

URINE DRUG PROCEDURES

### About This Document

The Forensic Lab Director / Quality Manager reviews this document at annually. If changes are made, analysts acknowledge the updated procedures. Obsolete procedures are archived and retained by the laboratory.

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## URINE DRUG PROCEDURES

### **Introduction & Scope**

Forensic urine drug analysis is defined as the practical application of specialized devices, instruments, and methods by trained laboratory personnel to qualitatively detect drugs/drug metabolites in samples of urine.

This document describes data acquisition protocols for the qualitative detection of drugs/drug metabolites in urine as well as the toxicology quality guidelines for the analysis, evaluation, acceptance procedures, and reporting of urine toxicology results.

The methods described in this document are for the qualitative detection of drugs and drug metabolites in urine. Deuterated recovery compounds are added to the urine samples. Compounds of interest and corresponding deuterated recovery compounds are then efficiently partitioned from the urine sample via a solid phase or liquid/liquid extraction technique and separated on a gas chromatograph (GC) column and are analyzed using a mass spectrometer (MS) utilizing select ion monitoring (SIM) and full scan monitoring (SCAN) methodology.

### **Evidence Handling and Preservation**

All laboratory personnel will handle submitted materials in a manner that ensures the integrity of the evidence. Prior to and during the processing of evidence, the analyst will employ the following practices:

- a. The work area will be clean and free of any excess debris.
- b. Countertops shall be cleaned when dirty. Should any biological spill occur, work will be stopped, the area cleaned, and the counter wiped with an appropriate agent such as 10% bleach solution, or a "Lysol-like" product designed to clean and disinfect.
- c. All glassware and tools to be used will be clean.
- d. Test tubes, autosampler vials and Pasteur pipettes shall only be used once, then discarded.
- e. To prevent cross contamination of samples, **only one case will be opened and aliquoted at a time.**
- f. Reagents and solvents will be kept in closed containers when not in use.
- g. During analysis, the evidence will be under constant control by the analyst.
- h. Evidence to be analyzed will be removed from evidence refrigerator/freezer and the chain of custody side of the pink Receipt/Contract for Examination Form will be filled out.

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- i. The analyst opening the kit will initial stickers bearing the HETL Identification Number. If the subject's name is not available at the time of log-in, the analyst will write the subject's name on the labels when the collection kit is opened.
- j. If a non-HETL approved collection kit or materials were submitted, the WO# field in STARLIMS folder metadata shall be filled in with N/A.
- k. The analyst will verify all identification numbers and names agree with the Receipt/Contract for Examination Form. The analyst will verify any kit information match the Receipt/Contract for Examination Form. If the kit, upon opening, is found to contain non HETL approved collection materials this shall be noted on the inventory form and STARLIMS metadata shall be updated appropriately.
- l. The analyst will verify and note in the case notes that the case information provided with the kit matches the HETL folder, the sample information from the Laboratory Urine Drug Analysis Request form submitted with the sample, and all Starlims labels.

|                                                                                                                                                                                                                    | Action needed:                                                                                        | Kit Inventory Form<br>Items Received Table:                                           | Starlims Metadata:                                                                                                                                                                                                                              | Starlims Items<br>Received Table:                         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|
| <b>Blood Tubes/Urine Container &amp; Paperwork Contain matching collection date &amp; times</b>                                                                                                                    | None                                                                                                  | Record collection date & time                                                         |                                                                                                                                                                                                                                                 |                                                           |
| <b>Blood Tubes/Urine Container &amp; Paperwork collection date &amp;/or times do not match (Major Discrepancy)<br/><i>Example: collection date/time prior to incident date/time.</i></b>                           | <b>Communicate discrepancy with submitted and obtain correct information prior to finalizing COA.</b> | Record collection date & time <b>from tubes/container (even if it is not correct)</b> | Record correct/verified collection date and time obtained from submitting agency.<br><br>If submitting agency does not provide information Record collection date & time <b>from tubes/container (even if it is not correct) or leave blank</b> |                                                           |
| <b>Blood Tubes/Urine Container &amp; Paperwork collection date &amp;/or times do not match (Minor Discrepancy)<br/><i>Examples: collection times within ~10mins, typos (2023 vs 2024, duplication of birth</i></b> | None                                                                                                  | Record collection date & time <b>from tubes/container</b>                             | Record collection date & time <b>from tubes/container</b><br>(In cases with multiple tubes/collection times any time may be used)                                                                                                               | Record collection date & time <b>from tubes/container</b> |



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|                                                                                                                                                                |                                       |                                                           |                                                         |  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|-----------------------------------------------------------|---------------------------------------------------------|--|
| <b>date/year, 24hr clock<br/>vs 12 hr clock)</b>                                                                                                               |                                       |                                                           |                                                         |  |
| <b>Blood Tubes/Urine<br/>Container are<br/>unlabeled or missing<br/>collection<br/>information.<br/>Paperwork contains<br/>collection date &amp;<br/>time.</b> | None                                  | <b>Collection Date: NA<br/>Collection Time: NA</b>        | Record collection date & time <b>from<br/>paperwork</b> |  |
| <b>Blood Tubes/Urine<br/>Container contains<br/>collection date &amp;<br/>time. Paperwork<br/>missing collection<br/>information</b>                           | None                                  | Record collection date & time <b>from tubes/container</b> |                                                         |  |
| <b>Blood Tubes/Urine<br/>Container are<br/>unlabeled or missing<br/>collection<br/>information.<br/>Paperwork missing<br/>collection<br/>information</b>       | None (reach<br>out or leave<br>blank) | <b>Collection Date: NA<br/>Collection Time: NA</b>        | <b>Leave blank</b>                                      |  |

- m. If a sample or urine collection box requires a more detailed description or photographic documentation, the analyst may document the description in the urine inventory form, or by taking a picture using a state controlled camera (not a personal camera or cell phone). If a picture is taken, a ruler shall be included in the photo. The photo shall be printed for the case file and have the following items documented on the printout: HETL case number, item number(s), analyst initials, and date.
- n) If a more detailed description is necessary, the analyst may document the item by making additional notations, or by taking a picture using a state-controlled camera (not a personal camera or cell phone). If a picture is taken, the following steps should be taken.

**Photography procedure**

- Using all appropriate PPE, place the item to be photographed on a distraction-free background.
- Ensure proper lighting is being used.



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- c. Take a photograph with a scale and a label containing the HETL case number, item number, and photographer's initials.
- d. Print the photograph(s) with the date and time stamp on it, in color and include it in the casefile for retention.

### **Photograph Retention**

- e. All photographs should be retained as a part of case documentation.
  - f. Original photographs should not be destroyed. All photographs, including poor quality or unintended photographs should remain a part of case documentation.
  - g. Photographs may be introduced as evidence and therefore need to be maintained to prevent loss and degradation.
- o) The analyst will fill out the urine inventory form, making any necessary notations. The analyst will document the HETL case number on the urine inventory form.
- o For continuity the volumes entered into the inventory form and subsequent StarLIMS items received table shall adhere to the following format:

| Sample Submitted                                           | Volume (mL)                            |
|------------------------------------------------------------|----------------------------------------|
| Visibly empty urine container, no label                    | 0 (leave collection date & time blank) |
| Visibly empty urine container, with label                  | 0                                      |
| Low volume of urine in container (unusable testing volume) | <1mL                                   |
| Usable testing volume of urine in container                | Approximate volume                     |

- p) The sample container from the collection kit will be labeled with the lab identification number and name of the subject. Letter designations (A, B, C, etc..) shall be added to each container for identification.
- q) A worksheet(s) with the sample identification number will be used and will follow the sample throughout the analysis. Each unique case identification number will be placed onto all of the testing forms used.
- r) After the initial screening, new aliquot(s) will be used for confirmation testing. Even if the sample screens negative on the Randox Evidence Investigator, a base extraction will be performed as a screen for compounds that are not screened for with the Randox Evidence Investigator.
- s) Samples and controls shall be fortified with recovery compound(s) and stock(s), respectfully, on the same day as the extraction.



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- t) Samples will be stored in a secure location (i.e. evidence refrigerator) while thawing or if work stops for any reason. (i.e., lunch, end of workday, etc).
- u) Samples to be confirmed by GC/MS will be stored in the refrigerator until analysis is complete.
- v) Samples will be confirmed by GC/MS procedures found elsewhere in this manual. Results of the confirmation testing will be recorded on the Urine Drug Result Worksheet form. The worksheets, chromatograms, immunoassay results, etc. will be retained in the case file.
- w) After analysis, the sample container with the remaining urine sample will be sealed with evidence tape and the seal initialed by the analyst and placed in an evidence freezer.

After analysis samples will be retained for at least six months. Chain-of-custody forms will be updated as necessary and retained. All completed case files will be securely stored either in the Forensic Chemistry Lab or Office, the File Storage Room, or the Evidence Room when not in the possession of the Analyst.

Once analysis has been completed for a urine sample, the evidence shall be destroyed no earlier than six months from the last analysis end date unless claimed by the officer or a responsible official. Sample disposal shall be performed in accordance with HETL hazardous waste disposal policies. The casefile shall be updated to indicate the sample has been destroyed.

The sample kit or original container will be stored in an appropriately labeled storage box, the location of the kit shall be noted on the chain of custody. This storage box will be retained until filled. All filled storage boxes will be placed in storage until returned to the submitter or destroyed.

### **Definitions**

**Unextracted Control:** Laboratory fortified sample that is not put through the extraction process, used to evaluate extraction process efficiency.

**Positive Controls:** Laboratory fortified matrix matched samples that are prepared from a certified reference material and put through the extraction process. Used to update retention times and qualifier ion ratios as a concurrently run certified reference material.

**Hydrolysis Controls:** Laboratory fortified matrix matched samples that are prepared from a conjugated certified reference material and put through the extraction process. Used to confirm effectiveness of hydrolysis process.

**Negative Controls:** An extracted matrix matched sample containing recovery compound, put through the extraction process. Used to confirm no compound of interest carry over from any step in the process and evaluate all reagents used in the analytical method for potential interference. May be referred to as Blanks with Recovery compound.



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Recovery compound(s): Most commonly a compound of interest in the panel matched deuterated equivalent. All quality controls and case samples are fortified with recovery compound at a consistent concentration. Recovery compound(s) are used to evaluate extraction process efficiency and matrix suitability. May be referred to as “surrogate compound”.

Reporting Limit (RL): The lowest concentration at which an analyte has been validated to be qualitatively reported by the laboratory as positive.

Lower Limit of Detection (LLOD): The lowest concentration at which an analyte has been validated. The LLOD must exhibit the presence of the qualifier ion within +/-20% of the positive control and have a signal to noise ratio of  $\geq 3.3$  and a retention time within 0.2 minutes of the positive control.

Decision Point Cut Off: A set concentration within the method equal to or greater than the LLOD that is a decided point that the laboratory will report a positive compound(s) of interest.

Select Ion Monitoring (SIM): A type of mass spectrometry where the intensities of one or more specific ion are recorded rather than the entire mass spectrum. Is more sensitive than SCAN methodology but does not allow for detection of “unknown” ions.

Full Scan Monitoring (SCAN): A type of mass spectrometry where a mass range beginning at the smallest mass of fragment ions to the highest mass expected for the fragments is recorded. Used in conjunction with a reference library for detection of “unknowns”, not as sensitive as SIM methodology.

Multiple Reaction Monitoring (MRM): A type of mass spectrometry where the intensities of one or more specific ion transitions (Parent>Fragments) are recorded.

Autotune: A tuning process that involves adjusting mass spectrometer parameters through the infusion of a tune solution.

Tune MSD: A GCMS tuning process that involves adjusting mass spectrometer parameters through the infusion of a tune solution. This tuning process uses the set parameters from the last Autotune and adjusts all the same parameters as a Autotune at the set temperatures (source: 300° and quad: 180°).

Quicktune: A GCMS tuning process that involves adjusting mass spectrometer parameters through the infusion of a tune solution. This tuning process only adjusts mass axis, peak width, and EM voltage, there is no adjustment of mass abundances.

Checktune (Generate Report): A tuning process that evaluates but does not adjust mass spectrometer parameters through the infusion of a tune solution.



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### **Quality Assurance**

#### **Equipment Maintenance and Calibration:**

Refer to GC/MS Operation and Procedures and the Quality Manual

#### **Equipment List:**

Volumetric flasks      various sizes  
Volumetric cylinders   various sizes  
Disposable glass tubes (silanized and non-silanized) and associated caps  
Autosampler vials, caps, and inserts  
Vortex mixer  
Disposable transfer pipettes  
Pipettes & tips- various  
Sample evaporator/Nitrogen Gas Supply  
Solid Phase Extraction (SPE) manifold and columns  
Pasteur pipets  
Centrifuge  
Refer to Specific Analytical Procedures for procedure specific equipment

#### **Safety Precautions and PPE:**

The solvents used in these analytical processes are considered toxic. Repeated or prolonged exposure can produce targeted organ damage. Proper PPE consisting of lab coats, gloves, and eye protection shall be used when handling solvents. When appropriate, a chemical fume hood shall be used. Due to the solvents utilized during the extraction process, all use of solid phase extraction (SPE) manifold(s), addition of derivatizing agents, and vialing of samples into autosampler vials shall be performed in a chemical fume hood.

#### **Waste Management:**

The most current approved version of the laboratory's hazardous waste plan shall be followed for the generating, labeling, and disposal of all hazardous waste generated by these methods. Maine Department of Environmental Protection rules Chapter 850: Identification of Hazardous Waste, Chapter: 851 Standards for Generators of Hazardous Waste, and Chapter 858: Universal Waste Rules shall be adhered to. Waste disposal for urine testing methods should follow the most recent waste stream evaluation forms on file in the section.

#### **Reagents, Standards, and Quality Control Materials:**

An evaluation of all new positive control stocks created shall be performed by, prior to any casework being performed, using this new stock and/or this new stock and the old stock (if available), to run a batch consisting of:

| <b>Control:</b>        | <b>Made with:</b>                            |
|------------------------|----------------------------------------------|
| UNEX (when applicable) | <i>Old Stock (if available) or New Stock</i> |



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|                      |                  |
|----------------------|------------------|
| PC                   |                  |
| NC                   |                  |
| HC (when applicable) |                  |
| Reporting Limit      | <i>New Stock</i> |

All acceptance criteria must be met for controls and the compounds in the reporting limit must meet all positive acceptance criteria for the new stock to be deemed acceptable to be used for casework.

For additional information refer to Quality Manual, SOP Manual and Urine Drug Testing Reagent Sheets.

### Quality Controls

The following control checks will be performed during the analytical process (See Specific Analytical Procedures for details):

#### Screening Testing:

Calibration (Randox Evidence Investigator) with new lot number or as indicated/needed.

Positive control

Negative control

#### Confirmatory Testing (GCMS):

Unextracted Control (UNEX)

Recovery compound

Negative Control (NC)

Positive Control (PC)

Hydrolysis Control (HC) if required by specific analytical procedure

Reinjected Control (Reinj PC/NC/HC)

#### Confirmatory Testing (LC-MS/MS):

Positive Control (PC)

Recovery compound

Negative Control (NC)

Reinjected Control (Reinj PC/NC/HC)

During the course of the analysis, appropriate controls and standards are used to ensure the validity of the analysis and that the procedure is working properly.

Refer to the Screening-Immunoassay Randox Evidence Investigator Acceptance Criteria and Screening and/or Confirmation GC/MS or LC-MS/MS Acceptance Criteria sections for quality control acceptance parameters and criteria.



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### **Case Documentation:**

All case notes, spectra and other data generated during analysis will bear the initials of the analysts and case number. All batch files and casefiles will undergo Technical and Administrative Review.

Each batch folder shall contain: *(Each piece of paper within a batch folder shall contain the analyst initials.)*

- Raw/Summary data from the instrument for all quality controls (Unextracted (if applicable), Positive Control, Hydrolysis Control (if applicable), and Negative Control
- All associated methanol/mobile phase blanks or sample negatives (where required)
- Urine Drug Screen, GC/MS, LC-MS/MS Review forms
- Randox Template
- Randox QC Summary Report Form
- Urine Extraction form
- LC Qualifier Ion Ratio excel worksheet (if applicable)
- Urine Drug Testing Bench sheet (for each extraction performed)
- Instrument Sequence Table
- Copy of associated tune report (GCMS only)

Each Sample folder shall contain: *(Each piece of paper within a casefile shall contain the Laboratory Identification Number (Sample #) and analyst initials.)*

- Laboratory Urine Drug Analysis Request Form
- Receipt/Contract for Examination Form
- Urine Kit Inventory form
- Urine Drug Results Worksheet
- Raw/Summary data from the instrument including reinjections if applicable
- Copy of the final Certificate of Analysis
- Any preliminary, supplementary or corrected reports
- Case Review Form

It is noted that photocopies of the Urine Drug Screen Form, GCMS Control Review Forms, and associated methanol/sample negatives may be included in applicable casefiles as a convenience. If these forms are photocopied and added to the casefile, the photocopying shall be performed once the associated batch has completed technical and administrative review, this is to ensure that all appropriate changes and documentation are captured.

