemoglobin produce an absorption spectrum of a doublet with maxima at 535 and 570 nm.

When this solution is reduced with sodium hydrosulfite, a relatively sharp singlet indicates the absence of carboxyhemoglobin. A well-resolved doublet indicates the presence of a high concentration of carboxyhemoglobin (in order of 50% saturation and higher). A definite doublet, not so well-resolved, indicates lower concentrations. A broad peak with a slight dip indicates saturation of approximately 15-25% saturation. Concentrations lower than 15% may be difficult to interpret.

The application of this procedure is intended to be for confirmation of quantity and identity of carboxyhemoglobin in blood specimens in which the primary method of analysis has been proven unsuitable or has produced an unacceptable result consisting of high methemoglobin.

**SPECIMEN REQUIRED:**

0.10 mL of blood or other appropriate specimen

**REAGENTS AND STANDARDS:**

CO-Oximeter Controls of known concentration

0.4% Ammonia solution

Sodium Hydrosulfite (sodium dithionite); ACS reagent Grade

**GENERAL SUPPLIES REQUIRED:**

Disposable Pasteur pipettes

**APPARATUS REQUIRED:**

A scanning/recording visible spectrophotometer.

1.0 cm cuvette

**CALIBRATION REQUIREMENTS:**

Controls of known concentrations should be analyzed prior to unknowns. Controls of approximately 20% Carboxyhemoglobin and 50% Carboxyhemoglobin should be analyzed as well as a negative control. Controls should be processed with each unknown and the resulting UV spectrums used for comparison with unknown samples.

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**PROCEDURE:**

1. Pour approximately 3 mL of 0.4% ammonia solution into the cuvettes.
2. Using a pasteur pipette, add 0.02 - 0.05 mL of blood to the cuvette and mix well.  
**NOTE:** The hemoglobin concentration varies markedly. Hence, the quantity of blood may have to be adjusted accordingly.
3. Place the cuvette in the spectrophotometer and scan in the visible mode from 700 nm to 400 nm. Absorbance should lie in the range of 0.5 to 1.5 for best results.
4. Add approximately 0.05 - 0.10 mg of sodium hydrosulfite to the cuvette. Mix by inversion approximately 15 - 20 times to allow proper mixing and reaction to occur.
5. Place the cuvette in the spectrophotometer and scan in the visible mode from 700 nm to 400 nm.
6. Evaluate the spectra and compare to the reference control spectrums.

**INTERPRETATION:**

A single absorption band after reduction indicates a low concentration or negative carboxyhemoglobin. A clear well resolved doublet which is little different from the first curve indicates a high concentration of carboxyhemoglobin. The spectrum from the unknown sample should be compared to the spectra of the controls. Acceptable reporting parameters are as follows:

1. <20% Carboxyhemoglobin
2. 20-50% Carboxyhemoglobin
3. >50% Carboxyhemoglobin

**DOCUMENTATION:**

A copy of all spectral data, including controls, should be placed in the permanent case jacket for future reference.

**REFERENCES:**

Kleinshoj, Feldstein, and Sprague, The Spectrophotometric Determination of Carbon Monoxide, J. Biol. Chem., 183, 1950.

Gossolin, Hodge, Smith, and Gleason, Clinical Toxicology of Commercial Products, 4th edition, 1976, The Williams and Wilkins Co., Baltimore.

Guyton, Textbook of Medical Physiology 5th Edition, 1976, Saunders, Philadelphia.

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**ANALYSIS OF TISSUE SPECIMENS**

**PURPOSE:**

To provide general procedures for the qualitative and quantitative analysis of tissue specimens.

**SPECIAL HANDLING:**

Universal precautions should be used when handling biological specimens.

**SPECIMEN REQUIRED:**

Tissue specimens  
Whole blood (screened)

**SUPPLIES REQUIRED:**

Large test tubes  
Small test tubes  
Forceps  
Scalpel

**APPARATUS REQUIRED:**

Analytical balance  
Biohomogenizer

**PROCEDURE:**

**SAMPLE PREPARATION:** Weigh out 3.0 g of tissue. (Do not cut tissue while frozen to reduce risk of injury.) Transfer the tissue to a large test tube (i.e. 20 x 150 mm) and add 9.0 mL of DI water. Homogenize the specimen using the Biohomogenizer. Transfer the tissue homogenate to a small test tube and cap the tube. Centrifuge the homogenate for approximately 10 minutes. Transfer 1 mL of the liquid portion of the homogenized tissue to a labeled test tube. Proceed as with blood.

Quantitative results can be performed by GC/MS or LC-MS/MS. If the standards used for the calibration are prepared with 1 mL of blood, then the concentration of analyte in the tissue is determined by **multiplying the result** obtained by the quantitative procedure **by 4** and expressing the results in units of ng/g of tissue. This multiplication takes into account that only 0.25 g of tissue is extracted and the calibrators contain 1 mL of blood.

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**NOTE:** It may not be practical or possible to perform this procedure as it is written due to limited sample. As long as the mass of the tissue and the volume of water used for the homogenization are known, this procedure can still be followed using the appropriate mathematical calculation for quantitative results. For example, if only 1g of tissue is available then only 3 mLs of DI can be used to make the homogenate. In this case the dilution factor remains the same. However, if only 1.0 g of tissue is used with 9.0 mL of water and the calibrators used are spiked 1 mL blood samples, then the final result should be multiplied by 10 in order to express in units of ng/g (since only 0.10 g of tissue is extracted). If a dilution other than  $\frac{1}{4}$  is used, the dilution should be documented in the case notes and retained for future reference.

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**SAMPLE CLEAN-UP, ACIDIC/NEUTRAL FRACTION**

**PRINCIPLE:**

Extracted biological may still contain large amounts of fatty acids and unwanted pigments. To minimize instrument downtime and improve the sensitivity of the existing GC/MS systems, a secondary liquid/liquid extraction/clean-up step has been provided for use when applicable.

**SPECIMEN REQUIRED:**

The following liquid/liquid extraction is provided as an optional clean-up step for use with sample extracts thought to contain acidic or neutral compounds.

**REAGENTS AND STANDARDS:**

Hexane  
80% Methanol in DI Water

**GENERAL SUPPLIES REQUIRED:**

16x100mm Clean Labeled Test Tube

**APPARATUS REQUIRED:**

Evaporation/Incubation Module  
Centrifuge  
Vortex

**CALIBRATION:**

None

**PROCEDURE:**

1. Add 1 mL of 80% methanol in DI water to the test tube which contains the evaporated acidic and neutral fraction.
2. Vortex sample.
3. Add 1 mL hexane.
4. Vortex.
5. Centrifuge sample for approximately 10 minutes or until the layers separate.
6. Discard hexane layer.
7. Evaporate under steady stream of nitrogen at temperatures not exceed 45 °C.

**NOTE:** All solvent volumes are approximate unless otherwise indicated.

**DOCUMENTATION:**

Worksheets should reflect the use of this procedure.

SAMPLE CLEAN-UP, ACIDIC/NEUTRAL FRACTION

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**REFERENCES:**

Clean Screen DAU Forensic Applications, World Wide Monitoring Inc., Horsham, Pennsylvania 19044, Revised 9/10/89.

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**SAMPLE CLEAN-UP, BASIC FRACTION**

**PRINCIPLE:**

Extracted biological samples may still contain large amounts of fatty acids and unwanted pigments. To minimize instrument down time and improve the sensitivity of the existing GC/MS systems, a secondary liquid/liquid extraction has been provided for use when applicable.

**SPECIMEN REQUIRED:**

The following method is to be performed on extracts of samples thought to contain compounds which are basic in nature.

**REAGENTS AND STANDARDS:**

Aqueous Ammonium Hydroxide pH 8.5-10  
Chloroform

**GENERAL SUPPLIES REQUIRED:**

16x100mm Clean, Labeled Test Tube  
Disposable Pasteur Pipette

**APPARATUS REQUIRED:**

Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 2 mL and 5 mL

**CALIBRATION:**

None.

**PROCEDURE:**

1. Add 3 mL of aqueous ammonium hydroxide (pH 8.5-10) to the test tube which contains the evaporated basic fraction.

**NOTE:** Substitution of aqueous ammonium hydroxide with pH 10-10.5 is necessary for optimum recovery of the various members of the opiate family.

2. Vortex sample.
3. Add 2-3 mL chloroform.
4. Vortex.
5. Centrifuge sample for approximately 10 minutes or until layers separate.
6. Transfer chloroform layer to a clean 16x100mm test tube.
7. Evaporate under steady stream of nitrogen at temperatures not exceed 45 °C.

**NOTE:** All solvent volumes are approximate unless otherwise noted.

SAMPLE CLEAN-UP, BASIC FRACTION

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**DOCUMENTATION:**

Worksheets should reflect the use of this procedure and include a list of the compound(s) found during subsequent analysis.

**REFERENCES:**

Clean Screen DAU Forensic Applications, World Wide Monitoring Inc., Horsham, Pennsylvania 19044, Revised 9/10/89.

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**GENERAL ACID/NEUTRAL and BASIC DRUG SCREEN BY MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of acid/neutral and basic drugs of concern by gas chromatography mass spectrometry (GCMS) and/or liquid chromatography tandem mass spectrometry (LC-MSMS). The samples are extracted using solid phase extraction techniques. The extracts are concentrated, injected into the GCMS for confirmation and may be subsequently re-prepared for analysis on LC-MSMS.

**SPECIMEN REQUIRED:**

1 mL of whole blood, urine, other biological fluids or tissue homogenates, (See Analysis of Tissue Specimens Protocol). Other non-biological fluids may be analyzed as well but consideration should be made to dilute samples appropriately if concentrated samples are expected.

**REAGENTS AND STANDARDS:**

Methamphetamine, Amitriptyline, Verapamil (Other Controls as needed)  
SKF-525A  
Hexobarbital  
1M Acetic Acid  
Methanol  
Phosphate Buffer (0.1M, pH 6.0)  
DI Water  
Hexane  
Elution Solvent A  
Elution Solvent B

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of controls. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the General extraction, prepare working solutions and a neat mix at 100 ng/mL/500 ng/mL as designated. Drugs for this mixture are selected based on physical characteristics and elution order. Mix may change as necessary. Examples for how to prepare working solutions are as follows (other dilutions and/or dilution solvents are acceptable if appropriately documented):

**Working Solutions:**

10 mg/L(SKF-525a), 50 mg/L(Hexobarbital), 100 mg/L(D4-Acetaminophen) Internal Standard Working Solution: Pipette 500 µL of 1 mg/mL Hexobarbital and 100 µL of 1 mg/mL SKF-525A and 1 mL of 1 mg/mL D4-Acetaminophen into appropriate container. To container, pipette 8.4 mL of Methanol. Seal and vortex.

10/50 mg/L Working Solution: Pipette 10 µL of 1 mg/mL Amitriptyline, 10 µL of 1 mg/mL Verapamil and 50µL of 1 mg/mL Methamphetamine into appropriate container. To container, pipette 930 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 50 µL of 20-30% Methanol in Ethyl Acetate, cap and vortex.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Comparison of the neat mix to the extracted control can be used to assess extraction efficiency.

**2. PREPARE CONTROLS:**

Negative Control will be included.

Positive Controls for both blood and urine will be prepared and will, at a minimum, be run at the beginning, middle and end of run. The middle control may be omitted if run contains fewer than 10 samples. Positive Controls will be prepared at 100/500 ng/mL by adding 10 µL of the 10/50 mg/L Working Solution to 1 mL of blank biological matrix.

**3. SAMPLE PREPARATION:**

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10 µL of 10 mg/L Internal Standard Working Solution will be added to all Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm.
4. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 3 of Extraction Procedure.

**URINE PREPARATION**

Due to the unknown nature of compounds present in urine samples, a pretreatment step designed to cleave the glucuronide conjugate is necessary to improve recovery of highly glucuronide bound compounds. Use of these steps should be notated on the appropriate extraction worksheet.

1. To 1 mL of urine, add 1.0 mL of β-Glucuronidase working solution (1M sodium acetate buffer containing 50 µL/mL β-Glucuronidase). Vortex sample.

**NOTE: Prepare β-Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately three hours at 60-65°C.
  3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.
- (Proceed to Step 1 of Sample Preparation reducing initial addition of DI water by 1 mL)

**4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:

- A. Pre-rinse column with 5 mL of hexane
- B. 3 mL methanol
- C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extractions use 0.1M Phosphate buffer to condition the column.**

- D. 1 mL 1.0M acetic acid
2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.
3. Add sample to buffer already in column reservoir and push/pull through column.
4. Wash Column:
  - A. 3 mL 0.1M phosphate buffer, pH 6.0
  - B. 1 mL 1.0M acetic acid

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- C. Dry column for a minimum of 5 minutes under maximum vacuum.
- D. 3 mL hexane
- 5. Elute acidic and neutral compounds with 3 mL of Elution Solvent A (50:50 ethyl acetate/hexane).
- 6. Rinse column with 2 mL methanol.
- 7. Allow column packing to dry under maximum vacuum for approximately 2 minutes.
- 8. Elute basic drugs with 3 mL of Elution Solvent B (78:20:2 methylene chloride:isopropanol:ammonium hydroxide).

**NOTE: Prepare elution solvent B fresh daily.**

- 9. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C.

**5. TRANSFER TO GC VIAL**

- 1. Add approximately 1 mL of 20-30% Methanol in Ethyl Acetate.
- 2. Vortex sample.
- 3. Transfer liquid to clean, labeled 11 mm GC vial.
- 4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.
- 5. Reconstitute samples in 50 µL 20-30% Methanol in Ethyl Acetate. (Other volumes may be appropriate if higher concentrations are expected.)
- 6. Cap and Vortex.

**6. ANALYSIS BY GC-MS**

Analysis should be performed on GC-MS using appropriate method. QUAL2 Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

General screen analysis is performed in SCAN mode.

**7. PREPARATION FOR ANALYSIS BY LC-MSMS**

Typically Blood/Tissue/Misc. generals have additional testing performed by LC-MSMS. Urines may be analyzed by this method if necessary.

- 1. Decrimp GC vials.
- 2. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

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3. Reconstitute samples with 250 µL DI Water. Other volumes may be appropriate based on concentrations/compounds expected. Volume should be notated on the appropriate worksheet.
4. Cap and vortex.

### 8. ANALYSIS BY LC-MSMS

Analysis should be performed on LC-MSMS using appropriate method. BLOOD GENERALS Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

General screen analysis is performed in MRM mode.

Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound            | Precursor Ion | Product Ions               | Internal Std.    |
|---------------------|---------------|----------------------------|------------------|
| Acetaminophen       | 152.1         | 110.0, 65.1, 39.1          | D4-Acetaminophen |
| 10-OH-carbamazepine | 255.1         | 194.1, 179.1, 165.1        | SKF-525A         |
| Aripiprazole        | 449.1         | 285.7, 98.1                | SKF-525A         |
| Buprenorphine       | 468.1         | 468.1, 414.2, 396.1, 55.2  | SKF-525A         |
| Buspirone           | 386.1         | 122.1, 148.1, 109.1        | SKF-525A         |
| Carbamazepine       | 237.1         | 193.1, 179.1, 164.7        | SKF-525A         |
| Carisoprodol        | 261.1         | 176.1, 158.1, 97.1         | SKF-525A         |
| Duloxetine          | 298.1         | 44.2, 154.1                | SKF-525A         |
| Fentanyl            | 337.1         | 188.2, 132.1, 105.1        | SKF-525A         |
| Gabapentin          | 172.1         | 55.1, 137.1, 95.1          | SKF-525A         |
| Guaifenesin         | 199.1         | 125.1, 163.1               | SKF-525A         |
| Hexobarbital        | 237.1         | 157.0, 81.1                | SKF-525A         |
| Hydromorphone       | 286.2         | 185.1, 157.1, 128.1        | SKF-525A         |
| Hydroxyzine         | 375.1         | 201.1, 165.1, 115.1        | SKF-525A         |
| Meprobamate         | 219.1         | 158.1, 97.1, 55.2          | SKF-525A         |
| Methocarbamol       | 242.1         | 118.1, 242.1, 199.1, 163.1 | SKF-525A         |
| Metoprolol          | 268.1         | 121.1, 91.1                | SKF-525A         |
| Mitragynine         | 399.2         | 174.1, 159.1, 130.1        | SKF-525A         |
| Modafinil           | 296.1         | 129.0, 296.1               | SKF-525A         |
| Oxcarbazepine       | 253.1         | 180.1, 236.1, 152.1        | SKF-525A         |
| Paliperidone        | 427.1         | 207.1, 110.1               | SKF-525A         |

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| Pregabalin       | 160.1 | 55.2, 125.1, 97.2   | SKF-525A |
| Quetiapine       | 384.1 | 253.1, 221.1, 139.1 | SKF-525A |
| Ramelteon        | 260.1 | 204.1, 159.1, 115.1 | SKF-525A |
| Risperidone      | 411.1 | 191.1, 55.2         | SKF-525A |
| Trazodone        | 372.1 | 176.1, 148.1, 78.1  | SKF-525A |
| Zaleplon         | 306.1 | 236.1, 209.1, 64.1  | SKF-525A |
| Ziprasidone      | 413.1 | 194.0, 130.1, 77.1  | SKF-525A |
| Zolpidem         | 308.1 | 235.1, 263.1, 92.1  | SKF-525A |
| Zonisamide       | 213.1 | 132.1, 77.1         | SKF-525A |
| Zopiclone        | 389.1 | 217.0, 345.1, 112.0 | SKF-525A |
| D4-Acetaminophen | 156.1 | 114.1, 43.1         |          |
| SKF-525A         | 354.1 | 167.1, 105.1        |          |

Other compounds may be added as necessary with approval from the departmental Lieutenant.

## **9. INTERPRETATION OF RESULTS**

Acetaminophen will not be reported if level is indicated as less than 10 µg/mL.

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

## **REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**LIQUID/LIQUID EXTRACTION, PRE-PREPARED EXTRACTION TUBES**

**PRINCIPLE:**

The following procedure provides an alternate method for the extraction of acidic/neutral and basic compounds from biological specimens or other samples of interest. These compounds are extracted from biological fluids using pre-prepared liquid/liquid extraction tubes (TOXI-TUBES® A and/or B and AMTOX TUBES A and/or B), which contain a mixture of solvents and buffering salts that extract basic and acidic/neutral drugs, respectively. The solvent extracts are concentrated by mild heat and evaporation..

**SPECIMEN REQUIRED:**

The pre-prepared extraction tubes are designed to use 3 or 5 mL of urine. As a result of more sensitive instrumentation available in the SLED Toxicology laboratory, only 1 mL of sample is typically used. Other volumes may be used in cases of limited, alternate specimen selection, or as needed for better analytical results (i.e. poor chromatography due to overloading the column with large amounts of drug). If amount of sample deviates from suggested volume, amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Methamphetamine, Amitriptyline, Verapamil (Other Controls as needed)  
SKF-525A  
Hexobarbital  
Other appropriate Internal Standards as needed  
DI Water

**GENERAL SUPPLIES REQUIRED:**

Pre-prepared extraction tube  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Inversion mixer  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of controls. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the General extraction, prepare working solutions and a neat mix at 100 ng/mL/500 ng/mL as designated. Drugs for this mixture are selected based on physical characteristics and elution order. Mix may change as necessary. Examples for how to prepare working solutions are as follows (other dilutions and/or dilution solvents are acceptable if appropriately documented):

**Working Solutions:**

10 mg/L(SKF-525a), 50 mg/L(Hexobarbital) Internal Standard Working Solution: Pipette 500 µL of 1 mg/mL Hexobarbital and 100 µL of 1 mg/mL SKF-525A. To container, pipette 9.4 mL of Methanol. Seal and vortex.

10/50 mg/L Working Solution: Pipette 10 µL of 1 mg/mL Amitriptyline, 10 µL of 1 mg/mL Verapamil and 50µL of 1 mg/mL Methamphetamine into appropriate container. To container, pipette 930 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 50 µL of 20-30% Methanol in Ethyl Acetate, cap and vortex.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Comparison of the neat mix to the extracted control can be used to assess extraction efficiency.

**2. PREPARE CONTROLS:**

Negative Control will be included.

Positive Controls for appropriate specimen type (ie. Urine, Blood, Non-biological) will be prepared and will, at a minimum, be run at the beginning, middle and end of run. The middle control may be omitted if run contains fewer than 10 samples. Positive Controls will be prepared at 100/500 ng/mL by adding 10 µL of the 10/50 mg/L Working Solution to 1 mL of blank biological or other appropriate matrix.

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**3. SAMPLE PREPARATION:**

10 µL of 10 mg/L Internal Standard Working Solution will be added to all Controls and Case samples.

1. To 1 mL of sample, add 4 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 Minutes (Blood only).
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm (Blood only).
4. Transfer supernatant to appropriately labeled extraction tube.

**4. EXTRACTION PROCEDURE:**

1. Mix gently to ensure dissolution of the buffer and continue mixing by gentle inversion for a minimum of 2 minutes.
2. Centrifuge sample at minimum speed of 2500 rpm for a minimum of 5 minutes. The colored (aqueous) layer should be on the bottom after centrifugation.

**5. TRANSFER TO GC VIAL**

3. Using a disposable Pasteur pipette carefully transfer the top, organic (non-colored) layer to a clean 11 mm GC vial.
4. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C.
5. Reconstitute samples in 100 µL 20-30% Methanol in Ethyl Acetate. (Other volumes may be appropriate if higher concentrations are expected.)
6. Cap and Vortex.

**6. ANALYSIS BY GC-MS**

Analysis should be performed on GC-MS using appropriate method. QUAL2 Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

General screen analysis is performed in SCAN mode.

NOTE: Additional sample preparation may be needed if sample requires derivatization or analysis by alternate technologies (ie. LC-MSMS).

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**7. INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed. A.C. Moffat, 3rd ed., London: The Pharmaceutical Press, 2004.

TOXI-LAB® Broad Spectrum Drug Detection Systems Instruction Manual, TOXI-LAB Inc., Irvine, Calif., 1989.

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**BARBITURATE QUANTITATION AND CONFIRMATION BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of Barbiturates and related compounds. The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GCMS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS:**

Butalbital, Phenytoin, Phenobarbital, Pentobarbital, Primidone and Thiopental  
d5-Butalbital, d10-Phenytoin, d5-Phenobarbital, d5-Pentobarbital, Hexobarbital  
1M Acetic Acid  
Methanol  
Phosphate Buffer (0.1M, pH 6.0)  
DI Water  
Hexane  
Elution Solvent A  
Butyl Iodide  
Tetramethylammonium Hydroxide (TMAH)  
Dimethyl Sulfoxide (DMSO)  
Isooctane  
0.1 M Hydrochloric Acid (HCl)

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Barbiturates, prepare working solutions and a neat mix at 1.0 µg/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

100 mg/L Internal Standard Working Solution: Pipette 50 µL each of 1 mg/mL d5-Butalbital, d10-Phenytoin, d5-Phenobarbital, d5-Pentobarbital, and Hexobarbital into appropriate container. To container, pipette 250 µL of Methanol. Seal and vortex.

100 mg/L Working Solution: Pipette 50 µL each of 1 mg/mL Butalbital, Phenytoin, Phenobarbital, Pentobarbital, Primidone and Thiopental into appropriate container. To container, pipette 200 µL of Methanol. Seal and vortex.

10 mg/L Working Solution: Pipette 50 µL of 100 mg/L Working Solution into appropriate container. To container, pipette 450 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 100 mg/L Internal Standard Working Solution and 10 µL of the 100 mg/L Working Solution to a test tube. Evaporate under nitrogen. Follow derivatizing procedure found in Step 5, Derivatization. Analyze derivatized sample using QUALG method on GCMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

0.10 µg/mL, 0.50 µg/mL, 1.0 µg/mL, 3.5 µg/mL, 5.0 µg/mL, 7.5 µg/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

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To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>( $\mu\text{g/mL}$ ) | Working Solution<br>Dilution | $\mu\text{L}$ to add |
|-------------|---------------------------------------|------------------------------|----------------------|
| CAL 1       | 0.10                                  | 10 mg/L                      | 10                   |
| CAL 2       | 0.50                                  | 10 mg/L                      | 50                   |
| CAL 3       | 1.0                                   | 100 mg/L                     | 10                   |
| CAL 4       | 3.5                                   | 100 mg/L                     | 35                   |
| CAL 5       | 5.0                                   | 100 mg/L                     | 50                   |
| CAL 6       | 7.5                                   | 100 mg/L                     | 75                   |

**CONTROLS:**

Negative Blood Control will be included. This should be blank biological matrix determined not to contain Butalbital, Phenytoin, Phenobarbital, Pentobarbital, Primidone or Thiopental.

Positive Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. See Quality Manual for additional information.

Positive Controls, appropriately spaced throughout the run and bracketing case samples will be prepared at 3.0  $\mu\text{g/mL}$ .

**3. SAMPLE PREPARATION:**

10  $\mu\text{L}$  of 100 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm.
4. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 3 of Extraction Procedure.

**4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. Pre-rinse column with 5 mL of hexane
  - B. 3 mL methanol

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C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extractions use 0.1M Phosphate buffer to condition the column.**

D. 1 mL 1.0M acetic acid

2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.

3. Add sample to buffer already in column reservoir and push/pull through column.

4. Wash Column:

A. 3 mL 0.1M phosphate buffer, pH 6.0

B. 1 mL 1.0M acetic acid

C. Dry column for a minimum of 5 minutes under maximum vacuum.

D. 3 mL hexane

5. Elute acidic and neutral compounds with 3 mL of Elution Solvent A (50:50 ethyl acetate/hexane).

6. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C.

**5. DERIVATIZATION**

1. To the test tube containing the dried extract, add 200 µL of a 1:20 mixture of 25% TMAH in DMSO. Vortex 3-4 seconds.

2. Allow to stand at room temperature for a minimum of 2 minutes to facilitate dissolution.

3. Add 200 µL of Butyl Iodide to sample tube. Vortex 3-5 seconds and allow sample to stand a minimum of 15 minutes at room temperature. With Barbiturates, solution should turn cloudy upon addition of Butyl Iodide. If it does not, add additional 200 µL and let stand until cloudiness appears.

4. To each tube add 200 µL of 0.1M HCl. Vortex 3-5 seconds.

5. To each tube add 2 mL of Isooctane. Vortex 3-5 seconds.

6. Transfer organic layer to a clean, labeled test tube.

7. Evaporate extract under nitrogen at a temperature not to exceed 45°C leaving approximately 0.5 mL.

**6. TRANSFER TO GC VIAL**

1. Transfer extract to a clean, labeled 11 mm GC vial.

2. Evaporate extract under nitrogen at a temperature not to exceed 45°C.

3. Reconstitute extract in approximately 400 µL of isooctane.

QC Worksheet and/or extraction log should reflect Butyl Iodide derivatization was performed.

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## **7. ANALYSIS**

Analysis should be performed on GC-MS using appropriate method. QUALG Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Barbiturates analysis is typically performed in SCAN mode. SIM mode may be allowed for additional sensitivity if required.

Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound         | Ions          |
|------------------|---------------|
| d5-Butalbital    | 268, 284, 298 |
| Butalbital       | 263, 279, 293 |
| d5-Phenobarbital | 151, 294, 321 |
| Phenobarbital    | 146, 289, 316 |
| d10-Phenytoin    | 189, 219, 318 |
| Phenytoin        | 180, 209, 308 |
| d5-Pentobarbital | 256, 273      |
| Pentobarbital    | 251, 268, 195 |
| Hexobarbital     | 277, 81       |
| Primidone        | 146, 117, 301 |
| Thiopental       | 283, 354, 321 |

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**8. INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

Knapp, Daniel R., Handbook of Analytical Derivatization Reactions, John Wiley & Sons, New  
York, 1979.

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**BENZODIAZEPINE QUANTITATION AND CONFIRMATION BY LIQUID  
CHROMATOGRAPHY-MASS SPECTROMETRY/ MASS SPECTROMETRY**

**PRINCIPLE:**

The physical characteristics of benzodiazepines differ widely from one drug to another. As a result, the general solid phase extraction tends to display poor recovery for the various members of the benzodiazepine class. As an alternative, this method has been accepted for use in this laboratory for the quantitation/confirmation of benzodiazepines. Biological samples are analyzed for the presence of benzodiazepines including but not limited to Clobazam, Norclobazam, Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Flurazepam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, and 7-Aminonimetazepam. The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the LC-MS/MS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol). Other sample volumes may be substituted in cases of insufficient sample or as needed for better analytical results (i.e. sample needs to be within the weighted quadratic quantitative range). If the amount of sample deviates from the suggested volume, the amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Clobazam, Norclobazam, Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Flurazepam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, and 7-Aminonimetazepam

<sup>13</sup>C<sub>6</sub>-Clobazam, D5-Chlordiazepoxide, D5-Diazepam, D5-Nordiazepam, D5-Temazepam, D5-Oxazepam, D5-Alprazolam, D5-Hydroxyalprazolam, D4-Clonazepam, D4-7-Aminoclonazepam, D4-Midazolam, D4-Triazolam, D4-Desalkylflurazepam, D7-7-Aminoflunitrazepam, D4-Lorazepam, D4-Norflunitrazepam, and D4-Phenazepam.

