Name of Procedure:

Toxicology
Operating and Safety Procedures for Use With The Seifert: Isovolt 320 DS-1 Industrial X-ray Unit

Suggested Uses:

This document addresses the rules and regulations applying to the Non-Healing Arts Application of irradiating bio-hazardous materials for the purpose of sterilization.

These instructions are provided to you so that we can comply with the state rules for radiation control. The North Carolina Division of Radiation Protection enforces the radiation rules in North Carolina. These rules require that our radiation machines meet specific requirements. The rules also require that certain procedures be followed and that certain records be kept. A copy of these rules is always available for you to read and review. It is entitled the North Carolina Regulations for Protection Against Radiation (NCRFPAR) and is located with the Radiation Safety Officer.

Items Needed to Perform Procedure:

Seifert: Isovolt 320 DS-1 Industrial X-ray Unit
Paste P8 (Identification # 9 018 32)
Water, 68 degrees Celsius or less.
Haskris Water Chiller / Recirculator
Radiation meter capable of measuring mR/hour

Instrument Maintenance:

1. Replace paste on the four voltage connector cones every three months as described in manual.

2. Change water as needed, or take necessary steps to prevent bacterial growth.

3. Perform annual radiation surveys and document results.

Application of Procedure on Evidence:

The following procedure has been registered with the North Carolina Division of Radiation Protection. To maintain the registration, the format of the submitted procedure has not been modified.

Literature References:

Operation Manual and Description - Fully Stabilized Industrial X-Ray Equipment - Isovolt 160/225/320/420/450 with control DS-1, Seifert and Co., 9/85.

North Carolina Regulations for Protection Against Radiation - 15A NCAC 11, NCDEHNR, July 1993.

"Inactivation of HIV by Ionizing Radiation in Body Fluids and Serological Evidence", **Journal of Forensic Sciences**, November 1989.

NORTH CAROLINA STATE BUREAU OF INVESTIGATION DRUG CHEMISTRY LAB: IRRADIATION OF BIO-HAZARDOUS MATERIALS

OPERATING AND SAFETY PROCEDURES FOR USE WITH THE SEIFERT: ISOVOLT 320 DS-1 INDUSTRIAL X-RAY UNIT

PURPOSE: This document addresses the rules and regulations applying to the Non-Healing Arts Application of irradiating bio-hazardous materials for the purpose of sterilization.

These instructions are provided to you so that we can comply with the state rules for radiation control. The North Carolina Division of Radiation Protection enforces the radiation rules in North Carolina. These rules require that our radiation machines meet specific requirements. The rules also require that certain procedures be followed and that certain records be kept. A copy of these rules is always available for employees to read and review. It is entitled the North Carolina Regulations for Protection Against Radiation (NCRFPAR) and is located with the Radiation Safety Officer.

The rules require that each X-ray facility be registered with the state. The Notification of Registration is located with the Radiation Safety Officer.

The intent of this document is to establish procedures to minimize radiation exposure of personnel. You, as an operator, are required to know the procedures and requirements in this manual and be able to demonstrate that you can use them properly. After reading this document and demonstrating that you can use the machine safely and correctly, you must sign and date the "Record for Individuals in Operating and Safety Procedure" located at the end of this document. The attached, "Safety and Procedural Guidelines in Industrial Applications", should be read by all x-ray unit operators.

All operators of x-ray machines are responsible for following the radiation safety procedures. Aaron Joncich is the radiation safety officer (RSO) for the North Carolina State Bureau of Investigation and has the responsibility and authority for overseeing matters relating to radiation protection. The RSO also confirms all training and serves as the contact person with the North Carolina Department of Environment, Health, And Natural Resources, which is responsible for regulating radiation safety. Employees should submit all radiation questions or concerns about radiation safety to the RSO.

There shall be posting of the State's "Standards for Protection Against Radiation Notices, Instruction and Reports to Workers, Inspections" located in areas where the x-ray unit operators and adjacent employees may read it. All areas where radiation may be emitted are to be posted as such, according to the NCRFPAR section .0411.

A copy of the operating procedures is to be posted near the x-ray equipment. A copy of the units most recent survey is to kept on the premises.

The general requirements for radiation safety and your rights and obligations as a radiation worker are found in NCRFPAR, Section .1600.

I. Emergency Procedure

If there is a need to turn off the unit in an emergency: TURN 'OFF' THE CIRCUIT BREAKER ON THE WEST WALL.

II. Excessive Exposure

If you suspect there has been an excessive exposure or a radiation incident, immediately notify Aaron Joncich, the RSO. The RSO will then notify the Division of Radiation Protection. The address is: Division of Radiation Protection, P.O. Box 27687, Raleigh, North Carolina, 27611-7687. The telephone number is 919-571-4141. The RSO will also notify the Assistant Director of the NCSBI Laboratory Section of the incident, in writing.

III. Personnel Monitoring

[Refer to the attached Regulatory Guide,"Appropriate Personnel Monitoring" for more information on personnel monitoring in the workplace.]

Always wear the personnel monitoring badge on your chest or collar when you are working with radiation producing equipment, and make sure it is the badge assigned to you.

When not in use, store badges in a low radiation area. The control badge shall also be stored in a radiation free area.
The RSO is responsible for the exposure records and exchanging the badges on a regular basis.

IV. Operation of the X-Ray Unit

The room in which the x-ray unit is located (Room #S430 at 121 East Tryon Rd, Raleigh, NC) is a restricted area at all times. Only authorized operators of the x-ray unit are allowed access to room # S430. Exceptions to the area restriction for maintenance, or other reasons, are to be authorized by the RSO. No pregnant women shall be allowed in Rm. S430.

Under no circumstances is anyone allowed to be in the shielded inner room inside Room S430 while the x-ray unit is in operation. No unauthorized operators shall be in the area of the instrument control panel at any time unless authorized by the RSO.

IMPORTANT: The x-ray unit is to be operated with the x-ray tube's circular window directed vertically down. Operation of the unit in any other direction may cause a radiation exposure risk.

Try to keep radiation exposure as low as possible. Never allow the lead shielded door to be open or any lead panels to be removed when operating the x-ray unit.

The x-ray equipment in this facility was installed following the manufacturer's specifications. Do not alter, tamper with, or remove any of the filters or collimator, shielding, or in any way cause needless radiation exposure.

V. Irradiation Procedures

Every activity involving the use of the x-ray unit will be logged in the activity log book for the x-ray unit. The log will include the date, time, operators initials, items irradiated, and system parameters used.

The following parameters have been determined to be effective for inactivation of the HIV virus, and will be used for the irradiation of contaminated materials.(Reference: Bigbee, et al., JFS, Nov. 1989)

RAD 25,000
Voltage 300 kV
Amperage 10 mA
Time 33 minutes

Distance 25.7 cm*

***This is the minimum distance a sample may be placed from the source of the x-rays. Increasing the distance two fold requires a four fold increase in the run time (e.g. at 51.4 cm the run time would be 132 minutes).**

Here are the calculations used to determine x-ray to sample distance and the time required to achieve the needed dose (based on measurements of the beam shape).

d = diameter (cm) of the top of the sample to be irradiated.
h = height (cm) of the sample.
X = distance (cm) from the x-ray focal point to the bottom of the sample. This is based on the approximate 20^{o} angle, from the normal, beam projection. (Subtract 12.2 cm to get the distance from the lens face to the bottom of the sample).

$$X = (d / 0.744) + h$$

T = Time (minutes) required to irradiate the sample with the correct dose.

$$T = X^2 / 25.7^2 * 33$$

A. Place evidence to be irradiated under the x-ray tube.
B. Exit room and close the door completely.
C. Turn on the water chiller / recirculator.
D. Turn on the unit's power.
   1. Turn on the power at the circuit breaker.
   2. Turn on the unit's power controller by turning the red knob on the front of the cabinet.
   3. Turn on the control unit using the on/off key-switch to the on position.
E. Select the warm up procedure according to the length of time the unit has been off: day
F. Enter the maximum kV to be used (i.e. 300kV).
G. Press start button to begin warm-up (monthly warm-up takes 30 minutes).
H. After the warm-up, use the keys to the right of the display (kV, mA, min) to enter the corresponding values.
I. Press the start button to begin the irradiation.

VI. System Security

The room that houses the system (Room S430) will be locked at all times. Access to Room S430 will be possessed by only those authorized to operate the x-ray unit and the Supervisor of the Drug Chemistry Section. Keys to the control box of the x-ray unit will be available to only those authorized to operate it. The door of the

inner chamber is connected to a fail-safe switch, which will not allow the operation of the unit if the inner door is open. There is a emergency-off button in the x-ray chamber. There is an audible alarm and a flashing light in the inner room, which are activated 10 seconds prior to the activation of the x-ray unit. There is an "x-ray on" light on the outside of the x-ray chamber.

VII. Inspections

Surveys of the x-ray unit's area must be performed annually, and after any maintenance that may affect the x-ray output or room shielding.

Appendix A

RECORD FOR INSTRUCTION OF INDIVIDUALS IN OPERATING AND SAFETY PROCEDURES FOR THE OPERATION OF THE SEIFERT: ISOVOLT 320 DS-1 INDUSTRIAL X-RAY UNIT

In accordance with NCRFPAR, these procedures have been made available to each individual who operates the x-ray equipment. I certify that each of the individuals listed has demonstrated to me, on the date indicated, that he/she is competent in these operating and safety procedures and can operate the x-ray equipment in a safe manner. This was demonstrated by operating the x-ray unit under my supervision.

_____ _____
RSO signature - Aaron Joncich date

Operator Statement:

I have read these procedures and agree to abide by them.

signature	date	RSO
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

CERTIFYING STATEMENT

These procedures have been developed to ensure safe radiological working conditions. Everyone must adhere to these procedures. Prior approval must be obtained for any deviation from these procedures.

In accordance with Rule .1603(c), the registrant shall annually review the radiation protection program content and implementation.

signature of registrant	date
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Name of Procedure:

Determination of Alcohol and Volatiles in Body Fluids and Other Dilute Solutions by Headspace Gas Chromatography.

Suggested Use:

To determine the alcohol concentration in human body fluids or other dilute solutions. May also be used to qualitatively determine other volatile compounds present in body fluid or dilute solutions.

Introduction:

Quantitative analysis by headspace gas chromatography is possible because of the relationship given by Henry's law, P/M = constant, which states that for dilute solutions the vapor pressure (P) of a volatile solute over the solution at a constant temperature is directly proportional to the mole fraction (M) of the solute. By utilizing two Gas Chromatograph columns with different retention indexes, the identification of analytes can be determined and verified. By using a series of calibration standards, the concentrations of ethanol, methanol, acetone, and isopropanol in body fluids and dilute solutions can be determined by headspace gas chromatography. Other volatiles may be identified by utilizing the retention times or relative retention times of standards chromatographed on both columns.