### **Urine Collection Kit:**

To evaluate collection kits, one kit from each lot number received is selected for quality control testing. The chemist performing the quality control testing will complete the appropriate collection kit QC form/reagent sheet listing all the lot numbers and expiration dates, if applicable, associated with the kit. Upon completion of testing the generated data and form is reviewed and approved. If the quality control testing shows no evidence of



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contamination, staff are notified the kits have been approved and can be stamped "Approved by Maine HETL".

Processing and testing procedure:

1. The lot number of the urine collection kit shall be recorded on the Urine Collection Kit QC Form.
2. The lot number of the negative urine used to test kit shall be recorded on the Urine Collection Kit QC Form and enough urine shall be placed into the urine collection cup to perform screening and confirmatory testing.
3. The urine collection cup sample shall be screened using the Randox Evidence Investigator Evidence Investigator and the Base GC/MS panel. All batch IDs shall be recorded on the Urine Collection Kit QC form.
4. The urine collection cup sample shall then be run using all GC/MS confirmatory panels, with the exception of the base, as it was run in the screening step. Confirmatory testing shall be performed since the limit of detection of Randox Evidence Investigator Evidence Investigator may be higher than GC/MS assays. All batch IDs shall be recorded on the Urine Collection Kit QC Form.
5. The urine collection cup sample must test negative for all target compounds to be approved.

Acceptable results exhibiting no interferences caused by manufacturing contaminants or interfering compounds are required prior to the lot number being distributed. Records containing the results, pass/fail status, and lot number of the collection kits shall be maintained.

### **Negative Urine Matrix:**

New lot numbers of negative urine matrixes may be concurrently screened with case samples as a negative control, screened independently of case samples, or tested as part of Collection Kit QC. All screening criteria must be deemed negative for the negative urine matrix to be used as a negative control for GC/MS extractions.

### **Sample Requirements:**

Only urine samples shall be analyzed using this method.

If a urine sample is received for testing in a non HETL approved urine collection kit/cup it shall be notated on the Urine kit inventory form.

Urine samples are collected and upon receipt, stored under refrigeration (0-8°C) or freezer (0-30°C) until analysis begins.

If a urine sample is received by the laboratory with volume not sufficient to perform standard OUI urine drug testing, samples may be screened using Randox Evidence Investigator and then selected confirmatory testing, volume permitting. Analysts will refer to any submitted case documentation for indicated or suspected drugs. Alternatively, if there is no documentation referring to any indicated or suspected drugs, the analyst may reach out to the investigating officer notifying them that the sample is QNS to perform the standard OUI



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urine drug panel and discuss course of testing. If the investigating officer does not respond the analyst may use their discretion to determine the best course of testing.

### **Specific Analytical Procedures**

#### **Screening by Immunoassay – Randox Evidence Investigator**

##### **Principles of the Procedure:**

The core of Biochip Array Technology is a solid state biochip onto which antibodies specific to different drug compounds are immobilized and stabilized and defined as discrete test sites. Competitive chemiluminescent immunoassays are employed for the biochip arrays. Light signal generated from each of the test regions on the biochip is simultaneously detected using digital imaging technology and compared to a calibration curve

The DOA Ultra Urine Assay from Randox is used for screening urine samples for drugs. For additional information refer to the Randox information provided electronically with each kit.

##### **Quality Controls:**

During the course of the analysis appropriate controls and standards are used to ensure the validity of the analysis and that the procedure is working properly. The following measures will be used:

A batch is considered a group of samples run at the same time. Each batch can use up to 6 Randox Evidence Investigator chips simultaneously. Quality controls (both positive and negative) will be run with each batch.

The controls used in each analysis will be documented in the batch folder

The results of all controls will be kept in the batch folder, which is also retained.

##### **Acceptance Criteria:**

Randox Evidence Investigator calibration controls are provided with the kit as well as manufactured quality controls (high and low controls).

Nine calibration points will be run to generate a calibration curve, the Randox Evidence Investigator software indicates if these calibration points are acceptable by pass/fail. The Randox Evidence Investigator software indicates if the curve is acceptable by pass/fail. A maximum of two calibration points can fail per category in a calibration curve. If greater than two calibration points fail the calibration curve is rejected, any data generated shall be rejected and the screen shall be performed again with a new calibration curve. Calibration curves should be run with at least each new kit lot number or if needed.

At least one positive control is run per batch shall be required, either Control I or a Control II. The Randox Evidence Investigator software indicates if the positive control is acceptable. Analysts will review the control data to determine if each category passes or fails based on the results of the control. If the value is colored red (>3 standard deviation) on the Randox Evidence Investigator



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software, the category fails. If the value is colored yellow (>2 standard deviation) or blue the category passes. At least one passing category between all positive controls is required for the screen batch to pass. If any category fails between all positive controls for a batch, any data generated shall be rejected and the screen shall be performed again. Alternatively a new calibration curve may be required.

One negative control per batch will be screened along with case samples. If any drug is detected in the negative control, all data generated shall be rejected and the screen shall be performed again.

A positive Randox screen result is indicated by the software based on the manufacturer's cutoff levels. Any results that are indicated as positive (+) or borderline (B) shall be considered a positive screen and move on to confirmation testing.

The Randox Evidence Investigator screens for more drugs/metabolites than are currently tested for in the GC/MS confirmation methods. If a sample screens positive for a category that there is no GC/MS confirmation method for (BUP, BARB, BENZ2, BENZ3, MBP) confirmation testing shall not be performed for that category. No screening results obtained from the Randox Evidence Investigator shall be reported out on the certificate of analysis reports.

### **Operation: Manufacturer's Instructions**

**Step by Step Randox Evidence Investigator Screening Sample Preparation Process:** The manufacturer recommends that reverse pipetting technique is used for this procedure:

How to Reverse Pipette:

1. Set the pipette to the desired volume.
2. Depress the plunger completely – past the first stop to the second (blowout) stop.
3. Immerse the tip in the liquid, and slowly release the plunger to full extension.
4. Dispense by pressing to the first stop.
5. A small volume of liquid will remain in the tip.

This procedure is to guide the Randox Evidence Investigator screening sample preparation process.

1. Put carrier securely into handling tray prior to adding liquids.
2. Pipette 220  $\mu$ L of assay diluent per well.
3. Pipette 10  $\mu$ L of calibrator/control/sample per well.
4. Pipette 120  $\mu$ L of conjugate per well. Mix by gently tapping the edge of the handling tray.
5. Secure the handling tray to the base plate of the thermoshaker. Incubate for 30 minutes at +37°C and 330 rpm.
6. Prepare Luminol/Peroxide solution, by adding peroxide to luminol, and place on rocker.
7. Ensure wash buffer is prepared. If preparation is necessary, add half a bottle of wash buffer to a 500mL DI wash squeeze bottle and fill to top with DI water. Wash buffer is stable for 30 days when stored at refrigerated temperatures.
8. Following incubation, remove the handling tray containing the carriers from the thermoshaker. Discard reagents into a waste container using a sharp, flicking action of the handling tray. All liquid waste generated shall be disposed of in the appropriate hazardous waste stream



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9. Immediately carry out 2 quick wash cycles. Using wash bottle with diluted wash buffer. add approx. 350  $\mu$ L wash buffer to each well, gently tapping the handling tray to release any reagents trapped below the biochip, and flick to waste with a sharp action. Take care not to overfill wells during washing in order to reduce potential for well-to-well contamination. Carry out a further six, 2 minute wash cycles, for each cycle gently tapping the handling tray for approximately 10 to 15 seconds, then leave the biochips to soak in wash buffer for 2 minutes.
10. After the final wash, fill wells with wash buffer and leave to soak protected from light until directly prior to imaging. No carrier should be left to soak for longer than 30 minutes.
11. IMAGING: Process carriers individually. Remove the first carrier to be imaged from the handling tray. Directly before addition of signal, remove wash buffer using a sharp, flicking action and tap the carrier onto lint free tissue to remove any residual wash buffer.
12. Add 250  $\mu$ L of 1:1 prepared Luminol:Peroxide solution to each biochip well and cover to protect from light.
13. Place the carrier into Evidence Investigator after 2 minutes ( $\pm$  10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time.
14. Images will be automatically captured after loading chips.

### **Step by Step Suggested Radox Evidence Investigator Screening Computer Setup Process:**

*(Start during incubation process to allow Radox instrument to get to temperature, instrument takes ~30 minutes to reach running temperature)*

1. Open software, login, select Sample Entry
  - a. New worklist-add name (ddmmyy & initials, example 050324EAF)
2. Sample Tab, select DOA, put sample names into wells and for controls right click and assign control (select-yes) accepted controls will turn green.
3. Accept carrier.
4. Highlight, right click and select profile.
5. Click apply and all entries will turn green.
6. Accept samples, select Ok twice.
7. The message "Please Open Door" will appear. (at this point stop until you are ready to place samples into the instrument)
8. Once ready select OK to open the door, and place carrier into instrument.
9. How to print reports
  - a. QC Display, select correct controls and lot number. Print QC Summary Report.
  - b. Result History, select correct batch
  - c. Individual Reports (separate), print all, opens as a PDF then print.

### **Documentation:**

Sample results are printed and stored in respective casefiles.

Calibration curves, positive control(s), QC Summary Report Form, and negative control are printed and stored in the batch folder.

Radox Evidence Investigator Templates are stored in the batch folder.

### **Screening and/or Confirmation by GC/MS**



## URINE DRUG PROCEDURES

### Principles of the Procedure:

Gas chromatograph mass spectrometer (GC/MS) works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas and into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule.

The detections of drugs should be confirmed (when possible) by a second technique based on a different instrument methodology, extraction method, and/or chemical principle. If a second technique is not possible then the confirmation must be performed on a different aliquot of the same sample.

If you are using the GCMS as a screen, and your initial undilute aliquot has an IS failure, specifically due to any issues arising from potential inference from a high concentration compound, the analyst shall indicate the compound(s) that is suspected to be causing the interference on the data and may then extract two separate diluted aliquots in the same batch to be used for screening and confirmation purposes. The compounds present in the dilution results must be consistent with the data from the initial undilute sample to be deemed acceptable. If differences are present, all data shall be rejected, and re-extractions shall be performed after review of the data.

Example: Using GCMS COC method to screen. Initially run undilute has low IS response and there is an overwhelming plateauing peak for Benzoylecgonine (the recovery compound Cocaethylene-d3 and Benzoylecgonine have similar retention times). Sample data is rejected due to the failing IS, but benzoylecgonine is indicated as the compound interfering with the IS. Two separate aliquots are diluted and extracted in the same batch to screen and confirm. If Benzoylecgonine is seen in both of these dilutions and meets all acceptance criteria, the dilution data can be accepted as a screen and confirmation.

Example: Using GCMS Base method to screen. Initially run undilute has low IS response and there is an overwhelming plateauing peak for PCP. Sample data is rejected due to the failing IS, but PCP is indicated as the compound interfering with the IS. Two separate aliquots are diluted and extracted in the same batch to screen and confirm. If PCP is not present with acceptable criteria in either dilution but fentanyl was present in both dilutions, all dilution data will be rejected and new undiluted and re-extractions shall be performed after review of the data.

### Quality Control Requirements & Acceptance Criteria:

**Unextracted Controls:** One unextracted positive control per extraction batch shall be created and derivatized as necessary. This unextracted control serves to evaluate extraction efficiency and all unextracted controls must meet/exhibit the following:

The recovery compound(s) must exhibit:

Acceptable chromatography

Recovery compound recovery response (surrogate percent recovery) is set to 100.0% (this is achieved by setting Unextracted control to Type: Cal, Level: 1. This can be set up in the instrument sequence or as needed in Masshunter Data Analysis)

S/N ratio  $\geq 3.3$



## URINE DRUG PROCEDURES

Retention time within 0.2 minutes of the concurrently run positive control  
Exhibit a minimum of two qualifier ion ratios within +/-20% of the concurrently run positive control.

If an recovery compound does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.

Each compound of interest must meet/exhibit the following:

Contains all compound(s) of interest

Acceptable chromatography

S/N  $\geq 3.3$

All retention times +/-0.20 min of (extracted) Positive Control

If the compounds of interest(s) does not meet the above parameters, any associated data generated shall be rejected for that compound(s) and any impacted samples shall be re-extracted.

It is noted that all of the data analysis methods are set to have a upper surrogate % recovery limit of 500%. This upper cap serves two purposes, one allows the data analysis method to calculate percent recovery for all samples in the batch and secondly serves as a indicator to further investigate the response of the UNEX control.

**Methanol Blanks:** Methanol blanks shall be run at the start of each batch and following each case sample extracted by a liquid-liquid extraction method to monitor for instrument carryover.

All methanol blanks must meet/exhibit the following:

Each compound of interest and recovery compound must meet one or more of the following to be considered negative:

No peak at expected RT

Peak response is below threshold (20% of positive control)

Unacceptable qualifier ion ratios

S/N <3.3

If a methanol blank does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.

**Negative Controls:** One batch negative control per extraction batch shall be extracted along with case samples. This batch negative control is to monitor for contamination of extraction consumables, reagents, and matrix. Additionally, each sample extracted using a SPE method (all except for THC-COOH\*) shall also have an associated sample negative control. This sample negative control shall be extracted and run preceding the sample to monitor for potential carryover from the SPE manifold or GCMS.

*\*As the THC-COOH method does not have associated sample negative controls, an associated methanol blank shall be run immediately after each sample to monitor for GCMS carryover.*

All negative controls must meet/exhibit the following:

The recovery compound(s) must exhibit:

Acceptable chromatography

Recovery at  $\geq 50\%$  of the concurrently run unextracted control

S/N ratio  $\geq 3.3$



## URINE DRUG PROCEDURES

Retention time within 0.2 minutes of the concurrently run positive control

Exhibit a minimum of two qualifier ion ratios within +/-20% of the concurrently run positive control.

Each compound of interest must meet one or more of the following to be considered negative:

No peak at expected RT

Peak response is below threshold (20% of positive control)

Unacceptable qualifier ion ratios

S/N <3.3

If the batch negative control does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.

For sample negative controls:

- If a sample negative control does not meet acceptability parameters for the recovery compound, and one or more of the bracketing samples are negative for all compounds of interest AND exhibit acceptable recovery compound then the sample may be reported out as negative.
- If a sample negative control does not meet the above parameters with regards to compounds of interest, the samples bracketing the rejected negative control shall be rejected for those compound(s). The sample(s) shall be re-extracted only if the sample(s) meets positive acceptance criteria for the compound(s) seen in the negative control. See below examples:

| Name         | Method     | Sample Positive For the Following: | Action Taken:                                                                        |
|--------------|------------|------------------------------------|--------------------------------------------------------------------------------------|
| UNEX         | Cocaines.M |                                    |                                                                                      |
| Batch Neg    | Cocaines.M |                                    |                                                                                      |
| PC1          | Cocaines.M |                                    |                                                                                      |
| Sample Neg 1 | Cocaines.M |                                    |                                                                                      |
| Sample 1     | Cocaines.M | Cocaine & Benzoylgonine            | <i>No action needed</i>                                                              |
| Sample Neg 2 | Cocaines.M |                                    |                                                                                      |
| Sample 2     | Cocaines.M | Benzoylgonine                      | <i>Source of contamination, results accepted</i>                                     |
| Sample Neg 3 | Cocaines.M | Benzoylgonine                      |                                                                                      |
| Sample 3     | Cocaines.M | Cocaethylene                       | <i>Bracketing samples does not contain possible contamination, results accepted.</i> |

| Name         | Method     | Sample Positive For the Following: | Action Taken:                                                                                |
|--------------|------------|------------------------------------|----------------------------------------------------------------------------------------------|
| UNEX         | Cocaines.M |                                    |                                                                                              |
| Batch Neg    | Cocaines.M |                                    |                                                                                              |
| PC1          | Cocaines.M |                                    |                                                                                              |
| Sample Neg 1 | Cocaines.M |                                    |                                                                                              |
| Sample 1     | Cocaines.M | Cocaine & Benzoylgonine            | <i>Bracketing sample contains possible contamination, must re-extract, may need dilution</i> |
| Sample Neg 2 | Cocaines.M | Benzoylgonine                      |                                                                                              |
| Sample 2     | Cocaines.M | Benzoylgonine                      | <i>Bracketing sample contains possible</i>                                                   |

Urine Drug Procedures: Doc # = 005

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## URINE DRUG PROCEDURES

|              |            |              |                                                          |
|--------------|------------|--------------|----------------------------------------------------------|
|              |            |              | <i>contamination, must re-extract, may need dilution</i> |
| Sample Neg 3 | Cocaines.M |              |                                                          |
| Sample 3     | Cocaines.M | Cocaethylene | <i>No action needed</i>                                  |

In addition, if any negative control does not meet the above parameters the batch shall be evaluated for the source of contamination or causation.

**Positive Controls:** One positive control per extraction batch will be extracted along with case samples. When applicable this control will be split/divided into two autosampler vials with one injected at the beginning of the batch and one injected at the end of the batch. The first positive control is used in the batch to update retention times and qualifier ion ratios (as a concurrently run certified reference material). This first positive control shall also be used as a calibrator point to allow the Masshunter Data Analysis software to flag low response results.