0.1 M Sodium Phosphate Buffer (pH 6.0)

Methanol

DI Water

5% v/v Acetonitrile in 0.1 M Phosphate Buffer

Hexanes

Ethyl Acetate containing 3% NH<sub>4</sub>OH

0.1% Formic Acid:Methanol

1 M Sodium Acetate Buffer

β-Glucuronidase

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**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes (Pasteur)  
GC vials  
Snap Cap Star Septa  
Micro-Centrifuge Tubes  
Disposable Macro Pipette Tips  
Disposable Pipette Tips (100-1000 µL) or equivalent

**APPARATUS REQUIRED:**

Vacuum Manifold or Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipettes (1 mL, 2 mL, 3 mL, and 5 mL)  
Adjustable Pipettes

**PROCEDURE:**

**1. VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix which is to be used for quantitative analysis.

To test the standards for the Benzodiazepine Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

**10 mg/L Internal Standard Working Solution:**

Pipette 10 µL of each 1.0 mg/mL deuterated drug internal standard and 100 µL of each 100 µg/mL deuterium-labeled or <sup>13</sup>C-labeled drug internal standard into appropriate container. To container, pipette appropriate volume of methanol for a final volume of 1000 µL. Seal and vortex.

**10 mg/L Working Solution:**

Pipette 10 µL of each 1.0 mg/mL drug standard and 100 µL of each 100 µg/mL drug standard into appropriate container. To container, pipette appropriate volume of methanol for a final volume of 1000 µL. Seal and vortex.

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**1 mg/L Working Solution:**

Pipette 25 µL of 10 mg/L Working Solution into appropriate container. To container, pipette 225 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Add 250 µL of DI H<sub>2</sub>O to vial. Cap and vortex. Analyze using the **BENZOS2** method on the LC-MSMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2. PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

10 ng/mL, 50 ng/mL, 100 ng/mL, 350 ng/mL, 500 ng/mL, 750 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution<br>Dilution | µL to add |
|-------------|--------------------------|------------------------------|-----------|
| CAL 1       | 10                       | 1.0 mg/L                     | 10        |
| CAL 2       | 50                       | 1.0 mg/L                     | 50        |
| CAL 3       | 100                      | 10 mg/L                      | 10        |
| CAL 4       | 350                      | 10 mg/L                      | 35        |
| CAL 5       | 500                      | 10 mg/L                      | 50        |
| CAL 6       | 750                      | 10 mg/L                      | 75        |

**CONTROLS:**

Negative Blood Control will be included. This should be blank biological matrix determined not to contain Clobazam, Norclobazam, Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxylalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Flurazepam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, or 7-Aminonimetazepam.

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Positive Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. See Quality Manual for additional information.

Positive Controls will be appropriately spaced throughout the run and bracket case samples. Positive Controls will be prepared at 300 ng/mL.

**3. SAMPLE PREPARATION:**

10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean test tube.  
for robotic extraction or carefully decant in step 2 of the extraction procedure.

**URINE PREPARATION**

A pretreatment step designed to cleave the glucuronide conjugate is necessary to improve recovery of benzodiazepines. The following steps should be used. 10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of urine, add 1.0 mL of β-Glucuronidase working solution (1M Sodium Acetate Buffer containing 50 µL/mL β-Glucuronidase). Vortex sample.

**NOTE: Prepare β-Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately one hour at 60-65°C.
3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.

**4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. 3 mL Methanol
  - B. 3 mL DI Water
  - C. 2 mL 0.1 M Sodium Phosphate Buffer, pH 6.0  
(allow approximately 1 mL to pass through column then remove from vacuum/positive pressure, leaving ~1 mL buffer in column)
2. Apply sample to column (additional buffer may be added to column to provide a less viscous sample).

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3. Wash Column:
  - A. 3 mL DI Water
  - B. 3 mL 5% v/v Acetonitrile in 0.1 M Phosphate Buffer
4. Dry column for a minimum of 5 minutes at maximum vacuum/pressure
5. Wash column with 2 mL of Hexanes
6. Elute benzodiazepines into a clean tube with 3 mL Ethyl Acetate containing 3% NH<sub>4</sub>OH
7. Completely evaporate eluent to dryness under a steady stream of Nitrogen at a temperature not to exceed 45°C.

**5. TRANSFER TO GC VIAL**

1. Add 1 mL of Ethyl Acetate containing 3% NH<sub>4</sub>OH to dried tube.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Completely dry down eluent under a steady stream of nitrogen at a temperature not to exceed 45°C.
5. Add 250 µL of DI H<sub>2</sub>O to vial.
6. Cap with Snap Cap Star Septa and vortex.

**6. ANALYSIS**

Analysis should be performed on an Agilent LC-MS/MS using appropriate method. BENZOS2 method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

Benzodiazepine analysis is typically performed in dynamic MRM mode.

Ions of interest available for use are as follows (all transition ions listed that were shown to have no interferences):

| Compound          | Precursor Ion | Transition Ions             |
|-------------------|---------------|-----------------------------|
| Clobazam          | 301.1         | 259, 224, 152.9, 118        |
| Norclobazam       | 287.1         | 244.9, 210, 181             |
| Chlordiazepoxide  | 300.1         | 255, 247.1, 227             |
| Diazepam          | 285.1         | 222.1, 193.1, 154.1         |
| Nordiazepam       | 271.1         | 243, 226, 165, 140.1        |
| Temazepam         | 301.1         | 255.1, 193, 177             |
| Oxazepam          | 287.1         | 269, 241, 104.1             |
| Alprazolam        | 309.1         | 281, 274.1, 205, 165, 151.1 |
| Hydroxyalprazolam | 325.1         | 297, 243, 215.9             |
| Clonazepam        | 316.1         | 270.1, 241, 214             |
| 7-Aminoclonazepam | 286.1         | 222.1, 194.1, 121.1         |

BENZODIAZEPINES II

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|                                        |       |                           |
|----------------------------------------|-------|---------------------------|
| Lorazepam                              | 321   | 275, 194, 163, 110.9, 100 |
| Midazolam                              | 326.1 | 291.1, 249.1, 223.1       |
| Hydroxymidazolam                       | 342.1 | 203, 168.1, 140.1         |
| Triazolam                              | 343.1 | 315, 308.1, 239           |
| Estazolam                              | 295.1 | 267, 240.9, 205.1, 203.1  |
| Phenazepam                             | 349   | 179.1, 151, 104.1         |
| Flurazepam                             | 388.1 | 315, 134, 107             |
| Desalkylflurazepam                     | 289.1 | 226, 165, 140             |
| 7-Aminoflunitrazepam                   | 284.1 | 256, 226, 135.1           |
| Desmethyflunitrazepam                  | 300.1 | 254.1, 225.1, 198.1       |
| Nimetazepam                            | 296.1 | 250, 221.1, 165.1, 151.1  |
| 7-Aminonimetazepam                     | 266.1 | 135.1, 104.1, 93.1        |
| <sup>13</sup> C <sub>6</sub> -Clobazam | 307   | 230, 152.9, 111, 265      |
| D5-Chlordiazepoxide                    | 305.1 | 288.1, 286.1, 232.1       |
| D5-Diazepam                            | 290.1 | 198.1, 154.1              |
| D5-Nordiazepam                         | 276.1 | 213.1, 165, 140           |
| D5-Temazepam                           | 306.1 | 288.1, 260.1              |
| D5-Oxazepam                            | 292.1 | 274.1, 246.1              |
| D5-Alprazolam                          | 314.1 | 286.1, 210.1              |
| D5-Hydroxyalprazolam                   | 330.1 | 302.1, 210.1              |
| D4-Clonazepam                          | 320.1 | 274.1, 245, 218.1, 154.1  |
| D4-7-Aminoclonazepam                   | 290.1 | 254.1, 226.1, 121.1       |
| D4-Lorazepam                           | 325.1 | 198, 106                  |
| D4-Midazolam                           | 330.1 | 295.1, 253.1, 226.1       |
| D4-Triazolam                           | 347.1 | 319, 312.1, 243.1         |
| D4-Phenazepam                          | 353   | 210, 209.5, 105, 104      |
| D4-Desalkylflurazepam                  | 293.1 | 230.1, 140                |
| D7-7-Aminoflunitrazepam                | 291.2 | 230, 138                  |
| D4-Norflunitrazepam                    | 304.1 | 258, 202                  |

## 7. INTERPRETATION OF RESULTS

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

## REFERENCES:

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**BENZODIAZEPINE QUANTITATION AND CONFIRMATION BY LIQUID  
CHROMATOGRAPHY-MASS SPECTROMETRY/ MASS SPECTROMETRY (Alternate)**

**PRINCIPLE:**

The physical characteristics of benzodiazepines differ widely from one drug to another. As a result, the general solid phase extraction tends to display poor recovery for the various members of the benzodiazepine class. As an alternative, this method has been accepted for use in this laboratory for the quantitation/confirmation of benzodiazepines. Biological samples are analyzed for the presence of benzodiazepines including but not limited to Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, and 7-Aminonimetazepam. The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the LC-MS/MS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol). Other sample volumes may be substituted in cases of insufficient sample or as needed for better analytical results (i.e. sample needs to be within the weighted quadratic quantitative range). If the amount of sample deviates from the suggested volume, the amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, and 7-Aminonimetazepam  
D5-Chlordiazepoxide, D5-Diazepam, D5-Nordiazepam, D5-Temazepam, D5-Oxazepam, D5-Alprazolam, D5-Hydroxyalprazolam, D4-Clonazepam, D4-7-Aminoclonazepam, D4-Midazolam, D4-Triazolam, and D4-Desalkylflurazepam  
0.1 M Sodium Phosphate Buffer (pH 6.0)  
Methanol  
DI Water  
20% v/v Acetonitrile in 0.1 M Phosphate Buffer  
Hexane  
Ethyl Acetate  
0.1% Formic Acid:Methanol  
1 M Sodium Acetate Buffer  
 $\beta$ -Glucuronidase

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**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes (Pasteur)  
GC vials  
Snap Cap Star Septa  
Micro-Centrifuge Tubes  
Disposable Macro Pipette Tips  
Disposable Pipette Tips (100-1000  $\mu$ L) or equivalent

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipettes (1 mL, 2 mL, 3 mL, and 5 mL)  
Adjustable Pipettes

**PROCEDURE:**

**2. VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Benzodiazepine Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

**10 mg/L Internal Standard Working Solution:**

Pipette 10  $\mu$ L of each 1 mg/mL deuterated drug internal standard (total of 8) and 100  $\mu$ L of each 0.1 mg/mL deuterated drug internal standard (total of 4) into appropriate container. To container, pipette 520  $\mu$ L of Methanol. Seal and vortex.

**10 mg/L Working Solution:**

Pipette 10  $\mu$ L of each 1 mg/mL drug standard (total of 18) and 100  $\mu$ L of each 0.1 mg/mL drug standard (total of 2) into appropriate container. To container, pipette 620  $\mu$ L of Methanol. Seal and vortex.

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1 mg/L Working Solution:

Pipette 25 µL of 10 mg/L Working Solution into appropriate container. To container, pipette 225 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Add 250 µL of DI Water to vial. Cap and vortex. Analyze using the **BENZOS\_dmm** method on the LCMSMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**6. PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

10 ng/mL, 50 ng/mL, 100 ng/mL, 350 ng/mL, 500 ng/mL, 750 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration (ng/mL) | Working Solution Dilution | µL to add |
|-------------|-----------------------|---------------------------|-----------|
| CAL 1       | 10                    | 1 mg/L                    | 10        |
| CAL 2       | 50                    | 1 mg/L                    | 50        |
| CAL 3       | 100                   | 10 mg/L                   | 10        |
| CAL 4       | 350                   | 10 mg/L                   | 35        |
| CAL 5       | 500                   | 10 mg/L                   | 50        |
| CAL 6       | 750                   | 10 mg/L                   | 75        |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain Chlordiazepoxide, Diazepam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, Hydroxalprazolam, Clonazepam, 7-Aminoclonazepam, Lorazepam, Midazolam, Hydroxymidazolam, Triazolam, Estazolam, Desalkylflurazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Phenazepam, Nimetazepam, or 7-Aminonimetazepam.

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Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. See Quality Manual for additional information. Positive Controls will be appropriately spaced throughout the run and bracket case samples. Positive Controls will be prepared at 300 ng/mL.

Positive Urine Controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

### **3. SAMPLE PREPARATION:**

10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 2 of the extraction procedure.

### **URINE PREPARATION**

A pretreatment step designed to cleave the glucuronide conjugate is necessary to improve recovery of benzodiazepines. The following steps should be used. 10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of urine, add 1.0 mL of β-Glucuronidase working solution (1M Sodium Acetate Buffer containing 50 µL/mL β-Glucuronidase). Vortex sample.

**NOTE: Prepare β-Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately one hour at 60-65°C.
3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.

### **4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. 3 mL Methanol
  - B. 3 mL DI Water
  - C. 1 mL 0.1 M Sodium Phosphate Buffer, pH 6.0

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2. Apply sample to column (additional buffer may be added to column to provide a less viscous sample).
3. Wash Column:
  - A. 2 mL DI Water
  - B. 2 mL 20% v/v Acetonitrile in 0.1 M Phosphate Buffer
4. Dry column for a minimum of 5 minutes at maximum vacuum
5. Wash column with 2 mL of Hexane
6. Elute benzodiazepines into a clean tube with 3 mL Ethyl Acetate
7. Evaporate eluant to dryness under a steady stream of Nitrogen at temperatures not to exceed 45°C.

NOTE: Due to the varying chemical properties of the benzodiazepines as a class, recoveries of certain benzodiazepines are less than optimal. For example, Flurazepam does not extract under these conditions; however, the metabolite Desalkylflurazepam extracts with high recovery.

**5. TRANSFER TO GC VIAL**

1. Add 1 mL of Ethyl Acetate to dried tube.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.
5. Add 250 µL for blood (300 µL for urine) of DI Water to vial. Cap with Snap Cap Star Septa and vortex.

**6. ANALYSIS**

Analysis should be performed on an LC-MS/MS using appropriate method. BENZOS\_dmm method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

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Benzodiazepine analysis is typically performed in MRM mode.

Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound               | Precursor Ion | Product Ions            |
|------------------------|---------------|-------------------------|
| Chlordiazepoxide       | 300.1         | 282.1, 247.1, 227, 89.1 |
| Diazepam               | 285.1         | 222.1, 193.1, 154.1     |
| Nordiazepam            | 271.1         | 165, 140.1, 77.1        |
| Temazepam              | 301.1         | 283, 255.1, 193         |
| Oxazepam               | 287.1         | 269, 241, 104.1         |
| Alprazolam             | 309.1         | 281, 205, 151.1         |
| Hydroxyalprazolam      | 325.1         | 297, 279.1, 243         |
| Clonazepam             | 316.1         | 270.1, 241, 214         |
| 7-Aminoclonazepam      | 286.1         | 222.1, 194.1, 121.1     |
| Lorazepam              | 321           | 275, 194, 163           |
| Midazolam              | 326.1         | 291.1, 249.1, 223.1     |
| Hydroxymidazolam       | 342.1         | 203, 168.1, 140.1       |
| Triazolam              | 343.1         | 315, 308.1, 239         |
| Estazolam              | 295.1         | 267, 205.1, 203.1       |
| Phenazepam             | 349           | 179.1, 151, 104.1       |
| Desalkylflurazepam     | 289.1         | 226, 165, 140           |
| 7-Aminoflunitrazepam   | 284.1         | 256, 226, 135.1         |
| Desmethylflunitrazepam | 300.1         | 254.1, 225.1, 198.1     |
| Nimetazepam            | 296.1         | 221.1, 165.1, 151.1     |
| 7-Aminonimetazepam     | 266.1         | 135.1, 104.1, 93.1      |
| D5-Chlordiazepoxide    | 305.1         | 288.1, 286.1, 232.1     |
| D5-Diazepam            | 290.1         | 198.1, 154.1            |
| D5-Nordiazepam         | 276.1         | 213.1, 165, 140         |
| D5-Temazepam           | 306.1         | 288.1, 260.1            |
| D5-Oxazepam            | 292.1         | 274.1, 246.1            |
| D5-Alprazolam          | 314.1         | 286.1, 210.1            |
| D5-Hydroxyalprazolam   | 330.1         | 302.1, 210.1            |
| D4-Clonazepam          | 320.1         | 274.1, 218.1            |
| D4-7-Aminoclonazepam   | 290.1         | 254.1, 226.1, 121.1     |
| D4-Midazolam           | 330.1         | 295.1, 253.1, 226.1     |
| D4-Triazolam           | 347.1         | 319, 312.1, 243.1       |
| D4-Desalkylflurazepam  | 293.1         | 230.1, 140              |

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**7. INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**BLOOD CANNABINOIDS CONFIRMATION AND QUANTITATION BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of  $\Delta^9$  Tetrahydrocannabinol (THC), and the metabolites 11-Hydroxy-tetrahydrocannabinol (OH-THC), and 11-Carboxy-tetrahydrocannabinol (THC-COOH). The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GC/MS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids, or tissue homogenates.

**REAGENTS AND STANDARDS:**

THC, OH-THC, and THC-COOH, 1 mg/mL  
d3-THC, d3-OH-THC, and d9-THC-COOH, 1 mg/mL or 0.1 mg/mL  
Acetonitrile  
0.1M sodium acetate buffer (pH 6)  
Methanol  
0.1M sodium acetate buffer with 5% methanol (pH 6)  
95:5 hexanes: ethyl acetate  
75:25 hexanes: ethyl acetate with 1% glacial acetic acid  
1:1 methanol: de-ionized water  
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane (TMCS)  
Ethyl acetate

**GENERAL SUPPLIES REQUIRED:**

Solid phase extraction columns (Bond Elut Certify II)  
16 x 100 mm test tubes  
Glass pipettes  
11mm GC vials

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the cannabinoid class, prepare working solutions and a neat mix at 2.0 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

1.0 µg/L Internal Standard Working Solution: Pipette 10 µL of 0.1 µg/mL d3-THC, 10 µL of 0.1 µg/mL of d3-OH-THC, and 10 µL 0.1 µg/mL of d9-THC-COOH into an appropriate container. To container, pipette 970 µL of methanol. Seal and vortex.

1.0 µg/L Working Solution: Pipette 10 µL of 0.1 µg/mL THC, 10 µL of 0.1 µg/mL of OH-THC, and 10 µL 0.1 µg/mL of THC-COOH into an appropriate container. To container, pipette 970 µL of methanol. Seal and vortex.

0.1 µg/L Working Solution: Pipette 100 µL of 1.0 µg/L Working Solution into an appropriate container. To container, pipette 900 µL of methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 1.0 µg/L Internal Standard Working Solution and 20 µL of the 0.1 µg /L Working Solution to a GC vial. Evaporate under nitrogen. Add 30 µL of BSTFA derivatizing agent and 30 µL of ethyl acetate to vial. Cap under nitrogen and vortex. Heat at 80 °C for 20 minutes and analyze using a version of the THCSIM method on GCMS with a 5sil-ms or other appropriate GC column.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels: 2 ng/mL, 5 ng/mL, 15 ng/mL, 25 ng/mL, 35 ng/mL, 50 ng/mL.

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

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| Calibrator: | Concentration<br>(ng/mL) | Working Solution Dilution | µL to add |
|-------------|--------------------------|---------------------------|-----------|
| CAL 1       | 2.0                      | 0.1 µg/L                  | 20        |
| CAL 2       | 5.0                      | 0.1 µg/L                  | 50        |
| CAL 3       | 15                       | 1.0 µg/L                  | 15        |
| CAL 4       | 25                       | 1.0 µg/L                  | 25        |
| CAL 5       | 35                       | 1.0 µg/L                  | 35        |
| CAL 6       | 50                       | 1.0 µg/L                  | 50        |

**CONTROLS:**

Negative Blood Control will be included. This should be blank biological matrix determined not to contain THC, OH-THC, and THC-COOH.

Positive Controls, appropriately spaced throughout the run and bracketing case samples, will be prepared at 30 ng/mL.

**3.) SAMPLE PREPARATION:**

10 µL of 1.0 µg/mL Internal Standard Working Solution will be added to all calibrators, controls and case samples.

1. Add approximately 2 mL cold acetonitrile dropwise while vortexing until protein precipitation is achieved.
2. Centrifuge samples for 10 minutes at a minimum of 2500 rpm.
3. Transfer supernatant to a clean, prelabeled test tube.
4. Add 7 mL of 0.1M sodium acetate buffer (pH 6) to each sample tube.

**4.) EXTRACTION PROCEDURE:**

1. Prepare the extraction column:
2. Pass the following solvents through the column sequentially:
  - a. 3 mL Methanol
  - b. 2 mL 0.1M sodium acetate buffer with 5% methanol

**NOTE: Turn off vacuum as soon as the buffer reaches the top of the sorbent bed to prevent column drying.**

3. Pour the sample into the column reservoir and draw the sample through the column slowly. The total volume of the sample may exceed the capacity of the column; therefore, sample may be added in multiple aliquots.

**NOTE: It should take at least three (3) minutes to pass the sample through the column.**

4. Pass through the column 2mL of 0.1 M acetate buffer (pH 6.0)
5. Allow column to dry under maximum vacuum for approximately five (5) minutes.

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6. Elute parent THC
  - a. Place a rack with labeled tubes into extraction manifold.
  - b. Pass through column and collect 2 mL 95:5 hexane:ethyl acetate.
7. Wash column by passing 5 mL of 50:50 methanol: de-ionized water through column.
8. Allow column to dry under maximum vacuum for approximately five (5) minutes.
9. Elute THC metabolites
  - a. Place a rack with labeled tubes into extraction manifold.
  - b. Pass through column and collect 2 mL 75: 25 hexane: ethyl acetate with 1% glacial acetic acid.

**5.) TRANSFER TO GC VIAL**

1. Dry down both collected fractions on evaporation manifold until approximately 1 mL of elution solvent is left in tube.
2. Transfer each fraction into a labeled 11mm GC vial.
3. Dry down samples to completeness under nitrogen at temperatures not to exceed 45 degrees C.

**6.) DERIVATIZATION**

1. To GC vial add 30 µL of BSTFA and 30 µL of ethyl acetate.
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 20 minutes at 80°C.

QC Worksheet and/or extraction log should reflect BSTFA (TMS) derivatization was performed and volumes/times used.

**7.) ANALYSIS**

Analysis should be performed on GC-MS using appropriate method. THCSIM method is typically used for this analysis. Method parameters are stored next to the instrument in the methods folder.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

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Ions of interest that may be used are as follows (optimal target ions are listed first):

| Compound                            | Ions                    |
|-------------------------------------|-------------------------|
| d3- $\Delta^9$ Tetrahydrocannabinol | 374, 389, 346, 306      |
| d3-11-Hydroxy-tetrahydrocannabinol  | 374, 462, 477           |
| d9-11-Carboxy-tetrahydrocannabinol  | 380, 479, 497, 361, 306 |
| $\Delta^9$ Tetrahydrocannabinol     | 371, 386, 343, 303      |
| 11-Hydroxy-tetrahydrocannabinol     | 371, 459, 474           |
| 11-Carboxy-tetrahydrocannabinol     | 371, 473, 488, 297, 355 |

### **8.) INTEPRETATION OF REULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

### **REFERENCES:**

Varian Sample Preparation Products, *Certify Methods Manual*, Varian, Harbor City, California.

Thurman, E. M. and Mills, M.S.; *Solid-Phase Extraction: Principles and Practice*. Vol. 147 in Chemical Analysis: A Series of Monographs on Analytical Chemistry, J.D. Winfordner, Ed.

Varian Sample Preparation Products, *Sorbent Extraction Technology Handbook*, Varian, Harbor City, California.

Foltz, Roger L. "Analysis of Cannabinoids in Physiological Specimens by Gas Chromatography/ Mass Spectrometry". *Advances in Analytical Toxicology*. Vol.1. R.C. Baselt, Ed., Biomedical Publications, Foster City, California, 1984.

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**URINE THC-COOH CONFIRMATION AND QUANTITATION BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

The primary metabolite of marijuana, 11-carboxy-THC (11-THC-COOH), may be extracted from urine or other specimens using a vacuum manifold, positive pressure manifold or an automated apparatus in conjunction with DAU solid phase extraction (SPE) columns.

**SPECIMEN REQUIRED:**

1 mL of urine

**REAGENTS AND STANDARDS:**

200 µL 10M NaOH

1 mL Glacial Acetic Acid

3 mL Methanol

1 mL 0.1M HCl

5 mL DI Water

2 mL 70:30 0.1M HCl/ Acetonitrile

200 µL Hexane

3 mL Elution Solvent A (50:50 hexane: ethyl acetate)

1 mL Sodium Acetate Working Solution (50 µL β-Glucuronidase in 1 mL of 1M Sodium Acetate Buffer)

**NOTE: All solvents should be ACS reagent grade or better unless otherwise noted.**

**GENERAL SUPPLIES REQUIRED:**

2 clean 16 x 100 mm test tubes

1 DAU SPE column

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold or Syringe Adapter

Evaporation/ Incubation Module

Centrifuge

Vortex

Macro Pipette, 2 mL

Macro Pipette, 5 mL

Pipette, 1 mL

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Cannabinoid Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

10 µg/mL Internal Standard Working Solution: Pipette 100 µL of 100 µg/mL d9-11-THC-COOH into an appropriate container. To container, pipette 900 µL of methanol. Seal and vortex.

10 µg/mL Working Solution: Pipette 100 µL of 100 µg/mL 11-THC-COOH into an appropriate container. To container, pipette 900 µL of methanol. Seal and vortex.

1 µg/mL Working Solution: Pipette 100 µL of 10 µg/mL Working Solution into an appropriate container. To container, pipette 900 µL of methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 µg/mL Internal Standard Working Solution and 10 µL of the 10 µg/mL Working Solution to a GC vial. Evaporate under nitrogen. Derivatize by adding 30 µL of BSTFA derivatizing agent and 30 µL of Ethyl Acetate to vial. Cap under nitrogen and vortex. Heat at 80 °C for 20 minutes and analyze using THCSCAN method on GC-MS. See alternate derivatization below for neat standard preparation with methyl iodide.

**2.) PREPARE CONTROLS**

Negative urine control will be included. This should be blank biological matrix determined not to contain 11-THC-COOH.

Positive urine controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3.) SAMPLE PREPARATION**

1. Into a clean, labeled, 16 x 100 mm test tube, pipette approximately 1 mL of urine.
2. Add 10 µL of the 10 µg/mL Internal Standard Working Solution
3. Base Hydrolysis
  - a. To 1 mL of urine add 100 µL of 10M NaOH
  - b. Mix/ Vortex sample
  - c. Heat for 20 minutes at approximately 60°C

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- d. Cool before proceeding
- e. Adjust pH of sample to  $3.5 \pm 0.5$  with 1.0 mL of glacial acetic acid.
4. Centrifuge sample for a minimum of 10 minutes at a minimum of 2500 rpm.

**ALTERNATE SAMPLE PREPARATION:**

1. Into a clean, labeled, 16 x 100 mm test tube, pipette approximately 1 mL of urine.
2. Add 10  $\mu$ L of the 10  $\mu$ g/mL Internal Standard Working Solution
3. a. To 1 mL of urine, add 1 mL of 1M sodium acetate working solution. Vortex sample.  
b. Incubate sample approximately 3 hours at 60-65 °C.
4. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.

**NOTE: Increased sensitivity from newer instrumentation has resulted in lower limits of detection for compounds such as 11-carboxy-THC. As a result, the hydrolysis of conjugated drug may not be necessary and the extraction procedure may be performed without step 3 of the sample preparation. If sodium acetate working solution is omitted, 1 mL 1M sodium acetate should be added to sample. However, any case where 11-carboxy-THC is not detected without the hydrolysis must be re-analyzed using one of the above methods of pretreatment.**

**4.) EXTRACTION PROCEDURE**

1. Prepare extraction column.
  - a. 3 mL methanol
  - b. 3 mL DI water
  - c. 1 mL 0.1 M HCl
2. Add sample to column reservoir and apply vacuum.
3. Wash column:
  - a. 2 mL DI water
  - b. 2 mL 70:30 0.1 M HCl: acetonitrile solution
  - c. Dry Column for 5 minutes.
  - d. 200  $\mu$ L Hexane
4. Elute compounds of interest:
  - a. 3 mL Elution Solvent A (50:50 hexanes:ethyl Acetate)
5. Dry down eluant under a steady stream of compressed nitrogen at a temperature not to exceed 45 °C.

**5.) TRANSFER TO GC VIAL**

1. Add approximately 1 mL of 50:50 hexanes: ethyl acetate
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial with glass Pasteur pipette.

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4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

**ALTERNATE SAMPLE EXTRACTION:**

Samples may be extracted by liquid/liquid extraction tubes following extraction method detailed in the Liquid/Liquid Extraction, Pre-Prepared Extraction Tubes protocol. Sample preparation and derivatization steps remain the same.

**6.) DERIVATIZATION**

1. To 11mm GC vial add 30 µL of BSTFA and 30 µL of ethyl acetate. Additional solvent may be added for dilution purposes.
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 20 minutes at 80°C.

QC Worksheet and/or extraction log should reflect BSTFA (TMS) derivatization was performed and volumes/times used.

**ALTERNATE DERIVATIZATION:**

1. Add 200µL of a 1:20 mixture of 25% tetramethyl ammonium hydroxide (TMAH) in dimethyl sulfoxide (DMSO). Vortex 3-5 seconds.
2. Allow to stand at room temperature for a minimum of two minutes.
3. Add 200µL of iodomethane to sample tube. Vortex 3-5 seconds and allow sample to stand at room temperature for a minimum of 15 minutes.
4. To each tube add 200µL of 0.1M HCl. Vortex for 3-5 seconds.
5. To each tube add 2mL iso-octane. Vortex 3-5 seconds.
6. Transfer organic layer to clean, labeled test tube.
7. Add a second 2mL aliquot of iso-octane to original tube.
8. Transfer 2<sup>nd</sup> aliquot of iso-octane to tube referenced in step six.
9. Evaporate iso-octane under a stream of nitrogen at a temperature not to exceed 45°C until approximately 1mL remains.
10. Transfer to 11mm GC vial.
11. Evaporate to dryness under nitrogen.
12. Reconstitute sample with 75mL iso-octane.