Apparatus and Chemicals Needed to Perform Procedure:

1: Headspace autosampler.
2: Gas chromatograph equipped with flame ionization detectors.
3: Restek BAC1 (Front) and BAC2 (Middle), 30m, 0.53 mm (i.d.) capillary columns.
4: Data Station.
5: Volumetric glassware:
Volumetric flasks - 100, 1000, and 2000 ml (TC) sizes.
Volumetric pipettes – 1, 5, 8, 10, 20, and 50 ml (TD) sizes.
6: Hamilton-Microlab 1000 plus, Hamilton 530B diluter/dispenser, or other appropriate liquid handler.
7: Headspace vials with sealing caps.
8: Crimper tool.
9: Ethanol, 200 proof USP Grade

10: n-Propanol
11: Isopropanol (IPA)
12: Acetone
13: Methanol
14: Helium gas
15: Hydrogen gas
16: Compressed Air
17: gas tight syringe

Formula for Preparing Reagents and Expiration Dates of Reagents:

1: Stock Solutions:

(A) Stock Calibrator Solution
Prepare a 1.000 gram/100 ml solution of ethanol, methanol, acetone, and isopropanol. Place the date on the container. Stock calibrator solution shall be refrigerated and prepared annually.

Example: Weigh out 10 g of ethanol, methanol, acetone, and isopropanol into a beaker. Decant each into a single 1000 ml volumetric flask. Dilute the flask to volume with deionized water.

(B) Stock Verification Solution - Prepare a 1.000 gram/100 ml solution of ethanol, methanol, acetone, and isopropanol. Place the date on the container. Stock verification solution shall be refrigerated and prepared annually.

Example: Weigh out 5 g of ethanol, methanol, acetone, and isopropanol into a beaker. Decant each into a single 500 mL volumetric flask. Dilute the flask to volume with deionized water.

2: Working Solutions:

(A) Calibrator Solutions - Prepare 0.010, 0.040, 0.080, 0.200, and 0.500 gram/100 ml calibrator solutions. Place the dates on the containers. Calibrator solutions shall be replaced every 3 months.

Example: Pipet 1, 4, 8, 20, and 50 ml respectively of the stock calibrator solution into 5 separate 100 ml volumetric flasks. Dilute each flask to volume with

deionized water.

(B) Internal Standard - Prepare a 0.050 gram/100 ml n-propanol internal standard solution. Place the date on the container. Internal standard solution shall be replaced every 3 months.

Example: weigh out 1 gram of n-propanol into a beaker. Decant into a 2000 ml volumetric flask. Dilute the flask to volume with deionized water.

(C) Verification Standard - Prepare a 0.100 gram/100 ml verification solution. Place the date on the container. Verification solution shall be replaced every 3 months.

Example: pipet 10 ml of stock verification solution into a 100-ml volumetric flask. Dilute the flask to volume.

(D) Commercial Verification Standard - An ethanol standard acquired from an outside commercial source with a concentration between 0.050 g/100 mL and 0.500 g/100 mL, inclusive.

Application of Procedure on Evidence and Quality Control Check:

1: Gas chromatograph set up:

Method Parameters for alcohol determinations:

Initial Column Temperature (40^{o}C) Isothermal
Injector Temperature (200^{o}C)
Detector Temperature (200^{o}C)
Column Flow 12.5 ml/min (constant flow). (Nominal)
Split 1:2
Run Time 4:00-5:00 minutes
Detector make up flow: 18 ml/min
Detector H2 flow: 30 ml/min
Detector Air Flow: 300 ml/min

Method parameters for determination of other volatiles:

A temperature programmed method may be used to determine qualitatively other volatiles by utilizing the retention times or relative retention times of standards chromatographed on both columns.

2: Headspace Autosampler set up (The following parameters are recommended starting parameters. Autosampler parameters may be adjusted to permit improved performance.):

Incubation Temperature - 70°C
Incubation Time – 6:00 minutes
Agitator Speed – set to appropriate value
Extraction 1
Default Runtime 4:00-5:00 minutes
Syringe heater temperature 70°C
Sample Volume 1000 ul
A syringe bakeout of at least 5 °C above the injection temperature and a gas flush of at least 1.5 minutes will be performed between each injection.

3: Data Station:

The peak retention time windows will be set to the peak retention time +/- (0.05 minutes + 0.5% of the peak retention time).

Report Writer

A custom report writer can be utilized in the method.

4: Allow reagents and blood to equilibrate to room temperature before analysis.

5: Calibration Step - Calibrate the gas chromatograph each time a new internal standard is prepared. With an appropriate liquid handler, deliver 0.20 ml of each of the calibrator solutions and 1.80 ml of the internal standard solution into separate headspace vials. Prepare each of the calibration solutions in duplicate. Chromatograph the calibration standards on the gas chromatograph to calibrate. The calibration curve will be fitted to a linear model with the origin included. The calibration curves for each analyte to be quantitated must show a correlation of determination (r^2) of 0.995 or greater.

The chromatographs produced from the calibration standards are reference materials and shall reviewed by another chemist and approved in the toxicology section object repository in FLAIRS with a file name beginning with "BACcal" and the date in yyyymmdd format with no space between them. (example: BACcal20080818).

6: Quality Control and Verification Step - After calibration, the verification standard followed by a single water blank, and the commercial verification standard followed by a single water blank are chromatographed to check the system for resolution and precision. With an appropriate liquid handler, deliver 1.80 ml of internal standard and 0.20 ml of, the verification standard, the commercial verification standard, and water, respectively into separate headspace vials. Prepare the verification standards in duplicate. Chromatograph and quantitate the verification standards. Any measured alcohol values exhibiting greater than 5% difference from targeted values will be rejected for that component. Due to its volatility, measured values of acetone that exhibit greater than 5% difference from the targeted value will be evaluated by the analyst. The water blanks must not show any identifiable amounts of methanol, ethanol, isopropanol, or acetone. This step shall be successfully completed before case analysis, and is applicable to cases injected within 24 hours of the injection of the first verification standard. The operator may run other verification standards or blanks, in addition to the ones described above, at other times to insure system performance. The chromatographs produced from the quality control samples are reference materials and shall reviewed by another chemist and approved in the toxicology section object repository of FLAIRS with a file name beginning with "BACver" and the date in yyyymmdd format with no space between them. (example: BACver20080818).

7: Blood Analysis - With an appropriate liquid handler, deliver 1.80 ml of internal standard and 0.20 ml of blood into a headspace vial. Prepare each blood sample in duplicate. Chromatograph each blood sample on each column.

8: Reporting - The alcohol or other volatile must be integrated at the expected retention time, or relative retention time, on both columns to be identified. The blood alcohol values shall be measured to the thousandths. The mean of the measured blood alcohol values is the blood alcohol concentration. Any blood alcohol values that exhibit greater than 5% difference as described by the equation; [({highest measured blood alcohol concentration minus lowest

measured blood alcohol concentration} divided by lowest measured blood alcohol concentration) multiplied by 100], shall be rerun. Blood samples where the highest measured alcohol value is 0.080 grams of alcohol or less per 100 milliliters of whole blood, do not need to be rerun if the other measured values are within 0.004 grams of alcohol per 100 milliliters of whole blood. For revocation reports, the blood alcohol concentration will be reported to the hundredths, and values between hundredths shall be reported to the lower hundredth. Blood alcohol concentrations less than 0.010 grams of alcohol per 100 milliliters of whole blood will be reported as zero. Clotted samples and samples where the analysts can only obtain a serum alcohol concentration or plasma alcohol concentration will be reported as an equivalent whole blood alcohol concentration by dividing the measured serum (plasma) alcohol concentration by 1.18 to compensate for the whole blood/serum (plasma) alcohol distribution ratio.

References:

1. James C. Garriott (Editor), **Medicolegal Aspects of Alcohol**, 3rd Ed., 1996.

2. Operation Manual(s) for the gas chromatograph.

3. Operation Manual(s) for the headspace autosampler.

4. Operation Manual(s) for the data system and applicable software.

Name of Procedure:

Toxicology
Use of the Hamilton - Microlab 1000 plus and Microlab 500 Liquid Handling Systems to Prepare Samples

Suggested Uses:

This procedure is designed to prepare most liquid samples for the analysis of some volatile substances (specifically low boiling point alcohols) by gas chromatography with headspace sampling. The Hamilton Microlab instruments may also be used for other sampling when necessary.

The use of an internal standard in gas chromatography can be done by delivering accurate amounts of the sample to be analyzed and of an internal standard. The use of an automated system for measuring and delivering liquids is comparable, if not better, in precision and accuracy to manual methods. The Hamilton - Microlab 1000 plus and Microlab 500 liquid handling systems improve speed and accuracy of preparing samples for analysis of volatile substances using headspace-gas chromatography methods.

Apparatus Needed to Perform Procedure:

Hamilton Microlab 1000 plus or Microlab 500 liquid handling system
Headspace sample vials
Magnetic seals
Hand crimper
Liquid waste container (suggested 250 mL beaker)
Kimwipes or equivalent
Volumetric flask
Reservoir for the diluent solution
Deionized water

Quality Control:

The Quality Control of the system is verified by use of known standards each time the system is utilized.

Instrument Set Up (for blood alcohol analyses):

A. Hamilton Microlab 1000 Plus

1. Start up:
   a. Turn power on. Switch is on the back of the unit at the bottom right. Selections on the display screen can be entered by pressing the "ENT/JA/YES" key. Different selections can be displayed or highlighted by pressing the "NEIN/NO" key. Values can be entered via the numeric keyboard.

2. Microlab 1000 plus program (If program has been entered skip this step):
   note - entries to be selected are in bold face type:
   a. Method? - **{enter method #}**
   b. *Function: - **Dil**
      - **Diluter**
   c. *Vol:
      Air gap? - **10 ul**
      Asp Automatic Air? - **yes**
      Sample Vol? - **200 ul**
      Diluent? - **ul**
      Diluent? - **1800 ul**
      Wash Vol? - **0 ul**
      Wash Step? - **1**
   d. *Syr:
      A - Syringe AD **2.5 ml**
      B - Syringe DAD **250 ul**
      Standard tubing - **yes**

   e. *Speed: (these are only suggested values)
      Asp sample **2** sec.
      Disp sample **2** sec.
      Asp diluent **4** sec.
      Disp diluent **3** sec.

3. Running the method:

   a. Select and enter "RUN".
   b. Choose method containing program.

c. Press enter to "zero" the syringes.
d. Place the diluent tubing, on the left of the Microlab 1000 plus, into the diluent solution.
e. Prime the system by pressing the "ENT" key at the prompt, "PRIME?". The system should be primed five times before use to clear the tubing of air and any residue. Once priming is done, press the "NO" key to continue.

B. Hamilton Microlab 500

1. Start up:
   a. Turn power on. Switch is on the front of the unit at the bottom right. Selections on the display screen can be entered by pressing the "Run/Stop" key. The arrow keys can be used to move from one data entry field to another. Values can be entered via the numeric keypad.