Until an extracted sample stability study is performed the laboratory shall inject a second (split) positive control at the end of the batch. This second (split) control is injected to ensure that the extracted samples are stable at room temperature over the entirety of the batch.

All positive controls must meet the following:

The recovery compound(s) must exhibit:

Acceptable chromatography

Recovery at  $\geq 50\%$  of the concurrently run unextracted control

S/N ratio  $\geq 3.3$

Contains all compounds of interest

Compounds of interest exhibit acceptable chromatography

Compounds of interest S/N  $\geq 3.3$

First Positive control only:

All qualifier ion ratios updated (set to  $100\% \pm 1\%$ )

All retention times updated (set to 0.0xx)

Set to Type: Cal, Level: 2 (this can be set up in the instrument sequence or as needed in Masshunter Data Analysis)

Second Positive control (when applicable):

Exhibit a minimum of two qualifier ion ratios within  $\pm 20\%$  of the concurrently run positive control.

Compound retention times  $\pm 0.20$  minutes of first positive control

Contains all compounds of interest of  $>20\%$  of first positive control

First Positive Control:

- If the first positive control does not meet the above parameters for all compounds of interest, any associated data generated shall be rejected and the batch shall be re-extracted (or reprocessed).



## URINE DRUG PROCEDURES

- For a positive control that does not meet the above parameters for one/a compound of interest, any associated data that is being confirmed for that compound shall be rejected and re-extracted. Any sample that is not being confirmed for that rejected compound may be reported out, as long as there is sufficient acceptable data for the absence of that compound from a prior analysis. Caution and care shall be taken as the Randox Evidence Investigator screens compounds in groups/categories so the Randox Evidence Investigator Cross Reactivity Table shall be consulted to ensure that the sample did not screen positive for a group/category that contains the rejected compound.

### Second Positive Control:

- The following testing methods do not require a second positive control run at the end of the batch as validation stability studies have been performed for these methods:
  - Amines
  - Base
- If the second positive control does not meet the above parameters for one or all compounds of interest/recovery compounds, each sample must be evaluated to determine if the data for that sample is rejected or accepted, as the compound(s) of interest may have already been determined in a previous passing extraction for that sample. As long as the results for the passing extraction are consistent with the data being evaluated, and there is no evidence to indicate any compound degradation, the sample data can be accepted and used towards the confirmation despite the failure of the second positive control.
- GCMS method used for screening only: All previously listed acceptance criteria must be met with the exception of qualifier ion ratios. Qualifier ion ratios for the compound(s) of interest of  $\geq \pm 20\%$  of the first positive control will be acceptable for screening.

**Hydrolysis Controls:** One hydrolysis control per hydrolysis extraction category (THC-COOH, Benzodiazepine, Narcotic) will be extracted along with case samples. This control evaluates the efficiency of the hydrolysis process. All hydrolysis controls must meet the following:

The recovery compound(s) must exhibit:

Acceptable chromatography

Recovery at  $\geq 50\%$  of the concurrently run unextracted control

S/N ratio  $\geq 3.3$

Retention time within 0.2 minutes of the concurrently run positive control

Exhibit a minimum of two qualifier ion ratios within  $\pm 20\%$  of the concurrently run positive control.

Compound(s) of interest must exhibit:

Acceptable chromatography

Exhibit a minimum of two qualifier ion ratios within  $\pm 20\%$  of the concurrently run positive control.

S/N  $\geq 3.3$

Retention times within  $\pm 0.20$  minutes of concurrently run Positive Control

Response  $\geq 20\%$  of concurrently run Positive control



## URINE DRUG PROCEDURES

If a hydrolysis control does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.

### **GC/MS Screening Sample Acceptance Criteria:**

The GC/MS Base method is used for sample screen for drugs/metabolites that are not included in the Randox Evidence Investigator panel.

The Randox Evidence Investigator screening detection levels are higher than GC/MS detection levels. Therefore compounds not included in the immunoassay screen or compounds included in the immunoassay screen but at a lower concentration levels may be detected during GC/MS data review.

In the event that the Randox Evidence Investigator is not able to be used for screening, all of the GC/MS methods may be run as a screen. Note: if this screening method is utilized confirmatory testing must be performed using a second aliquot extracted separately.

In these instances, GC/MS data may be used/defined as screening results. Screening conducted in this manner will meet the following:

Compound(s) present with acceptable chromatography and response  $\geq 20\%$  as compared to the concurrently run positive control.

The retention time of the compound(s) of interest being screened are within 0.2 minutes of the expected value as compared to the concurrently run positive control.

The signal to noise ratio shall be evaluated for the compound(s) of interest being screened and will be  $\geq 3.3$  ratio for the primary ion.

If a drug is detected with GC/MS data in this fashion, it must be re-extracted. Documentation in the case file will clearly indicate which data is a screen and which is a confirmation by adding the word "screen" or "confirmation" to the printout.

### **GC/MS Confirmation Sample Acceptance Criteria:**

1. All concurrently run positive control 1, hydrolysis control (if applicable) and negative control 1 shall be evaluated prior to data analysis being performed on a subject sample to confirm the controls meet acceptance criteria.
2. The recovery compound in a subject sample shall then be evaluated for the following to determine if the sample is suitable for comparison to concurrently run controls.
  - Presence of the recovery compound peak with acceptable chromatography
  - A recovery of greater than 50%
  - Signal to noise ratio of  $\geq 3.3$
  - Retention time  $\pm 0.2$  minutes
  - Minimum of two qualifier ion ratios within  $\pm 20\%$  of the concurrently run positive control.



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3. Once the subject sample has been deemed suitable for comparison, the analyst can determine the presence of a target compound. In order for a compound to be confirmed as positive, the following acceptance criteria must be met:

- Compound(s) present with acceptable chromatography and response  $\geq 20\%$  as compared to the concurrently run positive control.
- The retention time of the compound(s) of interest being confirmed are within 0.2 minutes of the expected value as compared to the concurrently run positive control.
- Ion ratios for the compound(s) of interest being confirmed shall be within  $\pm 20\%$  of a concurrently run standard for a minimum of two ions.
- The signal to noise ratio shall be evaluated for the compound(s) of interest being confirmed and will be  $\geq 3.3$  ratio for the primary ion.

### Screening and/or Confirmation by LC-MS/MS

#### **Principles of the Procedure:**

The methods described in this section are for the qualitative detection of drugs and drug metabolites in urine. Recovery compounds are added to the urine samples. Compounds of interest and the corresponding recovery compound are then efficiently partitioned from the urine sample via liquid/liquid extraction techniques, then separated on a liquid chromatograph (LC) column and are analyzed using tandem mass spectrometers (MS/MS) utilizing multiple reaction monitoring (MRM).

The detections of drugs should be confirmed (when possible) by a second technique based on a different instrument methodology, extraction method, and/or chemical principle. If a second technique is not possible then the confirmation must be performed on a different aliquot of the same sample.

#### **Quality Control Requirements & Acceptance Criteria:**

##### **Qualitative Quality Controls**

A corresponding Mix (positive control) shall be run with each new sequence. Mixes shall consist of blank matrix samples fortified at respective positive concentrations, extracted, and analyzed with each batch. Mixes function as both positive quality control to confirm the success of an extraction for the compounds of interest and as a calibration point in the creation of a one-point calibration curve to be used as a quantitative frame of reference for case samples. It is noted that the Qualitative A method A produces semi-quantitative results that are derived from a one-point calibration forced through zero curve, to achieve approximate quantitative results. The approximate quantitative values shall never be reported on any certificates of analysis.



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A qualitative batch shall consist of respective mixes and a negative quality control for every twenty samples.

**Qualitative Mix A (positive control) acceptance criteria:**

- Contains all compounds of interest and recovery compound.
- Compounds of interest and recovery compound exhibit acceptable chromatography.
- Compounds of interest S/N  $\geq 3.3$
- All retention times updated
- All qualifier ion ratios updated

**Second Positive Qualitative Mix A (positive control) acceptance criteria:**

- Contains all compounds of interest and recovery compound.
- Compounds of interest and recovery compound exhibit acceptable chromatography.
- Compounds of interest S/N  $\geq 3.3$
- All retention times within  $\pm 0.20$  minutes of first Mix A
- All qualifier ion ratios within  $\pm 30\%$  of first Mix A

**Qualitative Negative Quality Control** shall consist of a blank matrix fortified with only recovery compound and shall be extracted and analyzed with each batch after the Mix (positive control) in every batch to monitor for carryover. All Qualitative Negative Quality Controls must exhibit/meet the following:

- Recovery of recovery compound  $\geq 50\%$  of concurrently run Mix
- Recovery compound retention time  $\pm 0.20$  min of concurrently run Mix
- Recovery compound exhibits qualifier ion ratios within  $\pm 30\%$  of concurrently run Mix A
- Recovery compound S/N  $\geq 3.3$

If the batch negative control does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.

- Each compound of interest must meet one or more of the following to be considered negative:
  - No peak at expected RT
  - Calculated concentration is less than the decision point cut off
  - Unacceptable qualifier ion ratios
  - S/N  $< 3.3$

**Sample Mobile Phase Blanks:** shall consist of the reconstitution agent and shall be analyzed after each case sample monitor for carryover. All Sample Mobile Phase Blanks must exhibit/meet one or more of the following:

- No peak at expected RT
- Concentration of Benzodiazepine compounds  $< 10\text{ng/mL}$
- Concentration of Stimulant compounds  $< 20\text{ng/mL}$
- Unacceptable qualifier ratios
- S/N  $< 3.3$



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If a sample mobile phase blank does not meet the above parameters with regards to compounds of interest, the samples bracketing the rejected sample mobile phase blank shall be rejected for those compound(s). The sample(s) shall be re-extracted only if the sample(s) meets positive acceptance criteria for the compound(s) seen in the sample mobile phase blank.

### **LC-MS/MS Screening Sample Acceptance Criteria:**

The Randox Evidence Investigator screening and GCMS methods detection levels are higher than LC-MS/MS detection levels. Therefore, compounds not included in the immunoassay screen or compounds included in the immunoassay screen/GCMS methods but at a lower concentration levels may be detected during LC-MS/MS data review.

In the event that the Randox Evidence Investigator is not able to be used for screening, the LC-MS/MS method may be run as a screen. Note: if this screening method is utilized confirmatory testing must be performed using a second aliquot extracted separately.

In these instances, LC-MS/MS data may be used/defined as screening results. Screening conducted in this manner will meet the following:

Compound(s) present with acceptable chromatography and concentration greater than the decision point cutoff.

The retention time of the compound(s) of interest being screened are within 0.2 minutes of the expected value as compared to the concurrently run positive control.

The signal to noise ratio shall be evaluated for the compound(s) of interest being screened and will be  $\geq 3.3$  ratio for the primary ion.

If a drug is detected with LC-MS/MS data in this fashion, it must be re-extracted. Documentation in the case file will clearly indicate which data is a screen and which is a confirmation by adding the word "screen" or "confirmation" to the printout.

### **LC-MS/MS Confirmation Sample Acceptance Criteria:**

1. The concurrently run Mix A 1 (positive control 1) and negative control shall be evaluated prior to data analysis being performed on a subject sample to confirm the controls meet acceptance criteria.
2. The recovery compound in a subject sample shall then be evaluated for the following to determine if the sample is suitable for comparison to concurrently run controls.
  - Presence of the recovery compound peak with acceptable chromatography
  - A recovery of greater than 50% of concurrently run Mix
  - Signal to noise ratio of  $> 3.3$
  - Retention time  $\pm 0.2$  minutes
  - Qualifier ion ratios within  $\pm 30\%$  of the concurrently run Mix
3. Once the subject sample has been deemed suitable for comparison to concurrently run controls, the analyst can determine the presence of a target compound by making a comparison to



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the concurrently run certified reference materials. For a compound to be positive, the following acceptance criteria must be met:

- Compound(s) present with acceptable chromatography
- The retention time of the compound(s) of interest being confirmed is within 0.2 minutes of the expected value as compared to the concurrently run certified reference materials (Mix A control).
- The signal to noise ratio shall be evaluated for the compound(s) of interest being confirmed and will be greater than a 3.3 ratio for the primary ion.
- Concentration: For all compounds of interest within the Qualitative A method a decision point cut off concentration is used.
  - Screening: if all other acceptance criteria is met and a calculated approximated concentration is close to the respective decision point, cut off the compound of interest may be deemed a presumptive positive screen result based on analyst discretion.
  - Confirmation: Samples exhibiting compound(s) of interest at a concentration equal to or greater than the decision point cutoff shall be recorded as positive.
- Ion ratios for the compound(s) of interest shall be within +/- 30% of a concurrently run Mix.

### GC/MS & LC-MS/MS Testing Information

#### **Reporting of Confirmatory Drug Testing:**

Urine Drug test results are reported in the following manners (with words similar to, or having the same meaning):

“The following drugs were confirmed in this sample – [list drugs -or- No Drugs Detected]”

When confirming the presence of a drug in a urine Sample, LC-MS/MS and/or GC/MS will be used.

Any method deviations will be communicated to the customer on the report in the form of a comment.

If a urine sample is received by the laboratory with volume not sufficient to perform HETL OUI urine drug panel and only a portion of the confirmation testing menu can be confirmed for, then the following comment shall be included on the COA: “Unable to perform complete HETL OUI urine drug panel due to low sample volume.”

If a sample is unable to be tested or completed due to the sample matrix (inconclusive results), then the following comment shall be included on the COA: “Unable to perform complete HETL OUI urine drug panel due to sample matrix.”

An inventory table will be included on the report to indicate the number and types of containers received, the collection information, the approximate volume of each container, and an indication as to which containers were tested.



## URINE DRUG PROCEDURES

Expert witness letters will list all references used and contain, at minimum, the HETL case number and Subject's name, when applicable.

### **Carryover:**

Carryover may occur due to extremely high drug concentrations in biological samples and extreme caution is warranted when carryover is detected. To monitor for carryover, extracted sample negative controls shall be extracted in a bracketing formation to monitor for extraction carryover for every SPE method. Methanol/Mobile Phase blanks shall also be run at the start of each batch and following each case sample extracted by a liquid-liquid extraction method to monitor for instrument carryover.

The results of the methanol/Mobile Phase blanks and the sample negative controls shall be analyzed with the associated data analysis method to evaluate for carryover. If carryover is detected, the supervisor must be notified to provide guidance and review the analytical results.

### **Dilutions:**

Dilutions shall be noted with the dilution factor in the sample name or written on the generated data analysis report. The multiplication/dilution factor shall not be put into the sequence table as this will impact the surrogate recovery calculations in the data analysis methods.

Dilutions of case samples shall be performed as needed for confirmation testing by the analyst. Each extraction method has approved validated dilutions. New dilutions may be performed if the new dilution is within the range of already validated dilutions. If a dilution is needed that is greater than the validated dilution options, a validated dilution must be run to compare.

It is noted that the elevated presence of a compound of interest may impact the recovery compound (such as recovery compound qualifier ion ratios outside acceptance parameters) in a sample and even in a validated dilution. In these cases, all validated dilutions/the highest validated dilution must be evaluated prior to, or concurrently with, running a non-validated dilution.

The urine drug testing menu contains many compounds that are drugs and their associated metabolite (Examples: Cocaine & Benzoylecgonine or Methadone & EDDP), it is acceptable to only report out a drug or its associated metabolite instead of performing numerous dilutions to report out a drug and its associated metabolite. Care must be taken as there are some drugs and their associated metabolite in which the associated metabolite is also a drug (Example: Methamphetamine metabolizes to Amphetamine, but Amphetamine is also a drug in its own right), in these cases, sample volume permitting, appropriate dilutions must be found to report out these drugs.

Dilutions may need to be performed for, but are not limited to, two reasons:

Case samples with low sample volume: If a case sample does not contain enough volume to perform an extraction then a dilution may be performed to allow for extraction. Each extraction has been validated at a set sample volume. For example if 1mL is the set sample volume for a extraction and there is <1mL of sample, then a smaller amount of sample may be used as part of a validated dilution (example: 500ul of water + 500uL of urine sample) to allow for testing but must be brought up to full



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sample volume. The extraction methods have not been validated to test partial samples (example: only 500uL urine sample), as partial sample volume may impact extraction efficiency.

Case samples with high levels of compounds of interest or analyst discretion/experience: If a case sample screens high or is found to contain high levels of a compound of interest, then a dilution may be performed. Results from the preliminary screen may indicate the need to perform a dilution for confirmation testing. If a sample is run using the confirmatory method and some or all of the following are seen: qualifier ion ratios out, response significantly greater than the positive control, chromatography that exhibits tailing, or chromatography that appears to be saturating the detector (flat topped/plateau peaks) then a sample may need to be diluted. All samples must be brought up to the full volume of the extraction method.

If a case sample screens high or is found to contain high levels of a compound of interest or if the sample matrix is impacting the ability to confirm, a dilution may be performed to confirm presence without running a concurrent undiluted sample.

If there is a need for a sample dilution:

- If an undiluted sample indicates the need for a dilution, the sample shall be re-extracted with a dilution.
- If the results from the Randox Evidence Investigator indicates the need for a dilution the sample shall be run with a dilution as well as undiluted. The need for running a dilution and an undiluted sample is that the Randox Evidence investigator has higher reporting limits than the GC/MS and LC-MS/MS methods.