**7.) ANALYSIS**

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Analysis should be performed on GC-MS using appropriate method. THCSCAN Method is typically used for this analysis. Method parameters are stored next to the instrument in the methods folder.

Ions of interest to be used are as follows (optimal target ions are listed first) following BSTFA derivatization:

| Compound                           | Ions          |
|------------------------------------|---------------|
| 11-Carboxy-tetrahydrocannabinol    | 371, 488, 473 |
| d9-11-Carboxy-tetrahydrocannabinol | 380, 497, 479 |

Ions of interest to be used are as follows (optimal target ions are listed first) following methyl iodide derivatization:

| Compound                           | Ions          |
|------------------------------------|---------------|
| 11-Carboxy-tetrahydrocannabinol    | 313, 357, 372 |
| d9-11-Carboxy-tetrahydrocannabinol | 322, 363, 381 |

**8.) INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc.; Bristol, PA. Revised 2003.

Knapp, D. Handbook of Analytical Derivatizations. John Wiley & Sons; New York, 1979.

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**COCAINE, COCAETHYLENE and BENZOYLECGONINE QUANTITATION AND  
CONFIRMATION BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of Cocaine, Cocaethylene and Benzoyllecgonine. The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GCMS for confirmation and quantitation. Due to the similarity of extraction, derivatization and analysis, cocaine may be combined with opiates for routine qualitative/quantitative analysis.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS:**

Cocaine, Cocaethylene and Benzoyllecgonine  
d3-Cocaine, d8-Cocaethylene, d3-Benzoyllecgonine  
1M Acetic Acid  
Methanol  
Phosphate Buffer (0.1M, pH 6.0)  
DI Water  
Hexane  
Elution Solvent A  
Elution Solvent B  
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane (TMCS)  
Ethyl Acetate

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Cocaine Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

10 mg/L Internal Standard Working Solution: Pipette 10 µL of 1 mg/mL d3-Benzoylecgonine, d8-Cocaethylene and 10 µL of 1 mg/mL d3-Cocaine into appropriate container. To container, pipette 970 µL of Methanol. Seal and vortex.

10 mg/L Working Solution: Pipette 10 µL of 1 mg/mL Benzoylecgonine, 10 µL of 1 mg/mL Cocaine and 10 µL of 1 mg/mL Cocaethylene into appropriate container. To container, pipette 970 µL of Methanol. Seal and vortex.

1 mg/L Working Solution: Pipette 100 µL of 10 mg/L Working Solution into appropriate container. To container, pipette 900 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 60 µL of BSTFA derivatizing agent to vial. Cap under nitrogen and vortex. Heat at 80 °C for a minimum of 20 minutes and analyze using OPICOC method on GCMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:  
10 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 750 ng/mL, 1000 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

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To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution<br>Dilution | µL to add |
|-------------|--------------------------|------------------------------|-----------|
| CAL 1       | 10                       | 1 mg/L                       | 10        |
| CAL 2       | 50                       | 1 mg/L                       | 50        |
| CAL 3       | 100                      | 10 mg/L                      | 10        |
| CAL 4       | 500                      | 10 mg/L                      | 50        |
| CAL 5       | 750                      | 10 mg/L                      | 75        |
| CAL 6       | 1000                     | 10 mg/L                      | 100       |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain Cocaine, Cocaethylene, or Benzoylcegonine.

Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. See Quality Manual for additional information. Positive Controls, appropriately spaced throughout the run and bracketing case samples will be prepared at 400 ng/mL.

Positive Urine Controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3. SAMPLE PREPARATION:**

10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 3 of Extraction Procedure.

**OPTIONAL URINE PREPARATION**

Concentrations of Cocaine, Benzoylcegonine and Cocaethylene are often at concentrations in urine that allow for easy detection without glucuronide cleavage. However, a pretreatment step designed

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to cleave the glucuronide conjugate may be necessary to improve recovery if necessary. The following steps should be used when this is required. Use of these steps should be notated on the appropriate extraction worksheet.

1. To 1 mL of urine, add 1.0 mL of  $\beta$ -Glucuronidase working solution (1M sodium acetate buffer containing 50  $\mu$ L/mL  $\beta$ -Glucuronidase). Vortex sample.

**NOTE: Prepare  $\beta$ -Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately three hours at 60-65°C.
3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.  
(Proceed to Step 1 of Sample Preparation reducing initial addition of DI water by 1 mL)

**4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:

- A. Pre-rinse column with 5 mL of hexane
- B. 3 mL methanol
- C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extracting use 0.1M Phosphate buffer to condition the column.**

- D. 1 mL 1.0M acetic acid

2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.

3. Add sample to buffer already in column reservoir and push/pull through column.

4. Wash Column:

- A. 3 mL 0.1M phosphate buffer, pH 6.0
- B. 1 mL 1.0M acetic acid
- C. Dry column for a minimum of 5 minutes under maximum vacuum.
- D. 3 mL hexane

5. Elute acidic and neutral compounds with 3 mL of Elution Solvent A (50:50 ethyl acetate/hexane) to waste.

6. Rinse column with 2 mL methanol.

7. Allow column packing to dry under maximum vacuum for approximately 2 minutes.

8. Elute basic drugs with 3 mL of Elution Solvent B (78:20:2 methylene chloride:isopropanol:ammonium hydroxide).

**NOTE: Prepare elution solvent B fresh daily.**

9. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C

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**5. TRANSFER TO GC VIAL**

1. Add approximately 1 mL of 20-30% Methanol in Ethyl Acetate.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

**6. DERIVATIZATION**

1. To 11mm GC vial add 60 µL of BSTFA (Ethyl Acetate may be added for dilution purposes not to exceed 50% of total volume).
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 20 minutes at 80°C.

QC Worksheet and/or extraction log should reflect BSTFA derivatization was performed and volumes/times used.

**7. ANALYSIS**

Analysis should be performed on GC-MS using appropriate method. OPICOC Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Cocaine analysis is typically performed in SCAN mode. SIM mode may be allowed for additional sensitivity if required.

Ions of interest available for use are as follows (optimal target ions are listed first):

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| Compound            | Ions                   |
|---------------------|------------------------|
| d3-Cocaine          | 185, 85, 306, 275      |
| Cocaine             | 182, 82, 303, 272, 198 |
| d8-Cocaethylene     | 85, 220, 325, 204, 275 |
| Cocaethylene        | 82, 196, 317, 212, 272 |
| d3-Benzoyllecgonine | 85, 243, 364, 259      |
| Benzoyllecgonine    | 82, 240, 361, 256      |

**8. INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines

**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

Knapp, Daniel R., Handbook of Analytical Derivatization Reactions, John Wiley & Sons, New  
York, 1979.

Pierce, Alan E., Silylation of Organic Compounds, Pierce Chemical Company,  
Rockford, Illinois, 1982.

Crouch, Dennis J et. al., Analysis of Cocaine and Its Metabolites from Biological  
Specimens Using Solid-Phase Extraction and Positive Ion Chemical Ionization  
Mass Spectrometry. *Journal of Analytical Toxicology*, 19, 1995, 352-358.

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**ETHYLENE GLYCOL CONFIRMATION BY GAS CHROMATOGRAPHY/MASS  
SPECTROMETRY**

**PRINCIPLE**

Derivatization of diols such as ethylene glycol with phenylboronic acid to yield their respective boronate esters enhances their volatility and chromatographically yields symmetrical, non-tailing peaks. The method involves the addition of 1, 2-butanediol to blood as the internal standard, precipitation of the blood proteins using acetone, evaporation of the supernatant to dryness, and finally, derivatization of the glycols with phenylboronic acid. The derivatives are then analyzed and quantitated using a Gas Chromatograph (GC) equipped with a Mass Spectrometer (MS).

**SPECIMEN REQUIRED**

1 mL whole blood, biological fluid or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS**

Acetone  
Methylene Chloride  
1% Phenylboronic acid solution  
1, 2-Butanediol in acetone (5 mg/mL)  
Ethylene glycol solution (10 mg/mL)  
0.1 M Potassium Hydroxide  
Concentrated Hydrochloric Acid  
Sodium Sulfate, Anhydrous

**GENERAL SUPPLIES REQUIRED**

16 x 100 mm test tubes  
Glass pipettes  
11 mm GC vials and caps

**APPARATUS REQUIRED**

Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette; 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable pipette

**PROCEDURE**

**1.) VALIDATION OF STANDARDS**  
**Prepare Neat (at 0.50 mg/mL)**

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Pipette 50 µL ethylene glycol stock solution (10 mg/mL) into a test tube. Add 0.25 mL DI water followed by 25 µL of the 1, 2-butanediol internal standard solution, then vortex briefly. Add 5 mL of the 0.1% phenylboronic acid in acetone and vortex thoroughly (approx. 30 seconds). Evaporate to approximately 1.5 to 2 mL using the heating block under nitrogen at a temperature not to exceed 45°C. Proceed using extraction procedure below. Pipette 100 µL of the final methylene chloride layer in a GC vial and dilute with 400 µL stock methylene chloride. Cap the vial and vortex. Analyze using ETHGLYC.M method on GCMS.

## **2.) PREPARE CALIBRATION CURVES**

Prepare three calibration standards (in test tubes) at concentrations of 0.5, 1.25, and 5.0 mg/mL from known blank blood:

0.5 mg/mL: add 50 µL of ethylene glycol stock solution to 1.0 mL of whole blood.

1.25 mg/mL: add 125 µL of ethylene glycol stock solution to 1.0 mL of whole blood.

5.0 mg/mL: add 500 µL of ethylene glycol stock solution to 1.0 mL of whole blood.

### **Controls**

A negative blood control will be included. This should be blank biological matrix determined not to contain the compounds being analyzed.

Positive controls, appropriately spaced throughout the run and bracketing case samples will be prepared using UTAK whole blood control containing ethylene glycol at a target concentration of 80 mg/dL or 0.80 mg/mL.

## **3.) SAMPLE PREPARATION**

1. To each respective test tube (calibration standards, positive and negative controls, and case samples), add 0.25 mL of DI water followed by 25 µL of the 1, 2-butanediol internal standard solution, then vortex briefly.
2. Add 5 mL of the 0.1% phenylboronic acid in acetone to each test tube and vortex thoroughly (approx. 30 seconds).
3. Centrifuge at a speed of 2500 RPM for a minimum of 10 minutes and pour the supernatant into a clean labeled test tube and evaporate to approximately 1.5 to 2 mL using the heating block under nitrogen at a temperature not to exceed 45°C.

## **4.) EXTRACTION PROCEDURE**

1. To the aqueous solution in each test tube, add 2 mL of 0.1 M KOH and 3 mL of methylene chloride, vortex for 20-30 seconds, and centrifuge at a speed of 2500 RPM for a minimum of 10 minutes.

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2. Transfer the aqueous layer to a clean labeled test tube. Acidify the aqueous layer (pH of 4.0) with concentrated hydrochloric acid (increments of 100 to 200 µL; check pH) and extract with 3 mL of methylene chloride by vortexing for approximately 30 seconds.
3. Centrifuge at a speed of 2500 RPM for a minimum of 10 minutes.
4. The top aqueous layer goes to waste and the methylene chloride layer is dried briefly over anhydrous sodium sulfate (2 heaping spatulas full) in a clean labeled test tube. Vortex briefly and centrifuge at a speed of 2500 RPM for a minimum of 10 minutes.

**5.) TRANSFER TO GC VIAL**

1. Pipette 50 µL of the methylene chloride layer into a clean, labeled GC vial.
2. Add 200 µL stock methylene chloride.
3. Cap the vial, and vortex.

**6.) ANALYSIS**

Analysis should be performed on a GCMS using the ETHGLYC.M method. Method parameters may be found in the method folder associated with each instrument. Ions of interest and available for use for the target compound and the internal standard are listed in the table below (optimal target ions are listed first with qualitative ions following):

| Compound        | Ions         |
|-----------------|--------------|
| 1, 2-butanediol | 147, 176, 91 |
| ethylene glycol | 148, 118, 91 |

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

**7.) INTERPRETATION OF RESULTS**

Refer to the Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES**

McCurdy, H. Horton, and E. T. Solomons, "An Improved Procedure for the Determination of Ethylene Glycol in Blood." Journal of Analytical Toxicology, Vol 6, September/October (1982): p253-54.

ETHYLENE GLYCOL

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**FLURAZEPAM QUANTITATION AND CONFIRMATION BY LIQUID  
CHROMATOGRAPHY/MASS SPECTROMETRY/ MASS SPECTROMETRY**

**PRINCIPLE:**

The physical characteristics of benzodiazepines differ widely from one drug to another. As a result, the general solid phase extraction tends to display poor recovery for the various members of the benzodiazepine class. As an alternative, this method has been accepted for use in this laboratory for the quantitation/confirmation of Flurazepam. Certain Benzodiazepines may require an alternate elution (i.e. Diazepam, Alprazolam, Clonazepam, Lorazepam, Midazolam, or Triazolam). The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the LCMSMS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol). Other sample volumes may be substituted in cases of insufficient sample or as needed for better analytical results (i.e. sample needs to be within the weighted quadratic quantitative range). If the amount of sample deviates from the suggested volume, the amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Flurazepam  
D4-Desalkylflurazepam  
0.1 M Sodium Borate Buffer (pH 9.3)  
Methanol  
DI Water  
10% v/v Methanol in DI Water  
Elution Solvent B [Methylene Chloride:Isopropanol:Ammonium Hydroxide (78:20:2)]  
Concentrated Ammonium Hydroxide or Sodium Hydroxide  
0.1% Formic Acid:Methanol  
1 M Sodium Acetate Buffer  
β-Glucuronidase

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes (Pasteur)  
GC vials  
Snap Cap Star Septa  
Micro-Centrifuge Tubes  
Disposable Macro Pipette Tips  
Disposable Pipette Tips (100-1000 µL) or equivalent

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**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipettes (1 mL, 2 mL, 3 mL, and 5 mL)  
Adjustable Pipettes

**PROCEDURE:**

**1. VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for Flurazepam, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

**10 mg/L Internal Standard Working Solution:**

Pipette 10  $\mu$ L of 1 mg/mL D4-Desalkylflurazepam into appropriate container. To container, pipette 990  $\mu$ L of Methanol. Seal and vortex.

**10 mg/L Working Solution:**

Pipette 10  $\mu$ L of 1 mg/mL Flurazepam into appropriate container. To container, pipette 990  $\mu$ L of Methanol. Seal and vortex.

**1 mg/L Working Solution:**

Pipette 25  $\mu$ L of 10 mg/L Working Solution into appropriate container. To container, pipette 225  $\mu$ L of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10  $\mu$ L of the 10 mg/L Internal Standard Working Solution and 10  $\mu$ L of the 10 mg/L Working Solution to a GC vial. Add 250  $\mu$ L of 0.1% Formic Acid:MeOH to vial. Cap with Snap Cap Star Septa and vortex. Analyze using the BENZOS\_dmm method on the LCMSMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

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**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

10 ng/mL, 50 ng/mL, 100 ng/mL, 250 ng/mL, 500 ng/mL, 750 ng/mL.

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution<br>Dilution | μL to add |
|-------------|--------------------------|------------------------------|-----------|
| CAL 1       | 10                       | 1 mg/L                       | 10        |
| CAL 2       | 50                       | 1 mg/L                       | 50        |
| CAL 3       | 100                      | 10 mg/L                      | 10        |
| CAL 4       | 250                      | 10 mg/L                      | 25        |
| CAL 5       | 500                      | 10 mg/L                      | 50        |
| CAL 6       | 750                      | 10 mg/L                      | 75        |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain Flurazepam.

Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. Positive Controls will be appropriately spaced throughout the run and bracket case samples. Positive Controls will be prepared at 400 ng/mL.

Positive Urine Controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3. SAMPLE PREPARATION:**

10 μL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 4 mL of Sodium Borate Buffer. Vortex sample.

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2. Adjust pH of sample to  $8.5 \pm 0.5$  with several drops of concentrated Ammonium Hydroxide.
3. Allow sample to stand at room temperature for a minimum of 15 minutes.
4. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm.

**URINE PREPARATION**

A pretreatment step designed to cleave the glucuronide conjugate is necessary to improve recovery of Flurazepam. The following steps should be used. 10  $\mu$ L of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of urine, add 1.0 mL of  $\beta$ -Glucuronidase working solution (1M Sodium Acetate Buffer containing 50  $\mu$ L/mL  $\beta$ -Glucuronidase). Vortex sample.

**NOTE: Prepare  $\beta$ -Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately 3 hours at 58-65°C.
3. Adjust pH of sample to  $8.5 \pm 0.5$  with several drops of concentrated Ammonium Hydroxide.
4. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.

**3.) EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. 3 mL Methanol
  - B. 3 mL DI Water
  - C. 1 mL 0.1 M Sodium Borate Buffer, pH 9.3
2. Apply sample to column (additional buffer may be added to column to provide a less viscous sample).
3. Wash Column with 3 mL of 10% v/v Methanol in DI Water.
4. Dry column for a minimum of 5 minutes at maximum vacuum
5. Elute benzodiazepines into a clean tube with 3 mL of Elution Solvent B [Methylene Chloride:Isopropanol:Ammonium Hydroxide (78:20:2)]
6. Evaporate eluants to dryness under a steady stream of Nitrogen at a temperature not to exceed 45°C.

**NOTE:** Due to the varying chemical properties of the benzodiazepines as a class, recoveries of certain benzodiazepines are less than optimal. For example, if a case history suggests an unknown may contain Diazepam, Alprazolam, Clonazepam, Lorazepam, Midazolam, or Triazolam, an alternative extraction should be utilized.

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**4.) TRANSFER TO GC VIAL**

1. Add 1 mL of Ethyl Acetate to dried tube.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.
5. Add 250 µL of 0.1% Formic Acid:Methanol to vial. Cap and vortex.

**5.) ANALYSIS**

Analysis should be performed on an LC-MS/MS using appropriate method. BENZOS\_dmm method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

Benzodiazepine analysis is typically performed in MRM mode.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest available to be used are as follows (optimal target ions are listed first):

| Compound              | Precursor Ion | Product Ions  |
|-----------------------|---------------|---------------|
| Flurazepam            | 388.1         | 315, 134, 107 |
| D4-Desalkylflurazepam | 293.1         | 230.1, 140    |

**6.) INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

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**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**MISCELLANEOUS COMPOUND QUANTITATION AND CONFIRMATION BY GAS  
CHROMATOGRAPHY MASS SPECTROMETRY**

**PRINCIPLE**

Biological samples are analyzed for the presence of miscellaneous compounds or compounds that can be analyzed by GCMS and are not covered in other quantitations. The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the GCMS for confirmation and quantitation.

**SPECIMEN REQUIRED**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS**

Standards and Deuterated Standards (see table included)

DI Water

Phosphate Buffer (0.1 M, pH 6.0)

Hexane

1M Acetic Acid

Methanol

Elution Solvent A

Elution Solvent B

**GENERAL SUPPLIES REQUIRED**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)

16 x 100 mm test tubes

Glass Pipettes

11 mm GC vials and caps

**APPARATUS REQUIRED**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)

Evaporation/Incubation Module

Centrifuge

Vortex

Pipette, 1 mL, 2 mL, 3 mL, and 5 mL

Adjustable Pipettes

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**PROCEDURE**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the miscellaneous class of compounds, prepare working solutions and a neat mix. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Note: The compounds that will be required for the working solution may vary from week to week.**

**Internal Standard Working Solutions**

Internal Standards at 1mg/mL (Low Curve)

Pipette 10  $\mu$ L of each into appropriate container. To the container, pipette enough methanol to give a final total volume of 1000  $\mu$ L; yields a 10 mg/L solution. Seal and vortex.

Internal Standards at 100  $\mu$ g/mL or 100 mg/L (Low Curve)

Pipette 50  $\mu$ L of each into appropriate container. To the container, pipette enough methanol to give a final total volume of 500  $\mu$ L; yields a 10 mg/L solution. Seal and vortex.

Internal Standards at 1 mg/mL (High Curve)

Pipette 50  $\mu$ L of each into appropriate container. To the container, pipette enough methanol to give a final total volume of 500  $\mu$ L; yields a 100 mg/L solution. Seal and vortex.

Fentanyl Internal Standard at 100  $\mu$ g/mL or 100 mg/L (Fentanyl Curve)

Pipette 10  $\mu$ L into appropriate container. To the container, pipette enough methanol to give a final total volume of 1000  $\mu$ L; yields a 1 mg/L solution. Seal and vortex.

**Note: When fentanyl is to be analyzed, 10  $\mu$ L of fentanyl internal standard at 100  $\mu$ g/mL or 100 mg/L can be included in the low curve internal standard mix (with internal standards at 1mg/mL) above to avoid preparation of a separate mix.**

**Standard Working Solutions**

10 mg/L Working Solution (Low Curve)

Pipette 10  $\mu$ L of each 1 mg/L standard into appropriate container. To container, pipette enough methanol to give a final total volume of 1000  $\mu$ L. Seal and vortex.

1 mg/L Working Solution (Low Curve)

Pipette 50  $\mu$ L of 10 mg/L working solution above into appropriate container. To container, pipette 450  $\mu$ L of methanol. Seal and vortex.

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100 mg/L Working Solution (High Curve)

Pipette 50  $\mu$ L of each 1 mg/L standard into appropriate container. To container, pipette enough methanol to give a final total volume of 500  $\mu$ L. Seal and vortex.

10 mg/L Working Solution (High Curve)

Pipette 50  $\mu$ L of 100 mg/L working solution above into appropriate container. To container, pipette 450  $\mu$ L of methanol. Seal and vortex.

1 mg/L Fentanyl Working Solution (Fentanyl Curve)

Pipette 10  $\mu$ L of 1 mg/L fentanyl standard into appropriate container. To container, pipette 990  $\mu$ L methanol (10 mg/L solution). Seal and vortex.

Pipette 50  $\mu$ L of the 10 mg/L solution above into appropriate container. To container, pipette 450  $\mu$ L methanol (1 mg/L solution). Seal and vortex.

0.1 mg/L Fentanyl Working Solution (Fentanyl Curve)

Pipette 50  $\mu$ L of 1 mg/L working solution above into appropriate container. To container, pipette 450  $\mu$ L of methanol. Seal and vortex.

**Prepare Neat Mix**

To prepare the neat mix, add 10  $\mu$ L each of the 10 mg/L internal standard working solutions, 10  $\mu$ L of the 10 mg/L working solution, 10  $\mu$ L of the 100 mg/L working solution, and 10  $\mu$ L of the 1 mg/L fentanyl working solution to a GC vial. Evaporate under nitrogen. Add 100  $\mu$ L of mass solvent (20-30% methanol in ethyl acetate) to vial. Cap and vortex. Analyze using QUAL2.M method on GCMS. (Exception: fentanyl and haloperidol will also be analyzed in SIM using FENTSIM.M or FENTHALO.M methods respectively.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

Low Curve: 10 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 750 ng/mL, 1000 ng/mL

High Curve: 0.10  $\mu$ g/mL, 0.50  $\mu$ g/mL, 1.0  $\mu$ g/mL, 2.0  $\mu$ g/mL, 3.5  $\mu$ g/mL, 5.0  $\mu$ g/mL

Fentanyl Curve: 2.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 20 ng/mL, 35 ng/mL, 50 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. To prepare a calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

Low Curve:

| Calibrator | Concentration<br>(ng/mL) | Working<br>Solution Dilution<br>(mg/L) | $\mu$ L to add |
|------------|--------------------------|----------------------------------------|----------------|
| CAL 1      | 10                       | 1.0                                    | 10             |
| CAL 2      | 50                       | 1.0                                    | 50             |

GCMS MISC

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|       |      |    |     |
|-------|------|----|-----|
| CAL 3 | 100  | 10 | 10  |
| CAL 4 | 500  | 10 | 50  |
| CAL 5 | 750  | 10 | 75  |
| CAL 6 | 1000 | 10 | 100 |

High Curve:

| Calibrator | Concentration<br>( $\mu\text{g/mL}$ ) | Working<br>Solution Dilution<br>( $\text{mg/L}$ ) | $\mu\text{L}$ to add |
|------------|---------------------------------------|---------------------------------------------------|----------------------|
| CAL 1      | 0.10                                  | 10                                                | 10                   |
| CAL 2      | 0.50                                  | 10                                                | 50                   |
| CAL 3      | 1.0                                   | 100                                               | 10                   |
| CAL 4      | 2.0                                   | 100                                               | 20                   |
| CAL 5      | 3.5                                   | 100                                               | 35                   |
| CAL 6      | 5.0                                   | 100                                               | 50                   |

Fentanyl Curve:

| Calibrator | Concentration<br>( $\text{ng/mL}$ ) | Working<br>Solution Dilution<br>( $\text{mg/L}$ ) | $\mu\text{L}$ to add |
|------------|-------------------------------------|---------------------------------------------------|----------------------|
| CAL 1      | 2.0                                 | 0.10                                              | 20                   |
| CAL 2      | 5.0                                 | 0.10                                              | 50                   |
| CAL 3      | 10                                  | 1.0                                               | 10                   |
| CAL 4      | 20                                  | 1.0                                               | 20                   |
| CAL 5      | 35                                  | 1.0                                               | 35                   |
| CAL 6      | 50                                  | 1.0                                               | 50                   |

### **CONTROLS**

A negative blood control will be included. This should be blank biological matrix determined not to contain the compounds being analyzed.

Positive controls, appropriately spaced throughout the run and bracketing case samples will be prepared as follows: Low Curve: 400  $\text{ng/mL}$  (40  $\mu\text{L}$  of 10 $\text{mg/L}$ ), High Curve: 1.5  $\mu\text{g/mL}$  (15  $\mu\text{L}$  of 100  $\text{mg/L}$ ), Fentanyl Curve: 15  $\text{ng/mL}$  (15  $\mu\text{L}$  of 1  $\text{mg/L}$ ).

### **3. SAMPLE PREPARATION**

10  $\mu\text{L}$  of each internal standard working solution will be added to all calibrators, controls and case samples.

1. To each of the 1 mL samples, add 3 mL of DI water. Cap and vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.

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3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean labeled test tube for robotic extraction or carefully decant in step 3 for manual extraction.

**4. EXTRACTION PROCEDURE**

1. Prepare extraction column by pre-rinsing column with:

- A. 5 mL of hexane
- B. 3 mL methanol
- C. 3 mL DI water (or 0.1 M phosphate buffer)

**NOTE: Due to limitations related to the number of solvent positions available, for robotic extracting use 0.1 M phosphate buffer to condition the column.**

- D. 1 mL 1.0 M acetic acid

2. Add 3 mL of 0.1 M phosphate buffer to column. Do not push/pull through column.
3. Add supernatant sample to buffer already in column reservoir and push/pull through column.
4. Wash Column:
  - A. 3 mL 0.1 M phosphate buffer, pH 6.0
  - B. 1 mL 1.0 M acetic acid
  - C. Dry column for a minimum of 5 minutes under maximum vacuum.
  - D. 3 mL hexane
5. Elute acidic and neutral compounds with 3 mL of elution solvent A (50:50 ethyl acetate/hexane) and collect into a clean, labeled test tube.
6. Rinse column with 2 mL methanol to waste.
7. Allow column packing to dry under maximum vacuum for approximately 2 minutes.
8. Elute basic drugs with 3 mL of elution solvent B (78:20:2 methylene chloride:isopropyl alcohol:ammonium hydroxide) and collect into the test tube containing the acid and neutral eluant.

**NOTE: Prepare elution solvent B fresh daily.**

9. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C.

**5. TRANSFER TO GC VIAL**

1. Add approximately 1 mL of 20% methanol in ethyl acetate to each test tube.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

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5. Reconstitute samples by adding 100 µL of mass solvent (20-30% methanol in ethyl acetate) to each GC vial. Cap and vortex.

## **6. ANALYSIS**

Analysis should be performed on an Agilent GCMS using appropriate method. The QUAL2 method is typically used for the analysis of GC miscellaneous compounds with some exceptions such as fentanyl and haloperidol as stated above. Method parameters may be found in the method folder associated with each instrument.

GC miscellaneous compound analysis is typically performed in SCAN mode, with the exception of fentanyl and haloperidol which are also analyzed in SIM.