2. Microlab 500 program (If program has been entered skip this step):
   note - entries to be selected are in bold face type:
   a. Main Menu - **Create a new Method** or **Edit an existing Method**
      Select
      {Select Method Name}
      Select
   b. Method Title

Ratio	1: **9.0**
Dilution	1: **10.0**
Left Diluent Volume (uL):	**1800.0**
Right Air gap volume (uL):	**10.0**
Right Sample volume (uL):	**200.0**
Final Volume (uL):	**2000.0**
ACCEPT when complete	
Syringe Fill speed:	Left - **3**
Syringe Aspirate speed:	Right - **1**
Syringe dispense speed:	Left - **3** Right - **2**
Syringe Fill mode:	**AUTO**
Air gap mode:	**AUTO**
air gap delay:	**0.0**
ACCEPT when complete	
Wash volume (uL)	**0.0**
Left syringe fill speed	**4**

Left syringe dispense speed **4**
ACCEPT when complete
CONFIRM to Save Method, **ESCAPE** to cancel

3. Running the Method:

   a. From the main menu select Run an existing method.
   b. Highlight the appropriate method and press **SELECT**.
   c. Left syringe size (uL): 2500.0
   d. Right syringe size (uL): 250.0
   e. **CONFIRM**
   f. The system will purge.
   g. Place the left diluent tube in the diluent solution and prime the system.

Application of Procedure on Evidence:

1. Sample preparation using the Microlab 1000 plus:

   a. The following steps can be activated by two ways - press the "ENT" key or press the button on the wand.
   b. The sample to be analyzed is collected by placing the tubing, on the right side of the system, into the sample, and aspirating the sample by pressing the button.
   c. The outside of the tubing is wiped with a clean Kimwipe or equivalent to remove excess sample residue.
   d. The sample/diluent are dispensed into the proper vial by pressing the button again.
   e. The tubing is washed between each sample by aspirating an air sample and then dispensing the air/diluent into a waste container. (The washing procedure flushes the sample tubing with 1.8 mL of diluent).
   f. The sample tube is then wiped again, and the next sample is ready to be collected.
   g. Seal the vials, place the seal on top of the headspace vial, and crimp the seal with the hand crimper. The seal should not be loose enough to remove by hand.

2. Sample preparation using the Microlab 500:

   a. The following steps can be activated by two ways - press the “Run/Stop” key or press the button on the wand.
   b. Allow the left syringe to fill and the air gap on the right syringe to fill. The sample

to be analyzed is collected by placing the tubing, on the right side of the system, into the sample, and aspirating the sample by pressing the button.

c. The outside of the tubing is wiped with a clean Kimwipe or equivalent to remove excess sample residue.
d. The sample/diluent are dispensed into the proper vial by pressing the button again.
e. Seal the vials, place the seal on top of the headspace vial, and crimp the seal with the hand crimper. The seal should not be loose enough to remove by hand.
f. Allow the left syringe to fill and the air gap on the right syringe to fill before proceeding to the next sample.
g. The tubing is washed between each sample by aspirating an air sample and then dispensing the air/n-propanol into a waste container. (The washing procedure flushes the sample tubing with 1.8 ml of diluent).

Maintenance:

1. The tubing should be flushed with a Clorox solution, or equivalent, about once every few months to remove protein build-up and prevent bacterial growth in the tubing.

Comments:

1. The liquids being sampled should be warmed to room temperature before use.

2. Any liquids containing volatile substances should be covered or sealed to prevent evaporation of the volatiles. The reservoir containing the diluent solution should be closed, but not sealed, to prevent a vacuum from forming in the reservoir.

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, laboratory coat, eye protection, and work with the samples in a biosafety hood.

Literature References:

Hamilton Microlab 1000 plus User Manual; part number 610 370 / 01.

Hamilton User's Manual Microlab 500 B/C Series; part number 69176 (Rev. B).

Name of Procedure:

Toxicology
Extraction Procedure for Base Drugs Using United Chemical Technologies Clean Screen Extraction Columns[7]

Suggested Uses:

This is an extraction procedure for base drugs. The procedure is designed to extract base drugs from blood or urine for confirmation by mass spectrometry. It utilizes United Chemical Technologies Clean Screen Extraction Columns[7] and the Zymark RapidTrace SPE Workstation.

Apparatus Used to Perform Procedure:

Test tubes, 16 x 125, 13 x 100, 12 x 75
Test tube caps or stoppers
Vortexer
Centrifuge
pH meter
Eppendorf Pipettes
Pipet tips
Volumetric flasks
Zymark RapidTrace SPE Workstation or other SPE device
World Wide Monitoring Clean Screen Extraction Columns[7]
Zymark TurboVap LV or other evaporation device

Reagents Used:

Analytical Grade Internal Standard
Prazepam

Methylene chloride
Deionized water
Methanol
Hexane
Dimethylformamide
BSA (N,O-bis(trimethylsilyl)acetamide)

BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)
Heptafluorobutyric anhydride (HFBA)
Ethyl Acetate

Acetic Acid, 1.0M

a. To 400 mL deionized water add 28.6 mL glacial acetic acid.
b. Dilute to 500 mL with deionized water and mix.
c. Stability - 6 months at room temperature.

Methylene chloride (MeCl) : Isopropyl alcohol (4:1) with 2% Ammonium Hydroxide

a. Make a solution of MeCl:IPA (4 parts MeCl to 1 part IPA)
b. Add NH_4OH to the MeCl:IPA to make it 2% of the total volume.
c. Make fresh daily. MeCl:IPA may be made ahead of time and stored for several months.

Phosphate Buffer

a. Dissolve 1.70 g Na_2HPO_4 and 12.14 g NaH_2PO_4-H_2O in 800 mL DI water and dilute to 1L.
b. Adjust pH to 6.0 +/- 0.1 with monobasic sodium phosphate or dibasic sodium phosphate.
c. Stability: 1 month at 0-5 degrees Celsius (refrigerate when not in use). It is permitted to prepare less than one liter as long as the proportions are equivalent to the ones listed above.

Internal Standard Solution

a. <u>Internal standard</u>:
Prepare Base Drug Internal Standard Solution containing 2000 ng/mL of prazepam. Label the flask. Include on the label initials, date prepared, and the expiration date which is determined from the date listed on the acquired drug standard.

Procedure:

1. Blood Sample Preparation:

    a. To 2 mL of blood add 4.5 mL of deionized H_2O and 100 microliters of the internal standard solution
    b. Mix/Vortex and let stand for 5 minutes to lyse red blood cells.
    c. Mix/Vortex sample.
    d. Centrifuge for 10 minutes at >2000 RPM
    e. Decant liquid portion of the sample into 2 mL of 100 mM phosphate buffer (pH6). Mix.
    f. If needed, adjust pH to 6.0 ∀ 0.5 with 100 mM monobasic or dibasic sodium phosphate.

2. Urine and Liquid Sample Preparation:

    a. No sample preparation is usually needed for urine. Extract 5 mL of urine with 250 microliters of the internal standard added. Before extraction check the pH to see if it is 6.0 +/- 0.5. If it is not then adjust pH accordingly with monobasic or dibasic sodium phosphate. If a majority of the drug(s) of interest has formed a glucuronide complex, the glucuronide can be hydrolyzed utilizing one of the methods described below.
    b. Hydrolysis Method A:
        1. To 5 mL urine add 2 mL B-glucuronidase (5000 F units/mL Patella Vulgata in 1.0M acetate buffer.)
        2. Mix. Hydrolyze for 3 hours at 65 EC. Cool to room temp.
        3. Adjust pH to 6.0 +/- 0.5 with approximately 0.7 mL of 1.0 N NaOH.
        or
    c. Hydrolysis Method B:
        1. To 5 mL urine add 0.5 mL conc. HCl. Mix.
        2. Heat for 30 min. at 120 EC. Cool to room temperature.
        3. Add 1.0 mL 7.4 M NH_4OH. Mix.
        4. Adjust sample pH to 6.0 +/- 0.5 with 1 to 3 mL 500 mM phosphoric acid.

3. Extraction Procedure

    a. Condition column with 3 mL MeOH.

b. Rinse Column with 3 mL MeCl:IPA:NH_4OH
c. Condition column with 3 mL water.
d. Condition column with 1 mL phosphate buffer.
e. Load sample onto column.
f. Rinse column with 3 mL water.
g. Rinse column with 2 mL acetic acid, 1.0M
h. Rinse column with 3 mL MeOH.
i. Dry column for 5 minutes.
j. Collect basic drugs with 3 mL MeCl:IPA:NH_4OH

Reagent flow must be between 1 to 15 mL per minute, except for elution reagents and samples which cannot be more than 5 mL per minute.

Post Extraction Procedures:

Evaporation of the solvent from the collection test tube and reconstitution with an appropriate solvent is all that is required for analysis by mass spectrometry. The following derivatizations can be done to improve analysis by gas or liquid chromatography/mass spectrometry:

a. Silylation.

1. Evaporate the solvent.

2. Derivatize the extract in the collection tube or transfer to a vial using methanol and evaporate the methanol.

3. Derivatize by adding 50 microliters of BSA or BSTFA with 1% TMCS to the vial or test tube and capping. Mix and heat the vial or test tube for 30 minutes at 80 ^{o}C. Remove from heat source and allow the vial or test tube to cool before analysis.

4. Examine the extract using gas or liquid chromatography/mass spectrometry.

b. Acylation with propionic anhydride.

1. Add 50 microliters of 99% or greater purity propionic anhydride and 50 microliters of pyridine.

2. Cap the tubes, vortex, and heat at 80 ^{o}C for at least 30 minutes.

3. Remove the tubes from heat and evaporate at 40 ^{o}C until dry.

4. Reconstitute the residue with 50 microliters of acetonitrile.

5. Examine the extract using gas or liquid chromatography/mass spectrometry.

c. Acylation of amphetamine and methamphetamine with HFBA.

1. Add 50 microliters of dimethylformamide to the eluate.

2. Evaporate the solvent from the collection test tube.

3. Add 100 microliters of heptafluorobutyric anhydride to collection test tube and cap.

4. Heat for 30 minutes at 80 ^{o}C.

5. Remove the test tube from heat and evaporate to dryness in a fume hood using a flow of inert gas.

6. Reconstitute with 50 microliters of ethyl acetate.

7. Examine the extract using gas or liquid chromatography/mass spectrometry.

Quality Control:

Quality Control is verified for each extraction by utilizing an appropriate internal standard or standards. For each set of extractions a blood blank must be extracted as a negative control.

Safety Concerns:

1. When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

2. BSA, BSTFA with 1% TMCS, heptafluorobutyric anhydride should be handled in a fume hood, with gloves, and eye protection.