### **Reinjections of Samples Following Instrument Troubleshooting:**

Batches frequently can run over a significant amount of time (overnight). If a sample in a batch that has run overnight requires reinjection the following day, the instrument must be tuned (checktune or autotuned) prior to any reinjections and all controls (Unextracted (as applicable), First Positive Control, Hydrolysis Control (as applicable), Batch Negative, Sample Negative (as applicable), and Second Positive Control (as applicable)) must be reinjected. All reinjected data results shall be labeled as "reinjection". In order for a reinjected case sample to pass, the reinjected sample and reinjected corresponding controls must meet all acceptability and detection criteria. If one of the reinjected corresponding controls does not meet all acceptance criteria, the reinjected case sample shall be rejected and re-extracted.

If a reinjected sample is negative for the category that it screened positive for, the sample must be re-extracted. The re-extraction of reinjected samples with negative results is to eliminate the risk of compound breakdown in the reconstitution agent over time. In the event that a case sample is reinjected the unused original data shall be documented as rejected and information must be provided as to why it was unacceptable with analyst initials and date.

### **Documentation:**

All data analyzed shall have a Masshunter HETL Urine Report generated.



## URINE DRUG PROCEDURES

Any rejected data shall be documented by the notation “rejected” on the printout with the reason for rejection included with the date and analyst’s initials.

Case notes and comments shall be documented in the case file by the analyst. Minor and major deviations shall be authorized by the Supervisor and documented in the case file with a “Deviation Request Form”. Any deviations shall be documented within each case sample file in the affected batch and a comment shall be included on the report documenting a deviation from test method SOP.

Each case sample and each calibration and quality control batch shall have a technical and administrative review performed as described in the Quality Manual, these reviews shall be documented on the Case Review form (technical and administrative) and Batch Review form (technical).

### **Specific Extraction Procedures:**

#### **General Sample Setup**

The following sample preparation setup is to be utilized for all urine drug extractions:

- When aliquoting samples into extraction tube, the tube shall be segregated from the rest of the batch
- Repeat pipettors shall be used for adding recovery compound
- When not actively pipetting into an extraction tube, the tube shall be capped
- If a pipet tip/pipet syringe/transfer pipet touches a surface, (side of extraction tube, work counter, etc) it shall be discarded and a new pipet tip/syringe shall be used.

#### **General Method Limitations**

- All extraction methods utilizing Solid Phase Extraction (SPE) shall be extracted with sample negatives preceding each sample.
- Only one row of the SPE manifold shall be utilized for extractions.
- Only known drugs/metabolites are tested for using these GC/MS and LC-MS/MS methods, see Urine Drug Testing Menu in appendix for full list.



## URINE DRUG PROCEDURES

### THCCOOH in Urine

**Dilutions:** The following dilutions have been approved for this method.

| THC-COOH Dilutions |                       |                 |
|--------------------|-----------------------|-----------------|
| Dilution Factor    | Volume of Case Sample | Volume of Water |
| 1:2                | 500uL                 | 500uL           |
| 1:4                | 250uL                 | 750uL           |

**Limitations:** Silanized and non-silanized extraction tubes are approved for this method.

No validation stability study has been performed for this testing method therefore a second positive control must be run at the end of the batch.

**Sample Preparation:**

- Case samples shall be removed from refrigeration/freezer storage and allowed to completely thaw.
- Swirl each sample lightly to mix prior to sampling.
- Label all empty tubes with control name or sample number.

Unextracted Control Tube: Add 30uL THCCOOH Stock (500ng/mL) and 50uL of THCCOOH-d9 recovery compound (2000 ng/mL). Cover tube and vortex.

All Controls and Sample Tubes: Using a repeat pipet add 50uL of THCCOOH-d9 recovery compound (2000 ng/mL) and

Negative Control Tube: Add 1mL of negative urine. Cover tube and vortex.

Positive Control Tube: Add 30uL THCCOOH Stock B (500ng/mL) and 1mL of negative urine. Cover tube and vortex.

Hydrolysis Control Tube: Add 30uL THCCOOH Glucuronide Stock B (500ng/mL). Add 1mL of negative urine. Cover tube and vortex.

| ID                 | Target Concentration<br>THC-COOH (or<br>Gluronide)<br>ng/mL | Urine<br>(uL ) | Vol THC-COOH<br>Glucuronide<br>Stock B<br>(500ng/mL)<br>(ul) | Vol THC-<br>COOH<br>Stock B<br>(500ng/mL)<br>(uL ) | Vol THC-<br>COOH-D9 IS<br>(2000 ng/mL)<br>(uL ) | GC/MS sequence<br>sample type: |
|--------------------|-------------------------------------------------------------|----------------|--------------------------------------------------------------|----------------------------------------------------|-------------------------------------------------|--------------------------------|
| Unextracted        | 15                                                          |                |                                                              | 30                                                 | 50                                              | Cal, Level 1                   |
| Positive Control   | 15                                                          | 1000           |                                                              | 30                                                 | 50                                              | Cal, Level 2                   |
| Hydrolysis Control | 15                                                          | 1000           | 30                                                           |                                                    | 50                                              | Sample                         |
| Negative Control   |                                                             | 1000           |                                                              |                                                    | 50                                              | Sample                         |
| Sample(s)          |                                                             | 1000           |                                                              |                                                    | 50                                              | Sample                         |



## URINE DRUG PROCEDURES

### THCCOOH in Urine

#### Hydrolysis:

After all extraction samples have been fortified with appropriate stocks and recovery compounds:

1. Add 100µL 60% KOH to each tube and cap
2. Pulse vortex
3. Hydrolyze at 60°C for 15 minutes.
4. Remove from heat and allow to cool to room temperature

#### Liquid-Liquid Extraction:

1. In a fume hood, add 500 µL concentrated Glacial Acetic Acid to each tube and cap
2. Pulse vortex
3. In a fume hood, add 2 mL 90:10 Hexane: Ethyl acetate to each tube and cap.
4. Pulse vortex 1 minute
5. Centrifuge at high speed for ten minutes with no break
6. In a fume hood, remove the supernatant top layer and transfer into new labeled tubes
7. Evaporate to dryness under nitrogen gas.

#### Derivatization:

1. Add 50ul ethyl acetate followed by 50ul BSTFA with 1% TMCS, cap and mix/vortex
  - a. Apply Nitrogen to the BSTFA bottle and cap prior to closing.
2. Incubate at 70°C for 20 minutes.
3. Cool and transfer samples to GC/MS vials.
4. Run on GC/MS using THC instrument method.



Maine Center for Disease  
Control and Prevention  
*An Office of the  
Department of Health and Human Services*

## URINE DRUG PROCEDURES

*Urine Drug Procedures: Doc # = 005  
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## URINE DRUG PROCEDURES

### Qualitative A in Urine

**Dilutions:** The following dilutions have been approved for this method.

| Qualitative A Dilutions |                                                                                       |
|-------------------------|---------------------------------------------------------------------------------------|
| Dilution Factor         | Preparation                                                                           |
| 1:10                    | 100uL sample + 900uL water                                                            |
| 1:100                   | 10uL sample + 990uL water                                                             |
| 1:1000                  | 1mL Sample: 10uL Sample + 990uL water, vortex. Take 100uL of mixture + 900uL of water |

**Limitations:** Silanized and non-silanized tubes are approved for this method.

No validation stability study has been performed for this testing method therefore a second positive control must be run at the end of the batch.

This method utilizes a decision point cutoff of greater than the validated LLOD, only benzodiazepines  $\geq 10\text{ng/mL}$  and Stimulants  $\geq 20\text{ng/mL}$  will be reported on a COA.

This method utilizes some reagents and stocks from the blood drug testing program.

#### Sample Preparation:

- Case samples shall be removed from refrigeration/freezer storage and allowed to completely thaw.
  - Swirl each sample lightly to mix prior to sampling.
  - Label all empty tubes with control name or sample number.
1. Transfer 1mL of each case sample into a glass tube.
  2. Add 200 ul Mix A Working Stock to each tube
  3. Pulse vortex
  4. Add 50 $\mu\text{L}$  Recovery compound stock to each tube (Note: the same lot of recovery compound shall be used for all samples in an analytical batch.)
  5. Pulse vortex
  6. In a fume hood add 2 mL Chloroform to each tube and cap
  7. Pulse vortex ~1 minute
  8. Centrifuge at high speed for ten minutes
  9. In a fume hood remove the supernatant and discard, then remove the chloroform bottom layer using a glass pipet and transfer into new labeled glass tubes
  10. Dry down at room temp w/ Nitrogen
  11. Reconstitute in 200  $\mu\text{L}$  80:20 mobile phase Water w/0.1% Formic Acid and 5mM Ammonium Formate: Methanol
  12. Pulse vortex
  13. Transfer to an autosampler vial with insert for analysis



## URINE DRUG PROCEDURES



## URINE DRUG PROCEDURES

### Narcotics in Urine

**Dilutions:** The following dilutions have been approved for this method.

| Narcotics Dilutions |                       |                 |
|---------------------|-----------------------|-----------------|
| Dilution Factor     | Volume of Case Sample | Volume of Water |
| 1:2                 | 1mL                   | 1mL             |
| 1:20                | 100µL                 | 1.9mL           |
| 1:50                | 40µL                  | 1.96mL          |
| 1:100               | 20µL                  | 1.98mL          |

**Limitations:** Only silanized tubes are approved for this method, do not use non-silanized tubes for this method.

- High concentration levels of codeine or hydrocodone exhibit chromatography plateauing and retention time shifts. As these two compounds have the potential to interfere with each other if a high concentration level of codeine or hydrocodone are seen as exhibited by the some or all of the following: qualifier ion ratios out, response significantly greater than the positive control, chromatography that exhibits tailing, or chromatography that appears to be saturating the detector (flat topped/plateau peaks) then the analyst shall, sample volume permitting, re-extract the sample using a dilution to confirm codeine.
  - Hydrocodone is not currently included in the Urine Drug Testing Menu but does still have the ability to interfere with the detection of codeine as described above. If a sample exhibits the aforementioned attributes for codeine it shall be re-extracted with a dilution for confirmation.
- No validation stability study has been performed for this testing method therefore a second positive control must be run at the end of the batch.

#### Sample Preparation:

- Case samples shall be removed from refrigeration/freezer storage and allowed to completely thaw.
- Swirl each sample lightly to mix prior to sampling.
- Label all empty tubes with control name or sample number.

To Unextracted Control: Add 100uL of Narcotics Stock (varied ng/mL) and 50uL of 6-MAM-d6 Recovery compound Stock (4000 ng/mL). Cover tube and vortex.

All Control and Sample Tubes Being Extracted: Using a repeat pipet add 50uL of 6-MAM-d6 Recovery compound Stock (4000 ng/mL) to all tubes.



## URINE DRUG PROCEDURES

### Narcotics in Urine

Negative Control Tube: Add 2mL of negative urine. Cover tube and vortex.

Positive Control Tube: Add 100uL of Narcotics Stock (varied ng/mL) compound Stock and 2mL of negative urine. Cover tube and vortex.

Hydrolysis Control Tube: Add 100uL of Morphine Glucuronide Stock (3000 ng/mL) and 2mL of negative urine. Cover tube and vortex.

| ID                 | Target Concentration | Urine | Narcotics Stock (varied ng/mL) | Morphine Glucuronide Stock (3000ng/mL) | 6-MAM-d6 Recovery compound Stock (4000ng/mL) | GC/MS sequence sample type: |
|--------------------|----------------------|-------|--------------------------------|----------------------------------------|----------------------------------------------|-----------------------------|
|                    | ng/mL                | (uL ) | (uL )                          | (uL )                                  | (uL )                                        |                             |
| Unextracted        | Varied               |       | 100                            | --                                     | 50                                           | Cal, Level 1                |
| Positive Control   | Varied               | 2000  | 100                            | --                                     | 50                                           | Cal, Level 2                |
| Hydrolysis Control | 150                  | 2000  | --                             | 100                                    | 50                                           | Sample                      |
| Negative Control   | --                   | 2000  | --                             | --                                     | 50                                           | Sample                      |
| Sample(s)          | --                   | 2000  | --                             | --                                     | 50                                           | Sample                      |

### Enzyme Hydrolysis:

After all extraction samples have been fortified with appropriate stocks and recovery compounds:

Add 800 uL of Instant Buffer I to each tube.

Add 100 uL of BGTurbo to each tube.

Cover and Cover tube and vortex.

Incubate at 55°C for 75 minutes. If needed, centrifuge for 10 minutes and remove sample supernatant into new labeled tube.

### After Enzyme Hydrolysis:

Add 2 mL of 100 mM phosphate buffer (pH 6.0).

Cover tube and vortex.

**Solid Phase Extraction:** *United Chemical Technologies Clean Screen DAU Extraction Columns ZSDAU020*

**For column conditioning and washing, sample addition, and elution steps:** draw through on low vacuum – don't allow sorbent to dry.

1. Add 2 mL methanol to each column
2. Add 2 mL 100 mM phosphate buffer pH 6.0 to each column
3. Load sample with transfer pipette
4. Rinse columns with 2 mL DI water
5. Rinse columns with 3 mL 100mM acetic acid



## URINE DRUG PROCEDURES

### **Narcotics in Urine**

6. Rinse columns with 2mL methanol
7. Dry columns at full vacuum for 5 minutes.
8. Place new labeled glass tubes in rack.

### **Narcotics in Urine**

9. Elute with 2mL 78:20:2 dichloromethane/isopropanol/ammonium hydroxide - Made fresh daily as needed
10. Evaporate to dryness under nitrogen gas.

### **Derivatization:**

1. Add 50ul ethyl acetate followed by 50ul BSTFA with 1% TMCS, cap and vortex.
  - a. Apply Nitrogen to the BSTFA bottle and cap prior to closing.
2. Incubate at 70°C for 20 minutes.
3. Cool and transfer samples to GC/MS vials.
4. Run using NARCOTICS instrument method.



## URINE DRUG PROCEDURES

### Basic Drugs in Urine

**Dilutions:** The following dilutions have been approved for this method.

| Base Dilutions  |                       |                 |
|-----------------|-----------------------|-----------------|
| Dilution Factor | Volume of Case Sample | Volume of Water |
| 1:2             | 500 µL                | 500 µL          |
| 1:4             | 250µL                 | 750 µL          |

**Limitations:** Only silanized tubes are approved for this method, do not use non-silanized tubes for this method.

**The following compounds are not included in the Randox Evidence Investigator screen and if detected, a second aliquot must be extracted and analyzed for confirmation with GC/MS:**

- Norketamine
- Ketamine
- Sertraline
- Citalopram
- Flualprazolam

It is noted that, as seen in the validation, high concentration levels of Nortriptyline exhibited pronounced chromatography peak splitting at either side of the expected retention time. Due to this, if a high concentration level of Nortriptyline is seen as exhibited by the chromatography peak splitting and response significantly greater than the positive control, then the analyst shall, sample volume permitting, re-extract the sample using a dilution to confirm Nortriptyline.

**The following compounds can only be confirmed on TOX 2. TOX 1 may be used to screen:**

- Nortriptyline

A stability study has been performed for this method, extracted samples are stable at room temperature for 3 days and in freezer storage for 3 days.

### Sample preparation

Unextracted Control: Add 150µL of Base Stock (varied ng/mL) and 50µL of Recovery compound Stock (2000 ng/mL). Cover tube and vortex.

To all controls and sample tubes being extracted: Using repeat pipet add 50µL of Recovery compound Stock (2000 ng/mL) to all tubes

Negative Control: Add 1 mL of negative urine. Cover tube and vortex.



## URINE DRUG PROCEDURES

### Basic Drugs in Urine

Positive Control: Add 150uL of Base Stock (varied ng/mL) compound Stock and 1 mL of negative urine. Cover tube and vortex.

| ID               | Target Concentration | Urine | Base Stock (varied ng/mL) | Base Recovery compound Stock (2000ng/mL) | GC/MS sequence sample type: |
|------------------|----------------------|-------|---------------------------|------------------------------------------|-----------------------------|
|                  | ng/mL                | (uL ) | (uL )                     | (uL )                                    |                             |
| Unextracted      | Varied               |       | 150                       | 50                                       | <b>Cal, Level 1</b>         |
| Positive Control | Varied               | 1000  | 150                       | 50                                       | <b>Cal, Level 2</b>         |
| Negative Control | --                   | 1000  |                           | 50                                       | Sample                      |
| Sample(s)        |                      | 1000  |                           | 50                                       | Sample                      |

*After all extraction samples have been fortified with appropriate stocks and recovery compounds:*

Add 2 mL of 100 mM phosphate buffer (pH 6.0).

Cover tube and vortex.

### Solid Phase Extraction:

United Chemical Technologies ZSDAU020 SPE columns

Draw solvents and sample through slowly, on low vacuum.

Don't allow sorbent to dry.