For compound ions of interest, internal standards and the appropriate curve for each compound, see the table below. This list may be amended or added to with permission from the departmental Lieutenant.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest available for use are as follows (optimal target ions are listed first with qualitative ions following):

| Compounds          | Ions             | Curves   |
|--------------------|------------------|----------|
| D3-Chlorpromazine  | 61, 321, 89      | Low      |
| Chlorpromazine     | 58, 318, 86      | Low      |
| D3-Diphenhydramine | 61, 76           | Low      |
| Benztrapine        | 140, 96, 201     | Low      |
| Brompheniramine    | 247, 167, 72     | Low      |
| Chlorpheniramine   | 203, 167, 58     | Low      |
| Diphenhydramine    | 58, 73           | Low      |
| Doxylamine         | 58, 71, 180, 167 | Low      |
| Methylphenidate    | 84, 91, 150      | Low      |
| Orphenadrine       | 58, 165, 178     | Low      |
| D5-Fentanyl        | 250, 194         | Fentanyl |
| Fentanyl           | 245, 189, 202    | Fentanyl |

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|                                             |                    |      |
|---------------------------------------------|--------------------|------|
| D4-Haloperidol                              | 127, 169           | Low  |
| Haloperidol                                 | 123, 95, 165       | Low  |
| D3-Imipramine                               | 61, 283            | Low  |
| Clomipramine                                | 58, 85, 314        | Low  |
| Imipramine                                  | 58, 280, 85        | Low  |
| Desipramine                                 | 234, 266, 195      | Low  |
| D4-Ketamine                                 | 213, 142           | Low  |
| Ketamine                                    | 209, 138, 102      | Low  |
| <sup>13</sup> C <sub>3</sub> D3-Lamotrigine | 190, 261           | High |
| Lamotrigine                                 | 185, 255, 157      | High |
| D3(D6)-Levetiracetam                        | 129(132), 173(176) | High |
| Levetiracetam                               | 126, 170, 98       | High |
| D8-Mecizine                                 | 197, 293           | High |
| Mecizine                                    | 189, 285           | High |
| D4-Meperidine                               | 251, 222           | Low  |
| Meperidine                                  | 247, 218, 172      | Low  |
| D6-Metaxalone                               | 128, 227           | High |
| Metaxalone                                  | 122, 221, 135      | High |
| D9-Methadone                                | 78, 303, 226       | Low  |
| Amitriptyline                               | 58, 202, 215       | Low  |
| Cyclobenzaprine                             | 58, 215, 202       | Low  |
| Cyproheptadine                              | 287, 215, 96       | Low  |
| Doxepin                                     | 58, 165, 189       | Low  |
| Nordoxepin                                  | 44, 165, 178       | Low  |
| Loratadine                                  | 382, 266, 292      | High |
| Methadone                                   | 72, 294, 223       | Low  |
| Methorphan                                  | 271, 214, 171      | Low  |
| Methorphanin                                | 257, 228, 200      | Low  |
| Metoclopramide                              | 86, 99, 184        | High |
| Tapentadol                                  | 58, 221, 107       | Low  |
| Thioridazine                                | 98, 370, 126       | High |
| Verapamil                                   | 303, 151, 260      | Low  |
| Norverapamil                                | 289, 151, 260      | Low  |
| 13C, D3-Lacosamide                          | 78, 120, 254       | High |
| Lacosamide                                  | 74, 116, 250       | High |
| D4-Normeperidine                            | 237, 164           | Low  |
| Normeperidine                               | 233, 158, 204      | Low  |
| D5-Norpropoxyphene                          | 225, 210           | Low  |
| Norpropoxyphene                             | 220, 205           | Low  |
| D3-Nortriptyline                            | 47                 | Low  |
| Nortriptyline                               | 44                 | Low  |

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|                         |               |      |
|-------------------------|---------------|------|
| D6-O-Desmethyiltramadol | 64, 255       | Low  |
| O-Desmethyiltramadol    | 58, 249       | Low  |
| D6-Promethazine         | 78, 290       | Low  |
| Promethazine            | 72, 284       | Low  |
| D11-Propoxyphene        | 64, 213       | Low  |
| Propoxyphene            | 58, 208, 115  | Low  |
| D12-Topiramate          | 254, 241      | High |
| Topiramate              | 245, 229, 171 | High |
| D6-Tramadol             | 64, 269       | Low  |
| Tramadol                | 58, 263       | Low  |

**7. INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**GAMMA HYDROXYBUTYRATE (GHB) QUANTITATION AND SCREENING BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of Gamma Hydroxybutyrate (GHB). The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GC/MS for quantitation and screening.

**SPECIMEN REQUIRED:**

200 µL of whole blood, urine, or other biological fluids.

**REAGENTS AND STANDARDS:**

GHB Sodium Salt  
d6-GHB Sodium Salt  
Phosphate Buffer (0.1M, pH 6.0)  
Methanol  
DI Water  
Methanol:Ammonium Hydroxide (99:1)  
N,N-Dimethylformamide (DMF)  
Hexane saturated with DMF  
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1%  
Trimethylchlorosilane (TMCS)  
Ethyl Acetate

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen CSGHB or comparable)  
16x100 mm test tubes  
Glass Pipettes  
11 mm GC vials

**APPARATUS REQUIRED:**

Vacuum Manifold or Positive Pressure Manifold  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Adjustable Pipettes  
Pipette, 1 mL and 3 mL

GHB

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for GHB, prepare a working solutions and a neat mix at 10 µg/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

100 mg/L Internal Standard Working Solution: Pipette 100 µL of 1 mg/mL d6-GHB into appropriate container. To Container, pipette 900 µL of Methanol. Seal and vortex.

1000 mg/L Working Solution: The stock solution of GHB.

100 mg/L Working Solution: Pipette 100 µL of 1 mg/mL GHB into appropriate container. To Container, pipette 900 µL of Methanol. Seal and vortex.

10 mg/L Working Solution: Pipette 100 µL of 100 mg/L Working Solution into appropriate container. To container, pipette 900 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 20 µL of the 100 mg/L Internal Standard Working Solution and 20 µL of the 100 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 50 µL of BSTFA and 450 µL ethyl acetate to vial. Cap under nitrogen and vortex. Heat at 80°C for a minimum of 20 minutes and analyze using GHB method on GC/MS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES:**

Calibrators are to be prepared in whole blood at the following levels:

2 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepare working solution dilutions and amounts to add to spike the curve is as follows:

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To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 200  $\mu$ L of blank blood matrix:

| Calibrator: | Concentration<br>( $\mu$ g/mL) | Working Solution<br>Dilution | $\mu$ L to add |
|-------------|--------------------------------|------------------------------|----------------|
| CAL 1       | 2                              | 10 mg/L                      | 40             |
| CAL 2       | 5                              | 100 mg/L                     | 10             |
| CAL 3       | 10                             | 100 mg/L                     | 20             |
| CAL 4       | 25                             | 100 mg/L                     | 50             |
| CAL 5       | 50                             | 1000 mg/L                    | 10             |
| CAL 6       | 100                            | 1000 mg/L                    | 20             |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain GHB.

Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. Positive Controls, appropriately spaced through the run and bracketing case samples will be prepared at 40  $\mu$ g/mL.

Positive Urine Controls will be prepared at the threshold level of 50  $\mu$ g/mL and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3.) SAMPLE PREPARATION:**

20  $\mu$ L of 100 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 200  $\mu$ L of sample, add 100  $\mu$ L of 0.1 M phosphate buffer. Vortex sample.

**4.) EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. 3 mL methanol
  - B. 3 mL DI water
  - C. 0.5 mL 0.1 M phosphate buffer
2. Place test tubes in extraction manifold for collection.

GHB

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3. Add sample to column reservoir and push/pull through and collect into tubes. **NOTE: The sample loading and wash are both collected.**
4. Wash Column:
  - A. Add 1 mL Methanol:Ammonium Hydroxide (99:1) to original sample tube.
  - B. Decant wash onto column and collect.
5. Evaporate to dryness at 50°C under nitrogen.
6. Sample clean-up:
  - A. Add 0.5 mL DMF to dried sample extract.
  - B. Add 1 mL hexane saturated with DMF.
  - C. Mix by inversion for 5 minutes.
  - D. Centrifuge at 2500 rpm for 5 minutes.

**5.) TRANSFER TO GC VIAL:**

1. Transfer lower DMF layer to a clean, labeled 11 mm GC vial.
2. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 50°C.

**6.) DERIVATIZATION:**

1. To 11 mm GC vial, add 50 µL of BSTFA and 450 µL of ethyl acetate.
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 20 minutes at 80°C.

QC Worksheet and/or extraction log should reflect BSTFA derivatization was performed and volumes/times used.

**7.) ANALYSIS:**

Analysis should be performed on GC/MS using appropriate method. GHB method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

GHB analysis is typically performed in SCAN mode.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.

GHB

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3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound | Ions          |
|----------|---------------|
| D6-GHB   | 239, 240, 241 |
| GHB      | 233, 234, 235 |

#### **8.) INTERPRETATION OF RESULTS**

For urine samples, all results less than 50 µg/mL are to be considered negative. For blood samples taken from a living individual, all results less than 10 µg/mL are to be considered negative. For blood samples taken from a deceased individual, all results less than 50 µg/mL are to be considered negative. For reporting positive results, refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

#### **9.) REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**QUANTITATION OF MISCELLANEOUS DRUGS BY LIQUID CHROMATOGRAPHY-  
TANDEM MASS SPECTROMETRY**

**PRINCIPLE:**

There are classes of drugs which are not amenable to gas chromatography-mass spectrometry quantitation due to various chemical properties which interfere with acceptable chromatographic resolution or ion source formation. Analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers optimal resolution and source formation for a wide variety of drugs and enhanced sensitivity for lower limits of quantitation.

Biological samples are analyzed for the presence of the following drugs, but not limited to:

| <b>SSRI / SNRI</b>     | <b>Anti-seizure<br/>Anti-convulsant</b> | <b>Anti-<br/>psychotics</b> | <b>Sleep meds</b> | <b>Miscellaneous</b> |
|------------------------|-----------------------------------------|-----------------------------|-------------------|----------------------|
| Mirtazapine            | Gabapentin                              | Risperidone                 | Zolpidem          | Metoprolol           |
| Bupropion              | Pregabalin                              | Paliperidone                | Zopiclone         | Guaifenesin          |
| Hydroxybupropion       | Zonisamide                              | Ziprasidone                 | Zaleplon          | Methocarbamol        |
| Trazodone              | Carbamazepine                           | Quetiapine                  | Ramelteon         | Carisoprodol         |
| Venlafaxine            | 10-hydroxy-<br>carbamazepine            | Aripiprazole                |                   | Meprobamate          |
| O-desmethylvenlafaxine | Oxcarbazepine                           | Clozapine                   |                   | Diltiazem            |
| Citalopram             | Valproic acid                           | Olanzapine                  |                   | Hydroxyzine          |
| Norcitalopram          |                                         |                             |                   | Acetaminophen        |
| Paroxetine             |                                         |                             |                   | Modafinil            |
| Duloxetine             |                                         |                             |                   | Buprenorphine        |
| Fluoxetine             |                                         |                             |                   |                      |
| Norfluoxetine          |                                         |                             |                   |                      |
| Sertraline             |                                         |                             |                   |                      |
| Buspirone              |                                         |                             |                   |                      |

This list may be amended or added to with permission from the departmental Lieutenant. The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the LC-MS/MS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol). Other sample volumes may be substituted in cases of insufficient sample or as needed for better analytical results (i.e. sample needs to be within the weighted quadratic quantitative

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range). If the amount of sample deviates from the suggested volume, the amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Refer to Tables 2-6 for a list of drug standards and internal standards

1M Acetic Acid

Methanol

Concentrated Ammonium Hydroxide

Phosphate Buffer (0.1M, pH 6.0)

DI Water

Hexanes

Elution Solvent A

Elution Solvent B

Ethyl Acetate

0.1% Hydrochloric Acid (concentrated) in Methanol

0.1 % Formic Acid:Methanol (70:30)

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)

16x100 mm test tubes

Glass Pipettes

GC high-recovery vials

Snap Cap Star Septa

Micro-Centrifuge Tubes

Disposable Macro Pipette Tips

Disposable Pipette Tips (100-1000 µL) or equivalent

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)

Evaporation/Incubation Module

Centrifuge

Vortex

Pipette (1 mL, 2 mL, 3 mL, and 5 mL)

Adjustable Pipettes

**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

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To test the standards for the miscellaneous compounds of interest, prepare working solutions and a neat mix equivalent to the concentration of Calibrator 3 (i.e. 100 ng/mL, 1.0 µg/mL, 10 µg/mL, or 10 ng/mL). Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

**Internal Standard Working Solution:**

To prepare a 100 mg/L internal standard working solution, pipette 100 µL of stock internal standard into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 10 mg/L internal standard working solution, pipette 10 µL of 1.0 mg/mL stock internal standard into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 1.0 mg/L internal standard working solution, pipette 10 µL of 1.0 mg/mL stock internal standard into appropriate container. Pipette methanol to yield a final volume of 100 µL. Seal and vortex. From this dilution, pipette 10 µL into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

**100 mg/L and 10 mg/L Working Solutions:**

To prepare a 100 mg/L working solution, pipette 100 µL of stock drug standard into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 10 mg/L working solution, pipette 100 µL of 100 mg/L working solution into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

**10 mg/L and 1.0 mg/L Working Solutions:**

To prepare a 10 mg/L working solution, pipette 10 µL of stock drug standard into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 1.0 mg/L working solution, pipette 100 µL of 10 mg/L working solution into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

**1.0 mg/L and 0.10 mg/L Working Solutions:**

To prepare a 1.0 mg/L working solution, pipette 10 µL of stock drug standard into appropriate container. Pipette methanol to yield a final volume of 100 µL. Seal and vortex. From this dilution, pipette 10 µL into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 0.10 mg/L working solution, pipette 100 µL of 1.0 mg/L working solution into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

\*\*\*These dilutions are made assuming stock concentration of 1.0 mg/mL. If the stock concentration differs from 1.0 mg/mL, adjust volumes accordingly.

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**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the appropriate Internal Standard Working Solution and 10 µL of the 100 mg/L, 10 mg/L, 1.0 mg/L, and/or 1.0 mg/mL Working Solution to a high-recovery GC vial. Evaporate under nitrogen. Add 250 µL  $\text{d}_2\text{H}_2\text{O}$  to vial. Cap and vortex.

The results of the analysis of neat standards will most likely vary among quantitations, therefore making direct comparisons with previous neat standards unlikely. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels for the four calibration curves to be utilized:

- 1) 10 ng/mL, 50 ng/mL, 100 ng/mL, 350 ng/mL, 500 ng/mL, 750 ng/mL
- 2) 0.10 µg/mL, 0.50 µg/mL, 1.0 µg/mL, 3.5 µg/mL, 5.0 µg/mL, 7.5 µg/mL
- 3) 1.0 µg/mL, 5.0 µg/mL, 10 µg/mL, 20 µg/mL, 35 µg/mL, 50 µg/mL
- 4) 1.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 20 ng/mL, 35 ng/mL, 50 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepare working solution dilutions and amounts to add to spike the curve is as follows:

**Curve Level 1:**

To prepare the 0.01 ng/mL to 750 ng/mL calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution Dilution | µL to add |
|-------------|--------------------------|---------------------------|-----------|
| CAL 1       | 10                       | 1.0 mg/L                  | 10        |
| CAL 2       | 50                       | 1.0 mg/L                  | 50        |
| CAL 3       | 100                      | 10 mg/L                   | 10        |
| CAL 4       | 350                      | 10 mg/L                   | 35        |
| CAL 5       | 500                      | 10 mg/L                   | 50        |
| CAL 6       | 750                      | 10 mg/L                   | 75        |

Drugs that typically use this curve:

|                  |              |
|------------------|--------------|
| Aripiprazole     | Mirtazapine  |
| Bupropion/       | Olanzapine   |
| Buspirone        | Paliperidone |
| Hydroxybupropion | Paroxetine   |

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|               |                        |
|---------------|------------------------|
| Citalopram    | Quetiapine             |
| Norcitalopram | Sertraline             |
| Diltiazem     | Trazodone              |
| Duloxetine    | Venlafaxine            |
| Fluoxetine    | O-desmethylvenlafaxine |
| Norfluoxetine | Zolpidem               |
| Hydroxyzine   | Zopiclone              |
| Metoprolol    | Ziprasidone            |

**Curve Level 2:**

To prepare the 0.10 µg/mL to 7.5 µg/mL calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(µg/mL) | Working Solution Dilution | µL to add |
|-------------|--------------------------|---------------------------|-----------|
| CAL 1       | 0.10                     | 10 mg/L                   | 10        |
| CAL 2       | 0.50                     | 10 mg/L                   | 50        |
| CAL 3       | 1.0                      | 100 mg/L                  | 10        |
| CAL 4       | 3.5                      | 100 mg/L                  | 35        |
| CAL 5       | 5.0                      | 100 mg/L                  | 50        |
| CAL 6       | 7.5                      | 100 mg/L                  | 75        |

Drugs that typically use this curve:

|                     |
|---------------------|
| Acetaminophen       |
| Carbamazepine       |
| Carisoprodol        |
| Gabapentin          |
| Guaifenesin         |
| Meprobamate         |
| Modafinil           |
| Oxcarbazepine       |
| 10-OH-carbamazepine |
| Pregabalin          |
| Zonisamide          |

**Curve Level 3:**

To prepare the 1.0 µg/mL to 50 µg/mL calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(µg/mL) | Working Solution Dilution | µL to add |
|-------------|--------------------------|---------------------------|-----------|
|-------------|--------------------------|---------------------------|-----------|

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|       |     |           |    |
|-------|-----|-----------|----|
| CAL 1 | 1.0 | 100 mg/L  | 10 |
| CAL 2 | 5.0 | 100 mg/L  | 50 |
| CAL 3 | 10  | 1.0 mg/mL | 10 |
| CAL 4 | 20  | 1.0 mg/mL | 20 |
| CAL 5 | 35  | 1.0 mg/mL | 35 |
| CAL 6 | 50  | 1.0 mg/mL | 50 |

Drugs that typically use this curve:

|               |
|---------------|
| Valproic Acid |
|---------------|

**Curve Level 4:**

To prepare the 1.0 µg/L to 50 µg/L calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution Dilution | µL to add |
|-------------|--------------------------|---------------------------|-----------|
| CAL 1       | 1.0                      | 100 ng/mL                 | 10        |
| CAL 2       | 5.0                      | 100 ng/mL                 | 50        |
| CAL 3       | 10                       | 1.0 µg/mL                 | 10        |
| CAL 4       | 20                       | 1.0 µg/mL                 | 20        |
| CAL 5       | 35                       | 1.0 µg/mL                 | 35        |
| CAL 6       | 50                       | 1.0 µg/mL                 | 50        |

Drugs that typically use this curve:

|               |
|---------------|
| Buprenorphine |
|---------------|

**CONTROLS:**

Negative Blood Control will be included. This should be blank biological matrix determined not to contain compounds listed in Table 1.

Positive Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst.

Positive Controls, appropriately spaced throughout the run and bracketing case samples will be prepared at 300 ng/mL, 3.0 µg/mL, 15 µg/mL, or 15 ng/mL depending on curve range for the compound.

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**3. SAMPLE PREPARATION:**

10 µL of Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 6.

**BUPRENORPHINE QUANTITATION SAMPLE PREPARATION**

Because buprenorphine can be glucuronide bound in the blood, a pretreatment step designed to cleave the glucuronide conjugate may be necessary to improve recovery if necessary. The following steps should be used when this is required. Use of these steps should be notated on the appropriate extraction worksheet.

1. To 1 mL of blood, add 1.0 mL of β-Glucuronidase working solution (1M sodium acetate buffer containing 50 µL/mL β-Glucuronidase). Vortex sample.

**NOTE: Prepare β-Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately one hour at 60-65°C.
3. Add 2 mL DI water to sample. Vortex sample.
4. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.  
(Proceed to Step 1 of Sample Preparation reducing initial addition of DI water by 1 mL)

**4. EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. Pre-rinse column with 5 mL of hexane
  - B. 3 mL methanol
  - C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extracting use 0.1M Phosphate buffer to condition the column.**

- D. 1 mL 1.0M acetic acid
2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.
3. Add sample to buffer already in column reservoir and push/pull through column.
4. Wash Column:

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- A. 3 mL 0.1M phosphate buffer, pH 6.0
  - B. 1 mL 1.0M acetic acid
  - C. Dry column for a minimum of 5 minutes under maximum vacuum.
  - D. 3 mL hexane
- 5. Elute acidic and neutral compounds with 3 mL of Elution Solvent A (50:50 ethyl acetate/hexane) to waste.
  - 6. Rinse column with 2 mL methanol.
  - 7. Allow column packing to dry under maximum vacuum for approximately 2 minutes.
  - 8. Elute basic drugs with 3 mL of Elution Solvent B (78:20:2 methylene chloride:isopropyl alcohol:ammonium hydroxide).

**NOTE: Prepare elution solvent B fresh daily.**

- 9. Optional salt stabilization of basic compounds:

Add 100 µL of 0.1% hydrochloric acid in methanol to eluates to stabilize hydrochloride salts. **It is important that acidic/neutral compounds such as acetaminophen or valproic acid are not included in the quantitation.**

- 10. Dry down eluates under a steady stream of nitrogen at a temperature not to exceed 45°C.

**5. TRANSFER TO GC VIAL**

- 1. Add approximately 1 mL of 20-30% Methanol in Ethyl Acetate.
- 2. Vortex sample.
- 3. Transfer liquid to clean, labeled 11 mm high-recovery GC vial.
- 4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.
- 5. Add 250 µL  $d_4H_2O$  to vial. Cap and vortex.

**6. ANALYSIS**

Analysis should be performed on LC-MS/MS using appropriate method. LCMISC Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument. Compound transitions should be inputted for each compound, as well as expected retention times.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

- 1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.

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2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest to be used are as follows:

**Table 1. SSRI / SNRI**

| Compound Name          | Precursor Ion | Product Ions             | Internal Standard         | Precursor Ion | Product Ions      |
|------------------------|---------------|--------------------------|---------------------------|---------------|-------------------|
| Mirtazapine            | 266.2         | 195.1, 72.1, 65.1        | d3-mirtazapine            | 269.2         | 195.1, 75.1       |
| Bupropion              | 240.1         | 184.1, 130.1, 103.1      | d9-bupropion              | 249.1         | 185.1, 131.1      |
| Hydroxybupropion       | 256.1         | 238.1, 130.1, 77.1       | d6-hydroxybupropion       | 262.1         | 244.1, 77.1       |
| Trazodone              | 372.1         | 176.1, 148.1, 78.1       | d6-trazodone              | 378.1         | 182.1, 150.1      |
| Venlafaxine            | 278.2         | 260.2, 121.1, 58.2       | d6-venlafaxine            | 284.2         | 266.2, 121.1      |
| O-desmethylvenlafaxine | 264.2         | 246.2, 107.1, 58.2       | d6-O-desmethylvenlafaxine | 270.2         | 252.3, 58.1       |
| Citalopram             | 325.1         | 234.1, 116.1, 109.1      | d6-citalopram             | 331.2         | 234.1, 109.1      |
| Norcitalopram          | 311.1         | 234.1, 116.1, 109.1      |                           |               |                   |
| Paroxetine             | 330.2         | 192.1, 109.1, 70.1, 44.1 | d6-paroxetine             | 336.2         | 198.2, 76.1, 48.1 |
| Duloxetine             | 298.1         | 154.1, 44.2              | d7-duloxetine             | 305.1         | 154.1, 44.2       |
| Fluoxetine             | 310.1         | 148.1, 44.2              | d5-fluoxetine             | 315.2         | 153.2, 44.1       |
| Norfluoxetine          | 296.1         | 134.1, 30.1              | d5-norfluoxetine          | 301.1         | 139.1, 32.1       |
| Sertraline             | 306.1         | 275, 159, 123            | d3-sertraline             | 309.1         | 275, 159          |
| Buspirone              | 386.1         | 148.1, 122.1, 109.1      | d8-buspirone              | 394.1         | 154.2, 122.1      |

**Table 2. Anti-seizure / Anti-convulsant**

| Compound Name         | Precursor Ion | Product Ions        | Internal Standard        | Precursor Ion | Product Ions |
|-----------------------|---------------|---------------------|--------------------------|---------------|--------------|
| Gabapentin            | 172.1         | 154.1, 137.1, 95.1  | d4-gabapentin            | 176.1         | 158, 139     |
| Pregabalin            | 160.1         | 142.2, 83.2, 55.2   | d6-pregabalin            | 166.2         | 148.2, 58.1  |
| Zonisamide            | 213.1         | 132.1, 77.1, 51.2   | d4-zonisamide            | 217.1         | 136.1, 81.1  |
| Carbamazepine         | 237.1         | 193.1, 179.1, 164.7 | d10-carbamazepine        | 247.1         | 204.1, 187.1 |
| Oxcarbazepine         | 253.1         | 236.1, 180.1, 152.1 | d4-oxcarbazepine         | 257.1         | 212.1, 184.1 |
| 10-hydroxycarbazepine | 255.1         | 237.1, 194.1, 165.1 | d4-10-hydroxycarbazepine | 259.1         | 241.1, 198.1 |
| Valproic Acid         | 145.1         | 71, 43.1            | d6-valproic Acid         | 151.2         | 74.1, 46     |

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**Table 3. Anti-psychotics**

| Compound Name | Precursor Ion | Product Ions            | Internal Standard | Precursor Ion | Product Ions            |
|---------------|---------------|-------------------------|-------------------|---------------|-------------------------|
| Risperidone   | 411.1         | 191.1, 69.1, 55.2, 41.2 | d4-risperidone    | 415.2         | 195.2, 55.2, 72.2, 45.2 |
| Paliperidone  | 427.1         | 207.1, 110.1, 69.1      | d4-paliperidone   | 431.2         | 211.1, 114.1            |
| Ziprasidone   | 413.1         | 194, 130.1, 103.1, 77.1 | d8-ziprasidone    | 421.1         | 194, 130.1              |
| Quetiapine    | 384.1         | 253.1, 221.1, 139.1     | d8-quetiapine     | 392.2         | 257.7, 226.1            |
| Aripiprazole  | 449.1         | 286.1, 176.1, 98.1      | d8-aripiprazole   | 456.2         | 293.1, 106.2            |
| Clozapine     | 327.1         | 270.1, 192, 164         | d4-clozapine      | 331.2         | 272.1, 192.1            |
| Olanzapine    | 313.2         | 256.1, 198, 115.1       | d3-olanzapine     | 316.2         | 256.1, 198              |

**Table 4. Sleep meds**

| Compound Name | Precursor Ion | Product Ions        | Internal Standard | Precursor Ion | Product Ions |
|---------------|---------------|---------------------|-------------------|---------------|--------------|
| Zolpidem      | 308.1         | 263.1, 235.1, 220.1 | d6-zolpidem       | 314.2         | 263.1, 235.1 |
| Zopiclone     | 389.1         | 345.1, 217, 76.1    | d4-zopiclone      | 393.1         | 349.1, 217   |

| Compound Name | Precursor Ion | Product Ions        | Internal Standard | Precursor Ion | Product Ions |
|---------------|---------------|---------------------|-------------------|---------------|--------------|
| Metoprolol    | 268.1         | 121.1, 105.1, 103.1 | d7-metoprolol     | 275.2         | 133.1, 105.2 |
| Guaifenesin   | 199.1         | 163.1, 125.1        | d3-guaifenesin    | 202.1         | 166.1, 128.1 |
| Methocarbamol | 242.1         | 199.1, 163.1, 118.1 | d5-methocarbamol  | 247.1         | 204.1, 123.1 |
| Carisoprodol  | 261.1         | 176.1, 158.1, 97.1  | d7-carisoprodol   | 268.1         | 183.2, 165.2 |
| Meprobamate   | 219.1         | 158.2, 97.1, 55.2   | d7-meprobamate    | 226.1         | 165.2, 103.1 |

|           |       |                     |              |       |              |
|-----------|-------|---------------------|--------------|-------|--------------|
| Zaleplon  | 306.1 | 236.1, 209.1, 64.1  | d5-zaleplon  | 311.1 | 237.1, 64.1  |
| Ramelteon | 260.1 | 204.1, 159.1, 115.1 | d3-ramelteon | 263.2 | 204.2, 159.1 |

**Table 5. Miscellaneous**

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|       |                            |          |          |
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|               |       |                            |                  |       |                            |
|---------------|-------|----------------------------|------------------|-------|----------------------------|
| Diltiazem     | 415.1 | 178.1, 150.1, 109          | d3-diltiazem     | 418.1 | 178.1, 150.1               |
| Hydroxyzine   | 375.1 | 201.1, 165.1, 139.1, 115.1 | d8-hydroxyzine   | 383.2 | 201.1, 165.1, 115.1, 139.1 |
| Acetaminophen | 152.1 | 110.1, 65.1, 39.1          | d4-acetaminophen | 156.1 | 114.1, 43.1                |
| Modafinil     | 296.1 | 296.1, 129                 | d5-modafinil     | 301.1 | 301.1, 129                 |
| Buprenorphine | 468.1 | 468.1, 414.2, 396.1, 55.2  | d4-buprenorphine | 472.3 | 472.3, 400.3, 59.1         |

**INTERPRETATION OF RESULTS**

Acetaminophen will not be reported if indicated less than 10 µg/mL.

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

**REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**OPIATES QUANTITATION AND CONFIRMATION BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of Opiates. The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GCMS for confirmation and quantitation. Due to the similarity of extraction, derivatization and analysis, opiates may be combined with cocaine for routine qualitative/quantitative analysis.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS:**

Hydrocodone, Dihydrocodeine, Codeine, Hydromorphone, Oxycodone, Oxymorphone, Morphine and 6-Monoacetylmorphine (6-MAM)  
D3-Hydrocodone, D6-Dihydrocodeine, D3-Codeine, D3-Hydromorphone, D3-Oxycodone, D3-Oxymorphone, D3-Morphine and D3-6-Monoacetylmorphine (D3-6-MAM)  
1M Acetic Acid  
Methanol  
Phosphate Buffer (0.1M, pH 6.0)  
DI Water  
Hexane  
Elution Solvent A  
Elution Solvent B  
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane (TMCS)

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Opiates Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

10 mg/L Internal Standard Working Solution: Pipette 10 µL of each 1 mg/mL deuterated drug internal standard (total of 8) into appropriate container. To container, pipette 920 µL of Methanol. Seal and vortex.

10 mg/L Working Solution: Pipette 10 µL of each 1 mg/mL drug standard (total of 8) into appropriate container. To container, pipette 920 µL of Methanol. Seal and vortex.

1 mg/L Working Solution: Pipette 100 µL of 10 mg/L Working Solution into appropriate container. To container, pipette 900 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 60 µL of BSTFA derivatizing agent to vial. Cap under nitrogen and vortex. Heat at 80 °C for a minimum of 2 hours and analyze using OPICOC method on GCMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

10 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 750 ng/mL, 1000 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

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To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution<br>Dilution | µL to add |
|-------------|--------------------------|------------------------------|-----------|
| CAL 1       | 10                       | 1 mg/L                       | 10        |
| CAL 2       | 50                       | 1 mg/L                       | 50        |
| CAL 3       | 100                      | 10 mg/L                      | 10        |
| CAL 4       | 500                      | 10 mg/L                      | 50        |
| CAL 5       | 750                      | 10 mg/L                      | 75        |
| CAL 6       | 1000                     | 10 mg/L                      | 100       |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain Opiates.

Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. Positive Controls appropriately spaced throughout the run and bracketing case samples will be prepared at 400 ng/mL.

Positive Urine Controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3.) SAMPLE PREPARATION:**

1. 10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples. (Steps 2-4 for blood samples only)
2. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
3. Allow sample to stand at room temperature for a minimum of 20 minutes.
4. Centrifuge sample for a minimum of 10 minutes at the minimum speed of 2500 rpm. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 3 of Extraction Procedure.

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**OPTIONAL URINE PREPARATION**

Concentrations of Opiates are often at concentrations in urine that allow for easy detection without glucuronide cleavage. However, a pretreatment step designed to cleave the glucuronide conjugate may be necessary to improve recovery. The following steps should be used when this is required. Use of these steps should be notated on the appropriate extraction worksheet.

**OPTIONAL BLOOD PREPARATION**

Several Opiates are highly glucuronide bound in the blood and these bound compounds may also have pharmacological activity. In order to detect the bound drug, analysis of samples both treated with  $\beta$ -Glucuronidase to cleave the glucuronide and left untreated should be extracted and the results compared. This step may be necessary for Morphine, Hydromorphone and Oxycodone.

1. To 1 mL of urine or blood, add 1.0 mL of  $\beta$ -Glucuronidase working solution (1M sodium acetate buffer containing 50  $\mu$ L/mL  $\beta$ -Glucuronidase). Vortex sample.

**NOTE: Prepare  $\beta$ -Glucuronidase working solution fresh daily.**

2. Incubate urine sample for approximately three hours at 60-65°C.

**NOTE: Blood samples should be incubated at room temperature.**

3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.  
(Proceed to Step 1 of Sample Preparation reducing initial addition of DI water by 1 mL)

**4.) EXTRACTION PROCEDURE:**

1. Prepare extraction column:

- A. Pre-rinse column with 5 mL of hexane
- B. 3 mL methanol
- C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extracting use 0.1M Phosphate buffer to condition the column.**

- D. 1 mL 1.0M acetic acid

2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.

3. Add sample to buffer already in column reservoir and push/pull through column.

4. Wash Column:

- A. 3 mL 0.1M phosphate buffer, pH 6.0
- B. 1 mL 1.0M acetic acid
- C. Dry column for a minimum of 5 minutes under maximum vacuum.
- D. 3 mL hexane

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5. Elute acidic and neutral compounds with 3 mL of Elution Solvent A (50:50 ethyl acetate/hexane) to waste.
6. Rinse column with 2 mL methanol.
7. Allow column packing to dry under maximum vacuum for approximately 2 minutes.
8. Elute basic drugs with 3 mL of Elution Solvent B (78:20:3 methylene chloride:isopropanol:ammonium hydroxide).

**NOTE: Prepare elution solvent B fresh daily.**

9. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C.

#### **5.)TRANSFER TO GC VIAL**

1. Add approximately 1 mL of 20-30% Methanol in Ethyl Acetate.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

#### **6.) DERIVATIZATION**

1. To 11mm GC vial add 60 µL of BSTFA (Ethyl Acetate may be added for dilution purposes not to exceed 50% of total volume).
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 2 hours at 80°C.

QC Worksheet and/or extraction log should reflect BSTFA derivatization was performed and volumes/times used.

#### **7.) ANALYSIS**

Analysis should be performed on GC-MS using appropriate method. OPICOC Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

Opiates analysis is typically performed in SCAN mode. SIM mode may be allowed for additional sensitivity if required.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.

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2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound          | Ions          |
|-------------------|---------------|
| D3-Codeine        | 374, 181, 199 |
| Codeine           | 371, 234, 196 |
| D6-Dihydrocodeine | 379, 364      |
| Dihydrocodeine    | 373, 358, 315 |
| D3-Hydrocodone    | 374, 237      |
| Hydrocodone       | 371, 234, 184 |
| D3-Hydromorphone  | 432, 417, 237 |
| Hydromorphone     | 429, 234, 184 |
| D3-Oxycodone      | 462, 447      |
| Oxycodone         | 459, 444      |
| D3-Morphine       | 432, 417      |
| Morphine          | 429, 414, 401 |
| D3-Oxymorphone    | 520, 505      |
| Oxymorphone       | 517, 502      |
| D3-6-MAM          | 402, 290      |
| 6-MAM             | 399, 340, 287 |

## **8.) INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

## **REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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Knapp, Daniel R., Handbook of Analytical Derivatization Reactions, John Wiley & Sons, New York, 1979.

Pierce, Alan E., Silylation of Organic Compounds, Pierce Chemical Company, Rockford, Illinois, 1982.

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**SYMPATHOMIMETIC AMINES QUANTITATION AND CONFIRMATION BY GAS  
CHROMATOGRAPHY/MASS SPECTROMETRY**

**PRINCIPLE:**

Biological samples are analyzed for the presence of Sympathomimetic amines. The samples are extracted using solid phase extraction techniques. The extracts are concentrated, treated with a derivatizing agent and injected into the GCMS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol).

**REAGENTS AND STANDARDS:**

Methamphetamine, Amphetamine, Phenylpropanolamine, Ephedrine, Pseudoephedrine,  
Phentermine, Phenylephrine, MDMA and MDA  
D5-Methamphetamine, D8-Amphetamine, D3-Phenylpropanolamine, D3-Ephedrine, D3-  
Pseudoephedrine, D5-Phentermine, D3-Phenylephrine, D5-MDMA and D5-MDA  
1M Acetic Acid  
Methanol  
Phosphate Buffer (0.1M, pH 6.0)  
DI Water  
Hexane  
Elution Solvent B  
Heptafluorobutyric Anhydride (HFBA)  
Ethyl Acetate

**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen ZSDAU020 or comparable)  
16x100 mm test tubes  
Glass Pipettes

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette, 1 mL, 2 mL, 3 mL, and 5 mL  
Adjustable Pipettes

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**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the Sympathomimetic Amines Class, prepare working solutions and a neat mix at 100 ng/mL. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

10 mg/L Internal Standard Working Solution: Pipette 10 µL of each 1 mg/mL deuterated drug internal standard (total of 9) into appropriate container. To container, pipette 910 µL of Methanol. Seal and vortex.

10 mg/L Working Solution: Pipette 10 µL of each 1 mg/mL drug standard (total of 9) into appropriate container. To container, pipette 910 µL of Methanol. Seal and vortex.

1 mg/L Working Solution: Pipette 100 µL of 10 mg/L Working Solution into appropriate container. To container, pipette 900 µL of Methanol. Seal and vortex.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the 10 mg/L Internal Standard Working Solution and 10 µL of the 10 mg/L Working Solution to a GC vial. Evaporate under nitrogen. Add 50 µL of HFBA derivatizing agent to vial. Cap under nitrogen and vortex. Heat at 80 °C for a minimum of 15 minutes. Remove cap and evaporate under nitrogen. Add 100 µL of Ethyl Acetate and analyze using SMASIM (for blood) or SMASCAN (for urine) method on GCMS of choice.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels:

10 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 750 ng/mL, 1000 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepared working solution dilutions and amounts to add to spike the curve is as follows:

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To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration<br>(ng/mL) | Working Solution<br>Dilution | µL to add |
|-------------|--------------------------|------------------------------|-----------|
| CAL 1       | 10                       | 1 mg/L                       | 10        |
| CAL 2       | 50                       | 1 mg/L                       | 50        |
| CAL 3       | 100                      | 10 mg/L                      | 10        |
| CAL 4       | 500                      | 10 mg/L                      | 50        |
| CAL 5       | 750                      | 10 mg/L                      | 75        |
| CAL 6       | 1000                     | 10 mg/L                      | 100       |

**CONTROLS:**

Negative Control will be included. This should be blank biological matrix determined not to contain Methamphetamine, Amphetamine, Phenylpropanolamine, Ephedrine, Pseudoephedrine, Phentermine, Phenylephrine, MDMA or MDA

Positive Blood Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst. Positive Controls, appropriately spaced throughout the run and bracketing case samples will be prepared at 400 ng/mL.

Positive Urine Controls will be prepared at an appropriate level and run, at a minimum, at the beginning, middle and end of the run. The middle control may be omitted if run contains fewer than 10 samples.

**3.) SAMPLE PREPARATION:**

10 µL of 10 mg/L Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add 3 mL of DI water. Vortex sample.
2. Allow sample to stand at room temperature for a minimum of 20 minutes.
3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm. Transfer supernatant to a clean test tube for robotic extraction or carefully decant in step 3 of the Extraction Procedure.

SYMPATHOMIMETIC AMINES

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**OPTIONAL URINE PREPARATION**

Concentrations of Methamphetamine, Amphetamine, Phenylpropanolamine, Ephedrine, Pseudoephedrine, Phentermine, Phenylephrine, MDMA and MDA are often at concentrations in urine that allow for easy detection without glucuronide cleavage. However, a pretreatment step designed to cleave the glucuronide conjugate may be necessary to improve recovery if necessary. The following steps should be used when this is required. Use of these steps should be notated on the appropriate extraction worksheet.

1. To 1 mL of urine, add 1.0 mL of  $\beta$ -Glucuronidase working solution (1M sodium acetate buffer containing 50  $\mu$ L/mL  $\beta$ -Glucuronidase). Vortex sample.

**NOTE: Prepare  $\beta$ -Glucuronidase working solution fresh daily.**

2. Incubate sample for approximately three hours at 60-65°C.
3. Centrifuge sample for a minimum of 10 minutes at a minimum speed of 2500 rpm.  
(Proceed to Step 1 of Sample Preparation reducing initial addition of DI water by 1 mL)

**4.) EXTRACTION PROCEDURE:**

1. Prepare extraction column:

- A. Pre-rinse column with 5 mL of hexane
- B. 3 mL methanol
- C. 3 mL DI water(or 0.1M Phosphate buffer)

**NOTE: Due to limitations related to the number of solvents available, robotic extraction uses 0.1M Phosphate buffer to condition the column.**

- D. 1 mL 0.1M phosphate buffer, pH 6.0
2. Add 3 mL of 0.1M phosphate buffer to column. Do not push/pull through column.
  3. Add sample to buffer already in column reservoir and push/pull through column.
  4. Wash Column:
    - A. 3 mL DI water
    - B. 1 mL 1.0M acetic acid
    - C. 3 mL methanol
    - D. Dry column for a minimum of 5 minutes under maximum vacuum.
  5. Elute Sympathomimetic Amines with 3 mL of Elution Solvent B (78:20:2 methylene chloride:isopropyl alcohol:ammonium hydroxide).

**NOTE: Prepare elution solvent B fresh daily.**

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6. Dry down eluant under a steady stream of nitrogen at a temperature not to exceed 45°C to approximately 1 mL.

**5.) TRANSFER TO GC VIAL**

1. Transfer remainder of eluant to clean, labeled 11 mm GC vial..
2. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.

**6.) DERIVATIZATION**

1. To 11mm GC vial add 50 µL of HFBA.
2. Cover solvent layer with nitrogen and cap.
3. Vortex solution in GC vial.
4. Heat for a minimum of 15 minutes at 80°C.
5. Remove cap and evaporate under nitrogen at a temperature not to exceed 45°C.
6. Add 100-200 µL of Ethyl Acetate.

QC Worksheet and/or extraction log should reflect HFBA derivatization was performed and volumes/times used.

**7.) ANALYSIS**

Analysis should be performed on GC-MS using appropriate method. SMASIM (for blood) or SMASCAN (for urine) Method is typically used for this analysis. Method parameters may be found in the method folder associated with each instrument.

Blood Sympathomimetic Amine analysis is typically performed in SIM while Urine Sympathomimetic Amine analysis is typically performed in SCAN mode.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the blank before the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the blank before the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

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Ions of interest available for use are as follows (optimal target ions are listed first):

| Compound               | Ions              |
|------------------------|-------------------|
| D8-Amphetamine         | 243, 126, 96, 195 |
| Amphetamine            | 240, 118, 91, 192 |
| D5-Phentermine         | 96, 137           |
| Phentermine            | 91, 132, 117      |
| D3-Phenylpropanolamine | 243, 333          |
| Phenylpropanolamine    | 240, 330, 192     |
| D5-Methamphetamine     | 258, 213          |
| Methamphetamine        | 254, 210, 42      |
| D3-Ephedrine           | 257, 213          |
| Ephedrine              | 254, 210, 344     |
| D3-Pseudoephedrine     | 257, 213          |
| Pseudoephedrine        | 254, 210, 344     |
| D3-Phenylephrine       | 243, 213          |
| Phenylephrine          | 240, 210, 42      |
| D5-MDA                 | 167, 380          |
| MDA                    | 162, 375, 240     |
| D5-MDMA                | 258, 213          |
| MDMA                   | 254, 210, 389     |

## **8.) INTERPRETATION OF RESULTS**

In cases where both Ephedrine and Pseudoephedrine are identified, only one compound is to be reported if the response differs by 10X or greater. If the response difference is less than 10X, both compounds are to be reported if the compound meets reporting guidelines.

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines

## **REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

Knapp, Daniel R., Handbook of Analytical Derivatization Reactions, John Wiley & Sons, New York, 1979.

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**QUANTITATION OF SYNTHETIC CANNABINOIDS BY LIQUID CHROMATOGRAPHY-  
TANDEM MASS SPECTROMETRY**

**PRINCIPLE:**

An extraction and analysis method for the identification and quantitation of synthetic cannabinoids by liquid chromatography-tandem mass spectrometry is described. The method was developed to cover a broad range of synthetic cannabinoids. The method provides excellent ranges for quantitation when deuterated internal standards are available. Compounds for which there is no deuterated internal standard are typically qualitative only. Biological samples are analyzed for the presence of the following drugs, including, but not limited to:

|         |             |
|---------|-------------|
| JWH-200 | JWH-073     |
| JWH-081 | JWH-210     |
| JWH-015 | JWH-018     |
| RCS-04  | XLR-11      |
| RCS-08  | UR-144      |
| JWH-250 | JWH-019     |
| AM-2201 | JWH-022     |
| AM-2233 | JWH-203     |
| JWH-122 | WIN 55212-2 |

The samples are extracted using solid phase extraction techniques. The extracts are concentrated and injected into the LC-MS/MS for confirmation and quantitation.

**SPECIMEN REQUIRED:**

1 mL of whole blood, biological fluids or tissue homogenates (See Analysis of Tissue Specimens Protocol). Other sample volumes may be substituted in cases of insufficient sample or as needed for better analytical results (i.e. sample needs to be within the weighted quadratic quantitative range). If the amount of sample deviates from the suggested volume, the amount of sample used should be documented in the case notes and retained for future reference.

**REAGENTS AND STANDARDS:**

Refer to Table 1 for a list of drug standards and internal standards

Chilled Acetonitrile

Methanol

Phosphate Buffer (0.1M, pH 6.0)

DI Water

20% Acetonitrile in Phosphate Buffer (0.1 M, pH 6.0)

9:1 Ethyl Acetate:Methanol

Elution Solvent B

Methanol:0.1 % Formic Acid (75:25)

SYNTHETIC CANNABINOIDS, BLOOD

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**GENERAL SUPPLIES REQUIRED:**

Solid Phase Extraction Columns (UCT Clean Screen CSTHC206 or comparable)  
16x100 mm test tubes  
Glass Pipettes  
GC high-recovery vials  
Snap Cap Star Septa  
Micro-Centrifuge Tubes  
Disposable Macro Pipette Tips  
Disposable Pipette Tips (100-1000  $\mu$ L) or equivalent

**APPARATUS REQUIRED:**

Vacuum Manifold, Positive Pressure Manifold (or Robotic System)  
Evaporation/Incubation Module  
Centrifuge  
Vortex  
Pipette (1 mL, 2 mL, 3 mL, and 5 mL)  
Adjustable Pipettes

**PROCEDURE:**

**1.) VALIDATION OF STANDARDS**

Drug standards must be validated prior to preparation of calibrators. Working solutions of Standards and Internal Standards should be prepared and validated prior to usage. This is typically accomplished by analysis of a neat (non-extracted) drug mix.

To test the standards for the synthetic cannabinoids, prepare working solutions and a neat mix equivalent to the concentration of 1.0 ng/mL for compounds of interest and 10 ng/mL for internal standards. Examples for how to prepare working solutions are as follows (other dilutions are acceptable if appropriately documented):

**Working Solutions:**

**Internal Standard Working Solution:**

To prepare a 1.0  $\mu$ g/mL internal standard working solution, pipette 10  $\mu$ L of 1.0 mg/mL stock internal standard into appropriate container. Pipette methanol to yield a final volume of 100  $\mu$ L. Seal and vortex. From this dilution, pipette 10  $\mu$ L into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

**1.0  $\mu$ g/mL, 100 ng/mL, and 10 ng/mL Working Solutions:**

To prepare a 1.0  $\mu$ g/mL working solution, pipette 10  $\mu$ L of stock drug standard into appropriate container. Pipette methanol to yield a final volume of 100  $\mu$ L. Seal and vortex. From

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this dilution, pipette 10 µL into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 100 ng/mL working solution, pipette 100 µL of 1.0 µg/mL working solution into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

To prepare a 10 ng/mL working solution, pipette 100 µL of 100 ng/mL working solution into appropriate container. Pipette methanol to yield a final volume of 1.0 mL. Seal and vortex.

\*\*\*These dilutions are made assuming stock concentration of 1.0 mg/mL. If the stock concentration differs from 1.0 mg/mL, adjust volumes accordingly.

**Prepare Neat Mix:**

To prepare the Neat Mix, add 10 µL of the appropriate Internal Standard Working Solution and 10 µL of the 100 ng/mL Working Solution to a high-recovery GC vial. Evaporate under nitrogen. Add 75 µL of Methanol:0.1 % Formic Acid (75:25) to vial. Cap and vortex.

The results of the analysis of neat standards should be compared with the previous results of neat standards. Because similar concentrations are being used, peak area of internal standard and compounds of interest and ratio of compounds of interest to internal standard should be similar. Comparison of the neat mix to the extracted calibrator can be used to assess extraction efficiency.

**2.) PREPARE CALIBRATION CURVES**

Calibrators are to be prepared in whole blood at the following levels for the calibration curve to be utilized: 0.10 ng/mL, 0.50 ng/mL, 1.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL

Methods for preparing calibration curves should be documented on the Toxicology Quantitation Worksheet. An example for how to prepare working solution dilutions and amounts to add to spike the curve is as follows:

To prepare calibration curve, pipette the following volumes of the listed working solution into 16x100 mm test tubes containing 1 mL of blank blood matrix:

| Calibrator: | Concentration (ng/mL) | Working Solution Dilution | µL to add |
|-------------|-----------------------|---------------------------|-----------|
| CAL 1       | 0.10                  | 10 ng/mL                  | 10        |
| CAL 2       | 0.50                  | 10 ng/mL                  | 50        |
| CAL 3       | 1.0                   | 100 ng/mL                 | 10        |
| CAL 4       | 5.0                   | 100 ng/mL                 | 50        |
| CAL 5       | 10                    | 1.0 µg/mL                 | 10        |
| CAL 6       | 25                    | 1.0 µg/mL                 | 25        |
| CAL 7       | 50                    | 1.0 µg/mL                 | 50        |

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**CONTROLS:**

Negative Blood Control will be included. This should be blank biological matrix determined not to contain compounds of interest.

Positive Controls will be prepared and will bracket a maximum of 10 case samples. Positive controls should be prepared from a separate working solution mix and can be made by the same analyst if independent sources of standard are available (different manufacturer, lot# or opened date). If an independent source is not available, positive controls should be made by a different analyst.

Positive Controls appropriately spaced throughout the run and bracketing case samples will be prepared at 15 ng/mL.

**3.) SAMPLE PREPARATION:**

10 µL of Internal Standard Working Solution will be added to all Calibrators and Controls and Case samples.

1. To 1 mL of sample, add approximately 2 mL of chilled Acetonitrile dropwise while vortexing sample until protein precipitation is achieved.
2. Centrifuge sample for a minimum of 10 minutes at 2500 rpm or greater.
3. Transfer supernate to a clean, prelabeled test tube.
4. Add 3 mL of Phosphate Buffer (0.1 M, pH 6.0) to each sample tube. Vortex sample.

**4.) EXTRACTION PROCEDURE:**

1. Prepare extraction column:
  - A. 3 mL methanol
  - B. 3 mL DI water
  - C. 1 mL Phosphate buffer (0.1 M, pH 6.0)

**NOTE: Turn off vacuum as soon as the buffer reaches the top of the sorbent bed to prevent column drying.**

2. Pour the sample to the column reservoir and push/pull through column at approximately 1-2 mL/minute.
3. Wash Column:
  - A. 3 mL DI water
  - B. 3 mL 20% Acetonitrile in Phosphate buffer (0.1 M, pH 6.0)
  - C. Dry column for a minimum of 5 minutes under maximum vacuum.
4. Elute synthetic cannabinoids with two aliquots of 3 mL each 9:1 ethyl

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acetate:methanol.

5. Dry down eluates under a steady stream of nitrogen at a temperature not to exceed 45°C.

**5.) TRANSFER TO GC VIAL**

1. Add approximately 1 mL of 9:1 ethyl acetate:methanol.
2. Vortex sample.
3. Transfer liquid to clean, labeled 11 mm high-recovery GC vial.
4. Dry down eluants under a steady stream of nitrogen at a temperature not to exceed 45°C.
5. Add 250 µL Methanol:0.1 % Formic Acid (75:25) to vial. Cap and vortex.

**6.) ANALYSIS**

Analysis should be performed on LC-MS/MS using SYNCANN method. Method parameters may be found in the method folder associated with each instrument. Compound transitions should be input for each compound, as well as expected retention times.

If an analytical run stops due to instrument failure, the following parameters should be adhered to:

1. If failure is acknowledged within 6 hours, the run can be continued from the last unknown sample.
2. If failure is acknowledged within 24 hours, the run can be continued from the previous positive control.
3. If failure is acknowledged greater than 24 hours after the failure, the run should be restarted from the beginning.
4. The times and actions taken should be indicated on the quantitation worksheet.

Ions of interest to be used are as follows:

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**Table 1. Synthetic cannabinoids**

| Compound Name | Precursor Ion | Product Ions             | Internal Standard | Precursor Ion | Product Ions |
|---------------|---------------|--------------------------|-------------------|---------------|--------------|
| JWH-200       | 385.2         | 155, 127.1, 114.1        | d5-JWH-200        | 390.2         | 155.1, 114.1 |
| JWH-081       | 372.2         | 185, 127.1, 114.1        | d9-JWH-081        | 381.3         | 185, 114.1   |
| JWH-015       | 328.2         | 200.1, 155.1, 127.1      | d7-JWH-015        | 335.2         | 155, 127.1   |
| RCS-04        | 322.2         | 135, 107.1, 92.1         | d9-RCS-04         | 331.2         | 135.1, 107.1 |
| RCS-08        | 376.2         | 144.1, 121.1, 91.1, 65.1 |                   |               |              |
| JWH-250       | 336.2         | 130.1, 121.1, 91.1       | d5-JWH-250        | 341.2         | 121.1, 91.1  |
| AM-2201       | 360.2         | 232.1, 155, 127.1        | d5-AM-2201        | 365.2         | 155, 127.1   |
| AM-2233       | 459.1         | 112.1, 98.1, 76.1, 70.1  |                   |               |              |
| JWH-122       | 356.2         | 141.1, 214.1, 169, 115.1 | d9-JWH-122        | 365.3         | 223.2, 169   |
| JWH-073       | 328.2         | 200.1, 155, 127.1        | d7-JWH-073        | 335.2         | 155, 127.1   |
| JWH-210       | 370.2         | 183, 155.1, 153.1        | d9-JWH-210        | 379.3         | 223.2, 183   |
| JWH-018       | 342.2         | 214.1, 155, 127.1        | d9-JWH-018        | 351.2         | 155.1, 127.1 |
| XLR-11        | 330.2         | 232.1, 125.1, 97.1       |                   |               |              |
| UR-144        | 312.2         | 214.1, 125.1, 97.1       |                   |               |              |
| JWH-019       | 356.2         | 228.1, 155, 144.1, 127.1 |                   |               |              |
| JWH-022       | 340.2         | 212.1, 155.1, 127.1      |                   |               |              |
| JWH-203       | 340.2         | 214.1, 144, 125          |                   |               |              |
| WIN 55212-2   | 427.2         | 155, 127.1, 100.1        |                   |               |              |

## **7.) INTERPRETATION OF RESULTS**

Refer to Toxicology Quality Manual for quality control requirements and reporting guidelines.

## **REFERENCES:**

Solid Phase Extraction Applications Manual, United Chemical Technologies, Inc., Bristol, PA  
Revised 2003.

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**CYANIDE COLORIMETRIC DETERMINATION**

**PRINCIPLE:**

This colorimetric assay is designed to detect the presence of cyanide in a blood specimen. A positive result is indicated by the development of a violet color. It should be noted that this assay only serves as a screening tool, a positive result must be confirmed by subsequent testing.

**SPECIMEN REQUIRED:**

Minimum recommended specimen for the performance of this test is 500 µL of whole blood, serum, or plasma.

**SUPPLIES REQUIRED:**

10 mL volumetric flasks  
Disposable borosilicate test tubes  
Methyl Cellosolve (2-methoxyethanol)  
p-Nitrobenzaldehyde  
o-Dinitrobenzene  
Sodium Hydroxide (conc.)  
Potassium Cyanide  
250 mL amber glass bottle  
500 mL borosilicate bottle  
200 mL volumetric flask  
10 mL volumetric flask  
Vortex mixer  
Centrifuge

**PROCEDURE:**

**STANDARD PREPARATION:**

Cyanide 1 µg/mL working standard may be made from cyanide stock solution as follows:

1. Transfer 10 µL of the 1 mg/mL Cyanide stock std. into a 10 mL volumetric flask.
2. Add sufficient DI water to reach a final volume of 10 mL.

**NOTE:** Working solution should be prepared fresh just prior to use.

**TEST PROCEDURE:**

1. Setup and label the appropriate number of 12 X 75 mm disposable test tubes.
2. Add 100 µL of working cyanide standard to the appropriate tubes (i.e. Std and spiked case(s)).

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3. Prepare unknown samples by adding 100 µL of blood to the proper test tubes.
4. To each tube:
  - A. Add 1 mL of Cyanide test reagent
  - B. Vortex approximately 30 seconds
  - C. Centrifuge at maximum speed (approximately 2500 rpm)
  - D. Decant the supernatant to a clean test tube.
5. Add 50 µL of 0.1M NaOH (or 0.1M KOH) to each tube.
6. Vortex approximately 30 seconds.
7. Allow samples to stand in the dark for approximately 20 minutes.
8. Remove and observe for the development of a violet color.

**QUALITY CONTROL:**

All known control samples and unknown specimens should be prepared and processed in duplicate. The following quality control specimens should be included with any batch of cases processed:

1. 2 positive control samples containing 100 ng of potassium cyanide per sample.
2. 2 negative control samples.

**NOTE:** It is strongly recommended that a sample of actual case blood be spiked with 100 ng of sodium cyanide and processed along with the unspiked case blood to provide evidence that no inhibition of the assay has taken place.

**INTERPRETATION OF RESULTS:**

The development of a violet or purple color in test tube containing supernate is indicative of a positive test for cyanide.

**REFERENCES:**

Ellenhorn and Barceloux, Medical Toxicology; Diagnosis and Treatment of Human Poisoning, Elsevier Science Publishing Company, Inc., New York.

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**SEMI-QUANTITATIVE DETERMINATION OF CYANIDE BY COLORIMETRIC METHOD**

**PRINCIPLE:**

This semi-quantitative assay provides a method to confirm a positive result from the Cyanide Colorimetric Determination Assay. Cyanide may be liberated from biological fluids by Acidification. The evolved Hydrogen Cyanide gas is absorbed by the 0.1M Potassium Hydroxide (KOH) solution. After an incubation period, an aliquot of the KOH solution is removed and treated with the Cyanide Test Reagent for the development of a blue color. The intensity of the developed blue color is directly related to the concentration of Hydrogen Cyanide present, hence a darker blue color formation would indicate a higher Hydrogen Cyanide concentration. Realizing this to be true, it is possible to determine the concentration (within a range) of a particular sample by processing spiked standards of varying concentrations along with the case specimen.

**SPECIMEN REQUIRED:**

The following procedure is designed to be used with blood specimens which have been refrigerated or frozen and to which a preservative has been added. A minimum of 2.0 mL of blood is recommended for this analysis.

**SUPPLIES REQUIRED:**

8 (12 X 75 mm) Disposable test tube  
Labeling tape  
1.0 mg/mL Cyanide Stock Standard  
Cyanide Test Reagent  
0.25M Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) Solution  
0.1M Sodium Hydroxide (NaOH) or 0.1M Potassium Hydroxide (KOH) Solution

**APPARATUS REQUIRED:**

Conway Micro-diffusion dishes  
Adjustable Micropipette (10 - 100 µL)  
Macro Pipette (1.0, 2.0, and 5.0 mL)  
Vortex Mixer  
Centrifuge

**PROCEDURE:**

PREPARATION OF WORKING STANDARDS FROM THE 1.0 mg/mL CYANIDE STOCK STANDARD:

**NOTE:** It is recommended that at least 2 standards of known concentration and a blank be processed along with the case specimen. Examples of standards include;

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1. Take 10  $\mu\text{L}$  of cyanide stock solution and add to 2.0 mL of blood = 5.0  $\mu\text{g/mL}$
2. Take 10  $\mu\text{L}$  of cyanide stock solution and add to 4.0 mL of blood = 2.5  $\mu\text{g/mL}$
3. Blank Control = 2.0 mL of blood

**SEMI-QUANTITATIVE DETERMINATION OF CYANIDE:**

1. Properly label an appropriate number of Conway Micro-diffusion dishes (i.e. STD1, STD2, BLK, & case#).
2. Add 3.0 mL of 0.25M  $\text{H}_2\text{SO}_4$  to the middle ring of dish.
3. Add 1.0 mL of 0.1M NaOH (or 0.1M KOH) to the middle well.