Maintenance:

Zymark: RapidTrace SPE Workstation

1. Check reagent levels daily before using.
2. Clean protein build-up when needed.

Comments:

For an explanation of the operation of the RapidTrace refer to the operation manual.

Clean Screen7 Extraction Columns have been used in the Toxicology Unit to extract neutral, acidic and basic drugs and the metabolites of these drugs from whole blood and urine since 1995. Use of the Clean Screen7 Extraction Columns to extract neutral, acidic and basic drugs and the metabolites of these drugs has been validated through proficiency testing provided by College of American Pathologists.

Literature References:

RapidTrace SPE Workstation Installation and Quick Reference Manual, Zymark Co., revision 0, 1995.

Clean Screen7 Extraction Column Applications Manual, United Chemical Technologies Inc., Bristol, PA.

Kitchen, Chester J.; Telepchak Michael; and August, Thomas F.; AAn Automated Solid Phase Extraction Method for Thebaine, 6-Acetylmorphine and Other Opiates in Urine@, United Chemical Technologies.

Name of Procedure:

Toxicology
Evaporation and Concentration of Samples using the Zymark TurboVap LV Evaporator

Suggested Uses:

The TurboVap LV Evaporator is utilized to quickly evaporate multiple samples.

Apparatus needed to perform procedure:

TurboVap LV Evaporator
Fume hood
Gloves
Eye protection
A supply of drying gas
Deionized water
16 x 125, 13 x 100, or 12 x 75 mm glass test tubes
16 x 125, 13 x 100, or 12 x 75 mm TurboVap rack (depending on size of test used)
Antibacterial (suggested for water bath)

Calibration of Instrument:

Not Applicable

Application of Procedure on Liquids:

1. Turn TurboVap on with switch at lower left-hand side of instrument.

2. Turn on supply of drying gas.

3. Check water bath to make sure that the proper water level is present (if not add deionized water until the appropriate level is obtained).

4. Set water bath temperature with the TEMP numeric pushwheel and allow the bath to come up to temperature (TEMP light stops blinking).

5. Place sample tubes (up to 50) into the appropriate rack.

6. Set the TIME pushwheel to the desired drying time (usually 10 ml of an organic solvent, e.g., methylene chloride, at 35 degrees C should evaporate in approximately 30 minutes).

7. Select the row(s) containing the sample(s) by pressing its corresponding TUBE STATIONS push-button (choose the button that aligns horizontally with the row).

8. Press the START button.

9. Insure that the fume hood is on while the samples are drying.

10. The TurboVap LV will sound an alarm at the end of the drying time. At that time the test tubes ca

Safety Concerns:

The TurboVap LV does require 115VAC power; therefore, use caution not to disturb the water in the water bath, and avoid spillages on the TurboVap LV. The TurboVap is not classified as "Explosion Proof." Use the standard safety procedures with the solvent(s) that are being evaporated by the TurboVap.

Literature Reference:

TurboVap LV Evaporator Operator's Manual, Zymark Corporation, 1990.

Name of Procedure:

Toxicology
Solid Phase Extractions utilizing the Zymark RapidTrace SPE Workstation

Suggested Uses:

This procedure does not cover every aspect of the instrument used. The operator of the instrument should read the manual for the instrument before using this procedure.

This instrument is utilized to extract drugs from whole blood, urine, or other aqueous liquids using United Chemical Technologies Clean Screen Extraction Columns®.

Items Used to Perform Extractions:

Test tubes, 16 x 125, 13 x100, 12 x 75
Test tube caps or stoppers
United Chemical Technologies Clean Screen Extraction Columns®
Zymark: RapidTrace SPE Workstation and Controller
Nitrogen gas

Reagents Used:

2N NaOH
2N HNO_3

Calibration of Instrument:

No calibration is necessary.

Quality Control:

Quality Control is verified for each extraction by utilizing a control standard (Blank or Standard).

Sample Preparation:

Place the previously prepared blood, urine, or other aqueous liquids into separate 13 X 100 test tubes.

Extraction Procedure:

1. Turn power on at the rear of the Zymark RapidTrace SPE Workstation modules, and turn power for the workstation on at the designated location (top right corner).

2. From the Main Menu, double-click on the Rapid Trace Production Icon and wait until the screen appears.

3. Single-click on the Setup Racks from the Main Menu.

4. Select the module(s) that are going to be used for the extractions by placing an X in the box beside the module.

5. Assign the desired procedure to the appropriate sample(s) by the following steps:
   a. Single-click on the procedure
   b. Single-click on the appropriate sample(s)
   c. When the samples are selected single-click on the arrow button between the two boxes.

6. When finished with the setting up of the racks then single-click on the OK/Save button.

7. Place the 13 X 100 test tubes containing blood, urine, or other aqueous liquids into the designated positions on the rack.

8. Place the 12 X 75 collection tubes into the proper locations on the rack.

9. Insure that the appropriate reagent lines are in the proper reagents (check Reagent Setup for the appropriate locations).

10. Insert the rack into the module that it has been previously programmed for.

11. Place the designated extraction columns into the correct locations on the turret.

12. Single-click on Run/Monitor from the Main Menu.

13. This screen displays a box representing each module in the ten module workstation. The screen will show which module(s) you have made active. Insure that the correct modules are active. For each active module, check to verify that the proper

procedure is assigned to the correct sample. Insure that the gas supply is on and an adequate quantity exists.

14. If this screen is correct, then single-click on the Run button to start the corresponding module; however, if the screen is incorrect then Exit this screen and return to the Setup Racks screen (see step 3.).

15. Periodically monitor the controller to insure that the modules are running properly.

Post Extraction Procedure:

1. Evaporate the solvent from the collection test tubes.

2. Derivatization of some drugs may be necessary for analysis by gas chromatography - mass spectroscopy. Suggested derivative is the trimethylsilyl (TMS) by BSTFA or BSA. The following compounds are some that may need derivatization: consult the GC/MS operator.
   a. Some opiates
   b. Cocaine metabolites
   c. Some benzodiazepines and their metabolites

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

BSA and BSTFA should be handled in a fume hood, with gloves, and eye protection.

Maintenance:

1. Zymark: RapidTrace SPE Workstation
   a. Check reagent levels daily before using.
   b. Clean protein build up when needed by passing about 7.5 mL 2N NaOH and 2N HNO_3 through the cannula to the aqueous waste.

Comments:

Reagent flow should be between 1 to 15 mL per minute, except for elution reagents and samples which should be no more than 5 mL per minute.

Literature References:

RapidTrace SPE Workstation Installation and Quick Reference Manual, revision 0, Zymark Co., 1995.

Clean Screen® Extraction Column Applications Manual, United Chemical Technologies, Inc., Bristol, PA.

Name of Procedure:

Toxicology
pH Meter

Suggested Uses:

The pH meter is used to determine the pH of solutions.

Apparatus needed to perform procedure:

pH Meter
Electrode capable of performing pH determinations
Buffer solutions
Eye Protection
Beakers
Wash bottle
Thermometer

Calibration of Instrument:

1. Set the function selector to the pH position.

2. Obtain two buffer solutions with values that bracket the desired measuring range (e.g., pH 4.0 and pH 7.0 for samples that fall between ph 4 and 7.)

3. Place a beaker containing the buffer nearest in value to pH 7 in position and immerse the electrode and thermometer into the solution.

4. Standardize the pH meter to the buffer.

5. Remove the electrode and thermometer from the buffer solution.

6. Rinse the electrode and thermometer with deionized water

7. Place a beaker containing the second buffer in position, and immerse the electrode into the solution.

8. Standardize the pH meter to the buffer.

9. Remove the electrode from the buffer and rinse with distilled water.

Application of Procedure on Solutions:

1. Insure that the pH meter has been calibrated as described above.
2. Set the function selector to pH.
3. Immerse the electrode and thermometer into the sample solution.
4. Read the pH of the sample from the display and record value.
5. Remove the electrode and thermometer from the solution.
6. Rinse the electrode and thermometer with deionized water before proceeding with the next measurement.

Record Keeping:

In a log for the pH meter record calibrations, maintenance, and the use in preparation of reagents. Calibration entries will include the date, operator, lot number, expiration date, and pH of buffers used. Reagent preparation entries will include the date, the operator, and the reagent prepared.

Safety Concerns:

Always wear eye protection when handling buffer solutions.

Literature Reference:

pH Meter Operating Instructions; Fisher Scientific.

Name of Procedure:

Determination of Alcohol Concentration in Liquor Samples

Suggested Uses:

This procedure can be used for the determination of alcohol concentration (percent-by-volume) in samples containing alcohol concentrations between one and one hundred percent.

Items Needed to Perform Procedure:

Volumetric flask, 10 mL
Deionized water
100 μL pipet
Procedure used for Blood Alcohol Concentration (BAC) determination

Reagent Preparation Procedure:

Follow procedure used for BAC determination

Procedure:

1. Add 0.100 mL of sample to the volumetric flask.

2. Bring diluted sample to 10 mL. Mix.

3. Analyze diluted sample via the current BAC procedure, J-2.

4. Multiply the ethanol's grams-per-100 mL results by 1260 to obtain the original sample's ethanol concentration as percent-by-volume
   a. Explanation: Multiply by 100 to compensate for the dilution in step 1. Then multiply by 10 to compensate for the dilution with n-propanol internal standard solution. Then divide by 0.789 (density of ethanol) to convert from gm/mL to mL/mL.

Safety Concerns:

Solutions containing a high concentration of alcohol are flammable.

Literature Reference:

1. Moffat, Jackson, Moss and Widdop, "Clarke's Isolation and Identification of Drugs", 2nd edition, Volume 1, 1986.

Name of Procedure:

Toxicology
Extraction Procedure for Acid Drugs Using United Chemical Technologies Clean Screen Extraction Columns®

Suggested Uses:

This is an extraction procedure for acidic drugs using United Chemical Technologies Clean Screen Extraction Columns® and the Zymark RapidTrace SPE Workstation. This procedure is designed to extract acidic drugs for confirmation by mass spectrometry. After collection of the acidic fraction from the sample, a base fraction may be collected from the same sample (see procedure J-5 for details).

Apparatus Needed to Perform Procedure:

Test tubes, 16 x 125, 13 x 100, 12 x 75
Test tube caps or stoppers
Vortexer
Centrifuge
pH meter
Eppendorf Pipettes
Pipet tips
Volumetric flasks
Zymark RapidTrace SPE Workstation or other SPE device
World Wide Monitoring Clean Screen Extraction Columns® CSDAU203
Zymark TurboVap LV or other evaporation device

Reagents Needed:

Analytical Grade Drug Standard
    Hexobarbital
Methylene chloride
Deionized water
Methanol
Hexane
Ethyl Acetate

Acetic Acid, 1.0 M

a. To 400 mL deionized water, add 28.6 mL glacial acetic acid.
b. Dilute to 500 mL with deionized water and mix.
c. Stability - 6 months at room temperature.