1. Add 3 mL methanol to each column
2. Add 3 mL DI water to each column
3. Add 1 mL 100 mM phosphate buffer pH 6.0 to each column
4. Load sample with transfer pipette
5. Wash columns with 3 mL DI water
6. Wash columns with 1mL 100 mM acetic acid
7. Dry columns at full vacuum for 5 minutes
8. Wash columns with 2 mL hexanes
9. Wash with 3 mL hexane/ethyl acetate (50:50)
10. Wash columns with 3 mL methanol
11. Dry columns at full vacuum for 5 minutes
12. Place new labeled glass tubes in rack
13. Elute with 3 mL DCM/IPA/ Ammonium Hydroxide (78:20:2)-made fresh daily
14. Dry down all tubes under nitrogen until completely dry.

### Reconstitution:

1. Add 100 uL DCM to each tube, cap, and vortex.
2. Transfer to insert in autosampler vial
3. Run using BASE instrument method



## URINE DRUG PROCEDURES

### **References:**

ASB Standard 120, First Edition. 2019. Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Impaired Driving Investigations  
ASB Standard 098, First Edition. 2020. Standard for Mass Spectral Data Acceptance in Forensic Toxicology  
ASB Standard 054, First Edition. 2020. Standard for a Quality Control Program in Forensic Toxicology Laboratories  
ASB Standard 113, First Edition. 2020. Standard for Identification Criteria in Forensic Toxicology  
Journal of Analytical Toxicology, 2021; 45:529-536. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2021 Update  
ASB Standard 152, First Edition. 2021. Standard for the Minimum Content Requirements of Forensic Toxicology Procedures  
ASB Standard 053, First Edition. 2020. Standard for Report Content in Forensic Toxicology  
Randox Evidence Investigator Evidence Investigator DOA ULTRA URINE ARRAY (DOA ULTRA URN), Assay Protocol, INVESTIGATOR™ EV 4103, Randox Evidence Investigator Laboratories Ltd.  
Agilent GC/MS Maintenance, <https://community.agilent.com/technical/GC/MS/>

### **Appendix**

#### **Chromatography Integration Parameters & Examples:**

Auto Integration is set up in the instrument data analysis method to have the software correctly integrate most of the peaks. As this auto integration (Agile2) is set to integrate all compounds uniformly and is not tailored to specific compounds there are conditions in which the analyst shall need to use manual integration. Sound scientific principals shall be followed for correct peak integration to ensure that there is uniformity in data analysis.

Each individual chromatogram shall be evaluated in regard to but not limited to poor baseline resolution, chromatogram splitting, rider peaks, co-eluting interferences, misidentified chromatograms, poor chromatogram shape and symmetry, retention time shifts and if improper auto-integration was performed by the computer software as deemed by analyst experience then manual integration shall be utilized.

Each individual chromatogram shall be evaluated in comparison to it's corresponding primary or secondary ions. These corresponding ions, when possible, shall exhibit or be given comparable integration.

In the event that manual integration is required to be utilized then the following parameters shall be followed:

Manual integration shall be documented by chromatograms illustrating the integration as a variation of chromatogram shading or an asterisk on the Masshunter Urine Report.

Compounds within a sample or control shall not be manual integrated improperly to make the peak meet acceptance criteria.



## URINE DRUG PROCEDURES

A peak shall never be integrated unreasonably, generally speaking ~10%, below or above the baseline. (see examples of peak shaving or peak enhancing)

All samples and quality controls shall be integrated in the same manner.

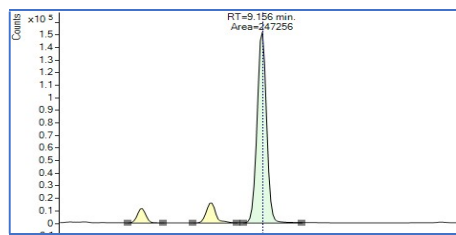
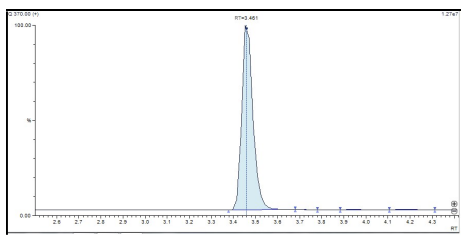
All compound ions within a sample shall be, when possible, integrated in a comparable manner.

All un-integrated batches shall be available for review in the data analysis software program.

The following illustrates commonly seen chromatography, suggested integrations, and some possible causes of poor chromatography:

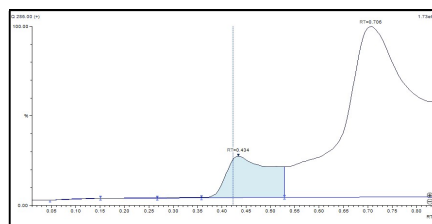
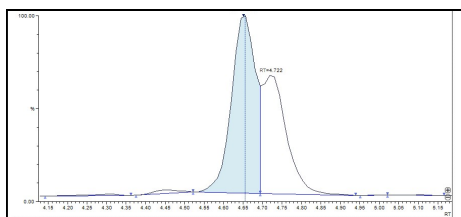
**Figure 1: Properly integrated single peak.**

The peak is symmetrically shaped and exhibits no indication of coelution, the baseline is flat and exhibits baseline to baseline integration that is normally integrated automatically by the software.

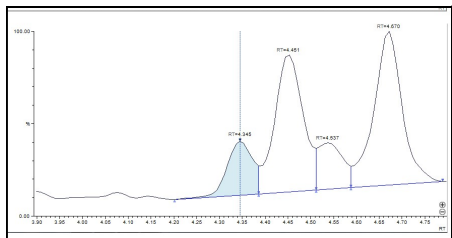


**Figure 2: Properly integrated coeluting peak.**

Proper integration of two peaks that are not completely resolved, meaning that the response does not return to the baseline between the two peaks. The lowest point between two peaks is the appropriate integration end point.



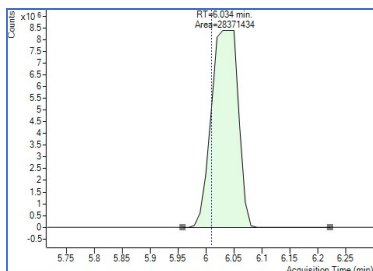
**Figure 2A: Properly integrated co-eluting peak with a rising baseline.**





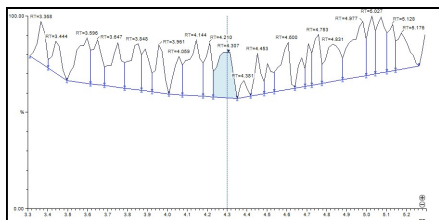
**Figure 3: Peak Plateauing**

Flat topped peaks or plateauing chromatography is a sign that the detector is being saturated. Since it is not capturing the entire peak this plateau peak may result in qualifier ion ratios being out due to high concentration.



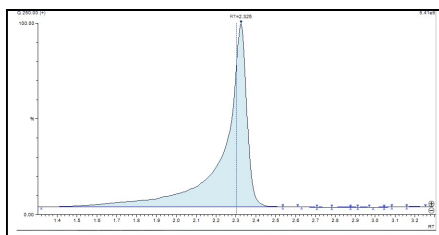
**Figure 4: Baseline Noise Example.**

This is an example of baseline noise as there are no definite peaks that distinguish themselves from the baseline and the 'peak' at the expected retention time has a signal to noise ratio of <3.3.



**Figure 5: Peak Fronting**

This is usually caused by an overloading of the column.

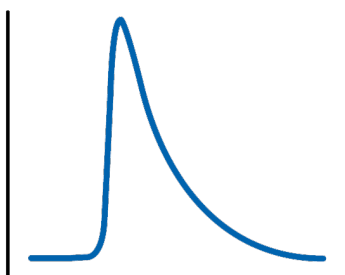


**Figure 6: Peak Tailing**

This is a limited example of peak tailing and could be caused by a number of factors including but not limited to: old column requiring maintenance, overloading of the column, interfering coelutions. If the issue is gross and persistent troubleshooting of the instrument may be required.

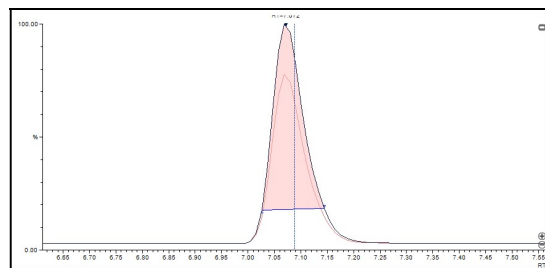
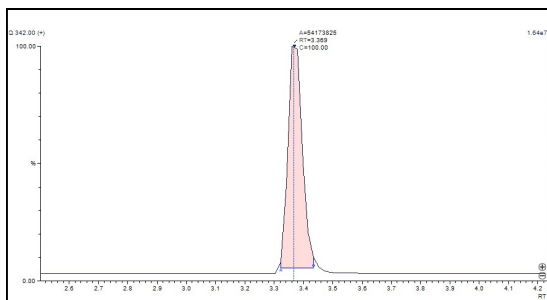


## URINE DRUG PROCEDURES



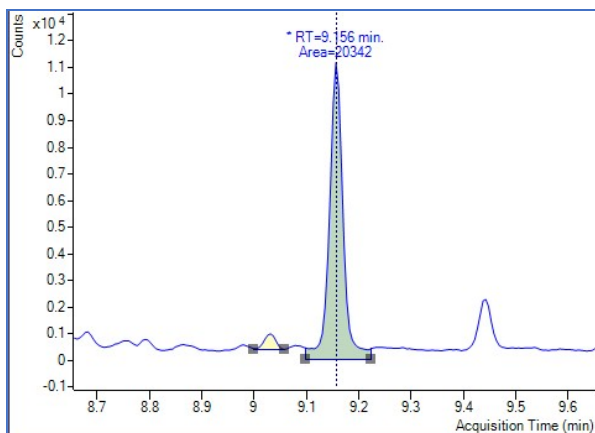
**Figure 7: Improper Peak Shaving**

Shaving is the exclusion of a large area of the peak, this includes: egregiously elevating the baseline so that the integration runs from peak side to peak side as opposed to baseline to baseline or eliminating the leading and trailing edges of the peak.



**Figure 8: Improper Peak Enhancing**

Enhancing is the integration of a large area that is not the target analyte peak, the following exhibits improper peak enhancement by integration including a large amount below the baseline.





## URINE DRUG PROCEDURES

### **Instrument Operation & Maintenance**

#### **Operation:**

#### **Instrument Sequence: Pre-Run Sequence Check**

Each auto sampler vial position and the performance of a daily tune shall be verified by a secondary individual in comparison to the instrument sequence, prior to starting your run. This shall be documented on the sequence check section of the Urine Extraction Form and the printed instrument sequence with the date and individual's initials.

Data analysis batches: TestDateInitials (THC080422EAF)

If sequence stops due to instrument error, an additional sequence and tune check is required the following day by a secondary individual to ensure sequence is acceptable and tune has been performed.

All analysts shall be responsible for placing their raw data files and data analysis batches on the KDrive (K:\Urine Data) (Masshunter data analysis files only)

- A sample negative control shall be run before each case sample extracted using a SPE method. These sample negative controls shall be labeled with the associated HETL case number (Example: 2220729 Negative).
- A methanol/Mobile Phase blank shall be run after each case sample extracted using a liquid/liquid method.

Both of these shall be analyzed in the data analysis batches to evaluate for carryover (instrument carryover/extraction carryover). At the start of each extraction batch a method matched blank shall be run. The following are examples of sequences for the SPE manifold and the instrument sequence illustrating the use of SPE sample negatives and Liquid/Liquid method matched blanks respectfully: Key: UNEX=Unextracted positive control, PC=Positive Control, HC=Hydrolysis Control, Neg=Negative Control, Methanol=Methanol/Mobile Phase blank.

| SPE Manifold Sequence Example #1 |    |                 |             |                 |             |                 |             |                 |             |                 |             |
|----------------------------------|----|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|
| Batch<br>NEG                     | PC | Sample<br>NEG 1 | Sample<br>1 | Sample<br>NEG 2 | Sample<br>2 | Sample<br>NEG 3 | Sample<br>3 | Sample<br>NEG 4 | Sample<br>4 | Sample<br>NEG 5 | Sample<br>5 |

| GC/MS Sequence Example #1 |        |                              |       |
|---------------------------|--------|------------------------------|-------|
| Sample Name               | Type   | Data<br>Acquisiton<br>method | Level |
| Methanol 1                | Sample | Narcotics.M                  |       |
| UNEX                      | Cal    | Narcotics.M                  | 1*    |
| Batch Neg                 | Sample | Narcotics.M                  |       |
| HC                        | Sample | Narcotics.M                  |       |
| PC 1                      | Cal    | Narcotics.M                  | 2*    |
| Sampl Neg 1               | Sample | Narcotics.M                  |       |
| Sample 1                  | Sample | Narcotics.M                  |       |

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|             |        |             |    |
|-------------|--------|-------------|----|
| Sampl Neg 2 | Sample | Narcotics.M |    |
| Sample 2    | Sample | Narcotics.M |    |
| PC 2        | Sample | Narcotics.M |    |
| Methanol 1  | Sample | THC-COOH.M  |    |
| UNEX        | Sample | THC-COOH.M  | 1* |
| PC1         | Sample | THC-COOH.M  |    |
| HC          | Sample | THC-COOH.M  | 2* |
| Batch Neg   | Sample | THC-COOH.M  |    |
| Sample 1    | Sample | THC-COOH.M  |    |
| Methanol 3  | Sample | THC-COOH.M  |    |
| PC2         | Sample | THC-COOH.M  |    |

\*If Cal levels are not assigned in the sequence table, they can be assigned in the Masshunter data analysis software.

**GCMS: Step by Step SIM batch review:** This procedure is to guide the review of a GC/MS SIM batch.

- Create a New Batch: File-New Batch
- Add Samples: File-Add Samples: Select desired samples and select OK.
- Apply Data Analysis Method: Method-Open-Open method from existing file and select desired data analysis method.
- Analyze Batch
- Review first Positive Control:
  - Update retention times for the batch (Click: Update-Update retention times. Then click Analyze Batch).
  - Update qualifier ion ratios (Click: Update-Update qualifier ion ratios. Then click Analyze Batch)

*Please note: if manual integration is performed on the first positive control after performing the retention time and qualifier ion ratio updates they must be re-updated.*

- Review remaining controls
- Review Subject Samples, review recovery compound first to evaluate if sample is suitable for analysis. Continue onto reviewing the compounds of interest.
- Once analysis has been completed, generate reports for documentation: Reports-Generate and select OK

If a data analysis batch requires correction or re-analysis, to ensure that the original batch is not saved over: Open the original batch, Save As: New Name (Example: THC122322EAF 2), make correction and print/generate reports.

The compounds Temazepam and Cocaethylene-D3 method has additional ions being collected. Only two qualifier ions print on the Masshunter generated reports. If a sample is being confirmed for Temazepam and the ions not on the report are being used to meet the two qualifier ion ratio acceptance criteria, the analyst must screen-shot and print the additional ions and place the printout in the casefile.

### GCMS: Software Installation



## URINE DRUG PROCEDURES

To select outlier flags: select the Select Outliers button this will pop up a window. Ensure the following selections are checked.