**NOTE:** Total volume of BASE solution must equal total volume of specimen.

4. Add 3.0 mL of 0.25M  $\text{H}_2\text{SO}_4$  and 1.0 mL specimen, blank, and standard to the inner ring of the appropriately labeled dish.

**NOTE:** Care must be taken not to mix the acid and blood until the cover is in place on the dish.

5. Gently swirl dish until the sample and acid in the inner ring are thoroughly mixed.

**NOTE:** Do not splash liquid into the middle well (i.e. Base Portion).

6. Once mixed, allow to incubate for at least 3 hours.
7. Take 0.10 mL of BASE from middle well and dispense into the appropriately labeled 12x75 mm disposable test tube.
8. Add 1.0 mL of Cyanide Test Reagent to each test tube.
  - A. Vortex for approximately 30 seconds to mix.
  - B. Centrifuge.
  - C. Decant supernatant into a clean disposable test tube.
9. Add 0.05 mL of 0.1M NaOH (or 0.1M KOH) to each test tube.
10. Allow samples to stand in the dark for approximately 20 minutes.
11. Remove samples and observe for development of a violet color.

**INTERPRETATION:**

The development of a violet color indicates the presence of cyanide in the sample. The intensity of the color development is directly related to the concentration of cyanide present (i.e. a darker color indicates a higher concentration of cyanide present). By using standards of known concentration it is possible to determine within a range the quantity of cyanide present in a case. The blank should exhibit no color development, the standard of highest concentration should exhibit the most intense color development.

When the assay is completed, the scientist will compare the case specimen to the standards and blank control. For example, if the case specimen color is more intense than the 2.5  $\mu\text{g/mL}$

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standard's color, but less intense than the 5.0 µg/mL standard's color, then it may be reasonable to indicate that the concentration of cyanide in the specimen is within the known concentration of these two standards.

CAUTION MUST BE TAKEN IN THE DISSEMINATION OF A POSITIVE RESULT UNTIL THE ORIGIN OF THE CYANIDE PRESENT IS DETERMINED. There are several possibilities - one of which might be the bacterial formation of Cyanide in a biological specimen. A method by which this could be excluded as being the Cyanide's origin is to retest the specimen bi-weekly over the course of a month noting any change in the Cyanide level present in the specimen. If bacterial formation is the origin of the Cyanide, it would stand to reason that the level of Cyanide present would increase with time resulting in a higher concentration from test to test.

**REFERENCES:**

Ellenhorn and Barceloux, Medical Toxicology; Diagnosis and Treatment of Human Poisoning, Elsevier Science Publishing Company, Inc., New York.

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**DETECTION OF HYDROCYANIC ACIDS AND CYANIDES**

**PRINCIPLE:**

The procedure described below is employed for the detection of hydrocyanic acid and cyanides in biological specimens, e.g. blood. The procedure may be qualitative or semi-quantitative in scope, depending on the needs of the analyst and the availability of an adequate volume of sample. This analysis utilizes CYANTESMO, a test paper which undergoes a colorimetric conversion (green to blue) in the presence of cyanide containing samples.

**SPECIMEN REQUIRED:**

Blood sample

**WARNING:** Cyanide may be generated in the postmortem sample both in vivo and in vitro. Spontaneous generation of cyanide has been documented to occur in the presence of Pseudomonas bacteria.

The presence of a preservative, e.g. NaF, appears to retard this process.

**SUPPLIES REQUIRED:**

Cyantesmo paper  
0.25M Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>)  
Disposable glass pipettes  
Sodium Cyanide

**APPARATUS REQUIRED:**

3 Conway Diffusion Discs with Covers

**PROCEDURE:**

1. To the inner well of each of three Conway Diffusion Discs, place a strip of CYANTESMO paper long enough to fit the diameter of the well (approximately 3.0 cm).
2. To the middle well of one Disc, place 1.0 mL of blood containing NO cyanide (negative control).
3. To the middle well of a second Disc, place 1.0 mL of blood containing a known concentration of sodium cyanide, e.g. 0.2 mg/L, the limit of detection for this procedure (positive control).
4. To the middle well of the third Disc, place 1.0 mL of the unknown blood sample.
5. To each of the middle wells of all three Discs, add 1-2 drops of 0.25M H<sub>2</sub>SO<sub>4</sub>.
6. To the outer wells of all three Discs, add approximately 3 mL of 0.25M H<sub>2</sub>SO<sub>4</sub>.
7. Carefully place a cover over each of the three Discs and allow to stand undisturbed during the incubation period.

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**NOTE:** The recommended sample volume for this procedure is 1 mL of whole blood, serum, or plasma. Smaller sample volumes may be substituted in cases of insufficient sample. Work sheets should reflect deviations from approved methods.

**INTERPRETATION:**

There will be no observed color change (pale green to shades of blue) in the negative control Disc during the incubation period, a minimum of 15 minutes reaction time. The positive control must be allowed to incubate for at least 15 minutes and there should be a change in color (pale green to blue) observed after this period. The unknown sample must be allowed to incubate for a minimum of 15 minutes and may, depending on the concentration of cyanide present, demonstrate no color change (negative sample) or very deep shades of blue if the sample contains high concentrations of cyanide. Very low concentrations may require incubation for 3-4 hours or overnight.

**REFERENCES:**

Dunn, W. A., and T. J. Siek, "A Rapid, Sensitive and Specific Screening Technique for Determination of Cyanide" Journal of Analytical Toxicology, Vol 14 (1990): 256.

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**FERRIC CHLORIDE TEST**

**PRINCIPLE:**

The ferric chloride test is a rapid, colorimetric test used to determine the presence of salicylates in urine samples.

**SPECIMEN REQUIRED:**

Urine

**SUPPLIES REQUIRED:**

5% ferric chloride solution

Glass test tubes

**APPARATUS REQUIRED:**

None.

**PROCEDURE:**

To 1 mL urine, add 3 drops of 5% ferric chloride solution.

**INTERPRETATION OF RESULTS:**

The development of a violet-purple color is presumptive for salicylates.

**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed., A. C. Moffat, 2nd ed., London: The Pharmaceutical Press, 1986.

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**FPN TEST**

**PRINCIPLE:**

The FPN colorimetric test is used in the qualitative screening for phenothiazines in urine samples.

**SPECIMEN REQUIRED:**

A minimum of 1 mL of urine is recommended for the performance of this test

**SUPPLIES REQUIRED:**

1 mL urine  
1 mL FPN solution  
Glass test tubes

**APPARATUS REQUIRED:**

None.

**PROCEDURE:**

1. To 1 mL urine, add 1 mL FPN solution.
2. The development of pink, red, orange, violet, blue colors is presumptive for phenothiazines.

**NOTE:** All volumes are approximate unless otherwise noted.

**REFERENCES:**

Skoutakis, Vasilios A., Clinical Toxicology of Drugs: Principles and Practice, Lea & Febiger, Philadelphia, 1982.

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**FUJIWARA'S SPOT TEST**

**PURPOSE:**

Fujiwara's colorimetric test is used to determine the presence of trichloro-compounds (i.e. chloroform, chloral hydrate, trichloroethanol) in whole blood, gastric and urine samples.

**SPECIMEN REQUIRED:**

1 mL of urine, blood, or gastric fluid.

**SUPPLIES REQUIRED:**

Pyridine  
1 mL Biological Fluid  
1 mL 10% Sodium Hydroxide (NaOH) solution  
Glass Test Tubes

**NOTE:** Directions for preparation of 10% NaOH solution can be found in the formulary section of this manual.

**APPARATUS REQUIRED:**

Boiling Water Bath  
Vortex Mixer

**CALIBRATION REQUIREMENTS:**

None.

**PROCEDURE:**

1. Mix 1 mL 10% NaOH and 1 mL pyridine.
2. Heat in boiling water bath for 2 minutes.
3. Add 1 mL blood, urine, or gastric fluid and shake gently.
4. Heat in boiling water bath for 2 minutes.

**INTERPRETATION OF RESULTS:**

Development of pink-red colors in pyridine layer (upper) is presumptive for trichloro-compounds.

**DOCUMENTATION:**

Work sheets should reflect the performance of this test, the date performed, the color observed, and the results of analysis (i.e. "+" or "-").

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**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed. A. C. Moffat, 2nd ed., London: The Pharmaceutical Press, 1986.

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**HYPOCHLORITE ION**

**PRINCIPLE:**

Hypochlorites, such as Sodium and Calcium Hypochlorite, are used as disinfectants. Domestic products contain relatively low concentrations, whereas industrial products are much more concentrated and produce greater toxicity if ingested. Hypochlorites are often added to urine samples in an attempt to defraud the analysis. This spot test is an indicator of the presence of hypochlorite ion.

**SPECIMEN REQUIRED:**

1 mL urine or other suitable sample.

**SUPPLIES REQUIRED:**

Test tubes  
Blank urine for positive and negative controls  
Bleach to add to urine for positive control  
0.185 g diphenylamine  
Concentrated Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>)

**APPARATUS REQUIRED:**

None.

**PROCEDURE:**

1. Prepare 1% w/w diphenylamine solution by adding 0.185g diphenylamine to 10 mLs concentrated sulfuric acid. Mix well.
2. Prepare a test tube for each sample and for the positive and negative control by labeling and adding 1 mL of the 1% diphenylamine solution to each tube.
3. Add several drops of the positive control to the appropriate test tube until a blue color is given. Add the same number of drops of sample to the appropriately labeled tube and watch for the immediate blue.
4. Repeat these steps with the negative control (no color should develop) and each consecutive case sample.

**NOTE:** Other strong oxidizing agents will also react.

**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed. A. C. Moffat et al.,  
3<sup>rd</sup> ed., London: The Pharmaceutical Press, 2004.

HYPOCHLORITE SPOT TEST

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**KUNKEL'S TEST**

**PRINCIPLE:**

Kunkel's Test is a rapid qualitative test used in the determination of carbon monoxide in whole blood.

**SPECIMEN REQUIRED:**

The following procedure is recommended for use with approximately 0.2 mL of whole blood.

**SUPPLIES REQUIRED:**

Disposable pasteur pipettes  
Glass micro test tubes or cuvettes  
Distilled water  
10% Sodium Hydroxide Solution (NaOH)

**APPARATUS REQUIRED:**

None.

**PROCEDURE:**

1. Add distilled water to 3/4 volume of the cuvette or test tube.
2. Add 2-3 drops of whole blood to the water in the test tube or cuvette.
3. Add 10% w/v NaOH solution drop wise to each tube until negative control turns brown.

**QUALITY CONTROL:**

A positive (CO>20%) and negative carboxyhemoglobin control should be processed along with each batch of case samples.

**INTERPRETATION:**

The continued presence of a cherry red color following the addition of 10% w/v NaOH is presumptive of carboxyhemoglobin binding (>20%). The development of a straw-gold to brown color indicates a negative test.

Positive tests should be confirmed by AVOXimeter or by Ammonia Method for confirmation of carbon monoxide.

**REFERENCES:**

Brookes, Vincent J., Hubert N. Alyea. Poisons, Their Properties, Chemical Identification, Symptoms, and Emergency Treatments. New York: D. Van Nostrand Company, Inc., 1946.

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**REINSCH TEST**

**PRINCIPLE:**

The Reinsch test provides a rapid qualitative screening technique used in the identification of various heavy metals. The Reinsch test may be used to detect/identify the following analytes: mercury, bismuth, arsenic, antimony, selenium, tellurium, and sulfur.

**SPECIMEN REQUIRED:**

20 mLs of urine or an aliquot of homogenized tissue extract, gastric lavage, vomitus, other liquids, powders, tablets, or residues dissolved or suspended in 20 mLs of water.

**SUPPLIES REQUIRED:**

Small Beaker, Erlenmeyer Flask, or Large Test Tube  
Hydrochloric Acid  
DI Water  
Copper Strip or a spiral of copper wire  
2.5 M Nitric Acid  
10% Potassium Cyanide Solution

**APPARATUS REQUIRED:**

Hot Plate or Water Bath

**PROCEDURE:**

1. Wash the copper strip or spiral with 2.5 M nitric acid, then rinse with ethanol and dry.
2. Place 20 mLs of urine or other appropriate specimen into suitable glass container. Add 4 mLs of concentrated hydrochloric acid.

**NOTE:** Smaller specimen volumes may be used at the analyst's discretion but should be noted.

3. Add freshly washed copper strip or copper spiral to the container.
4. Heat the solution gently for 1 hour.
5. Remove the copper, wash gently with water and examine the surface. If no deposit is observed, return copper to solution and continue heating for another hour. Remove the copper, wash gently with water and examine the surface.
6. If a deposit is noted, place the coated copper into approximately 2 mLs of 10% potassium cyanide solution.

**NOTE:** Record solubility in cyanide solution. A black deposit, which is soluble in cyanide solution, indicates the presence of arsenic.

Appropriate positive and negative controls should be run in order to accurately interpret results and insure the test is working properly.

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**INTERPRETATION OF RESULTS:**

A silver deposit indicates the presence of mercury. A shiny black deposit, which is insoluble in potassium cyanide solution, indicates the presence of bismuth. A dull black deposit, which is soluble in potassium cyanide solution, indicates the presence of arsenic. A purple deposit, which is insoluble in potassium cyanide solution, indicates the presence of antimony. Dark deposits may indicate selenium or tellurium, whereas a speckled discoloration may indicate high concentrations of sulfur.

**LIMITATIONS:**

All cases/specimens screening positive for the presence of heavy metals should be confirmed by an alternate procedure, which has been SLED laboratory approved.

Clark's Isolation and Identification of Drugs, lists the limit of detection for this test as follows:

|          |           |
|----------|-----------|
| Arsenic  | 0.5 µg/mL |
| Antimony | 1.0 µg/mL |
| Bismuth  | 1.0 µg/mL |
| Mercury  | 2.5 µg/mL |

**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed. A. C. Moffat,  
2<sup>nd</sup> ed., London: The Pharmaceutical Press, 1986.

Methodology for Analytical Toxicology, Volume I, ed. Irving  
Sunshine, CRC Press, Boca Raton, Florida, 1975.

**REINSCH TEST**

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**TRINDER'S TEST**

**PRINCIPLE:**

Trinder's colorimetric test is a rapid qualitative test, which may be used to detect the presence of salicylates and/or acetaminophen in whole blood.

**SPECIMEN REQUIRED:**

Approximately 1 mL whole blood

**SUPPLIES REQUIRED:**

5 mL Trinder's reagent  
Glass test tubes

**APPARATUS REQUIRED:**

Centrifuge  
Vortex mixer

**CALIBRATION REQUIREMENTS:**

None.

**PROCEDURE:**

1. Add 5 mL of Trinder's reagent to 1 mL whole blood.
2. Vortex sample for 5-15 seconds.
3. Centrifuge for approximately 5 minutes at approximately 2000 rpm.

**INTERPRETATION OF RESULTS:**

Specimens which are positive for the presence of salicylates and/or acetaminophen will present with a violet colored supernatant.

**QUALITY CONTROL:**

A negative and positive control should be run with each batch of unknown specimens.

**REFERENCES:**

Clark's Isolation and Identification of Drugs, ed. A.C. Moffat, 2nd ed., London: The Pharmaceutical Press, 1986.

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**CONTROLLED SUBSTANCE HANDLING**

**PRINCIPLE:**

The possession of controlled substances for testing purposes requires specific procedures for registration, security, ordering, and record keeping.

**REGISTRATION REQUIREMENTS:**

In order for law enforcement agency laboratories to obtain controlled substances for use in chemical analysis, such laboratories must annually obtain both a State and Federal registration to conduct chemical analysis. Such laboratories shall be exempted from payment of a fee for registration according to Drug Enforcement Administration Code of Federal Regulations section 1301.26(d). Laboratory personnel, when acting in the scope of their official duties, are deemed to be officials exempted by section 1301.26(d) and within activity described in section 515(d) of the ACT (21 U.S.C. 885(d)). The Department of Health and Environmental Control also considers law enforcement agency laboratories to be exempt from payment of a fee for registration by Regulation 61-4 section 111(d).

**SECURITY REQUIREMENTS:**

Schedules I, II, III, IV, and V controlled substances should be stored in a locked substantially constructed cabinet that cannot be readily removed. This cabinet shall be located in an area restricted to public access. Controlled substances that need refrigeration shall be stored in a locked refrigerator. Such cabinet and refrigerator will be located in the SLED Forensics Laboratory, department of Toxicology. Controlled substances should be accessible only to an absolute minimum number of specifically authorized employees, other than the Registrant and his appointed power of attorney.

The Narcotic and Drug Control Division of DHEC should be notified of any theft or significant loss of any controlled substance upon discovery of such theft or loss. The registrant shall also notify the Field Division Office of the Drug Enforcement Administration in his area and complete DEA Form 106 regarding such loss or theft.

The registrant shall not employ, as an employee who has access to controlled substances, any person who has been convicted of a felony offense relating to controlled substances or who has had an application with the DEA denied or had a DEA registration revoked. It is the position of the DEA and of the Narcotic and Drug Control Division of DHEC that the obtaining of certain information by non-practitioners is vital to fairly assess the likelihood of an employee committing a drug security breach.

The following questions will become part of an employer's comprehensive employee screening program:

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(1) Within the past five years, have you been convicted of a felony or misdemeanor or are you presently formally charged with committing a criminal offense? (Do not include any traffic violations, juvenile offenses or military convictions, except by general court-martial). If the answer is yes, furnish details of conviction, offense, location, date and sentence.

(2) In the past three years, have you ever knowingly used any narcotics, amphetamines, or barbiturates, other than those prescribed to you by a physician or other practitioner? If the answer is yes, furnish details.

An authorization, in writing, that allows inquiries to be made of courts and law enforcement agencies for possible pending charges or convictions must be executed by a person who is allowed to work in an area where access to controlled substances clearly exists. A person must be advised that any false information or omission of information will jeopardize his or her position with respect to employment. The application for employment should inform a person that information furnished or recovered as a result of any inquiry will not necessarily preclude employment, but will be considered as part of an overall evaluation of employment practices, the protection of the person's qualifications. The maintaining of fair employment practices, the protection of the person's right to privacy, and the assurance that the results of such inquiries will be treated by the employer in confidence will be explained to the employee.

**ORDERING:**

DEA Form 222 is required for each order of a schedule I or II controlled substance. Order forms may be obtained only by the registrant or his appointed power of attorney, who are registered to handle Schedule I and II controlled substances. An order form may be used only by registrant and only if his/her registration has not been suspended or revoked. The registrant should be the Toxicology supervisor.

Each order form is to be filled out with name and address of supplier and items (up to 10 per order form). Items are to be listed one per line and should include finished form (e.g. 10mg tablet), volume or number of units, number of containers ordered, and name and quantity per unit. Order forms are to be signed and dated by a person authorized to sign a requisition for order forms on behalf of the purchaser. The name of the purchaser, if different from the individual signing the order form, is also to appear in the signature space.

Additional forms may be required for ordering controlled substances for importation for example DEA form 357.

**Power of Attorney:**

The registrant may authorize another individual to obtain and execute order forms on his behalf by executing a power of attorney.

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**RECORD KEEPING:**

All Schedule I and II controlled substances will be recorded on a Standards Register – TOX 043. For this register, all controlled substances will be given a unique identifier. The Lot Number may serve as this identifier however if multiple lots of the same compound exist, they must be uniquely identified. When the standards register is created, the weight of the substance and container will be recorded. Any time an amount of substance is removed from the container, the starting and final weight will be recorded along with a reason for the removal (for example for preparation of a drug standard), the date and the analyst's initials. An inventory/audit of all controlled substances on hand shall be taken yearly.

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- 71. 9:1 Ethyl Acetate:Methanol
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1.) 5% w/v Ferric Chloride Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mLs:

Dissolve 5 g of ferric chloride in approximately 80 mL of DI water. Add DI water for a total of 100 mL.

**NOTE:** Solution may be used for 6 months from date of preparation

2.) 50% v/v Nitric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

Carefully add 50 mL HNO<sub>3</sub> to 50 mLs DI water.

3.) 30% v/v Sulfuric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

Carefully add 30 mL H<sub>2</sub>SO<sub>4</sub> to 70 mL DI water. Mix well.

4.) 0.01% w/v Tertiary Butanol (t-Butanol) Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 2L:

1. Remove t-butanol from refrigerator. Allow substance to warm to room temperature (until completely liquefied).
2. Add 254 µL t-butanol to approximately 1900 mL of distilled water
3. Add 1.3 g Sodium Azide.
4. Mix well.
5. Add sufficient amount of distilled water to final volume of 2L.
6. Transfer solution to collapsible reservoir.
7. Repeat steps 1-5 to make a total of 10L.

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5.) 1M Acetic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. To a 1 L volumetric containing approximately 900 mL of DI water
2. Add 57.2 mL of glacial acetic acid.
3. Mix Well.
4. Dilute to 1000 mL final volume with DI water.

6.) 0.25M Sulfuric Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mls:

1. Slowly add 7 mls of concentrated Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ) to approximately 300 mls of distilled water. Mix well.
2. Add sufficient amount of distilled water to a final volume of 500 mls. Mix well.
3. Transfer the solution to the screw top glass bottle for long term storage.

**NOTE:** This solution can be kept and used up to 1 year from date of preparation.

**NOTE:** When preparing acid solutions from concentrated acid, one should always add concentrated acid to water.

7.) 0.05M Sulfuric Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mls:

1. Slowly add 1.4 mls of concentrated Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ) to approximately 300 mls of distilled water. Mix well.
2. Add sufficient amount of distilled water to a final volume of 500 mls. Mix well.
3. Transfer the solution to the screw top glass bottle for long term storage.

**NOTE:** This solution can be kept and used up to 1 year from date of preparation.

**NOTE:** When preparing acid solutions from concentrated acid, one should always add concentrated acid to water.

8.) 80% v/v Sulfuric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mls:

Carefully add 80 mls  $\text{H}_2\text{SO}_4$  to 20 mls distilled water as water is cooling in an ice bath.

**WARNING:** This solution becomes VERY HOT - PROCEED WITH CAUTION.

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9.) Trinder's Reagent

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

1. Weigh 4 g of mercuric chloride and dissolve with stirring and heating in 85 mL of DI water. Cool.
2. Add 12 mL 1M HCL and 4 g ferric nitrate. Dissolve with gentle stirring.
3. After dissolution, adjust total volume to 100 mL with DI water.

10.) 10% v/v Hydrochloric Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 10 mls:

Add 1 mL concentrated HCL to 9 mL DI water. Mix well.

11.) 10% w/v Sodium Hydroxide (Kunkel's Reagent)

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mLs:

Dissolve 10 g of sodium hydroxide in approximately 90 mLs of distilled water. Mix well.  
Add sufficient amount of distilled water to final volume of 100 mLs.

12.) 0.1M Sodium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mL:

Sodium Hydroxide, 0.1M (from NaOH pellets):

1. Add 1 g of sodium hydroxide pellets to 100-150 mL of water in a 250 mL volumetric flask.
2. Dilute to 250 mL mark.
3. Mix thoroughly to dissolve.

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13.) Aqueous Ammonium Hydroxide, pH 8.5-10.5

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

1. To a clean glass beaker add 500 mL of DI water.
2. While monitoring the pH of the solution, add concentrated ammonium hydroxide drop wise with stirring to reach desired pH.
3. Store in an amber, tightly stoppered glass container. Refrigerate for best life.

**NOTE:** Solution will expire in 120 days.

14.) 0.4% w/v Ammonium Hydroxide Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mls:

1. Add 1.5 mL of concentrated ammonium hydroxide (NH<sub>3</sub>) with approximately 80 mL of distilled water. Mix thoroughly.
2. Add sufficient amount of water to final volume of 100 mL.
3. Mix thoroughly.
4. Transfer the solution to the glass bottle.

**NOTE:** This solution can be kept and used for 1 year from the date of preparation.

15.) 1% w/v Phenylboronic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

Dissolve 1.0 g of phenylboronic acid in 100 mL of acetone.

**NOTE:** Solution is stable for 1 year when stored at 2-8°C.

16.) 1,2-Butanediol in Acetone

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

Dissolve 100 mg of 1,2-Butanediol in 20 mL of acetone.

**NOTE:** The concentration of the solution is 5 mg/mL. Solution is stable for 1 year stored at 2-8°C.

17.) Ethylene Glycol Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 50 mL:

Add 250 mg of ethylene glycol to 25 mL volumetric flask and QS with DI water.

**NOTE:** The concentration of the solution is 10 mg/ml. Solution is stable for 1 year when stored at 2-8°C.

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18.) 1.0M Sodium Acetate Stock Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. Dissolve 82 g of anhydrous Sodium Acetate in approximately 950 mL of DI water.
2. Adjust pH of buffer to approximately 5.2 with addition of glacial acetic acid.
3. Dilute to a final volume of 1000 mL with de-ionized water.
4. Refrigerate upon completion.

19.) Sodium Acetate Working Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mLs:

1. Measure 100 mL of 1.0M Sodium Acetate Stock Solution.
2. Add 5 mL of  $\beta$ -Glucuronidase and mix gently. Do not shake vigorously.

**NOTE:** Solution will expire in 24 hours.

20.) Sodium Phosphate Buffer, pH 6.0

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. In appropriate glassware add 13.8 g of sodium phosphate monobasic.
2. Dissolve in approximately 950 mL of DI water.
3. Mix well.
4. Adjust pH of solution to approximately 6.0 with the addition of concentrated sodium hydroxide.
5. Dilute to 1000 mL with DI water. Refrigerate upon completion.

21.) Elution Solvent A

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 500 mL of solution:

1. Add 250 mL of Ethyl Acetate to a suitable mixing container.
2. Add 250 mL of Hexane to container.
3. Mix well.
4. Transfer to dark container for storage.

**NOTE:** Solution expires after 180 days.

22.) Elution Solvent B

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly,

1. To a clean amber glass container add 78 mL of GC/MS grade Methylene Chloride
2. In a graduate cylinder measure 20 mL of Isopropyl Alcohol.
3. Add 2 mL concentrated Ammonium Hydroxide to the isopropyl alcohol.

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4. Add the IPA/Ammonium Hydroxide mixture to the Methylene Chloride
5. Mix vigorously. Check to make sure two layers have not formed.
6. Store in a tightly stoppered, amber, glass container.

**NOTE:** pH of the solvent should be approximately 11-12 to ensure proper elution of all compounds of interest. Additional concentrated Ammonium Hydroxide may be required to adjust pH.

**NOTE:** Solvent will expire in 3 days.

23.) 0.1M Potassium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mL:

1. Dissolve 2.8 g KOH in approximately 450 mL of DI water.
2. Add sufficient amount of distilled water to final volume 500 mL.

24.) Cyanide Test Reagent

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 200 mL:

1. Dissolve 0.756 gm of p-Nitrobenzaldehyde and 0.840 gm of o-Dinitrobenzene in 200 mL of DI Water.
2. Mix thoroughly.

**NOTE:** Store reagent in tightly capped amber bottle.

**NOTE:** Solution expires in six months.

25.) Potassium Cyanide Stock Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

1. Dissolve 0.1 g of potassium cyanide in 60-80 mL of 0.1M sodium hydroxide.
2. Add sufficient 0.1M sodium hydroxide to reach a final volume of 100 mL.
3. Mix thoroughly.
4. Store solution in a suitable screw-capped plastic container (preferably polyethylene).

**NOTE:** Solution expires in 3 months.

26.) 1:20 25% TMAH:DMSO

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

1. To a clean glass beaker, add 20 mL of dimethylsulfoxide.
2. Add 1 mL of tetramethyl ammonium hydroxide.
3. Stir well.
4. Store in amber bottle at room temperature.

**NOTE:** Solution will expire in 120 days.

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27.) Chloroform/IPA Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 liter:

1. Add 250 mL of GC/MS grade Isopropanol to 750 mL of GC/MS grade chloroform.
2. Mix well.
3. Upon completion, store in a tightly sealed container at room temperature.

28.) Diphenylamine Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 150 mL:

1. Dissolve 1 g of diphenylamine in 50 mL concentrated sulfuric acid.
2. Mix well and store in tightly sealed container.

**NOTE:** This solution expires six months from date of preparation.

29.) 50% v/v Acetic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

1. To a 100 mL volumetric containing approximately 45 mL of DI water
2. Add 50 mL of glacial acetic acid.
3. Mix Well.
4. Dilute to 100 mL final volume with DI water.

30.) 0.6M Hydrochloric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly. This recipe utilizes HCl that is approximately 37% pure. The purity of the stock HCl should be checked (on label) and volumes adjusted accordingly.

For 1 liter:

1. Add approximately 900 mL of water to a 1 L volumetric flask.
2. Slowly add 49 mL concentrated HCl to flask.
3. Dilute to mark with additional DI water.

31.) 0.5M Sodium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 liter:

1. Dissolve 20 g NaOH in approximately 800 mLs of DI water.
2. Add appropriate amount of distilled water to final volume of 1 liter.
3. Store in suitable container.

**NOTE:** Sodium hydroxide pellets should be weighed and transferred to solution as rapidly as possible due to its hygroscopic nature.