Phosphate Buffer

a. Dissolve 1.70 g Na_2HPO_4 and 12.14 g NaH_2PO_4-H_2O in 800 mL DI water and dilute to 1L.
b. Adjust pH to 6.0 +/- 0.1 with monobasic sodium phosphate or dibasic sodium phosphate.
c. Stability: 1 month at 0-5 degrees Celsius (refrigerate when not in use). It is permitted to prepare less than one liter as long as the proportions are equivalent to the ones listed above.

Internal Standard Solution

a. Acid Drug Internal Standard: Prepare an internal standard solution that contains 40 micrograms/mL (40,000 ng/mL) of hexobarbital. Label the flask "Acid Drug Internal Standard". Include on the label initials, date prepared, and the expiration date which is determined from the date listed on the acquired drug standard. Example: From a drug standard containing 1.0 mg/mL of hexobarbital dilute 1.0 mL with methanol to 25 mL in a volumetric flask.

Procedure:

1. Blood Sample Preparation:
   a. To 2 mL of blood add 4.5 mL of DI H_2O and 100 microliters of the acid drug internal standard (add an appropriate base drug internal standard if a basic fraction is to be collected).
   b. Mix/Vortex and let stand for 5 minutes to lyse red blood cells.
   c. Mix/Vortex sample.
   d. Centrifuge for 10 minutes at >2000 RPM
   e. Decant liquid portion of the sample into 2 mL of 100 mM phosphate buffer (pH6). Mix.
   f. If needed, adjust pH to 6.0 $\pm$ 0.5 with 100 mM monobasic or dibasic sodium phosphate.

2. Urine and Liquid Sample Preparation:
   a. No sample preparation is usually needed for urine. Extract approximately 5 mL of urine with 500 microliters of the acid drug internal standard added. Before extraction, check the pH to see it if it is 6.0 +/- 0.5. If it is not, then adjust pH accordingly with monobasic or dibasic sodium phosphate.

3. Extraction Procedure

   a. Condition column with 3 mL MeOH.
   b. Condition column with 3 mL water.
   c. Condition column with 1 mL phosphate buffer.
   d. Load sample onto column.
   e. Rinse column with 3 mL water.
   f. Rinse column with 1 mL acetic acid, 1.0M
   g. Dry column for 5 minutes with nitrogen.
   h. Rinse the column with 2 mL of hexane
   i. Collect acidic drugs with 6 mL methylene chloride

   Add the following three steps to collect a basic fraction from the same sample.

   j. Rinse the column with 3 mL of methanol
   k. Dry column for 2 minutes with nitrogen
   l. Collect basic drugs with 3 mL of MeCL:IPA:NH_4OH

Reagent flow must be between 1 to 15 mL per minute, except for elution reagent and samples, which cannot be more than 5 mL per minute.

Quality Control:

Quality control is verified for each extraction by utilizing the appropriate internal standard. For each set of extractions a blank must be extracted as a negative control.

Post Extraction Procedures:

Evaporation of the solvent from the collection test tube and reconstitution with an appropriate solvent is all that is required for analysis by full scan mass spectrometry. Examine the collected fraction utilizing an appropriate method on the gas chromatograph or liquid chromatograph/mass spectrometer. Follow the post extraction procedures in J-5 if a basic fraction was collected.

Safety Concerns:

1. When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

2. Ethyl acetate should be handled in a fume hood with eye protection.

Maintenance:

Zymark: RapidTrace SPE Workstation

1. Check reagent levels daily before using.
2. Clean protein build-up when needed

Comments:

For an explanation of the operation of the RapidTrace refer to the operation manual.

Clean Screen® Extraction Columns have been used in the Toxicology Unit to extract neutral, acidic and basic drugs and the metabolites of these drugs from whole blood and urine since 1995. Use of the Clean Screen® Extraction Columns to extract neutral, acidic and basic drugs and the metabolites of these drugs has been validated through proficiency testing provided by College of American Pathologists.

Literature References:

RapidTrace SPE Workstation Installation and Quick Reference Manual, Zymark Co., revision 0, 1995.

Clean Screen® Extraction Column Applications Manual, United Chemical Technologies Inc., Bristol, PA.

Name of Procedure:

Toxicology
Extraction of Gamma Hydroxybutyric Acid (GHB) in Blood, Urine and Other Fluids

Suggested Uses:

This is an extraction procedure for Gamma Hydroxybutyric Acid (GHB) in blood, urine, and other fluids. This procedure is designed to extract Gamma Hydroxybutyric Acid for confirmation by gas chromatography/mass spectrometry. The extraction procedure targets Gamma Hydroxybutyric Acid at a concentration of 1.0 microgram/mL in a 1.0 mL blood sample, at 5.0 micrograms/mL in a 0.2 ml urine sample, or another fluid at an appropriate volume. Calibration standards must be utilized for quantitative analysis. Additional calibration standards at concentrations not listed may be used. Samples with GHB concentrations that exceed the upper level of the calibration curve may be reanalyzed after dilution with the proper matrix to bring them within the calibration range.

Apparatus Needed to Perform Procedure:

Test tubes, 16 x 125, 13 x 100, 12 x 75
Test tube caps or stoppers
Vortexer
Test tube rocker
Centrifuge
Eppendorf pipettes
Pipet tips
Pasteur pipets
Volumetric flasks
Zymark TurboVap LV
Nitrogen

Reagents Needed:

Analytical Grade Drug Standard
Gamma Hydroxybutyric Acid, Na (GHB)
Internal Standard
Gamma Hydroxybutyric Acid-D_6 , Na (GHB-D_6)
Sulfuric acid

Deionized water
Methanol
Ethyl Acetate
Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA w/1% TMCS)

Sulfuric Acid 0.1N

a. To approximately 75 mL of deionized water add 280 microliters concentrated sulfuric acid.
b. Dilute to 100 mL with deionized water and mix.
c. Stability - indefinite.

Internal Standard Solution

Gamma Hydroxybutyric Acid-D_6 (GHB-D_6) 100 micrograms/mL: Purchase or prepare an internal standard solution that contains 100 micrograms of GHB-D_6/mL. Label the flask "100 micrograms/mL GHB-D_6 Internal Standard." Include on the label initials, the date prepared, and the expiration date obtained from the ampule.

Example: From an ampule that contains 1.0 mg/mL GHB-D_6, add 1.0 mL to a 10.0 mL volumetric flask, and dilute the flask to volume with methanol.

Calibration Solutions

a. GHB Stock Calibrator Solution: Prepare or purchase a GHB stock calibrator solution that contains 1.0 milligram of GHB/mL in methanol. Label this solution "1.0 mg/mL GHB Stock Calibrator" and include initials, the date prepared, and the expiration date which is determined from the expiration date of the acquired drug standard or one year from date of preparation if prepared from solid GHB-sodium.

   Example of preparation: Add 10 milligrams of GHB Sodium to a 10 ml volumetric flask and dilute to volume with methanol.

   A GHB stock verification solution must be prepared using the same procedure to prepare the stock calibrator. Label this solution appropriately.

b. GHB Working Calibrator Solutions: From the GHB Stock calibrator solution

perform dilutions to obtain solutions that contain 100 micrograms/mL of GHB in methanol, 50 micrograms/mL of GHB in methanol, and 10 micrograms/mL of GHB in methanol. Label these solutions "(concentration) GHB Working Calibrator" and include initials, the date prepared, and the expiration date which is determined from the expiration date of the stock calibrator solution. Purchased commercial standards of GHB may be used at any of these concentrations.

Examples: 100 micrograms of GHB/mL-Dilute 1.0 milliliter of the GHB stock solution to 10.0 milliliters with methanol. 50 micrograms of GHB/mL-Dilute 500 microliters of the GHB stock solution to 10.0 milliliters with methanol. 10 micrograms of GHB/mL-Dilute 100 microliters of the GHB stock solution to 10.0 milliliters with methanol.

Working verification solution(s) must be prepared using the same procedure to prepare the working calibrators. Label these solution(s) appropriately.

c. Blood Calibration/Verification Standards:
   1. 1.0 microgram GHB/mL: add 100 microliters of the 10 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40^{o}C or less. Add 1.0 mL of blood to the test tube and vortex.
   2. 5.0 micrograms GHB/mL: add 100 microliters of the 50 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40^{o}C or less. Add 1.0 mL of blood to the test tube and vortex.
   3. 10.0 micrograms GHB/mL: add 200 microliters of the 50 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40^{o}C or less. Add 1.0 mL of blood to the test tube and vortex.
   4. 25.0 micrograms GHB/mL: add 250 microliters of the 100 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40^{o}C or less. Add 1.0 mL of blood to the test tube and vortex.
   5. 50.0 micrograms GHB/mL: add 500 microliters of the 100 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40^{o}C or less. Add 1.0 mL of blood to the test tube and vortex.
   6. 100 micrograms GHB/mL: add 1.0 milliliter of the 100 micrograms of GHB/mL working calibrator and 200 microliters of the GHB-D_6 internal

standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 1.0 mL of blood to the test tube and vortex.

d. Urine Calibration/Verification Standards:

1. 1.0 microgram GHB/mL: add 20 microliters of the 10 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
2. 5.0 micrograms GHB/mL: add 100 microliters of the 10 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
3. 10 micrograms GHB/mL: add 200 microliters of the 10 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
4. 20 micrograms GHB/mL: add 400 microliters of the 10 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
5. 50 micrograms GHB/mL: add 200 microliters of the 50 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
6. 100 micrograms GHB/mL: add 400 microliters of the 50 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.
7. 200 micrograms GHB/mL: add 400 microliters of the 100 micrograms of GHB/mL working calibrator and 40 microliters of the GHB-D_6 internal standard to a clean test tube. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine and 0.8 mL of deionized water to the test tube and vortex.

Procedure:

1. Sample Preparation:

Blood - To a clean test tube add 200 microliters of the 100 micrograms GHB-D_6/mL internal standard solution. Evaporate to dryness at 40°C or less. Add 1.0 mL of blood to the test tube and vortex.

Urine - To a clean test tube add 40 microliters of the GHB-D_6/mL internal standard solution. Evaporate to dryness at 40°C or less. Add 0.2 mL of urine or other liquid and 0.8 mL of deionized water to the test tube and vortex.

2. Extraction Proceedure:
   a. Add 250 microliters of cold 0.1N H_2SO_4 to the urine or blood sample.
   b. Mix/vortex.
   c. Add 4 milliliters of ethyl acetate and cap the test tube securely.
   d. Place the test tube(s) on the test tube rocker and allow to mix for 10 minutes. After removing the test tube(s) from the rocker centrifuge the test tubes for 5 minutes.
   e. Transfer the organic layer (upper) into a clean test tube.
   f. To the test tube containing the blood or urine, add 4 milliliters of ethyl acetate and cap the test tube securely.
   g. Repeat steps d and e.
   h. Transfer the organic layer into the test tube that contains the previously transferred organic layer from step e.
   i. Evaporate the organic layers to dryness at 40°C or less. This test tube will contain the extracted material.