Outliers

- ☒ Peak Result
  - ☒ Alternative Peak
  - ☐ Capacity Factor
  - ☒ Peak Not Found
  - ☐ Peak Resolution Front
  - ☐ Peak Resolution Rear
  - ☐ Plates
  - ☐ QValue
  - ☐ SureMass Ratio
  - ☒ Retention Time
  - ☐ Relative Retention Time
  - ☒ Integration Metric
  - ☐ Symmetry
  - ☐ Full Width Half Maximum
  - ☐ Purity
  - ☒ Signal To Noise Ratio
  - ☐ Limit Of Detection
  - ☒ Limit Of Quantitation
  - ☐ Method Detection Limit
- ☒ Qualifier
  - ☐ Qualifier Coelution Score
  - ☒ Qualifier Peak Not Found
  - ☐ Qualifier Peak Resolution Front
  - ☐ Qualifier Peak Resolution Rear
  - ☐ Qualifier SureMass Ratio
  - ☒ Qualifier Signal To Noise Ratio
  - ☒ Qualifier Ratio
  - ☒ Qualifier Integration Metric
  - ☐ Qualifier Peak Symmetry
  - ☐ Qualifier Peak Full Width Half Maximum
- ☐ ISTD
- ☐ Sample
- ☐ Blank
- ☐ Calibration
  - ☐ Average Response Factor RSD
  - ☐ Relative Standard Error
  - ☒ Accuracy
  - ☐ Average Response Factor
  - ☐ Calibration Range
  - ☐ Curve Fit R2
  - ☐ Relative Response Factor
  - ☐ Response Factor
- ☐ QC
- ☒ CC
  - ☐ CC Average Response Factor
  - ☐ CC ISTD Response Ratio
  - ☐ CC Relative Response Factor
  - ☐ CC Response Ratio
  - ☒ CC Retention Time
  - ☐ CC Time
- ☐ Matrix
- ☒ Surrogate
  - ☐ Surrogate
  - ☒ Surrogate Percent Recovery
- ☐ Response Check
- ☐ Mass
- ☐ Custom

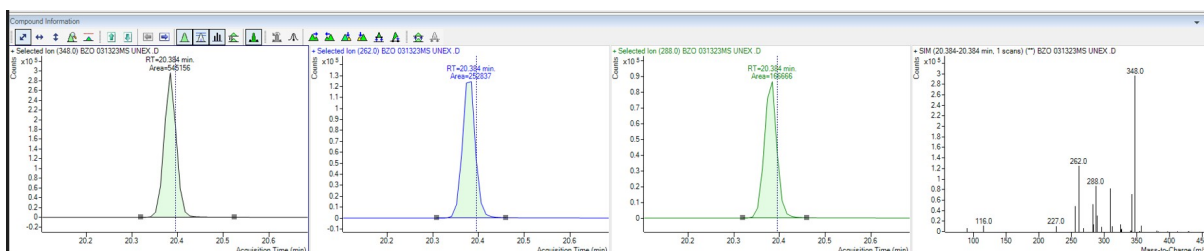
Batch Table: ensure the following columns are visible

| Batch Table                                                                                                   |              |                                                                                     |         |                    |    |             |                      |                                                                                                              |       |                                                                                             |       |                     |       |                     |  |
|---------------------------------------------------------------------------------------------------------------|--------------|-------------------------------------------------------------------------------------|---------|--------------------|----|-------------|----------------------|--------------------------------------------------------------------------------------------------------------|-------|---------------------------------------------------------------------------------------------|-------|---------------------|-------|---------------------|--|
| Sample:  BZO 031323MS UNEX |              |  |         | Sample Type: <All> |    |             |                      | Compound:  TEMAZEPAM D5 |       | ISTD:  |       |                     |       |                     |  |
| Compound Method                                                                                               |              | BZO 031323MS UNEX                                                                   |         |                    |    |             |                      |                                                                                                              |       | Qualifier 1 Resu...                                                                         |       | Qualifier 2 Resu... |       | Qualifier 3 Resu... |  |
| ID /                                                                                                          | Name         | RT                                                                                  | RT Dif. | Resp.              | MI | Calc. Conc. | Surrogate % Recovery | S/N                                                                                                          | Ratio | MI                                                                                          | Ratio | MI                  | Ratio | MI                  |  |
|  0                         | TEMAZEPAM D5 | 20.384                                                                              | 0.011   | 545.               |    | 100.0000    | 100.0                |                                                                                                              | 46.4  |                                                                                             | 30.6  |                     |       |                     |  |

Chromatograms: ensure the “show/hide chromatogram”, “show/hide qualifiers” and “show/hide spectrum” are clicked on and the windows below are visible. To show all ions right click on ions and select Properties, Compound Information (2), Max. # of panes per row and set number to 10.



## URINE DRUG PROCEDURES



### LC-MS/MS Starting a sequence from a template:

- Open Labsolutions go to Main tab / Real time Batch
- File, new batch, select your template
- Change: Sample names, Sample IDs, Sample type (standard, control, unknown), Level #s, Dilution factor (if any), comments
- Check: method file, vial #s (you can right click fill down or fill in series)
- Add lines if needed (right-click, add line- it will prompt for how many)
- Delete any extra lines (highlight, right-click delete)
- Batch settings (found on top drop down or from right clicking)- change folder to today's date with the appropriate test panel. If you do not want to shut down after this batch, then go to shutdown tab and unselect the "shutdown" button. When you leave settings, the software will prompt you to create the new folder for the new folder for this date.
- File- save batch as today's date under the new folder for the date using the format: TestDateInitials
- Start Real time Batch
- Edit batch while running: under real time batch, hit "edit batch/restart" button, do editing then make sure to hit the button again to restart.
- You can watch the MS data file in Realtime by clicking on Acquisition/Instrument Parameters and the MS tab. Click on instrument parameters again to return.

### LC-MS/MS Data Analysis

- Open Insight software and enter username and password.
- File/open and select your batch files. All files associated with the batch will be opened. You can sort by clicking the top column heading.
- Then File/Save as and name the LCMS method file (\*.lcm) and DAML project file (\*.damlp) with the analysis panel code + the analysis date + analyst initials. For example: NAR050119NMI.
- Start with the calibration or mix sample. Review each peak and update retention times and ion ratios using a mid-level calibrator for each compound (right click each compound and select: Update- RT & ion ratio then click on the apex of the peak).
- Analyze each data file and each compound.
- To edit a peak: right click on the chromatogram
  - Manual identification and click on the correct integrated peak if the peak was misidentified.
  - Manual identification-horizontal to draw from baseline to baseline.



## URINE DRUG PROCEDURES

- Manual integration-new baseline to draw a new baseline or horizontal to use existing baseline.
- Review/Accept- This will accept each compound as complete.
- File/ Save again.
- Report/ HETL Batch report for all checked files and HETL sample report for a single file. The software will pull up the report for review before printing.

### LC-MS/MS Maintenance

This section describes LC-MS/MS instrument maintenance protocols for the daily prior to analyzing samples maintenance and the as needed maintenance for LC-MS/MS methodology. All daily and as needed maintenance shall be documented on the LCMSMS Shimadzu Maintenance Log, any additional maintenance shall be recorded in the maintenance notes section of the respective Maintenance Log with the date performed and analysts' initials.

#### **Daily Maintenance:**

Daily Maintenance shall be performed daily prior to sample analysis; if instrument is not used for sample analysis on a particular day, then Daily Maintenance shall not be performed for that day. When Daily Maintenance is performed it shall be recorded on the Maintenance Log.

- **Mobile Phase Levels:** Mobile phase levels shall be checked to confirm that there is adequate volume to run the intended samples.
- **Check Rinse Line:** The Agilent autosampler rinse line is a small diameter line that draws from Mobile Phase B, this line shall be checked to ensure that the end is submerged in the bottle.
- **Cleaning Electrospray Chamber& Shield-Daily:** The daily cleaning of the electrospray chamber and shield shall be performed prior to activating the MS to maintain optimal instrument performance.
  - Confirm that the MS is not activated.
  - Rinse interior of electrospray chamber with 50:50 HPLC grade 2-propanol: HPLC grade water.
  - Wipe interior of electrospray chamber with a clean Kimwipe taking care to avoid the nebulizing needle.
  - Rinse the area around the spray shield with 50:50 HPLC grade 2-propanol: HPLC grade water. (Do not spray directly toward the end of the capillary. This can cause pressure surges in the vacuum system).
  - Wipe the area around the spray shield with a clean Kimwipe taking care to avoid the nebulizing needle.
  - Rinse nebulizing chamber door and wipe with a clean Kimwipe.
  - Reattach door to chamber and close chamber.
- **Gas Level Check:** The Shimadzu Argon gas level shall be checked to ensure that a level of 70-110 psi is being maintained, if 70-110 psi is not being maintained then remedial action such as adjusting the regulator or changing the tank shall be performed. The Agilent Nitrogen gas level shall be checked to ensure that a level of ~20 psi is being maintained, if ~20 psi is not being maintained then remedial action such as adjusting the regulator or changing the tank shall be performed.



## URINE DRUG PROCEDURES

- **Pump Level Check:** The pump pressure shall be recorded on the Maintenance Log to be monitored since pump pressure is an indicator of the condition of the LC system.
- **IG Vacuum Level & High Vacuum Level:** The Shimadzu IG Vacuum level shall be checked to confirm that it is maintaining a vacuum of  $\sim 3 \times 10^{-3}$  when the MS is on, if the MS is not maintaining a vacuum of  $\sim 3 \times 10^{-3}$  then a Shimadzu service engineer shall be called to troubleshoot the issue. The Agilent High Vacuum level shall be checked to confirm that it is maintaining a vacuum of  $2.0-5.0 \times 10^{-5}$  when the MS is on, if the MS is not maintaining a vacuum of  $2.0-5.0 \times 10^{-5}$  then a Agilent service engineer shall be called to troubleshoot the issue.
- **Check for Leaks:** Once the LC pump has been turned on the operator shall use a clean dry Kimwipe to check the jointures of the lines and capillary lines in the LC stack to ascertain that there are no slow leaks that could affect instrument performance.
- **Check Waste Level:** The instrument waste level bottle shall be checked and once the bottle is approximately half filled with waste then the bottle shall be emptied into the appropriate hazardous waste disposal container.
- **Checktune:** Performed to check that optimal instrument detection performance is being maintained and shall be performed daily prior to sample analysis. If the Checktune does not meet parameters, then action is required.

**Shimadzu:** Shimadzu does not have any pre-set/provided acceptance criteria for their LC-MS/MS instrument, historical review during the initial validations found that monitoring the Q1 and Q3 Scan positive ions allowed for optimal instrument monitoring. Acceptance criteria consist of Q1 and Q3 Scan positive ion 168.10, 256.15 and ion 300.20 having an intensity of >50% of the respective intensities from the most recent Autotune.

- Activate MS.
- Attach red end of tuning capillary to bottle of Tuning Solution, both located in tuning bottle chamber.
- Remove capillary that attaches the LC to the MS and replace with the beige end of the tuning capillary.
- In LabSolutions Data Acquisition Main Tab select Tuning to open the tuning menu.
- Select Manual Tune and then the Standard Sample button this will start the flow of Tuning Solution, once peaks become visible and show acceptable chromatography select Tuning Results and select "No" to saving this file this will bring you back to the Autotune menu.
- Once you are back in the Autotune menu select Autotuning Conditions and deselect all parameters and select OK, this ensures that none of the parameters will be changed only checked.
- Select AutoTuning Start (and confirm that positive mode (+) is selected, this will start the Checktune. Upon completion print the Checktune file, labeling the document as a Checktune and return to the main data acquisition window in Labsolutions and select "No" to saving this file as the Checktune is automatically printed upon completion.
- Remove Tune Solution bottle and replace with bottle of methanol.
- Select Manual Tune and then the Standard Sample button this will start the flow of the methanol, once peaks disappear the tuning capillary has been flushed and is



## URINE DRUG PROCEDURES

ready for storage. Select Tuning Results and select "No" to saving this file this will bring you back to the Autotune menu.

- Disconnect the tuning capillary and place into storage in the Tune Solution bottle chamber.
- Reconnect the LC flow capillary to the MS.

### Bi-Weekly Maintenance:

Bi-Weekly Maintenance shall be performed as defined as a minimum of once every two weeks. When Bi-Weekly Maintenance is performed it shall be recorded on the Maintenance Log.

- **Ballast Pump:** The Shimadzu ballast pump requires draining on a bi-weekly basis as defined as a minimum of every two weeks. If the oil level is low, post ballast pump drain or oil looks discolored, pump maintenance shall be performed per manufacturer's recommendation. The Agilent pump is self-ballasting and does not need to be manually ballasted.

### As Needed Maintenance:

As Needed maintenance shall be performed on an as needed basis as indicated by instrument performance, frequency of instrument use, or by visual inspection by the operator.

- **Cleaning Electrospray Chamber/Shield-As Needed:** The As Needed cleaning of the Electrospray Chamber and Shield shall be performed as needed when indicated by visual inspection of the Electrospray Chamber and Shield by the operator. This maintenance is performed on the MS to maintain optimal instrument performance.
  - Confirm that the MS is not activated.
  - Rinse interior of electrospray chamber with 50:50 HPLC grade 2-propanol: HPLC grade water.
  - Wipe interior of electrospray chamber with a clean Kimwipe.
  - Remove spray shield.
  - Gently clean the spray shield with 6000 and 8000 abrasive paper moistened with 50:50 HPLC grade 2-propanol: HPLC grade water.
  - Rinse spray shield with 50:50 HPLC grade 2-propanol: HPLC grade water and wipe with a clean Kimwipe.
  - Replace spray shield.
  - Rinse area around the spray shield with 50:50 HPLC grade 2-propanol: HPLC grade water (Do not spray directly toward the end of the capillary. This can cause pressure surges in the vacuum system) and wipe area around the spray shield with a clean Kimwipe.
  - Rinse nebulizing chamber door with 50:50 HPLC grade 2-propanol: HPLC grade water and wipe with a clean Kimwipe.
  - Close chamber.
- **Change Column Guard Filter:** The column guard filter shall be changed on an as needed basis dependent upon instrument performance and frequency of instrument use at the discretion of the operator. This is performed to protect the integrity and longevity of the LC column.
  - Confirm that the LC pumps are off.
  - Unscrew the line connecting the column module to the column guard.
  - Unscrew the column guard from the column.



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- Using the appropriate wrenches unscrew the column guard and remove the filter.
- Place a new filter in the column guard.
- Using the appropriate wrenches screw the two pieces of the column guard back together.
- Screw the column guard back into the column.
- Screw the line connecting the column guard to the column module back in.
- Turn the LC pumps on and using a clean dry Kimwipe confirm that there are no leaks at any of the jointures in the column module.

### Autotune

Performed as needed such as if a Checktune shows marked decreased signal or following extensive instrument maintenance.

#### Shimadzu:

- Activate MS.
- Attach red end of tuning capillary to bottle of Tuning Solution, both located in tuning bottle chamber.
- Remove capillary that attaches the LC to the MS and replace with the beige end of the tuning capillary.
- Select Manual Tune and then the Standard Sample button this will start the flow of Tuning Solution and peaks should become visible, once peaks become visible and show acceptable chromatography select Tuning Results and select "No" to saving the tune file at this time.
- In LabSolutions Data Acquisition File drop down tab select New Tune File and then save as "AutotuneDate" Example: Autotune061720
- In LabSolutions Data Acquisition Main Tab select Autotuning Conditions to confirm that "positive tune only" is selected and all remaining parameters are selected.
- Once you are back in the Autotune window select Autotuning Start (and confirm positive mode (+) is selected) this will start the Autotune, this runs for ~20-30 minutes. Upon completion save and print the tune file.
- Remove Tune Solution bottle and replace with a bottle of methanol.
- Select Manual Tune and then the Standard Sample button, this will start the flow of the methanol, once peaks disappear the tuning capillary has been flushed and is ready for storage. Select Tuning Results and select "No" to saving this file, this will bring you back to the Autotune menu.
- Disconnect the tuning capillary and place it into storage in the Tune Solution bottle chamber.
- Reconnect the LC flow capillary to the MS.

### Venting & Shutting Down: Shimadzu 8030 LCMSMS

- Confirm that the LC stack and MS are shut down.
- In LabSolutions 'Main' left tab select 'System Control' then 'Advanced' options.
- Close CID valve.
- Place DL plug into DL hole in electrospray chamber (DL plug is stored in tune bottle chamber & has the appearance of an Allen wrench).
- Stop Turbo pump, allow instrument to sit for one hour.



## URINE DRUG PROCEDURES

- Stop Rotary pump
- Open Vent valve.
- In LabSolutions 'File' tab select 'Exit'
- Once all computer windows are closed, shut down the computer, switch LC stack and MS off (MS switch located on back of instrument) and unplug everything.

### **Starting the MS from "cold": Shimadzu 8030 LCMSMS**

- Plug all equipment in, turn on computer and switch LC stack, and MS on.
- Confirm DL plug is in DL hole in electrospray chamber.
- In LabSolutions, 'Main' left tab select 'System Control' then 'Advanced' options.
- Close Vent valve.
- Start Rotary pump
- Once the PG vacuum has reached  $\times 10^{-1}$  start the Turbo pump, the light next to the start button will stop flashing when the Turbo pump is up to the correct speed.
- Once the Turbo pump light has stopped flashing and is a solid green, open the CID valve and remove the DL plug.

### **GCMS Maintenance**

Refer to TOX GC/MS Instrument Log for routine maintenance, non routine maintenance shall be documented in the comments section of the form or the general instrument maintenance form, if more space is needed.

Performance checks: Performance checks shall be performed following major maintenance Example: replacing the GC column shall require the running of at least one unextracted control to check RTs.

### **Weekly Maintenance**

Check foreline oil level

### **As Needed Maintenance**

Clean source & Tune

Change gold seal

Trim Column/Guard Column

Change liner, septum and o-ring

Clean syringe

Change helium tank

Change gas traps and purifiers

Change Column

Change Gold Seal

Data is transferred from instrument to the K:Drive for back-up purposes

### **Every 6 Months**

Check calibration vial

Replace foreline pump oil

**Every 12 Months:** Performed during PM, when possible

Urine Drug Procedures: Doc # = 005

Approved by: Forensic Lab Director – Lauren Niskach

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**Refer to SharePoint for the most current version**



## URINE DRUG PROCEDURES

Replace traps and filters

### **GCMS Tuning**

A tune report is generated daily (when in use), prior to running casework and/or following maintenance. If a batch of casework runs overnight, into the next day the run does not need to be stopped and the instrument tuned, but the instrument must be tuned prior to a new batch of samples being put on the instrument to run.

If a tune report does not meet all of the acceptance parameters, then a Quicktune or Tune MSD may be performed.

An Tune MSD is performed using the ATUNE\_300.U file following MS maintenance, or as needed.