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32.) 2.25M Sodium Phosphate Buffer

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mL:

1. Dissolve 77.5 g Sodium Phosphate (monobasic) in water.
2. Add appropriate amount of distilled water to final volume of 250 mL.
3. Store in a suitable container.

**NOTE:** Solution expires six months from date of preparation.

33.) Chloroform/IPA Solution (9:1)

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mL:

1. To a suitable container add 450 mL GC/MS grade chloroform.
2. Add 50 mL of GC/MS grade Isopropanol.
3. Mix well.
4. Upon completion, store in a tightly sealed container at room temperature.

34.) Chloroform/IPA (3:1)

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 400mL:

1. To a suitable container add 300 mL chloroform.
2. Add 100 mL of isopropanol.
3. Mix well.
4. Upon completion, store in a tightly sealed container at room temperature.

35.) 0.1M Hydrochloric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 liter:

1. To a suitable container, add approximately 900 mL of DI water.
2. Slowly add 8.4 mL concentrated HCl.
3. Add appropriate amount of DI water to final volume of 1 liter.

36.) 0.1M Acetic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. To a 1 L volumetric containing add approximately 900 mL of DI water
2. Add 57.5 mL of glacial acetic acid.
3. Mix Well.
4. Dilute to 1000 mL final volume with DI water.

**NOTE:** Solution will expire in 180 days.

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37.) 0.1M Sodium Phosphate Buffer, pH 6.0

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. In suitable container add 3.22 g of sodium phosphate dibasic heptahydrate (or 1.7 g sodium phosphate dibasic anhydrous)
2. Add 12.14 g sodium phosphate monobasic
3. Dissolve in approximately 800 mL of DI water
4. Mix well.
5. Adjust pH of solution to approximately 6.0 with the addition of 0.1M monobasic sodium phosphate (lowers pH) or 0.1M dibasic sodium phosphate (raises pH).

**NOTE:** Suitable acids or bases may be substituted for 0.1M Monobasic Sodium Phosphate or 0.1M Dibasic Sodium Phosphate during pH adjustment.

6. Dilute to 1000 mL with DI water.

**NOTE:** Refrigerate upon completion.

38.) 0.1M Sodium Phosphate Monobasic

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly. This recipe utilizes anhydrous sodium phosphate. Other formulations such as hydrates of sodium phosphate may also be used. However, the amount used must be adjusted accordingly.

For 200 mL:

1. Using a suitable container, add 2.76g of sodium phosphate monobasic.
2. Dissolve in 160 mL of DI water.
3. Mix well.
4. Dilute to 200 mL with DI water.

**NOTE:** Refrigerate upon completion.

39.) 0.1M Sodium Phosphate Dibasic

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly. This recipe utilizes anhydrous sodium phosphate. Other formulations such as hydrates of sodium phosphate may also be used. However, the amount used must be adjusted accordingly.

For 200mL:

1. Using a suitable container, add 2.84 g of sodium phosphate dibasic.
2. Dissolve in 160 mL of DI water.
3. Mix well.
4. Dilute to 200 mL with DI water.

**NOTE:** Refrigerate upon completion.

40.) 0.1M Sodium Acetate, pH 6.0

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

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1. Dissolve 1.36g of sodium acetate • 3 H<sub>2</sub>O (or 0.82 g anhydrous sodium acetate) in approximately 80 mLs of DI water. Mix thoroughly.
2. Adjust pH to 6.0 with the addition of glacial acetic acid.
3. Dilute to 100 mLs with DI water.
4. Confirm pH of final solution.

**NOTE:** Store at room temperature in glass or plastic. Buffer is stable for six (6) months.

41.) 0.1M Sodium Acetate, pH 6.0 w/5% Methanol

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

1. Dissolve 1.36 grams of sodium acetate • 3 H<sub>2</sub>O (or 0.82 g anhydrous sodium acetate) in approximately 80 mLs of DI water. Mix thoroughly.
2. Add 5 mLs of methanol.
3. Adjust pH to 6.0 with the addition of glacial acetic acid.
4. Dilute to 100 mLs with DI water.
5. Confirm pH of final solution.

Or

1. Combine 95 mLs of 0.1 M sodium acetate with 5 mLs of methanol
2. Mix thoroughly.

**NOTE:** Store at room temperature in glass or plastic. Buffer is stable for six (6) months.

42.) 95:5 Hexane:Ethyl Acetate

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

1. To 95 mLs hexanes add 5 mLs ethyl acetate.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass or plastic.

43.) 75:25 Hexane:Ethyl Acetate

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

1. To 75 mLs hexane, add 25 mLs ethyl acetate.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass or plastic.

44.) 75:25:1 Hexane:Ethyl Acetate:Acetic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

1. To 75 mLs hexane, add 25 mLs ethyl acetate, and 1 mL of glacial acetic acid.

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2. Mix thoroughly.

Or

1. To 100 mLs of 75:25 hexane:ethyl acetate, add 1 mL glacial acetic acid.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass or plastic.

45.) 2.5M Nitric Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

1. Dissolve 15 mLs of concentrated nitric acid in approximately 60 mLs of DI water.
2. Add DI water for a total of 100 mLs.
3. Mix thoroughly.

46.) 10% w/v Potassium Cyanide Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mL:

1. Dissolve 10 g of potassium cyanide in approximately 80 mL of DI water.
2. Add DI water for a total of 100 mL.
3. Mix thoroughly.

**NOTE:** A magnetic stirrer may be used to ensure adequate mixing.

47.) 0.01M Hydrochloric Acid Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 1 liter:

1. Add 0.833 mLs of concentrated hydrochloric acid (37%) to approximately 800 mLs of DI water.
2. Dilute to 1000 mLs with addition of DI water.
3. Mix thoroughly.

Or alternately

1. Add 100 mLs of 0.1 M hydrochloric acid solution to approximately 800 mLs of DI water.
2. Dilute to 1000 mLs with additional DI water.
3. Mix thoroughly.

48.) 1:1 MTBE:Toluene

1. Determine approximate amount of mixture necessary to process specimens that are ready for extraction.

**NOTE:** The barbiturate extraction will require approximately 5 mLs of solvent per sample being extracted.

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2. Combine 1 part MTBE and 1 part toluene for a final volume sufficient to extract specimens, controls, and calibrators.
3. Mix thoroughly.

49.) Hexane Saturated with Methanol

1. Determine the approximate of reagent necessary to process specimens that are ready for extraction.

**NOTE:** The barbiturate extraction will require approximately 0.5 mLs of reagent per specimen processed.

2. Place sufficient amount of hexane to process specimens in graduated cylinder or other suitable container.
3. Add methanol to container in 1 mL aliquots until saturation is achieved (note separation into two layers).
4. Mix gently to assure methanol layer remains separated.

**NOTE:** If methanol layer disappears, resume adding aliquots of methanol until layer remains with mixing.

50.) Minilyser Buffer, 0.1M Sodium Phosphate Buffer with Preservative

**NOTE:** Weights/volumes may be adjusted accordingly to prepare other volumes.  
For 1L:

1. Weigh 4.8 g Sodium Phosphate Monobasic (FW 138)
2. Weigh 17 g Sodium Phosphate Dibasic Heptahydrate (FW 268) or 11.3 g Potassium Phosphate Dibasic anhydrous (FW 174)
3. Weigh 9 g Sodium Chloride (FW 58.5)
4. Measure 1 mL of ProClin<sup>®</sup> 300 (10 ug/mL activity) or an antimicrobial agent containing **no Sodium Azide**.
5. Mix all the above in a 1 L volumetric flask containing 900 mL of DI water
6. Bring to pH 7.0 with the addition of 6 M Sodium Hydroxide
7. Add DI water to bring up to 1 L.

51.) 70:30 0.1M Hydrochloric Acid:Acetonitrile Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.  
For 250 mL:

1. To a suitable container, add 175 mL 0.1M HCl.
2. Add 75 mL Acetonitrile.
3. Mix Well.

52.) 10M Sodium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.  
For 250 mL Sodium Hydroxide, 10M (from NaOH pellets):

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1. Add 100 g of sodium hydroxide pellets to 100-150 mL of water in a 250 mL volumetric flask.
2. Dilute to 250 mL mark.
3. Mix thoroughly to dissolve.

53.) 10% Methanol in DI Water

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mLs:

1. Add 25 mL Methanol to
2. 225 mL DI water. Mix well.

54.) 20% Acetonitrile in 0.1M Sodium Phosphate Buffer, pH 6.0

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mL:

1. To a suitable container, add 50 mL Acetonitrile.
2. Add 200 mL 0.1M Sodium Phosphate Buffer, pH 6.0.
3. Mix Well.

55.) 1:1 Methanol:DI Water

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mLs:

1. Add 125 mL Methanol to
2. 125 mL DI water.
3. Mix well.

56.) 99:1 Methanol:Ammonium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mLs:

1. Measure 99 mL Methanol.
2. To Methanol add 1 mL Ammonium Hydroxide.
3. Mix well.

Solution should be made fresh daily

57.) Hexanes saturated with DMF

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

1. Place approximately 300 mL of hexane in 500mL amber bottle or other suitable container.
2. Add DMF to container in 1 mL aliquots until saturation is achieved (note separation into two layers).
3. Mix gently to assure DMF layer remains separated.

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**NOTE:** If DMF layer disappears, resume adding aliquots of DMF until layer remains with mixing.

58.) 80% Methanol in DI water

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mLs:

1. Add 200 mL Methanol to
2. 50 mL DI water. Mix well.

59.) 9:1 Hexane:Isopropyl Alcohol

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mLs:

1. Measure 225 mL Hexane.
2. Add to Hexane 25 mL Isopropyl Alcohol.
3. Mix well.

60.) 0.1M Sodium Borate Buffer, pH 9.3

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 100 mL:

1. In a 100 mL volumetric, dissolve 3.81 g Sodium Borate in 90 mL water.
2. Add appropriate amount of distilled water to final volume of 100 mL.
3. Store in a suitable container.

**NOTE:** Solution expires six months from date of preparation.

61.) 30% Methanol in Ethyl Acetate

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mL:

1. To 30 mL methanol, add 70 mL ethyl acetate.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass container.

62.) 20% Methanol in Ethyl Acetate

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mL:

1. To 20 mL methanol, add 80 mL ethyl acetate.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass container.

63.) Ammonium Acetate Triethylamine Buffer, pH 5.2

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

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For 1L:

1. In appropriate glassware add 3.85g ammonium acetate.
2. Add 2 mL glacial acetic acid.
3. Add 2 mL triethylamine.
4. Dilute to 1000 mL with DI water.
5. Mix well. Refrigerate upon completion.

**NOTE:** Buffer will be valid for 6 months from the date of preparation.

64.) 50:50 Acetone:Chloroform

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mL:

1. Add 250 mL of Acetone to 250 mL of chloroform.
2. Mix well.
3. Upon completion, store in a tightly sealed container at room temperature.

65.) 15:85 Methanol:Chloroform

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mL

1. Add 75mL of Methanol to 425 mL of chloroform.
2. Mix well.
3. Upon completion, store in a tightly sealed container at room temperature.

66.) 62:38 Methanol:Ammonium Acetate Triethylamine Buffer

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 500 mL:

1. In appropriate glassware add 310 mL Methanol.
2. Add 190 mL Ammonium Acetate Triethylamine Buffer.
3. Mix well.

**NOTE:** Solution will be valid for 6 months from the date of preparation. Unused portion should be refrigerated.

67.) 1.0M Ammonium Formate Stock Solution

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1L:

1. Dissolve 63 g of anhydrous Ammonium formate in approximately 950 mL of DI water.
2. Mix well.
3. Dilute to a final volume of 1000 mL with de-ionized water.
4. Refrigerate upon completion.

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68.) 20mM Ammonium Formate Buffer

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 2 L:

1. Add 40 ml of 1.0 M ammonium formate to approximately 1900 mL of DI water.
2. Adjust pH of buffer to approximately 3 with addition of 4 ml of formic acid.
3. Dilute to a final volume of 2000 mL with de-ionized water.

69.) Mixed Volatile Check Mix

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mLs:

1. To approximately 80 mLs of DI water in a 100 mL volumetric flask add 32  $\mu$ L of acetaldehyde, 63  $\mu$ L of methanol, 63  $\mu$ L of acetone, 64  $\mu$ L of isopropanol, and 63  $\mu$ L of ethanol.
2. Add DI water for a total of 100 mLs.
3. Mix thoroughly.
4. Refrigerate upon completion.

70.) 0.1% v/v Formic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 L:

1. Add 1130  $\mu$ L of formic acid to approximately 900 mL of DI water.
2. Dilute to a final volume of 1000 mL with DI water.

**NOTE:** Store solution at room temperature in glass container.

**NOTE:** This solution can be kept and used up to 1 year from date of preparation.

**NOTE:** When preparing acid solutions from concentrated acid, one should always add concentrated acid to water.

71.) 9:1 Ethyl Acetate:Methanol

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

To prepare 100 mL:

1. To 10 mL methanol, add 90 mL ethyl acetate.
2. Mix thoroughly.

**NOTE:** Store solution at room temperature in glass container.

72.) 75:25 Methanol:0.1% v/v Formic Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 L:

Add 75 mL Methanol to 25 mL 0.1% v/v formic acid.

**NOTE:** Store solution at room temperature in glass container.

**NOTE:** This solution can be kept and used up to 1 year from date of preparation.

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73.) 0.1% v/v Hydrochloric Acid in Methanol

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 10 mls:

Add 10  $\mu$ L concentrated HCL to 10 mL Methanol. Mix well.

74.) 1M Sodium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mls:

Add 10 g Sodium Hydroxide to a 250 mL volumetric flask. Dilute to final volume of 250 mL with DI water. Mix well.

75.) 1 M Hydrochloric Acid

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 1 liter:

1. To a suitable container, add approximately 800 mL of DI water.
2. Slowly add 84 mL concentrated HCl.
3. Add appropriate amount of DI water to final volume of 1 liter.

76.) 0.25M Sodium Hydroxide

**NOTE:** Other volumes may be prepared by adjusting amounts accordingly.

For 250 mls:

Add 2.5 g Sodium Hydroxide to a 250 mL volumetric flask. Dilute to final volume of 250 mL with DI Water. Mix well.

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**APPROVED ABBREVIATIONS**

**PRINCIPLE:**

This section lists the abbreviations and their definitions that are approved for use on notes, worksheets and forms in the Toxicology department.

**PROCEDURE:**

The following abbreviations may be used to signify the corresponding translation on Toxicology documentation:

| <b>Translation:</b>                               | <b>Abbreviation:</b>  |
|---------------------------------------------------|-----------------------|
| 2-Ethyl-5-methyl-3,3-diphenylpyrroline            | emdp                  |
| 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine | eddp                  |
| Absorbance                                        | abs                   |
| Acetaminophen                                     | aceta, acetamin, apap |
| Acetonitrile                                      | acn                   |
| Acid and Base Fractions                           | oh-/h+, h+/oh-        |
| Acidic Fraction                                   | h+                    |
| Add Diluent to Reach Volume                       | qs                    |
| Administrative                                    | admin                 |
| Admission                                         | admiss, adm           |
| Alprazolam                                        | alpraz                |
| Amitriptyline                                     | amitrip               |
| Amount                                            | amt                   |
| Amphetamine                                       | amp, amphet           |
| Antidepressants                                   | ad                    |
| Antipsychotic                                     | anti-psy              |
| Antiseizure Anticonvulsant                        | asac                  |
| Aortic                                            | ao                    |
| Autopsy                                           | aut                   |
| Barbiturate                                       | barb                  |
| Basic Fraction                                    | oh-                   |
| Benzodiazepine                                    | benz, benzo           |
| Benzoyllecgonine                                  | be, bze               |
| Beta-Glucuronidase                                | bglu                  |
| Bicyclist                                         | bicyc                 |
| Bile                                              | bi                    |

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|                                       |                             |
|---------------------------------------|-----------------------------|
| Bis(trimethylsilyl)trifluoroacetamide | bstfa                       |
| Blood                                 | b, bl                       |
| Blood Alcohol                         | ba                          |
| Blood Alcohol Concentration           | bac                         |
| Blood Drug Screen (ELISA)             | bdes                        |
| Blood Drug Screen (FPIA)              | bds                         |
| Blood Drug Screen Quantitation FPIA   | bqds                        |
| Blood/Urine Collection Kit            | b/u kit, b/u coll kit, buck |
| Blue Top                              | bt                          |
| Boating Under the Influence           | bui                         |
| Bottle                                | bot                         |
| Brain                                 | br                          |
| Buprenorphine                         | bup                         |
| Butalbital                            | butal                       |
| Calculate                             | calc                        |
| Calibrator                            | cal, calib                  |
| Cannabinoids                          | cannab, cannabs             |
| Carbon Monoxide                       | co                          |
| Carboxy                               | -cooh                       |
| Carboxyhemoglobin                     | cohb                        |
| Carboxytetrahydrocannabinol           | cooh-thc, the-cooh          |
| Carisoprodol                          | cari                        |
| Check Mix                             | ck mix, √ mix               |
| Child Fatality                        | cf                          |
| Clonazepam                            | clonaz                      |
| Clotted                               | cl                          |
| Cocaine                               | coc                         |
| Collection                            | coll, collect               |
| Concentration                         | c, conc, [ ]                |
| Confirmation                          | conf, confirm               |
| Container                             | cont, cnt                   |
| Contents                              | conts                       |
| Control                               | ctrl, cntl                  |
| Co-oximeter                           | co-ox                       |
| Coroner                               | cor                         |
| Criminal Sexual Conduct               | csc                         |
| Cyclobenzaprine                       | cyclobenz                   |
| Data Acceptance Worksheet             | DAW                         |

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|                                       |                    |
|---------------------------------------|--------------------|
| Decomposition                         | decomp             |
| Deionized Water                       | di h2o, di water   |
| Derivatize(-ing)                      | der, deriv, derive |
| Deuterated                            | d                  |
| Diazepam                              | diaz               |
| Diethyltoluamide                      | deet               |
| Difference                            | dif, diff          |
| Driving Under the Influence           | dui                |
| Driving Under the Influence Blood     | dui-b              |
| Driving Under the Influence Urine     | dui-u              |
| Drug Screen                           | ds                 |
| Embalmed                              | emb                |
| Embalming Fluid                       | embfl              |
| Enzyme Linked Immunosorbant Assay     | ELISA              |
| Ephedrine                             | eph, ephed         |
| Ethanol                               | etoh               |
| Ethyl Acetate                         | etoac, etac        |
| Evidence                              | ev                 |
| Evidence Bag                          | ebag               |
| Expiration                            | exp                |
| Extended                              | ext                |
| Felony Boating Under the Influence    | fbui               |
| Felony DUI                            | fdui               |
| Fluid                                 | fl                 |
| Fluorescence Polarization Immunoassay | FPIA               |
| Flying Under the Influence            | fui                |
| Freedom of Information                | foi                |
| Gamma-butyrolactone                   | gbl                |
| Gamma-hydroxybutyrate                 | ghb                |
| Gas Chromatography                    | GC                 |
| Gastric                               | gast               |
| General Screen                        | gen                |
| Gold Top                              | gold t             |
| Gray Top                              | gt                 |
| Green Top                             | grn t              |
| Gun Shot                              | gs                 |
| Gun Shot Wound                        | gsw                |
| Half Life                             | t 1/2              |

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|                                     |                   |
|-------------------------------------|-------------------|
| Headspace                           | hs                |
| Heart                               | ht                |
| Heart Related                       | hr                |
| Heat Sealed Pouch                   | hsp               |
| Hemoglobin                          | hb                |
| Heptafluorobutyric acid             | hfba              |
| Hexobarbital                        | hexo, hexobarb    |
| Hospital                            | hosp              |
| Hydroxy                             | -oh               |
| Hydroxytetrahydrocannabinol         | oh-thc, thc-oh    |
| Iliac                               | il                |
| Iliac Vein Blood                    | ivb               |
| Include                             | incl              |
| Inferior Vena Cava Blood            | ivcb              |
| Instrument                          | inst, instru      |
| Insufficient                        | ins, insuff       |
| Internal Standard                   | is, istd, int std |
| Isopropanol                         | ipa               |
| Kidney                              | k, kid            |
| Lavender                            | lav               |
| Lavender Top                        | lav t             |
| Left                                | l                 |
| Left Ventricle Blood                | lvb               |
| Library                             | lib               |
| Light                               | lt                |
| Limit of Detection                  | lod               |
| Limit of Quantitation               | loq               |
| Liquid                              | liq               |
| Liquid Chromatography               | lc                |
| Liver                               | li, liv           |
| Lysergic Acid Diethylamide          | lsd               |
| Manual                              | man               |
| Manufacturer                        | manufac           |
| Mass Spectrometry                   | ms                |
| Mass Spectrometry Mass Spectrometry | MSMS, MS/MS       |
| Matrix Blank                        | mb                |
| m-Chlorophenylpiperazine            | m-cpp             |
| Metabolite                          | metab             |

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|                                      |                        |
|--------------------------------------|------------------------|
| Methamphetamine                      | ma, met, meth, methamp |
| Methanol                             | meoh                   |
| Methemoglobin                        | methb                  |
| Methylenedioxyamphetamine            | mda                    |
| Methylenedioxymethamphetamine        | mdma                   |
| Midazolam                            | midaz                  |
| Molecular                            | mol, molec             |
| Monoacetylmorphine                   | mam                    |
| Monoamine oxidase                    | mao                    |
| Monoamine oxidase inhibitors         | maoi                   |
| Monoethylglycinexylidide             | megx                   |
| Motorcyclist                         | motorcyc               |
| Natural Death                        | nat d, nat death       |
| N-Benzylpiperazine                   | bzp                    |
| Negative                             | neg, "-", ø            |
| No Analysis Performed                | nap                    |
| Non-Steroidal Anti-Inflammatory Drug | nsaid                  |
| Nordiazepam                          | nordiaz                |
| Not Detected                         | nd                     |
| Ocular                               | o, oc                  |
| Ocular Alcohol                       | oa                     |
| O-desmethyltramadol                  | odt                    |
| O-desmethylvenlafaxine               | odv                    |
| Officer                              | off                    |
| Opiate                               | opi, opt               |
| Opioid                               | opd                    |
| Orange Top                           | ot                     |
| Oxycodone                            | oxy                    |
| Oxyhemoglobin                        | o2hb                   |
| Panel                                | p                      |
| Panel Negative                       | pn                     |
| Passenger                            | pass                   |
| Pedestrian                           | ped, pedes             |
| Peripheral                           | per                    |
| Phencyclidine                        | pcp                    |
| Phenobarbital                        | pheno, phenobarb       |
| Phenylpropanolamine                  | ppa                    |
| Phenytoin                            | pheny                  |
| Pink Top                             | pink t                 |
| p-Methoxyamphetamine                 | pma                    |

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|                                                        |                 |
|--------------------------------------------------------|-----------------|
| p-Methoxymethamphetamine                               | pmma            |
| Positive                                               | pos, "+"        |
| Positive Pressure Manifold                             | PPM             |
| Prepared                                               | prep            |
| Propoxyphene                                           | propox, ppox    |
| Pseudoephedrine                                        | pse, pseudo     |
| Purple Top                                             | pt, pur top     |
| Qualitative                                            | qual            |
| Quality Control                                        | qc              |
| Quantitative                                           | quant           |
| Red Top Tube                                           | rtt             |
| Refrigerator                                           | refrig          |
| Relative                                               | rel             |
| Relative Retention Time                                | rrt             |
| Request                                                | req             |
| Retention                                              | ret             |
| Retention Time                                         | rt              |
| Revalidated                                            | reval           |
| Right                                                  | r               |
| Robot                                                  | rob             |
| Salicylates                                            | sal, salic      |
| Sample                                                 | samp            |
| Selective Serotonin Norepinephrine Reuptake Inhibitors | ssnri           |
| Selective Serotonin Reuptake Inhibitors                | ssri            |
| Separator                                              | sep             |
| Serotonin Norepinephrine Reuptake Inhibitors           | snri            |
| Serum                                                  | s, ser          |
| Serum Separator                                        | ssep            |
| Solid                                                  | sol             |
| Spectrometry                                           | spec            |
| Standard                                               | std             |
| Subclavian                                             | subclv, subclav |
| Subject                                                | sub, subj       |
| Sulfhemoglobin                                         | sulfhb          |
| Sympathomimetic Amines                                 | sma             |
| Table of Contents                                      | toc             |
| Technical                                              | tech            |
| Temperature                                            | temp            |
| Tetrahydrocannabinol                                   | thc             |
| Threshold                                              | thres, thresh   |
| Tiger Top                                              | tt              |
| Tissue                                                 | tiss            |
| Total Ion Chromatogram                                 | tic             |

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|                             |               |
|-----------------------------|---------------|
| Toxicology                  | tox           |
| Traffic Fatality            | tf            |
| Tramadol                    | tram          |
| Tricyclic Antidepressants   | tca           |
| Trimethylsilyl              | tms           |
| Trimethylsilyl (x2)         | ditms         |
| Tube or Test Tube           | t             |
| Underivatized               | under         |
| Urine                       | u, ur         |
| Urine Alcohol               | ua            |
| Urine Alcohol Concentration | uac           |
| Urine Drug Screen           | udrs          |
| Valproic Acid               | val, valproic |
| Victim                      | v, vic, vict  |
| Vitreous                    | vit           |
| Volatile                    | vol           |
| Volume of Distribution      | vd            |
| Weight                      | wt            |
| Whole Blood                 | wb            |
| World Wide Monitoring       | wwm           |
| Yellow Tiger Top            | ytt           |
| Yellow Top                  | yt            |
| Zolpidem                    | zol           |

ABBREVIATIONS

|                                 |                 |
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## **TOXICOLOGY TRAINING MANUAL**

### Training Objectives

- The purpose of this manual is to define the training program for technicians and toxicologists of the Toxicology Department of the South Carolina Law Enforcement Division. This training manual is to serve as a minimum standard of competency for members of the department.
- Technicians will be defined as laboratory personnel responsible for Level I analyses, evidence inventory, and all other laboratory duties as assigned. Technicians will not be responsible for interpretation of results.
- Toxicologists will be defined as laboratory personnel responsible for Level II analyses and report writing and interpretation.
- This manual is divided into modules that represent various aspects of toxicology. Each module consists of general outlines of a topic, suggested reference material, training time guidelines, study questions and modes of evaluation.
- Each trainee is responsible for the modules that pertain to his or her actual work duties. For example, a technician is not responsible for extractions whereas a toxicologist must complete the specific module. Also, it is not necessary to follow the exact sequence in which these modules are listed.
- All trainees must become familiar with SLED policies, safety manual and ISO 17025 compliant laboratory policies.

### Administration

- Toxicology supervisor will assign a training officer to each trainee.
- The trainee must be available for periodic meetings with the training officer or supervisor to discuss his/her training progress.
- The training officer should be prepared to provide periodic written and/or oral training updates to the supervisor when requested.
- The supervisor will assess the progress of each trainee and determine the pass/fail status of each module, with the exception of written tests. A minimum score of 80% must be attained in order to pass. If a trainee fails to pass any evaluation portion, the trainee will be allowed ample time for remedial training, not to exceed 1 month, before retaking the evaluation.

### Guidelines

- A trainee is required to complete each module of training in order to be deemed competent to perform work in a particular area.
- The modules of training do not have to be completed in any particular order.
- If an employee cannot successfully complete a module, then he or she must remain in training for that module for an additional amount of time, to be determined by the training officer and the

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supervisor. At the end of the time allotment, the trainee will be required to successfully complete the area of the module that he or she could not previously.

- If a new analyst to the Toxicology Laboratory has previous experience, the training module for appropriate categories may be modified to meet the level of training necessary for the analyst to be deemed competent. The competency/evaluation portion of the training module will remain the same.
- If a current analyst must undergo remedial training as a part of Corrective Action Procedures, the training manual specific to the area of testing in question will be used or modified. Because each corrective action is different, the requirements will be different in order to satisfy the corrective action. Requirements may include, but are not limited to:
  - Retraining on a specific module of the Training Manual to include all requirements
  - Retraining on a specific module of the Training Manual, modified to fit the Corrective Action.
  - Successful completion of a Competency Evaluation, to include a written report that may have to be orally defended.

## MODULES

- [Safety](#)
- [Evidence Handling](#)
- [Balance Operation](#)
- [Pipette Operation](#)
- [Preparing Solutions and Buffers](#)
- [Immunoassay Screening](#)
- [Extractions](#)
- [Robotics](#)
- [Gas Chromatography – Mass Spectrometry](#)
- [Liquid Chromatography – Mass Spectrometry/Mass Spectrometry](#)
- [Quantitative/Qualitative Mass Spectral Analysis](#)
- [Lab Information Management System](#)
- [Pharmacology](#)
- [Mathematics and Statistics](#)
- [Courtroom Preparation and Testimony](#)
- [Volatile Analysis](#)
- [Carbon Monoxide Analysis](#)
- [Spot Tests](#)
- [Data Review and Case Examination](#)

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**SAFETY**

**OBJECTIVES:**

- The employee should complete the New Employee Laboratory Safety Checklist to include reviewing the following required reading/viewing:
  - [Forensics Laboratory Chemical Hygiene Plan and Safety Manual](#)
  - Required Safety Postings on the 1st Floor Communication Board
  - Safety Videos on:
    - Ergonomics, Bloodborne Pathogens, Office Safety, Hazard Communication, Others as appropriate and specified by the Safety Officer.
  - [SLED Bloodborne Pathogen Exposure Control Plan](#)
  - [SLED Fire and Safety Plan](#)
  - [Forensics Laboratory Written Hazard Communication Program](#)
- SLED Exposure – Injury Procedures
- Be aware of Hepatitis B vaccination program
- The trainee should be able to:
  - Explain the handling, storage and disposal of biological materials.
  - Be made aware of safe transport of large solvent containers (4L transport vessels).
  - Demonstrate and understand the importance of daily use of essential laboratory safety equipment.
  - Possess a working knowledge of and demonstrate the proper emergency shutdown procedure for electrical equipment and outlets in the laboratory.
  - Understand how to interpret SDS sheets associated with the Forensic Toxicology Department and know the location of departmental SDS sheets.
  - Become familiar with all emergency safety equipment in the Forensic Toxicology Department (fire extinguishers, eyewash stations, showers, fire blankets, etc.).
  - Become familiar with the location of all safety related bulletin boards and emergency evacuation routes, first aid kits, location of emergency Numbers.
  - Demonstrate and explain the proper use of chemical and biological hoods and their limitations.