Post Extraction Procedure:

1. Add 50 microliters of BSTFA w/1% TMCS to each test tube and cap securely.

2. Heat for 15 minutes at 80°C.

3. Remove from heat and allow the mixture to cool before analysis.

4. Examine the collected fraction utilizing the ghbblood, ghburine, ghbfs or other appropriate methods on the gas chromatograph/mass spectrometer.

Quality Control:

Quality control is verified for each extraction by utilizing the appropriate internal standard. For each set of extractions an appropriate blank must be extracted as a negative control. Reported quantitative values must be based on a calibration curve using at least four calibration standards, and at least one verification standard must be

extracted to verify the calibration curve. The verification standard must quantify within +/- 20% of the target for quantitative values to be reported.

Personnel Safety Concerns:

1. When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

2. Sulfuric acid, ethyl acetate, and methanol should be handled in a fume hood with eye protection.

Literature References:

Fiona J. Couper and Barry K. Logan; "Determination of Gamma-Hydroxybutyrate (GHB) in Biological Specimens by Gas Chromatography-Mass Spectrometry," **Journal of Analytical Toxicology**, Vol. 24, January/February 2000, pp. 1-7.

Rachel R. McCusker, Helen Paget-Wilkes, Chris W. Chronister, Bruce A. Goldberger, and Mahmoud A. ElSohly; "Analysis of Gamma-Hydroxybutyrate (GHB) in Urine by Gas Chromatography-Mass Spectrometry," **Journal of Analytical Toxicology**, Vol. 23, September 1999, pp. 301-305.

Randall C. Baselt and Robert H. Cravey, **Disposition of Toxic Drugs and Chemicals in Man**, Fourth Edition, Chemical Toxicology Institute, Foster City, CA, 1995.

Name of Procedure:

Toxicology
ELISA Screen for Methamphetamine and 3,4-Methylenedioxymethamphetamine (MDMA) Using the Immunalysis Methamphetamine Direct ELISA Kit

Suggested Uses:

This procedure does not cover every aspect of the instruments used. The operator of the instrument should read the manual for the instruments before using this procedure.

The procedure can be used to screen blood and urine samples for methamphetamine and 3,4-Methylenedioxymethamphetamine (MDMA).

The cutoff for blood case samples is 100 ng/ml d-methamphetamine, and the cutoff for urine case samples is 200 ng/ml d-methamphetamine.

This is only a screening test. Any positive result must be confirmed using a more specific analysis, such as gas or liquid chromatography/mass spectrometry before it can be reported. There are some cases of uncommon substances causing false positives using this test, which must be kept in mind when evaluating the results of this test.

Items Used to Perform Procedure:

Reagent Kit for Methamphetamine
- 96 well coated Microplates
- 50 ng/ml Urine d-Methamphetamine Standard
- Urine Negative
- Enzyme Conjugate
- TMB Substrate Reagent
- Stop Reagent

Automatic pipets, 1 mL, 100 uL, 0.010 - 1.0 mL adjustable
Test tubes
Adjustable microchannel pipet (15-200 uL)
Volumetric flasks 100 mL
Interval Timer
Microplate Washer
Microplate Reader capable of reading at 450 nm and 630 nm

Reagents Used in Procedure:

Analytical Grade Drug Standard - Methamphetamine, 1 mg/mL
Methanol

1,000 ng/ml Stock Methamphetamine Verification Standard:

To a 100 mL volumetric flask, add 100 microliters of a 1 mg/ml methamphetamine standard and dilute the flask to volume with methanol. Label the solution. Include on the label initials, the date prepared, and an expiration date determined by the expiration date of the acquired drug standard.

100 ng/ml Working Methamphetamine Verification Sample:

Prepare a 100 ng/ml working methamphetamine verification sample by adding 100 microliters of the 1,000 ng/ml stock methamphetamine verifier solution to a clean test tube. Add 0.90 mL of blood to the test tube and vortex. This sample should be prepared fresh on the same day that it is used for the assay.

Microplate Washer:

1. Read and follow all manufacturer instructions for use of the microplate washer.

Microplate Reader:

1. Read and follow all manufacturer instructions for use of the microplate reader.

Application of Procedure on Evidence:

Allow all reagents and samples to equilibrate to room temperature before use.

1. Urine case sample must be diluted with 3 volumes of deionized water or negative urine to one part urine case sample before their use in this procedure.
2. Add 10 microliters of the of the urine negative, 50 ng/ml d-methamphetamine urine standard, working verification sample, and case sample(s) to each well in duplicate.
3. Add 100 microliters of the enzyme conjugate to each well. Keep the time differences in the addition of enzyme conjugate to each well at a minimum. Tap the sides of the plate holder to ensure proper mixing.
4. Incubate the plate for 60 minutes after addition of enzyme conjugate to the last well. Incubate at room temperature in the dark.
5. Wash each well 6 times with 350 microliters of deionized water.
6. Invert the wells and vigorously slap dry on absorbent paper or towel to ensure that all residual moisture is removed.

7. Add 100 microliters of the TMB substrate reagent to each well and tap sides of the plate holder to ensure proper mixing. Keep the time differences in the addition of substrate reagent to each well at a minimum.
8. Incubate the plate for 30 minutes after addition of the substrate reagent to the last well. Incubate at room temperature in the dark.
9. Add 100 microliters of the Stop Solution to each well. Keep the time differences in the addition of stop solution to each well at a minimum. The color should change from blue to yellow.
10. Measure the absorbance at a dual wavelength of 450 nm (measuring filter) and 630 nm (background reference filter).

Results:

A reciprocal relationship exists between absorbance and concentration.

The adjusted negative control value is defined as the average value of the negative controls run minus 0.300.

Blood case samples that have at least one absorbance value at or below the average value of the 100 ng/ml blood working methamphetamine verification standards are considered positive. Blood case samples that have all absorbance values above the average value of the 100 ng/ml blood working methamphetamine verification standards, but are at or below the adjusted negative blood control are considered elevated. Blood case samples that are above the adjusted negative blood control will be considered negative.

Diluted urine case samples that have at least one absorbance value at or below the average value of the 50 ng/ml urine working d-methamphetamine standards (200 ng/ml cutoff) are considered positive. Diluted urine case samples that have all absorbance values above the average value of the 50 ng/ml urine working methamphetamine standards, but are at or below the adjusted negative urine control are considered elevated. Diluted urine case samples that are above the adjusted negative urine control will be considered negative.

Data Record Keeping:

The following information must be recorded for each sample that was run using this procedure.

1. Lot numbers of reagents and 50 ng/ml d-methamphetamine urine standard.

2. Expiration dates of reagents, 50 ng/ml d-methamphetamine urine standard, and the stock methamphetamine verification standard.
3. Results of the urine negative, 50 ng/ml d-methamphetamine urine standard, working methamphetamine verification sample, and case sample(s).

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

The stop reagent is corrosive.

Maintenance:

Perform the rinse procedure on the microplate washer after each use.

Literature Reference:

Methamphetamine Direct ELISA Kit Insert, Immunalysis Corporation, Version 5/2001.

Name of Procedure:

Use of the Dade Behring Viva Jr. EMIT Analyzer as a Drug Screen

Suggested Uses:

This procedure does not cover every aspect of the instrument used. The operator of the instrument should read the manual for the instrument before using this procedure.

The EMIT analyzer can be used to screen blood, serum, and urine samples for the presence of the following classes of compounds: cocaine and its metabolites, benzodiazepines, barbiturates, opiates, methadone, and metabolites of delta-9-THC.

The EMIT analyzer is designed to analyze urine, but can test blood / serum extracts.

This is only a screening test. Samples that test positive for one or more classes of drugs by this test must have the drugs confirmed by mass spectrometry to be reported as an identification. There are some cases of uncommon substances causing false positives using this test, which must be kept in mind when evaluating the results of this test.

Items Needed to Perform Procedure:

Test tubes
Stoppers or caps
Vortexer
Centrifuge
Reservoir Filters
Glass boiling beads
Zymark TurboVap LV or other evaporator
Dade Behring Viva Jr. EMIT Analyzer
2 mL analyzer cups and caps
Appropriate EMIT Reagent kits for the Viva Jr. EMIT Analyzer
Appropriate EMIT calibrators
Automatic pipets, 1mL, 250 uL, 0.010 - 5 mL adjustable

Reagents Used in Procedure:

Phenobarbital, 1 mg/mL
Nordiazepam, 1 mg/mL
Morphine, 1 mg/mL
Benzoylecgonine, 1 mg/mL
Methadone, 1 mg/mL
Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (THC-COOH), 5 µg/mL
Deionized water
Acetone
Methanol

Stock Calibration Solution:

1. To a 10 mL volumetric flask, add the following:
   0.5 mL Phenobarbital (1 mg/mL),
   0.025 mL Nordiazepam (1 mg/mL),
   0.025 mL Morphine (1 mg/mL),
   0.025 mL Benzoylecgonine (1 mg/mL),
   0.025 mL Methadone (1 mg/mL),
   2.5 mL Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (5 µg/mL),
   dilute the flask to volume with methanol.

Stock Verification Solution:

Prepare a second solution identical to the stock calibrator solution and label this solution the “Stock Verification Solution”.

Calibration Sample:

1. Add 0.100 mL of the stock calibration solution to the test tube.

2. Add 4.9 mL of a drug free matrix equivalent to the analyte(s) (water, blood, etc.) to the test tube.

3. Cap and vortex the test tube.
4. The concentration of the calibration sample is 1000 ng/mL phenobarbital, 50 ng/mL nordiazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 50 ng/ml methadone, 25 ng/mL THC-COOH.

Verification Sample:

Prepare a verification sample(s) identical to the calibrator sample using the stock verification solution.

Reagents:

1. Fill the appropriate reagent bottles. Mark bottles with the appropriate expiration dates and lot numbers.

2. When the instrument is not in use cap the bottles.

Instrument Set Up:

1. Check fluid levels of the system solution bottle in the instrument's cabinet and refill as needed. Ensure that the waste bottle is empty.

2. Check the paper and add paper if needed.

Calibration of Instrument:

1. Order Calibration/QC.
2. Choose the appropriate calibration/control method.
3, Place the appropriate calibrators/controls in the proper location.
4. Start Measurement.

Sample Preparation:

1. Urine samples and clear liquid samples:

   a. Pipet at least 0.250 mL of sample into appropriate sample cup.