All tunes generated are initialed by the analyst and retained. All generated tunes must meet the following acceptance criteria prior to running casework:

1. Relative abundances should be 100% for 69, >40% for 219, and >1% for 502. If 219 becomes the base peak 69 should be > 70% of 219.
2. Isotope Ratios should be within +/- 25% of their expected value: 0.81-1.35 for 70 m/z (expected 1.08 m/z), 3.24-5.40 for 220 m/z (expected 4.32 m/z), and 7.57-12.61 for 503 m/z (expected 10.09 m/z)
3. Peak masses should be 69, 219, and 502 +/- 0.2 amu
4. Pw50 should be 0.60 +/- 0.1 amu for all peaks
5. The m/z 28 (nitrogen, "air") and m/z 18 (H<sub>2</sub>O, "water") must be less than 5% of the base peak. Note: If the m/z 28 is greater than 5% the system should be checked for leaks.
6. The tune file being used must be ATUNE\_300.U
7. Temperatures: MS Source: 300° and MS Quad: 180°

If an Autotune does not meet all of the acceptance parameters the following may be performed: the instrument is manually tuned, instrument maintenance is performed, or the instrument is put out of service.

Original tune printout shall be placed into tune binder. A copy of the tune shall be placed in associated batch file. It is noted that the instrument automatically saves all autotunes performed, checktunes are not automatically saved by the instrument.

### **Cleaning an Agilent GC/MS Ion Source:**

This procedure is to guide the cleaning of the Agilent GC/MS Ion Source.

GC/MS sources require periodic cleaning and is part of normal user maintenance. This can be indicated by:

- Loss in analyte response not improved by normal inlet and column maintenance.
- Poor calibrant ion peak shapes during tuning, especially for the 502 ion.
- Escalating tune repeller voltage.
- Escalating tune Electron Multiplier voltage.

1. Vent instrument following vendor's instructions.



## URINE DRUG PROCEDURES

2. Wearing appropriate PPE, including gloves, remove the source and disassemble.
3. Cleaning steps:
  - a. Make a thick paste with DI water and Aluminum oxide. (Use DI water to make the abrasive paste so that you do not have to work in a hood.)
  - b. Use the cotton-tipped swabs.
  - c. Clean everywhere. Fronts, edges, backs, in the holes, everywhere. (You are NOT trying to remove metal, only surface contamination. Mechanically, this happens quickly.)
4. Rinse steps:
  - a. DI water – Water removes salts
  - b. Methanol – this step removes the water
  - c. Acetone
  - d. Hexane – this step removes any hydrocarbon residue
5. Use a separate beaker for each different type of solvent – four beakers. Use tweezers to transfer the parts to leave as much residue in the previous solvent as possible. Do not allow the parts to dry in between solvents! This list goes from most polar to least polar on purpose.
6. Suggested sonication time: Three to five minutes in each solvent.
7. Donning clean gloves, reassemble the source and return to instrument, following vendor's instructions.
8. Pump down instrument for a minimum of two hours. Perform air and water check to assess if more time is needed before performing tune.

### Installing a GC Column:

This procedure is to guide the installation of a capillary column.

Warning: The GC and MS operate at high temperatures. Do not touch any parts of GC and MS until you are sure they are cool.

Always wear safety glasses when handling capillary columns. Use care to avoid puncturing your skin with the end of the column.

Tip: Always wear clean gloves to prevent contamination while handling any parts that go inside the GC or the analyzer chambers.

1. Vent the MS. Open the vent valve by turning the knob counterclockwise.
2. Install a capillary guard column in the inlet. See GC Inlet Maintenance for details.
3. Condition the column by turning on the inlet pressure flow to clear any debris from column.
4. Installing the column in the MS:
5. Open the analyzer chamber door enough to ensure that the end of the GC/MS interface is visible.
6. Slide the column into the GC/MS interface. The tapered end of the ferrule must point towards the nut.
7. Adjust the column by pushing the column through the ferrule, cut the column, then pull back until it extends the following distance from GC/MS interface: 1 to 2 mm.
8. Tip: The cut end of the column must be flat with no cracks or jagged edges. Use a flashlight and magnifying loupe to see the end of the column. Do not use your finger to feel for the column end.



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9. Tighten the nut: Finger tighten the nut then use a spanner to tighten the nut 1/4 to 1/2 turn. Check the nut's tightness after one or two heat cycles and tighten as appropriate.  
Caution: Over-tightening the nut may damage the column or block the column flow.
10. Check the GC oven to be sure that the column does not touch the oven walls.
11. Check the alignment of the ion source and the interface tip seal. When the ion source is aligned correctly, the front analyzer chamber can be closed. Don't use the shipping screw to close the door.
12. Close the analyzer chamber door and vent valve.
13. Turn on MS followed by GC. Load software and pump down. Pump down instrument for a minimum of two hours. Perform air and water check to assess if more time is needed before conditioning the column. If air and water check is acceptable use Conditioning.M method to condition the column before use.

Note: If the GC/MS interface/transfer line connection needs to be replaced or if in the event it is broken and needs to be reset, the MS must be vented to protect the MS and allow access to the MS to insure the column extends 1 to 2mm from the GC/MS interface.

### Installing a GC Guard Column:

This procedure is to guide the installation of a capillary guard column.

Warning: The GC and MS operate at high temperatures. Do not touch any parts of GC and MS until you are sure they are cool.

Always wear safety glasses when handling capillary columns. Use care to avoid puncturing your skin with the end of the column.

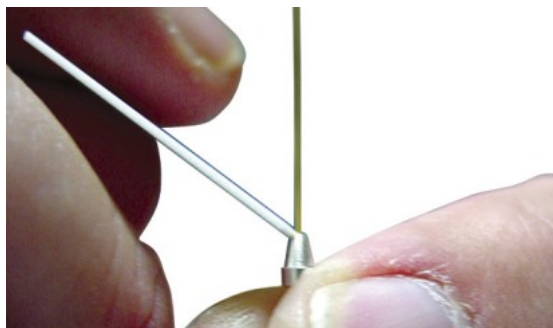
Tip: Always wear clean gloves to prevent contamination while handling any parts that go inside the GC or the analyzer chambers.

Tip: Recommend that this task is performed with a secondary individual for assistance, as one person needs to weave the column guard onto the holder and the second person holds the remainder of the column guard and ensuring it does not tangle.

1. Remove column guard from Ultimate union and GC column nut from inlet.
2. Discard old column guard-save the internal nut, this can be reused.
3. Put internal nut on column guard and then ferrule
4. Using the wrench and ferrule pre-swaging tool, tighten the nut a little at a time, occasionally checking to see if the ferrule is gripping the tube. When the ferrule just starts to grip, notice position of the nut and then tighten by turning 45 to 60 degrees of rotation, but no more than 60 degrees (one flat). If you can pull the column free, it is not tight enough.
5. Remove the swaging tool
6. Using a ceramic column cutter, trim the tubing at the small end of the ferrule leaving approximately 0.3 mm of tubing extending beyond the ferrule. See below image for angle to hold column cutter at to perform cut.



## URINE DRUG PROCEDURES



7. Check the end of the tube with a magnifier. The end of the tube need not be perfectly square, but should not have cracks which extend under the ferrule. See below image for acceptable cut and amount of tubing extending beyond the ferrule.



8. Connect columns to the Ultimate union Finger-tighten the nuts. Further tighten with a wrench only 15 to 20 degrees.
9. Unwind column guard and place Ultimate union into holder and secure using wire bracket.
10. Gently feed column guard onto column holder using an over and under pattern to match the column. Feed until column guard is almost completely on the holder. Leave ~12 inches free to secure into the inlet port.
11. Install a capillary guard column in the inlet. See GC Inlet Maintenance for details.

### Cleaning Autosampler Needle:

This procedure is to guide the cleaning of the autosampler needle.

1. First draw an appropriate solvent into the syringe and expel it onto a laboratory tissue or waste container.
2. Wipe the tip of the needle with a laboratory tissue.
3. Draw the solvent into the syringe, wipe the syringe and plunger with a laboratory tissue and appropriate solvent. Repeat as needed.
4. Reassemble the syringe, expel any remaining solvent onto a laboratory tissue or waste container. Again, wipe the needle with a laboratory tissue.

### GC Inlet Maintenance:

This procedure is to guide GC inlet maintenance.

Reduce the inlet, oven, and AUX temperature to 40C. (Leaving the inlet on allows the injection port fan to continue to operate thus, aiding in cooling the injection port.)

After the inlet has cooled sufficiently (at least 70C), turn the inlet flow off.



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*These steps can be performed from the GC front panel or the Chemstation software depending on which instrument is being used.*

1. Remove autosampler tower. While wearing appropriate safety apparel, remove the nut that covers the GC septa and liner. Remove the septa and liner completely from the GC using forceps.
2. Place new liner, O-ring, and septa into GC using forceps and replace nut (finger tight) and autosampler tower.
3. Loosen and remove the GC column nut from the inlet.
4. Remove the insulator and the gray reducing nut that houses the gold seal and washer from the bottom of the inlet.
5. Reassemble the inlet with a new gold seal, washer, and a new ferrule on the column. Make sure that the column is re-cut using the ceramic cutter, examine cut column under magnification to make sure it is cut straight, and adjusted to 4-6mm past the ferrule prior to installation (you may use a septa as a guide).
6. Reload method or manually changes values to turn on inlet, oven and AUX temperatures and inlet flow.
7. An air and water check or a tune may be performed to check for leaks.

### **Data Acquisition and Data Analysis Methods:**

All GC/MS instruments shall use the following data acquisition parameters:

1. Ultra-inert liner with glass wool
2. Ultimate Plus deactivated fused silica tubing guard column with ultimate union junction, 5m x 0.25 mm
3. Agilent analytical column DB-5MSA UI (30m x 0.25mm x 0.25 um)
4. Masshunter Quantitative Data Analysis Software V10.2 Build 10.2.733.8 on GC/MS 1 and GC/MS 2
5. Masshunter Data Acquisition v10.0 on GC/MS 2 and v10.1.49 on GC/MS 1
6. Source temperature and transfer line temperature of 300°C
7. Quadrupole temperature of 180°C
8. Dwell time of 20 msec for each ion.
9. Injection volume of 2uL

All LC-MS/MS instruments shall use the following data acquisition parameters:

1. Shimadzu 8030 Tandem Mass Spectrometer and LC system (or equivalent)
2. Shimadzu Labsolutions Data Acquisition Version 5.93 and Insight Data Analysis Version 4.5.28381.1
3. LC oven 40°C
4. LC Guard Cartridge – Phenomenex C18
5. LC Column- Phenomenex Kinetex C-18 2.6 um 50 mm, 2.1 ID
6. Qualitative A Flow rate 0.5 mL/Min



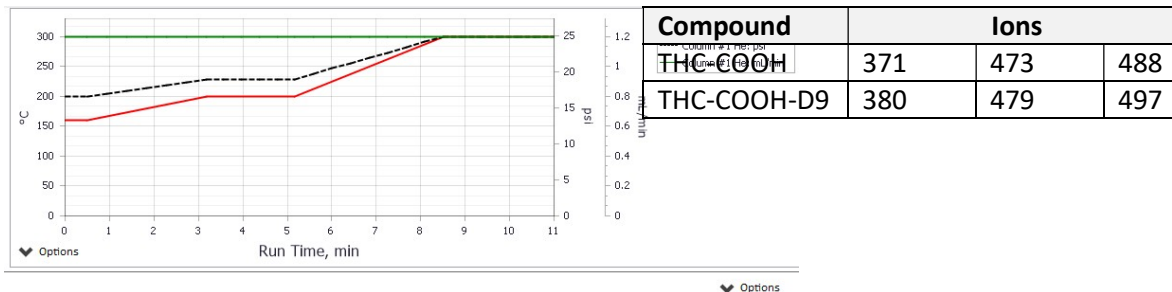
## URINE DRUG PROCEDURES

7. Qualitative A Mobile phase A: Water with 0.1% Formic Acid and 5mM Ammonium Formate
8. Mobile Phase B: Methanol
9. Injection volume 20  $\mu$ L

The following are specific instrument Data Acquisition parameters for each testing method:

### THCCOOH

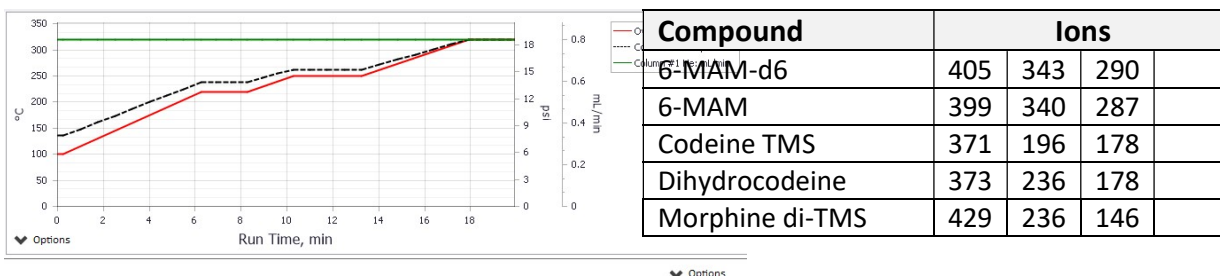
The following is the GC temperature gradient and ions detected for the THCCOOH data acquisition method:



|             | Rate<br>°C/min | Value<br>°C | Hold Time<br>min | Run Time<br>min |
|-------------|----------------|-------------|------------------|-----------------|
| ► (Initial) |                | 150         | 0.5              | 0.5             |
| Ramp 1      | 15             | 200         | 2                | 5.1667          |
| Ramp 2      | 30             | 300         | 2.5              | 11              |
| *           |                |             |                  |                 |

### Narcotics

The following is the GC temperature gradient and ions detected for the Narcotics data acquisition method:



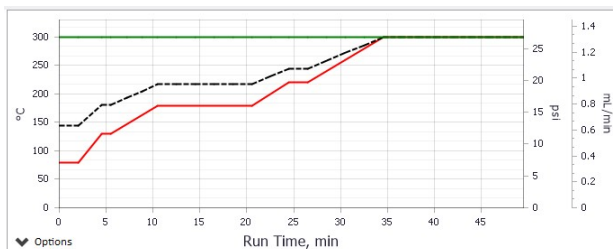
|             | Rate<br>°C/min | Value<br>°C | Hold Time<br>min | Run Time<br>min |
|-------------|----------------|-------------|------------------|-----------------|
| ► (Initial) |                | 100         | 0.25             | 0.25            |
| Ramp 1      | 20             | 220         | 2                | 8.25            |
| Ramp 2      | 15             | 250         | 3                | 13.25           |
| Ramp 3      | 15             | 320         | 2                | 19.917          |
| *           |                |             |                  |                 |



## URINE DRUG PROCEDURES

### Base

The following is the GC temperature gradient and ions detected for the Base data acquisition method:



|             | Rate<br>°C/min | Value<br>°C | Hold Time<br>min | Run Time<br>min |
|-------------|----------------|-------------|------------------|-----------------|
| ▶ (Initial) |                | 80          | 2                | 2               |
| Ramp 1      | 20             | 130         | 1                | 5.5             |
| Ramp 2      | 10             | 180         | 10               | 20.5            |
| Ramp 3      | 10             | 220         | 2                | 26.5            |
| Ramp 4      | 10             | 300         | 15               | 49.5            |

| COMPOUND        | IONS |     |     |  |
|-----------------|------|-----|-----|--|
| Norketamine     | 166  | 195 | 168 |  |
| Ketamine        | 180  | 209 | 152 |  |
| Diphenhydramine | 58   | 165 | 167 |  |
| PCP             | 200  | 242 | 186 |  |
| Tramadol        | 58   | 263 | 135 |  |
| Norfentanyl     | 96   | 189 | 82  |  |

|                         |     |     |     |  |
|-------------------------|-----|-----|-----|--|
| N-Desmethyl<br>Tramadol | 189 | 202 | 135 |  |
| EDDP                    | 277 | 262 | 115 |  |
| Methadone               | 72  | 165 | 223 |  |
| Dextromethorphan        | 271 | 150 | 214 |  |
| Amitriptyline           | 58  | 202 | 215 |  |
| Nortriptyline           | 44  | 202 | 203 |  |
| Cyclobenzaprine         | 58  | 215 | 202 |  |
| Sertraline              | 274 | 276 | 262 |  |
| Citalopram              | 58  | 238 | 208 |  |
| Acetyl Fentanyl         | 231 | 146 | 188 |  |
| Fentanyl                | 245 | 146 | 189 |  |
| Zolpidem                | 235 | 219 | 307 |  |
| Flualprazolam           | 297 | 257 | 222 |  |
| Estazolam               | 259 | 239 | 205 |  |
| Alprazolam              | 279 | 204 | 308 |  |
| Etizolam                | 342 | 266 | 313 |  |
| PCP D5                  | 205 | 246 | 190 |  |
| Fentanyl D5             | 250 | 151 | 194 |  |



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Qualitative A (LC-MS/MS)

**Table: Qualitative A LC Pump Gradient**

| Time  | % A: Water with 0.1% Formic Acid and 5mM Ammonium Formate | % B: Methanol |
|-------|-----------------------------------------------------------|---------------|
| 0.01  | 95                                                        | 5             |
| 0.01  | To MS                                                     | To MS         |
| 9.00  | 5                                                         | 95            |
| 12.00 | To Waste                                                  | To Waste      |
| 12.00 | 5                                                         | 95            |
| 12.01 | 95                                                        | 5             |
| 14.00 | Stop                                                      | Stop          |