**ESTIMATED TIME:**

Estimated time for this module is 3 days.

**STUDY QUESTIONS:**

What should you do if a large bottle of organic solvent is dropped and broken?

What are the dangers of derivatizing agents and how should these be handled?

Where are the fire extinguishers located in the department?

What PPE should be worn daily?

Are their locations in the laboratory where additional PPE is required?

How does the biological laminar flow hood protect?

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**EVALUATION:**

Trainee will be tested with completion of a written test.

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**EVIDENCE HANDLING**

**OBJECTIVES:**

- The trainee must describe the procedures followed for the intake of toxicology evidence at Evidence Control and transfer to the Toxicology Department.
- The trainee must describe the flow of evidence within the Toxicology Department from start to finish of analysis.
- The trainee must understand chain of custody, as it pertains to LIMS and evidence integrity.
- The trainee must be familiar with storage parameters of biological samples.
- The trainee must be familiar with the associated risks and dangers associated with handling biological samples.
- The trainee must be familiar with evidence intake and inventory. At a minimum, the trainee should spend 5 days assisting with conducting inventory of evidence. The trainee should receive and inventory at least 20 death investigation cases and at least 20 DUI/FDUI or police investigation cases. List should be maintained for training file.

**ESTIMATED TIME:**

Estimated time for this module is 4 weeks.

**REQUIRED READING:**

- SLED Evidence Submission Manual
- [SLED Forensic Services Laboratory Quality Manual](#)
- Toxicology Quality Manual
- Section 17-28-300 of the SC Code of Laws

**STUDY QUESTIONS:**

Describe the procedural steps involving evidence from receiving to final disposition.

Define the following terms: chain of custody, evidence transfer, tamper evident packaging, LIMS, SLED Laboratory case number, Item number.

Who has access to the Evidence Control Evidence Room? Toxicology Department and Department Refrigerators?

Who has access to your work area?

What actions are taken to ensure the proper preservation of evidence?

At what temperature(s) are biological specimens stored?

How is evidence sealed?

Describe the steps of proper evidence inventory.

How are biological samples handled that are differentiated based on collection site or collection time?

Describe the process to send a sample of blood from a Toxicology case to DNA for testing.

Define a proper seal.

What should happen if evidence is received and the seal is compromised?

What should happen if evidence is received and the specimen container is leaking?

Describe the disposition of evidence following toxicological analysis.

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When is evidence returned to the submitting agency?

**EVALUATION:**

Trainee will be tested with completion of a practical evaluation.

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**BALANCE OPERATION**

**OBJECTIVES:**

- The trainee must be familiar with the operation of any analytical balance used to prepare solutions or reference material for the Toxicology Department.
- The trainee must be familiar with maintenance, validation and record keeping of balances.
- The trainee must be familiar with the concept of traceability.
- The trainee must be able to demonstrate proper weighing practices.

**ESTIMATED TIME:**

Estimated time for this module is 1 day.

**REQUIRED READING:**

- ASCLD/LAB Policy on Measurement Traceability (Available Online)
- Toxicology Protocol on Balance Verification

**RECOMMENDED READING:**

- User manual for various balances  
Example: [Mettler Toledo XS Analytical Balances Operating Instructions](http://us.mt.com/dam/product_organizations/laboratory_weighing/WEIGHING_SOLUTIONS/PRODUCTS/XS/MANUALS/en/XS-Analytical_OI_en_11781099A.pdf) - HYPERLINK  
"http://us.mt.com/dam/product\_organizations/laboratory\_weighing/WEIGHING\_SOLUTIONS/PRODUCTS/XS/MANUALS/en/XS-Analytical\_OI\_en\_11781099A.pdf"  
[http://us.mt.com/dam/product\\_organizations/laboratory\\_weighing/WEIGHING\\_SOLUTIONS/PRODUCTS/XS/MANUALS/en/XS-Analytical\\_OI\\_en\\_11781099A.pdf](http://us.mt.com/dam/product_organizations/laboratory_weighing/WEIGHING_SOLUTIONS/PRODUCTS/XS/MANUALS/en/XS-Analytical_OI_en_11781099A.pdf)

**STUDY QUESTIONS:**

What does NIST traceable mean?

How often must balances be calibrated?

What is the proper way to handle weights used for balance verifications?

How often are balance verifications performed and how are they documented?

**EVALUATION:**

Trainee will be tested with a oral evaluation as part of their pipette and buffers training.

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**PIPETTE OPERATION**

**OBJECTIVES:**

- The trainee must be familiar with the operation of various manual and automated pipettes used in the toxicology laboratory.
- The trainee must be familiar with the maintenance and validation of pipettes used in the toxicology laboratory.
- The trainee must show proficiency in both accuracy and precision in making aliquots of various matrices.

**ESTIMATED TIME:**

Estimated time for this module is 3 days.

**REQUIRED READING:**

- [User manual for Microlab 500/600](#)  
([http://www.hamiltoncompany.com/downloads/Microlab\\_600\\_Basic\\_Technical\\_Manual\\_RevF.pdf](http://www.hamiltoncompany.com/downloads/Microlab_600_Basic_Technical_Manual_RevF.pdf))
- [User manual for Eppendorf Reference](#) ([www.eppendorfna.com](http://www.eppendorfna.com))
- Toxicology Protocol on Pipette Verification
- Toxicology Protocol on MLA Pipette Maintenance

**STUDY QUESTIONS:**

What is the allowable variance for pipette validation?

What liquid is used for gravimetric pipette checks?

How often are pipettes calibrated?

What levels need to be checked when verifying pipettes? How many replicates?

How do you know when a calibration expires?

Where are calibration certificates stored?

**EVALUATION:**

Trainee will be tested with a practical evaluation.

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**PREPARING SOLUTIONS AND BUFFERS**

**OBJECTIVES:**

- The trainee must be able to define the following terms and concepts:
  - Molarity
  - Normality
  - Percent weight/volume (%w/v)
  - Percent weight/weight (%w/w)
  - Buffers and solvents
- The trainee must become proficient in solution preparation and documentation.
- The trainee must become proficient in pH meter usage and documentation.

**ESTIMATED TIME:**

Estimated time for this module is 3 days.

**REQUIRED READING:**

- Toxicology Protocol on Corning 320 pH Meter
- Toxicology Quality Manual Section on Reagent Preparation and Reagent Verification

**RECOMMENDED READING:**

- User manual for pH meter

**STUDY QUESTIONS:**

What is a serial dilution?

What is the molarity of a solution containing 4.1g of anhydrous sodium acetate in 500mL of DI water?

Where is the reagent preparation log stored?

**EVALUATION:**

Trainee will be given a written test.

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**IMMUNOASSAY SCREENING**

**OBJECTIVES:**

- The trainee will understand and be able to explain immunoassay including the following terms:
  - Antigen
  - Antibody
  - Threshold
  - Cross-reactivity
- The trainee will be able to prepare biological samples for ELISA screening.
- The trainee will be able to perform manual and automated ELISA screening.
- The trainee will be able to implement and understand the quality control standards as described by the departmental QA program.
- The trainee will be able to perform routine maintenance and documentation of automated system.
- The trainee will be able to interpret screening results according to instrument printouts.
- The trainee will participate in the screening of 100 case samples.

**ESTIMATED TIME:**

Estimated time for this module is 4 weeks.

**REQUIRED READING:**

- Levine, Barry. *Principles of Forensic Toxicology*.
- Manufacturer test kit inserts for various test kits
- Toxicology Protocol on ELISA

**STUDY QUESTIONS:**

Define the role of the antibody/antigen relationship in immunoassay.

What are the limitations of quantitation using ELISA?

Define the term “threshold”.

Which panels are run for each case type?

What goes into a quality control pack?

Define False Positive and False Negative?

Name the chemical compound that is the primary target of the antibody in each of the ELISA assays.

Find the cross-reactivity of Alprazolam for the Benzodiazepine Panel.

Explain B/B<sub>0</sub>. How is it calculated?

Explain the relationship between absorbance and the concentration of the drug being determined.

What is the practice when one screen of the duplicate tests is positive and one is negative?

**EVALUATION:**

Trainee will be given a written test and must complete a practical evaluation and mock court.

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**EXTRACTIONS**

**OBJECTIVES:**

- The trainee will become familiar with sample preparation prior to extractions including the following:
  - Dilutions
  - Tissue homogenates
  - Protein precipitation
- The trainee will become familiar with the theory of liquid/liquid extractions including the following:
  - Partition coefficient
  - Henderson-Hasselbach equation
  - Acid/Base/Neutral extractions
  - Back extraction/clean up
- The trainee will become familiar with the theory of solid phase extractions including the following:
  - Normal phase, reverse phase, ion exchange and mix mode
  - Extraction column prep, washes and elutions
- The trainee will be able to perform multiple types of extractions.

**ESTIMATED TIME:**

Estimated time for this module is 4 weeks.

**RECOMMENDED READINGS:**

- [Current Applications manual from United Chemical Technologies](#)
- Levine, Barry. *Principles of Forensic Toxicology*.
- Juhascik, M. and Jenkins, A. "Comparison of Liquid/Liquid and Solid Phase Extraction for Alkaline Drugs" *Journal of Chromatographic Science* 47 (2009): 553-557.

**REQUIRED READING:**

- Toxicology Protocol Manual

**STUDY QUESTIONS:**

At what pH will an acid with a pKa of 5.3 be 99% ionized?

If a slightly basic drug is treated with a strong acid, will it be partitioned into the aqueous or organic phase of a liquid/liquid extraction?

List and explain the typical steps of a SPE procedure.

**EVALUATION:**

Trainee will be given a written test and must complete a practical exam as part of the final competency evaluation.

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**ROBOTICS**

**OBJECTIVES:**

- The trainee must become familiar with the SOP for robotic extractions.
- The trainee must be familiar with daily and monthly maintenance and record keeping of robotic systems.
- The trainee must demonstrate a basic understanding of troubleshooting techniques for the robotic systems.

**ESTIMATED TIME:**

Estimated time for this module is 2 weeks.

**REQUIRED READING:**

- Toxicology Protocol Manual

**RECOMMENDED READING:**

- Zymark Zymate User Manual

**STUDY QUESTIONS:**

What sample preparation steps are required prior to placing samples on the Zymark robot?

Where are the monthly logs archived?

Where are Robot maintenance records stored?

**EVALUATION:**

Trainee will be observed preparing the robot for extraction as well as cleaning the robot after extraction.

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**GAS CHROMATOGRAPHY – MASS SPECTROMETRY**

**OBJECTIVES:**

- The trainee will become familiar with the basic principles of gas chromatography.
- The trainee will be able to define the following terms as they relate to GC:
  - Resolution
  - HETP and Van Deemter
  - Area Under Curve
  - Signal to Noise ratio
- The trainee will be able to perform and properly document basic maintenance to GC including:
  - GC column maintenance
  - GC inlet maintenance
  - Replace Syringes
- The trainee will be familiar with the basic principles of mass spectrometry.
- The trainee will be able to define the following terms as they relate to mass spectrometry including:
  - Ionization
  - Quadrupole
  - TIC
  - Mass to charge ratio
  - SIM
  - Derivatization
- The trainee will be able to perform and properly document basic maintenance to Mass Spectrometer including:
  - Clean source
  - Replace filaments
  - Vacuum pump maintenance
- The trainee must demonstrate a thorough understanding of the system's software, troubleshooting techniques, principles and applications of quantitative analysis.
  - Types of Columns
  - Define Base Peak, Molecular Ion
  - Acquiring and evaluating Mass Spectra
  - Use of libraries and databases

**ESTIMATED TIME:**

Estimated time for this module is 5 weeks.

**REQUIRED READING:**

- Toxicology Operations Manual
- Levine, Barry. *Principles of Forensic Toxicology*.

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**RECOMMENDED READINGS:**

- [Gas Chromatography – Mass Spectrometry](#)  
[http://courses.chem.indiana.edu/a315/documents/GCMSHandout\\_000.pdf](http://courses.chem.indiana.edu/a315/documents/GCMSHandout_000.pdf)

**STUDY QUESTIONS:**

What carrier gas is used by the toxicology lab for GC-MS analysis?  
Name three reasons why samples are derivatized.  
Why are different derivatizing agents used?  
What does BSTFA, HFBA stand for?  
Where are the libraries stored for the Toxicology instruments?  
With BSTFA, Which functional groups are derivatized in a compound?  
What is PFTBA?  
Explain how a tune is evaluated?  
How often are tunes performed?  
What voltage is applied to the filament?  
Explain LOD and LOQ in GC-MS analysis.  
Where are GCMS methods stored?  
How can you tell if the electronic method matches the printed method?  
How do you optimize a SIM method for the appropriate ions and retention time windows?  
What is an acceptable correlation coefficient for calibration curves?

**EVALUATION:**

Trainee will be given a written test and must complete a practical exam as part of the final competency evaluation.

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**LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY/MASS  
SPECTROMETRY**

**OBJECTIVES:**

- The trainee will become familiar with the basic principles of liquid chromatography.
- The trainee must be able to define the following terms as they relate to LC-MS/MS including:
  - Reverse phase
  - Capacity
  - Gradient
  - Ion suppression
  - MRM
- The trainee will become familiar with the SOP for instrumental operation.
- The trainee must demonstrate a thorough understanding of the system's software, troubleshooting techniques, principles and applications of quantitative analysis.
- The trainee will be able to implement and understand the quality control standards as described by the departmental QA program.

**ESTIMATED TIME:**

Estimated time for this module is 5 weeks.

**REQUIRED READING:**

- Toxicology Protocol Manual

**RECOMMENDED READING:**

- [LC-MS/MS user manual, Agilent](#)
- *Clarke's Analysis of Drugs and Poisons*

**STUDY QUESTIONS:**

How does changing the solvent gradient affect the retention time of a compound?

Explain the significance of ion suppression and internal standards.

What are some advantages and disadvantages of LC-MS compared to GC-MS?

When should an autotune or a check tune be performed?

**EVALUATION:**

Trainee will be given an oral exam.

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**QUANTITATIVE/QUALITATIVE MASS SPECTRAL ANALYSIS:**

**OBJECTIVES:**

- The trainee must possess a working knowledge and perform a qualitative batch.
- The trainee must possess a working knowledge and perform quantitation in SCAN and SIM mode.
- The trainee must understand how to prepare a Qualitative or Quantitative Quality Control Packet
- The trainee will be familiar with the following terminology:
  - Positive Controls
  - Negative Controls
  - Matrix Blanks
  - Solvent Blanks
  - Curve Fit
  - Curve Acceptance
  - Calibrators
  - Internal Standards

The trainee must understand the Measurement Uncertainty for each.

**ESTIMATED TIME:**

Estimated time for this module is 3 months.

**REQUIRED READING:**

- Toxicology Protocol Manual
- Toxicology Quality Manual

**STUDY QUESTIONS:**

What is the criterion for positive control acceptability?

What is the maximum number of samples bracketed by positive controls?

What goes into a Qualitative QC Packet?

What goes into a Quantitative QC Packet?

Where are QC Packets stored?

Explain the SOP for rejecting MS data as it pertains to ion ratios.

How do you select an internal standard?

What is an acceptable Relative Retention Time agreement to make a positive identification?

Explain the reporting Procedure for dilutions above and below the linear range of the calibration curve in SCAN and SIM mode.

**EVALUATION:**

Trainee will be given a practical evaluation as part of their final competency evaluation.

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**LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS)**

**OBJECTIVES:**

- The trainee will become familiar with the policies and procedures regarding LIMS.
- The trainee will be able to perform LIMS functions routinely used in the Toxicology Department. These functions include but are not limited to:
  - Search for cases based on case information
  - Identify important case information regarding case history
  - Transfer evidence between locations
  - Create items or sub-items
  - Enter results and generate reports
- The trainee will be able to operate the laboratory cameras in order to photo document evidence and link it to LIMS.

**ESTIMATED TIME:**

Estimated time for this module is 2 weeks.

**REQUIRED READING:**

- Forensic Technology Operations Manual
- BEAST User Guide (found on the BEAST Home Page)
- Toxicology Module (found on the BEAST Home Page)

**STUDY QUESTIONS:**

How do you subitem blood and urine from a single parent item?

How do you subitem multiple blood types from a single parent item?

Where are photographs stored?

What is the proper way to transfer evidence?

**EVALUATION:**

Trainee will be given a practical exam as part of their competency evaluations.

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**PHARMACOLOGY**

**OBJECTIVES:**

- The trainee will develop a working knowledge of the various classes of drugs encountered in toxicological analysis. Drugs of interest may fall into the following classes:
  - Cocaine
  - Opiates and Opioids
  - Benzodiazepines
  - Sympathomimetic amines
  - Cannabinoids
  - Antidepressants
  - Antihistamines
  - Antipsychotics
- The trainee will understand the difference in interpretation of drug concentrations in death investigation cases, DUI cases and CSC cases.
- The trainee will understand the basic principles of pharmacokinetics including:
  - Administration of drugs
  - Distribution of drugs throughout the body
  - Metabolic pathways of drugs
  - Elimination of drugs
- The trainee will be able to explain the pharmacodynamic effects of drugs at therapeutic, toxic and lethal levels and as they pertain to motor vehicle operation.
- The trainee will become familiar with potential drug interactions.
- The trainee will become familiar with postmortem redistribution.

**ESTIMATED TIME:**

Estimated time for this entire module is 12 months; however, trainees may become proficient in individual drug classes intermittently.

**RECOMMENDED READING:**

- Levine, Barry. *Principles of Forensic Toxicology*.
- Goodman and Gilman. *The Pharmacologic Basis of Therapeutics*.
- Garriott, James. *The Medicolegal Aspects of Alcohol*.
- Baselt Randall C. *Drug Effects on Psychomotor Performance*.
- "Drugs and Human Performance Fact Sheets" National Highway Safety Traffic Administration, 2004
- "The Effects of Drugs on Human Performance Behavior" Forensic Science Review 14 Jan. 2002.

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**STUDY QUESTIONS:**

What are the common routes of administration of cocaine?

What are the main metabolites of cocaine and their respective half-lives (t<sub>1/2</sub>)?

How does cocaine affect the CNS?

How does cocaine impair one's ability to operate a motor vehicle?

**EVALUATION:**

The trainee will be given a written test and an oral evaluation as part of the final competency evaluation.

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**MATHEMATICS AND STATISTICS**

**OBJECTIVES:**

- The employee should have a thorough understanding of the following:
  - Sources of error in quantitative analysis
  - Types of error
  - Mean and standard deviation
  - Confidence limits of the mean
  - Propagation of errors
  - Calibration graphs in instrumental quantitation
    - Linear regression
    - Weighted regression
    - Curvilinear regression
  - Method of standard additions
  - Calculation of concentration
  - Limits of detection
  - Limits of quantitation
  - Percent error
  - Percent difference
  - Calculations related to dilution of standards and preparation of controls and calibration samples
  - Significant figures
- The trainee should be able to:
  - Calculate percent difference of replicate samples
  - Calculate percent error for standards of known concentration
  - Describe for a non-scientist the process of instrument calibration (Internal Standard quantitation) and how quantitation is performed on unknown samples
  - Describe uncertainty of measurement such that a non-scientist understands that all measurements have a certain amount of uncertainty due to random and systematic error.
  - Describe methods for the calculation of measurement uncertainty used by this laboratory
  - Calculate the amount of drug stock solution required to prepare calibration samples of various concentrations.

**ESTIMATED TIME:**

Estimated time for this module is 14 days.

**SUGGESTED READING:**

Miller, James N. and Miller Jane C. *Statistics for Analytical Chemistry*.

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**STUDY QUESTIONS:**

To investigate the reproducibility of a method for the determination of selenium in foods, nine measurements were made on a single batch of brown rice with the following results:

| Sample | Selenium, $\mu\text{g/g}$ |
|--------|---------------------------|
| 1      | 0.07                      |
| 2      | 0.07                      |
| 3      | 0.08                      |
| 4      | 0.07                      |
| 5      | 0.07                      |
| 6      | 0.08                      |
| 7      | 0.08                      |
| 8      | 0.09                      |
| 9      | 0.08                      |

Calculate the mean, standard deviation and relative standard deviation of these results.

What is the percent error calculated for a 0.08 ethanol standard that has a result of 0.084 g/dL ethanol by HS/GC?

Calculate the percent difference for replicate samples that have readings of 0.1480 and 0.1720 g/dL ethanol?

What is the minimum acceptable  $r^2$  value for a non weighted linear regression calibration curve?

What is the minimum number of calibrators suggested for a quantitation performed using the method of standard addition?

Define precision and accuracy.

Can a method demonstrate good precision but poor accuracy?

What mathematical tool(s) are used to define imprecision and inaccuracy?

What is the allowable percent error for measurements using an Eppendorf 100-1000  $\mu\text{L}$  adjustable pipette?

**EVALUATION:**

Trainee will complete written study questions.

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**COURTROOM PREPARATION AND TESTIMONY**

**OBJECTIVES:**

- The trainee will learn the general courtroom procedures for all levels of court (civil, magistrate and general sessions) in South Carolina.
- The trainee will become familiar with courtroom etiquette.
- The trainee will properly prepare curriculum vitae and be able to answer voir dire questions.
- The trainee will understand the implication of Daubert v. Merrell Dow Pharmaceutical, Frye v. United States and other significant court rulings as it pertains to expert witness testimony.
- The trainee will know how to prepare a litigation packet to take with him/her to court.
- The trainee will accompany a senior toxicologist to court and observe testimony on multiple subjects including alcohol and drugs.

**ESTIMATED TIME:**

Estimated time for this module is 3 weeks.

**RECOMMENDED READING:**

- Matson, Jack V. *Effective Expert Witnessing*.
- "Preparing the Expert Witness" SOFT 2011, San Francisco.
- Barnette, Barry. "Case Law on Driving Cases in South Carolina." Aug. 2013

**STUDY QUESTIONS:**

- What is to be included in a litigation packet?
- What is the definition of an expert witness?
- Which court takes precedent when multiple subpoenas exist for the same time?
- Are you allowed to give your opinion during testimony? If so, based on what?

**EVALUATION:**

The trainee will be tested in a mock court setting.

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**VOLATILE ANALYSIS**

**OBJECTIVES:**

- The trainee will become familiar with the theory and application of headspace gas chromatography and mass spectrometry as it relates to headspace analysis.
- The trainee will be able to prepare samples for analysis.
- The trainee will be able to operate the HS-GC and HS-GC/MS for quantitative and qualitative analysis of volatiles.
- The trainee should be trained in calibration of the Headspace GC/FID for Ethanol, Methanol, Acetone and Isopropanol.
- The trainee will be able to interpret results by examination and explanation of chromatograms.
- The trainee should understand the use of Internal Standards.
- The trainee should demonstrate proficiency by analyzing 500 previously analyzed specimens.
- The trainee should perform at least 5 analyses on beverage/moonshine type samples.
- The trainee should perform at least 5 analyses of volatiles other than ethanol. This can be from previous proficiency tests or prepared samples if necessary. Understand the Measurement Uncertainty and how it is calculated and explained in court.

**ESTIMATED TIME:**

Estimated time for this module is 2 months.

**LECTURES:**

- Chemistry and Instrumental Analysis
- Legal Issues
- Pharmacology and Interpretation
- Extended volatiles (Data processing)

**STUDY QUESTIONS:**

**Volatile Training Questions:**

How often are pipettes calibrated?

Why do we use Whole blood for our analysis?

What is in a gray top tube?

What is in a red top tube?

What is in a purple top tube?

How often are the instruments calibrated?

Describe how an FID works.

Describe Henry's Law.

Demonstrate how to calculate percent difference.

What is the associated error in the measurements?

How are samples positive for volatiles confirmed?

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**Volatile Interpretation Questions:**

Be able to describe impairment at different levels of BAC - typical observations and the continuum of effects. (Dubowski chart)

What is an acceptable average elimination rate for average individuals?

What would be an acceptable range of elimination rates?

Be able to calculate amount of Ethanol consumed based on Widmark Equation

What is an acceptable whole blood/serum conversion factor?

What is the Melanby Effect?

What is a typical "endogenous" ethanol range?

Why is Methanol Toxic?

What is appropriate treatment for methanol poisoning?

Describe the metabolites of Ethanol.

Where is most of the ingested Ethanol Absorbed?

How long does it take, post consumption, to reach peak BAC? (range)

What type of kinetic profile does alcohol elimination follow?

Why do we test vitreous humor in post mortem cases?

What effect would food have on peak BAC?

Is arterial or venous blood better for measuring BAC? Why?

In the post absorptive state, is arterial or venous blood higher in concentration? Why?

Show the difference between first order kinetics and zero order kinetics?

Describe Michaelis Menton kinetics.

What are drugs that are given to combat alcoholism and give a basic description for how they work?

What are the assumptions that must be made prior to performing retrograde extrapolation? Why?

**EVALUATION:**

Trainee will be tested by a series of written tests, a practical test and a mock court.

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**CARBON MONOXIDE ANALYSIS**

**OBJECTIVES:**

- The trainee will become familiar with the relationship between hemoglobin, oxygen and carbon monoxide.
- The trainee will be able to identify toxic and lethal carboxyhemoglobin levels.
- The trainee will be able to document and perform routine quality control for the AVOXimeter.
- The trainee will understand and be able to explain the operational theory of the AVOXimeter.
- The trainee will be able to analyze case samples on the AVOXimeter.

**ESTIMATED TIME:**

Estimated time for this module is 3 weeks.

**REQUIRED READING:**

- Toxicology Protocol Manual
- Toxicology Quality Manual

**RECOMMENDED READING:**

- [AVOXimeter 4000 user manual](#)
- Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*.

**STUDY QUESTIONS:**

What is the affinity of hemoglobin to carbon monoxide relative to oxygen?

How long are control standards viable after being opened for analysis?

**EVALUATION:**

Trainee will be given a written test accompanied by a practical examination.

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**SPOT TESTS**

**OBJECTIVES:**

- The trainee will become familiar with the advantages and limitations of spot tests.

The spot tests of interest include:

- Cyanide Colorimetric Determination
- Cyanide Semiquantitative Determination
- Cyanide and Hydrocyanic Acids
- Ferric Chloride
- FPN
- Fujiwara
- Hypochlorite Ion
- Kunkel
- Reinsch
- Trinder

**ESTIMATED TIME:**

Estimate time for this module is 3 weeks.

**RECOMMENDED READING:**

- Levine, Barry. *Principles of Forensic Toxicology*.
- Clarke's *Analytical Forensic Toxicology*.
- Jungreis, Ervin. *Spot Test Analysis*.
- Toxicology Operations Manual

**STUDY QUESTIONS:**

What compounds may be indicated by a positive Reinsch Test?

Chlorine bleach, an oxidizer, yields what color in the Diphenylamine test?

When should a Kunkel's Test be performed?

**EVALUATION:**

Trainee will be given a written test.

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**DATA REVIEW AND CASE EXAMINATION**

**OBJECTIVES:**

- The trainee will become familiar with the process and documentation involved in data review.
- The trainee will be able to accept or reject case analysis data including:
  - HS-GC-FID and HS-GC-MS
  - Immunoassay
  - GC-MS
  - GC-MS/MS
- The trainee will become familiar with the process and documentation involved with case examination.
- The trainee will understand the work flow for various types of toxicology cases including:
  - DUIB and DUIU
  - Natural and Pending Toxicology death investigations
  - Traffic Fatalities and Homicide and Suicides
  - Criminal Sexual Conduct cases

**ESTIMATED TIME:**

Estimated time for this module is 8 weeks.

**REQUIRED READING:**

- Toxicology Operations Manual

**RECOMMENDED READING:**

- Garriott, James C. *The Medicolegal Aspects of Alcohol*.
- Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*.
- Levine, Barry. *Principles of Forensic Toxicology*.

**STUDY QUESTIONS:**

Is a general required on a fire death?

At what point does analysis cease on a blood DUI?

Will a blood sample be analyzed if taken 24 hours after the incident? If so, under what circumstances?

Which drugs need to be quantitated in a pending toxicology case?

**EVALUATION:**

Trainee will be given an oral examination as part of final competency evaluation.

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**APPROVAL**

Department Supervisor:  
(Issuing Authority)



Dustin W. Smith

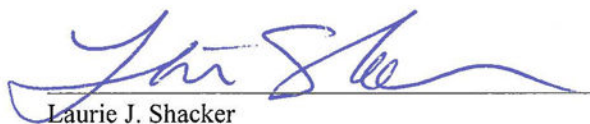
Date: 7/25/17

Technical Leader:

N/A

Date: N/A

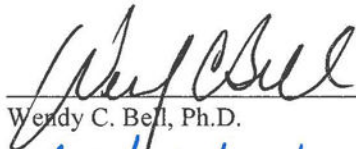
Quality Manager:



Laurie J. Shacker

Date: 8/22/17

Captain:



Wendy C. Bell, Ph.D.

Date: 8/10/17

Laboratory Director:



C. Todd Hughey, Ph.D.

Date: 8/25/17  
(Effective Date)

**APPROVAL**