2. Whole blood and Serum extraction

   Extract calibration samples, blanks, and verification sample(s) with case samples.

a. Pipet 2.5 mL of acetone into a disposable glass test tube (preferably 16 x 125 mm).
b. Pipet 1 mL of whole blood (case, std., or control) into the appropriate tube. Add the blood directly to the acetone. Do not run the blood down the side of the tube.
c. Cap the tube with a stopper and vortex for approximately 10 seconds.
d. Allow the tubes to stand for approximately 10 minutes and repeat the vortex step.
e. Centrifuge the tubes for ten minutes at a minimum of 2500 rpm.
f. If the supernatant contains particulate then place a 4 mL reservoir containing a frit into a clean test tube. If the supernatant does not contain significant particulate then decant the supernatant directly into a clean test tube, add a glass boiling bead to each test tube, and skip to step j.
g. Decant the supernatant from the previous step into the reservoir and allow it to completely drain into the test tube.
h. Add 0.5 mL acetone to each reservoir and allow it to drain into the tube.
i. If a reservoir was used then remove the reservoir and add a glass boiling bead to each tube.
j. Ensure that water level is adequate in the TurboVap LV. Place the tubes in the TurboVap LV set at 50 ^{o}C and evaporate the liquids until the tubes are completely dry.
k. If the specimens are not going to be analyzed that day, they can be sealed and placed in the refrigerator.
l. Immediately prior to analysis, reconstitute the residues with 0.25 mL of methanol.
m. Vortex each tube.
n. Centrifuge the tubes for 10 minutes and transfer the supernatant to an EMIT analyzer cup with a disposable glass Pasteur pipet.
o. Cap the cups until the rack is placed on the analyzer to prevent evaporation of the methanol.

Application of Procedure on Evidence:

1. Sample Programming:

   a. Create a Sample List by ordering the appropriate samples.
   b. Place the samples in the appropriate tray location.
   c. Start Measurement.

2. Quality Control:

At least one blank and one verifier must be run during the analysis.

3. Data Record Keeping:

   Ensure that the following data are documented.
   a. Lot numbers of reagents and calibrators.
   b. Expiration dates of reagents and calibrators.
   c. Results of the calibrators, verification samples, blanks, and samples.

   Original calibration, control, or case data that is transcribed to a worksheet will be reviewed by another chemist.

   The data produced from calibration standards and quality control samples are reference materials and shall be stored in the toxicology section object repository of FLAIRS with a file name made of "EMITQC" and the date in yyyymmdd format with no space between them. (example: EMITQC20080818) Original calibration, control, or case data that is transcribed to a worksheet will be reviewed by another chemist.

4. Reporting Criteria

   An immunoassay result that is at or above the cutoff control minus 0.015 dAbs/m (0.005 for the opiate assay) will be considered positive. An immunoassay result that is not at least 0.015 dAbs/m (0.005 dAbs/m for the opiate assay) above the negative control will be considered negative for the analyte tested for that assay. An immunoassay result that is at least 0.015 dAbs/m (0.005 dAbs/m for the opiate assay) above the negative control, but less than 0.015 dAbs/m (0.005 dAbs/m for the opiate assay) below the cutoff control will be considered elevated.

Safety Concerns:

Insure that the instrument cover is in the down position when the instrument is in use.

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

Maintenance:

The EMIT maintenance log book will be filled out at the completion of any set of EMIT runs. Any other maintenance or service performed on this instrument will be documented in the log book.

Literature References:

Viva Jr. Operator's Manual.

Blood extraction procedure acquired from Georgia Bureau of Investigation.

Name of Procedure:

Hewlett-Packard/Agilent GC interfaced to the Hewlett-Packard/Agilent 5973 series MSD for Toxicology Analysis

Suggested Uses:

The gas chromatograph / quadrupole mass selective detector / data system is used to qualitate and quantitate compounds present in items of evidence.

The gas chromatograph separates mixtures of compounds and the mass spectra of the compounds of interest are examined. The mass spectrum of a compound is compared to reference spectra for confirmation. If necessary, mass spectral libraries can be searched through computer-based matching software to aid in identifying unknown compounds.

Apparatus Used to Perform Procedure:

Hewlett-Packard/Agilent Gas Chromatograph (GC)
Hewlett-Packard/Agilent 5973 series Mass Selective Detector (MSD)
Hewlett-Packard/Agilent Automatic Liquid Sampler
PC with HP Analytical MSD Productivity ChemStation Software, or equivalent
Output Device
Methanol
Ethyl Acetate
Sample vials and caps
crimper tool
10μl syringe
DB-5 column (or other appropriate column)
Helium Gas
Perfluorotributylamine [PFTBA]

Calibration of the Hewlett Packard 5973 GC/MSD/DS:

1. The GC-MS system is kept on at all times.
2. Calibration is performed daily when in use with the Autotune program, using the Autotune option.
3. This procedure uses Perfluorotributylamine (PFTBA) as a tuning standard and the resulting data file is kept in a notebook near each instrument.

4. Compare this tune file to previous ones and address any major variations which may indicate instrument problems. The tune file should conform to the manufacturer's requirements.

Procedure:

These procedures do not cover every aspect of the instrument. The operator of the instrument should consult the manual(s) for the instrument.

A. Sample Preparation (suggested):

1. Solid Phase Extraction residues: reconstitute with the appropriate solvent or derivatizing agent and transfer to injection vial.
2. Syringes: Wash with methanol and extract if necessary (if excessive quantities of blood or other liquids are present in syringe then an extraction is required.

B. GC/MS Methods (The listed methods are for specific applications. Other methods may be developed and used as needed.)

1. ACIDFS.M
   Initial Temperature 90 ^{o}C hold for 1 minute
   90 ^{o}C - 125 ^{o}C @ 40 ^{o}C/minute hold for 1.00 minute
   125 ^{o}C - 285 ^{o}C @ 17 ^{o}C/minute hold for 7.71 minutes
   Total time of run: 20.00 minutes

2. AUTO.M
   Initial Temperature 70 ^{o}C hold for 1.00 minute
   70 ^{o}C - 125 ^{o}C @ 40 ^{o}C/minute hold for 1.00 minute
   125 ^{o}C - 285 ^{o}C @ 17 ^{o}C/minute hold for 12.00 minutes
   Total time of run: 24.79 minutes

3. BENZOFS.M
   Initial Temperature 120 ^{o}C hold for 1 minute
   120 ^{o}C - 210 ^{o}C @ 30 ^{o}C/minute hold for 2.00 minutes
   210 ^{o}C - 300 ^{o}C @ 40 ^{o}C/minute hold for 11.75 minutes
   Total time of run: 20.00 minutes

4. CANSIM.M
   Initial Temperature 150 ^{o}C hold for 1 minute
   150 ^{o}C - 235 ^{o}C @ 50 ^{o}C/minute hold for 2.00 minutes
   235 ^{o}C - 300 ^{o}C @ 15 ^{o}C/minute hold for 5.97 minutes
   Total time of run: 15.00 minutes

5. GHBBLOOD, GHBURINE, and GHBFS
   Initial temperature - 60 ^{o}C hold for 4.00 minutes
   60 ^{o}C - 180 ^{o}C @ 15 ^{o}C/minute hold for 0.00 minute
   180 ^{o}C - 250 ^{o}C @ 35 ^{o}C/minute hold for 4.00 minute
   Total time of run: 18.00 minutes
   GHBFS - full scan from 50-350 amu
   GHBBLOOD and GHBURINE - scan from 230-245 amu

6. 70METH
   Initial temperature - 70 ^{o}C hold for 1.00 minute
   70 ^{o}C - 125 ^{o}C @ 40 ^{o}C/minute hold for 1.00 minute
   125 ^{o}C - 285 ^{o}C @ 17 ^{o}C/minute hold for 12.00 minutes
   Total time of run: 24.79 minutes

C. Injection of Sample:

1. Obtain a chromatogram of a blank solvent injection prior to the analysis of the sample.
2. Dilute the sample with the appropriate solvent, if needed, before injecting the sample.
3. After the data system has collected the data, examine the chromatogram and spectra for the peaks of interest, print all necessary data and spectra.
4. The syringe must be flushed at least 10 times with clean solvent between injections to insure the sample integrity between injections and that no sample transfer is made between sample vials.

D. Reporting:

The requirements for analyte identification using the GC/MS system are described in the Toxicology Criteria for Identification of Analytes.

E. Activity Log:

A log of all injections and maintenance will be kept. The log will include the date, sample identification, initials of operator, GC/MS method used, and comments.

Safety Concerns:

1. Avoid syringe punctures of hand and fingers.
2. Use extreme caution handling organic solvents to avoid contact with skin and eyes.
3. Use extreme caution dismantling/installing/transporting compressed gas cylinders.
4. Caution: Gas Chromatograph and Mass Spectrometer may be extremely hot.

Literature References:

Moffat, Jackson, Moss and Widdop**, "Clarke's Isolation and Identification of Drugs"**; 2nd Ed., Vol. 1, 1986.

Pfleger, Maurer, and Weber, "**Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites"**; 2nd. Ed., Vols. 1-3, 1992.

Telepchak, Long, and Moore, **"Determination of Delta-9-Tetrahydrocannabinol (THC) and its Metabolite 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid (THCA) in Whole Blood"**; United Chemical Technologies, Inc.

"Distinguishing Sympathomimetic Amines from Amphetamine and Methamphetamine in Urine by Gas Chromatography/Mass Spectrometry," **Journal of Analytical Toxicology**; Vol. 16, January/February 1992, pp. 19-27.

Name of Procedure:

Toxicology
THC and THC-COOH Extraction Procedure Using United Chemical Technologies Styre Screen Extraction Columns[7]

Suggested Uses:

This procedure is an extraction of delta-9-tetrahydrocannabinol (THC) and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) from blood using United Chemical Technologies Styre Screen Extraction Columns[7]. This procedure is designed to extract THC and THC-COOH for confirmation by mass spectrometry. The procedure targets THC and THC-COOH in a 1.0 mL blood sample. Calibration standards may be utilized for quantitative analysis.

Items Used to Perform Procedure:

Test tubes, 16 x 125, 13 x 100, 12 x 75
Test tube caps or stoppers
Vortexer
Centrifuge
Pipettes
Pipette tips
Volumetric flasks
World Wide Monitoring Styre Screen Extraction Columns[7]
Zymark RapidTrace SPE Workstation or other SPE device
Zymark TurboVap LV or other evaporation device

Reagents Used:

Acquired drug standards
- Delta-9-tetrahydrocannabinol (THC)
- 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)

Acquired deuterated drug standards
- Delta-9-tetrahydrocannabinol (THC)-D_3
- 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid-D_3

Deionized water
Hexane
Ethyl Acetate

Acetonitrile
Concentrated Ammonium Hydroxide
Glacial Acetic Acid
Deionized Water **:** Acetonitrile **:** Concentrated Ammonium Hydroxide (84:15:1) mixture
(prepare reagent on the same day of extractions)
Hexane **:** Ethyl Acetate **:** Glacial Acetic Acid (49:49:2) mixture
(prepare reagent on the same day of extractions)
BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamine with 1% trimethylchlorosilane)

Internal Standard Solution

a. Internal Standard: Prepare an internal standard solution that contains 1.0 μg/mL (1,000 ng/mL) of THC-D_3 and THC-COOH-D_3. Label this solution "Cannabinoid Internal Standard" and include on the label initials, date prepared, and the expiration date which is determined from earliest date listed on the acquired drug standards.
Example: From separate drug standards containing 100 μg/mL of THC-D_3 and THC-COOH-D_3, transfer 1.0 mL from each standard to the same 100 mL volumetric flask. Dilute the flask to volume with methanol.

Calibration and Verification Solutions

a. Cannabinoid Calibrator Solution: from the individual drug standards prepare a solution that contains 1.0 μg/mL (1,000 ng/mL) of THC and THC-COOH. Label this solution "Cannabinoid Calibrator Solution" and include on the label initials, the date prepared and the expiration date which is determined from the earliest date listed on the acquired drug standards.
Example: Dilute 1.0 mL of a 1.0 mg/mL THC standard to 10 mL with methanol. Place 1.0 mL of this solution in a 100 mL volumetric flask and add 1.0 mL of a 100 μg/mL THC-COOH standard. Dilute the flask to volume with methanol.

b. Cannabinoid Verification Solution must be prepared using the same procedure to prepare the calibrator solution.

c. 50 ng/mL cannabinoid calibration/verification standard: Add 50 μL of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add 100 μL of the internal standard solution. Vortex the mixture.
d. 100 ng/mL cannabinoid calibration/verification standard: Add 100 μL of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add

100 μL of the internal standard solution. Vortex the mixture.

e. 200 ng/mL cannabinoid calibration/verification standard: Add 200 μL of the cannabinoid calibration/verification standard to 1.0 mL of drug free blood. Add 100 μL of the internal standard solution. Vortex the mixture.

Procedure:

1. Blood Sample Preparation:
   a. To 1 mL of blood slowly add 2 mL of cold acetonitrile and 100 μL of the internal standard.
   b. Mix/Vortex samples and let stand for 5 minutes.
   c. Mix/Vortex samples.
   e. Centrifuge for 10 minutes at >2000 RPM
   f. Decant liquid portion of the sample into a clean test tube and add 2.0 mL of deionized water.

2. Extraction Procedure

   a. Load sample onto column.
   b. Rinse column with 1.0 mL of the Water:Acetonitrile:Concentrated Ammonium Hydroxide reagent. The flow rate should be between 1 and 15 mL per minute.
   c. Dry column for 15 minutes.
   d. Collect cannabinoids with 3 mL of the Hexane:Ethyl Acetate:Glacial Acetic Acid reagent. The flow rate should not exceed 5 mL per minute.

Note: The method of extraction (RapidTrace SPE vs. vacuum box) is discretionary.

Post Extraction Procedure:

1. Evaporate the solvent from the collection test tube.

2. Derivatize by adding 50 μL BSTFA with 1% TMCS to the vial or collection test tube and capping. Mix and heat the vial or collection test tube at 80^{o} C for 30 minutes. Remove from heat source and allow the vial or collection test tube to cool before analysis.

3. Examine the extract utilizing gas or liquid chromatography/mass spectrometry.

Quality Control:

Quality control is verified for each extraction by utilizing the internal standard. For each set of extractions a blood blank must be extracted as a negative control. At least one verifier must be extracted as a positive control. Reported quantitative values must be based on a calibration curve using at least three calibration standards, and at least one verification standard must be extracted to verify the calibration curve. The verification standard must quantify within +/- 20% of the target for quantitative values to be reported.

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

BSTFA with 1% TMCS should be handled in a fume hood, with gloves, and eye protection.

Maintenance:

Zymark: RapidTrace SPE Workstation

a. Check reagent levels daily before using.
b. Clean protein build-up when needed.

Comments:

For an explanation of the operation of the RapidTrace refer to the operation manual.

Literature References:

RapidTrace SPE Workstation Installation and Quick Reference Manual, revision 0, Zymark Co., 1995.

Styre Screen7 Extraction Column Applications Manual, United Chemical Technologies, Inc., Bristol, PA.

Name of Procedure:

Use of the Immunalysis Tecan Freedom EVO 75 Workstation as a Drug Screen

Suggested Uses:

This procedure does not cover every aspect of the instrument used. The operator of the instrument should read the manual for the instrument before using this procedure.

The ELISA analyzer can be used to screen blood, serum, and urine samples for the presence of the following classes of compounds: barbiturates, benzodiazepines, carisoprodol, cocaine metabolite, metabolites of delta-9-THC, methamphetamine, methadone, 3,4-methylenedioxymethamphetamine, opiates, tramadol, and zolpidem.

The cutoff for blood case samples is 1000 ng/mL phenobarbital, 50 ng/mL nordiazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 50 ng/mL methadone, 25 ng/mL THC-COOH, 50 ng/mL tramadol HCl, 100 ng/mL methamphetamine, 50 ng/mL carisoprodol, and 50 ng/mL zolpidem.

The ELISA analyzer is primarily designed to analyze blood samples, but can test urine and serum extracts.

This is only a screening test. Samples that test positive for one or more classes of drugs by this test must have the drugs confirmed by mass spectrometry to be reported as identification. There are some cases of uncommon substances causing false positives using this test, which must be kept in mind when evaluating the results of this test.

The data obtained from the ELISA analyzer is measured to 5 significant figures. Any trailing digits that are zero are not printed by the analyzer software.

Items Needed to Perform Procedure:

Reagent kits for barbiturates, benzodiazepines, carisoprodol, cocaine and its metabolites, metabolites of delta-9-THC, methamphetamine, methadone, opiates, tramadol, and zolpidem.

96 well coated microplates
Enzyme Conjugate that is matched to the appropriate microplates
TMB Substrate Reagent
Stop Reagent
Stoppers or Caps
Vortex Mixer
Tecan/Immunalysis Freedom EVO ELISA Analyzer
Test tubes
Volumetric Flasks, 10 mL
Appropriate ELISA Reagent kits for the Freedom EVO ELISA Analyzer
Appropriate ELISA calibrators
Automatic pipettes, 1mL, 100 µL, 0.010 - 1.0 mL adjustable

Reagents Used in Procedure:

Benzoylecgonine, 1 mg/mL
Carisoprodol, 1 mg/mL
Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (THC-COOH), 5 µg/mL
(+-)-Methadone, 1 mg/mL
(+-)-Methamphetamine, 1mg/mL
Morphine, 1 mg/mL
Nordiazepam, 1 mg/mL
Phenobarbital, 1 mg/mL
Cis-Tramadol HCl, 1 mg/mL
Zolpidem, 1 mg/mL
Deionized water
Methanol
Negative blood
Negative urine

Stock Calibration (Positive) Solution:

1. To a 10 mL volumetric flask, add the following:
   0.025 mL Benzoylecgonine (1 mg/mL),
   0.025 mL Carisoprodol (1 mg/mL),
   2.5 mL Delta-9-carboxy-11-nor-delta-9-tetrahydrocannabinol (5 µg/mL),
   0.025 mL Methadone (1 mg/mL),
   0.050 mL Methamphetamine (1 mg/mL),

0.025 mL Morphine (1 mg/mL),
0.025 mL Nordiazepam (1 mg/mL),
0.500 mL Phenobarbital (1 mg/mL),
0.025 mL Tramadol HCl (1 mg/mL),
0.025 mL Zolpidem (1 mg/mL),
Then dilute the flask to volume with methanol.

Stock Verification Solution:

Prepare a second solution identical to the stock calibrator (positive) solution and label this solution the "Stock Verification Solution".

Working Calibration Standard:

1. Add 0.100 mL of the stock calibration solution to the test tube.

2. Add 4.9 mL of a drug free matrix equivalent to the analyte(s) (blood, urine, etc.) to the test tube.

3. Cap and vortex the test tube.

4. The concentration of the calibration sample is 1000 ng/mL phenobarbital, 50 ng/mL nordiazepam, 50 ng/mL morphine, 50 ng/mL benzoylecgonine, 50 ng/mL methadone, 25 ng/mL THC-COOH, 50 ng/mL tramadol HCl, 100 ng/mL methamphetamine, 50 ng/mL carisoprodol, and 50 ng/mL zolpidem.

Working Verification (Positive) Standard:

1. Add 0.200 mL of the stock verification solution to the test tube.

2. Add 4.8 mL of a drug free matrix equivalent to the analyte(s) (blood, urine, etc.) to the test tube.

3. Cap and vortex the test tube.

4. The concentration of the verification sample is 2000 ng/mL phenobarbital, 100 ng/mL nordiazepam, 100 ng/mL morphine, 100 ng/mL benzoylecgonine, 100 ng/mL methadone, 50 ng/mL THC-COOH, 100 ng/mL tramadol HCl, 200 ng/mL

methamphetamine, 100 ng/mL carisoprodol, and 100 ng/mL zolpidem.

Negative Standard:

Prepare a negative standard as described under Sample Preparation using negative blood and /or urine as applicable.

Reagents:

1. Fill the appropriate reagent troughs with either TMB or stop reagent. Check and fill appropriate conjugate vials.

Instrument Set Up:

1. Check system liquid container and fill with deionized water when necessary. Ensure that the waste bottle is empty.

2. Check the printer and add paper if needed.

Sample Preparation:

1. Preparation of Urine and Blood Standards, and Urine and Blood Samples
   a. Pipette 0.25 mL of Urine or Blood into each of two (minimum) disposable glass test tubes.
   b. Pipette 2.5 mL of Deionized Water into each of the sample tubes.
   c. Cap the tube with a stopper and vortex for approximately 10 seconds.
   d. Arrange sample tubes into appropriate sample racks. Each sample, positive standard, negative standard, and verification standard must be prepared in duplicate, at a minimum, and run concurrently.

Application of Procedure on Evidence:

1. Sample Programming:
   a. Follow steps outlined in book titled “Immunalysis Freedom EVO 75 Workstation Operating Procedure”

Results:

A reciprocal relationship exists between absorbance and concentration.

The adjusted negative standard value is defined as the average value of the negative standard minus 0.300.

The set of absorbance values for each sample and standard will be averaged. Case samples that have an average absorbance value at or below the average value of the working calibration standards are considered positive. Case samples that have an average absorbance value above the average value working calibration standards, but are at or below the adjusted negative standard are considered elevated. Case samples that have an average absorbance value at or above the adjusted negative standard will be considered negative.

Quality Control:

The set of absorbance values for the verification standard will be averaged. The average value of the verification standard must produce a positive result for the assay that is utilized.

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

Maintenance:

The Immunalysis Tecan Freedom EVO 75 Workstation maintenance log book will be filled out at the completion of any set of ELISA runs. Any other maintenance or service performed on this instrument will be documented in the log book.

Literature References:

Immunalysis Tecan Freedom EVO 75 Workstation Operating Manual
Immunalysis Tecan Freedom EVO 75 Workstation Operating Procedures