**Table: Instrument Parameters for Target Analytes**

| Target Analyte  | Precursor Ion ( <i>m/z</i> ) | Product Ion |     |
|-----------------|------------------------------|-------------|-----|
| Amphetamine     | 136                          | Quant       | 91  |
|                 |                              | Qualifier   | 119 |
| Methamphetamine | 150                          | Quant       | 91  |
|                 |                              | Qualifier   | 119 |
| MDMA            | 194                          | Quant       | 163 |
|                 |                              | Qualifier   | 105 |
| Norketamine     | 224                          | Quant       | 207 |
|                 |                              | Qualifier   | 125 |
| Ketamine        | 238                          | Quant       | 125 |
|                 |                              | Qualifier   | 207 |
| Cocaethylene    | 318                          | Quant       | 82  |
|                 |                              | Qualifier   | 196 |
| Methylphenidate | 234                          | Quant       | 84  |
|                 |                              | Qualifier   | 56  |
| Cocaine         | 304                          | Quant       | 182 |
|                 |                              | Qualifier   | 82  |
| Benzoylecgonine | 290                          | Quant       | 168 |
|                 |                              | Qualifier   | 105 |
| PCP             | 244                          | Quant       | 159 |
|                 |                              | Qualifier   | 91  |

| Target Analyte | Precursor Ion ( <i>m/z</i> ) | Product Ion |     |
|----------------|------------------------------|-------------|-----|
| 7-             | 286                          | Quant       | 222 |

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## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

|                             |     |           |     |
|-----------------------------|-----|-----------|-----|
| Aminoclonazepam             |     | Qualifier | 121 |
| Zolpidem                    | 308 | Quant     | 235 |
|                             |     | Qualifier | 263 |
| 7-Aminoflunitrazepam        | 284 | Quant     | 135 |
|                             |     | Qualifier | 227 |
| Lorazepam                   | 321 | Quant     | 275 |
|                             |     | Qualifier | 321 |
| $\alpha$ -hydroxyalprazolam | 325 | Quant     | 297 |
|                             |     | Qualifier | 216 |
| Clonazepam                  | 316 | Quant     | 270 |
|                             |     | Qualifier | 214 |
| Oxazepam                    | 287 | Quant     | 241 |
|                             |     | Qualifier | 104 |
| Alprazolam                  | 309 | Quant     | 281 |
|                             |     | Qualifier | 205 |
| Flunitrazepam               | 314 | Quant     | 268 |
|                             |     | Qualifier | 239 |
| Nordiazepam                 | 271 | Quant     | 140 |
|                             |     | Qualifier | 165 |
| Temazepam                   | 301 | Quant     | 255 |
|                             |     | Qualifier | 177 |
| Diazepam                    | 285 | Quant     | 154 |
|                             |     | Qualifier | 193 |
| Etizolam                    | 343 | Quant     | 314 |
|                             |     | Qualifier | 259 |

| Target Analyte | Precursor Ion ( $m/z$ ) | Product Ion |     |
|----------------|-------------------------|-------------|-----|
| Zolpidem-d6    | 314                     | Quant       | 235 |
|                |                         | Qualifier   | 314 |



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Data Analysis Methods

All GC/MS instrument shall use the following data analysis parameters:

- Retention Time Setup: Criteria-Close RT, right and left deltas minimum 0.3 minutes (with the exception of compounds that consistently exhibit elongated tailing that could be cut off in the 0.3 minute window)
- Compound Calibration setup: Compounds of interest set to level 2, recovery compounds set to level 1.
- Compound Concentration Setup: CF-Linear, CF-Origin Force, CF Weight-None.
- Compound Qualifier and Mass Extraction Setup: Uncertainty-Relative, 20.
- Compound Noise and Smoothing Setup: Noise Alg. ASTM, Smoothing-None
- Compound Integration Parameters: Integrator-Agile2
- Outlier Setup:
  - Retention Time: RT Window-0.4, RT Units-Minutes
  - Signal-to-Noise Ratio: Min S/N-3.3
  - Limit of Quantitation: 20% of compounds positive control concentration.
  - Surrogate Percent Recovery: Surrogate Conc Limit Low-50% recovery compound concentration, Surrogate Conc Limit High-500, Surrogate Concentration-concentration of recovery compound.
    - It is noted that all of the data analysis methods are set to have a upper surrogate % recovery limit of 500%. This upper cap serves two purposes, one allows the data analysis method to calculate percent recovery for all samples in the batch and secondly serves as a indicator to further investigate the response of the UNEX control.

All LC-MS/MS methods shall use the following data analysis parameters:

- Retention Time Setup: Criteria-Close RT
- Compound Calibration/Positive Control setup:

|     |         |
|-----|---------|
| Mix | Level 1 |
|-----|---------|
- Compound Concentration Setup: CF-Linear, CF-Origin Force, CF Weight 1/x.
- Compound Qualifier and Mass Extraction Setup: Uncertainty-Relative, 30.
- Outlier Setup:
  - Retention Time: RT Window-0.4, RT Units-Minutes (Except for Lorazepam: RT Window-0.2)
  - Signal-to-Noise Ratio: Min S/N-3.3



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Relative Retention times:

The following tables contain retention times for all of the drugs/drug metabolites in the urine drug testing program based on the validated data acquisition methods above. These retention times were found during the initial validation study, due to routine column maintenance they are subject to change though they shall remain the same in relation to each other. The following retention times may be used to guide the data analysis process. Retention times shall always be updated on concurrently run certified reference material (positive control). Highlighted compounds are not included in the Randox Evidence Investigator screen and if detected a second aliquot must be extracted and analyzed for confirmation on GC/MS and or LC-MS/MS.

| Base                                                                                                                                          | RT     | Narcotics       | RT     | Qualitative A           | RT   |
|-----------------------------------------------------------------------------------------------------------------------------------------------|--------|-----------------|--------|-------------------------|------|
| PCP-d5                                                                                                                                        | 18.405 | 6-MAM-D6        | 15.66  | Zolpidem-d6             | 3.97 |
| Fentanyl-d5                                                                                                                                   | 33.639 | Dihydrocodeine  | 13.87  | MDMA                    | 2.29 |
| Norketamine                                                                                                                                   | 16.156 | Codeine TMS     | 14.613 | Methamphetamine         | 2.12 |
| Ketamine                                                                                                                                      | 17.132 | Morphine di-TMS | 15.08  | Amphetamine             | 1.93 |
| Diphenhydramine                                                                                                                               | 17.628 | 6-MAM           | 15.725 | PCP                     | 4.31 |
| PCP                                                                                                                                           | 18.504 |                 |        | Benzoylcegonine         | 3.07 |
| Tramadol                                                                                                                                      | 20.887 | THCCOOH         | RT     | Cocaine                 | 3.48 |
| N-desmethyl tramadol                                                                                                                          | 22.232 | COOH-d9         | 10.551 | Methylphenidate         | 3.48 |
| EDDP                                                                                                                                          | 23.259 | COOH            | 10.592 | Cocaethylene            | 4.08 |
| Methadone                                                                                                                                     | 25.530 |                 |        | Ketamine                | 3.09 |
| Dextromethorphan                                                                                                                              | 25.646 |                 |        | Norketamine             | 3.04 |
| Amitriptyline                                                                                                                                 | 26.933 |                 |        | Zolpidem                | 4.00 |
| Nortriptyline                                                                                                                                 | 27.367 |                 |        | 7-Aminoclonazepam       | 3.24 |
| Cyclobenzaprine                                                                                                                               | 27.931 |                 |        | 7-Aminofluinazepam      | 3.86 |
| Sertraline                                                                                                                                    | 29.834 |                 |        | Etizolam                | 6.33 |
| Citalopram                                                                                                                                    | 30.462 |                 |        | Diazepam                | 6.62 |
| Acetyl Fentanyl                                                                                                                               | 33.191 |                 |        | Temazepam               | 6.18 |
| Fentanyl                                                                                                                                      | 33.657 |                 |        | Nordiazepam             | 6.37 |
| Zolpidem                                                                                                                                      | 34.339 |                 |        | Flunitrazepam           | 5.69 |
| Flualprazolam                                                                                                                                 | 35.075 |                 |        | Alprazolam              | 6.03 |
| Estazolam                                                                                                                                     | 35.327 |                 |        | Oxazepam                | 5.97 |
| Alprazolam                                                                                                                                    | 35.686 |                 |        | Clonazepam              | 5.62 |
| Etizolam                                                                                                                                      | 36.565 |                 |        | Alpha-hydroxyalprazolam | 5.77 |
|                                                                                                                                               |        |                 |        | Lorazepam               | 6.03 |
| Highlighted compounds are not screened for on the Randox Evidence Investigator, for confirmation a second aliquot shall be extracted and run. |        |                 |        |                         |      |



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Urine Drug GC/MS Testing Menu, Reporting Limits, & Positive Control Levels:

| Compound              | Randox Preliminary Screen Decision Point Cutoff (ng/mL) | GCMS/LCMSMS Method Lower Reporting Limit/ Decision Point Cutoff (ng/mL) | GCMS/LCMSMS Method Positive Control Level (ng/mL) |
|-----------------------|---------------------------------------------------------|-------------------------------------------------------------------------|---------------------------------------------------|
| THC-COOH              | (THC) 30*                                               | 5                                                                       | 15                                                |
| Benzoyllecgonine      | (BZG) 150                                               | 20                                                                      | 80                                                |
| Cocaethylene          | (BZG) 150                                               | 20                                                                      | 80                                                |
| Cocaine               | (BZG) 150                                               | 20                                                                      | 80                                                |
| Amphetamine           | (AMPH) 200                                              | 20                                                                      | 80                                                |
| Methamphetamine       | (MAMP) 200                                              | 20                                                                      | 80                                                |
| MDMA                  | (AMPH) 200                                              | 20                                                                      | 80                                                |
| Methylphenidate       | (AMPH) 200, (MAMP) 200, (PCP) 25                        | 20                                                                      | 80                                                |
| PCP                   | (PCP) 25                                                | 10 (GCMS), 20 (LCMSMS)                                                  | 30 (GCMS), 80 (LCMSMS)                            |
| Norketamine           | Not included in Randox                                  | 100 (GCMS), 20 (LCMSMS)                                                 | 300 (GCMS), 80 (LCMSMS)                           |
| Ketamine              | Not included in Randox                                  | 100 (GCMS), 20 (LCMSMS)                                                 | 300 (GCMS), 80 (LCMSMS)                           |
| 7-amino flunitrazepam | (BENZ2) 100 & (BENZ3) 25                                | 10                                                                      | 40                                                |
| 7-aminoclonazepam     | (BENZ3) 25                                              | 10                                                                      | 40                                                |
| a-Hydroxyalprazolam   | (BENZ1) 100                                             | 10                                                                      | 40                                                |
| Clonazepam            | (BENZ2) 100 & (BENZ3) 25                                | 10                                                                      | 40                                                |
| Diazepam              | (BENZ1) 100                                             | 10                                                                      | 40                                                |
| Flunitrazepam         | (BENZ1) 100                                             | 10                                                                      | 40                                                |
| Lorazepam             | (BENZ1 & BENZ2) 100                                     | 10                                                                      | 40                                                |
| Nordiazepam           | (BENZ1) 100                                             | 10                                                                      | 40                                                |
| Oxazepam              | (BENZ1 & BENZ2) 100                                     | 10                                                                      | 40                                                |
| Temazepam             | (BENZ1) 100                                             | 10                                                                      | 40                                                |
| Zolpidem              | (ZOL) 10                                                | 50 (GCMS), 10 (LCMSMS)                                                  | 150 (GCMS), 40 (LCMSMS)                           |
| Alprazolam            | (BENZ1) 100                                             | 50 (GCMS), 10 (LCMSMS)                                                  | 150 (GCMS), 40 (LCMSMS)                           |
| Etizolam              | (BENZ1) 100                                             | 100 (GCMS), 10 (LCMSMS)                                                 | 300 (GCMS), 40 (LCMSMS)                           |
| 6-MAM                 | (OPD/Opiate) 100                                        | 25                                                                      | 75                                                |
| Codeine               | (OPD/Opiate) 100                                        | 50                                                                      | 150                                               |
| Dihydrocodeine        | (OPD) 100                                               | 50                                                                      | 150                                               |
| Morphine              | (OPD/Opiate) 100                                        | 50                                                                      | 150                                               |
| Diphenhydramine       | (TCA) 100                                               | 50                                                                      | 150                                               |
| Tramadol              | (TRM) 5                                                 | 50                                                                      | 150                                               |
| N-Desmethyl Tramadol  | (TRM) 5                                                 | 100                                                                     | 300                                               |
| EDDP                  | (MDONE) 300                                             | 50                                                                      | 150                                               |

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### Forensic Chemistry Section

|                  |               |     |     |
|------------------|---------------|-----|-----|
| Methadone        | (MDONE) 300   | 50  | 150 |
| Dextromethorphan | (DMP) 20      | 100 | 300 |
| Amitriptyline    | (TCA) 100     | 100 | 300 |
| Nortriptyline    | (TCA) 100     | 100 | 300 |
| Cyclobenzaprine  | (TCA) 100     | 10  | 30  |
| Sertraline       | See GCMS Base | 25  | 75  |
| Citalopram       | See GCMS Base | 100 | 300 |
| Acetyl Fentanyl  | (FENT) 1      | 10  | 30  |
| Fentanyl         | (FENT) 1      | 10  | 30  |
| Flualprazolam    | See GCMS Base | 25  | 75  |
| Estazolam        | (BENZ1) 100   | 50  | 150 |

\*HETL assigned



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

**Randox Evidence Investigator Cross Reactivity Table:** *Highlighted compounds are not able to be confirmed using the GC/MS methods.*

| OXYC 1                      | OXYC 2                     | OPDS                         | Opiate                           | DMP                        |
|-----------------------------|----------------------------|------------------------------|----------------------------------|----------------------------|
| Hydrocodone (132.6)         | Oxycodone (100)            | Hydrocodone (1057)           | 6-MAM ( 1168)                    | Dextromethorphan (100)     |
| Oxycodone (100)             | Oxymorphone (22.9)         | Ethyl Morphine (339)         | 6-Acetyl-Codeine (430.3)         |                            |
| Noroxycodone (29)           | <b>AMPH</b>                | Codeine (287)                | Heroin (353.6)                   |                            |
| <b>MBP</b>                  | MDA (323.3)                | 6-Acetyl-Codeine(166.8)      | Desomorphine (159.9)             | <b>MAMP</b>                |
| Meprobamate (100)           | (S) Amphetamine (100)      | Dihydrocodeine (103.5)       | Codeine (112.2)                  | PMMA HCl (291)             |
| Carisoprodol (88)           | PMA HCl (292.8)            | Hydromorphone (102.5)        | Morphine (100)                   | MDMA (114.4)               |
|                             | BDB (120.6)                | Oxycodone (100)              | Morphine Glucuronide (68.4)      | (S) Methamphetamine (100)  |
|                             | +/- Amphetamine (49.6)     | Morphine Glucuronide( 35.1)  | Hydromorphone (50.8)             | +/- Methamphetamine (69.8) |
|                             | Phentermine (25.4)         | Heroin (29.5)                | Hydrocodone (38.4)               | Methylphenidate (<1)       |
|                             | R-Amphetamine (16.6)       | Morphine (26.3)              |                                  |                            |
|                             | Methylphenidate (<1)       | 6-MAM (21.2)                 |                                  |                            |
| BARB                        | BENZ 1                     | BENZ 2                       | BENZ 3                           |                            |
| Secobarbital (371)          | Temazepam (382)            | Lorazepam (100)              | Desmethyflunitrazepam (128)      |                            |
| Butabarbital (166)          | Flubromazepam (326)        | Delorazepam (79.2)           | Clonazepam (100)                 |                            |
| Pentobarbital (151)         | Nordiazepam (317)          | Phenazepam (72.8)            | Delorazepam (41)                 |                            |
| Alphenal (117)              | Alpha-OH-Alprazolam (310)  | Clonazepam (28.2)            | 7-Aminoclonazepam (40.6)         |                            |
| Cyclopentobarbital (70.1)   | Alprazolam (258)           | Flubromazepam (25.9)         | Phenazepam (29.7)                |                            |
| p-OH-phenobarbital (64)     | Diazepam (256)             | Lorazepam Glucuronide (24.8) | 7-Aminoflunitrazepam (2.7)       |                            |
| Butalbital (51.1)           | Estazolam (253)            | Oxazepam (13)                | <b>BZG</b>                       |                            |
| Amobarbital (44)            | Clobazam (204)             | Meclonazepam (12.8)          | Cocaine (103.8)                  |                            |
| Barbital (33.3)             | Nitrazepam (194)           | <b>TCA</b>                   | Benzoyllecgonine (100)           |                            |
| <b>MDONE</b>                | Brotizolam (191)           | Imipramine N Oxide (1127)    | m-hydroxybenzoyllecgonine (95.6) |                            |
| Methadone (100)             | 2-OH-Ethylflurazepam (188) | Imipramine (294)             | Cocaethylene (54.4)              |                            |
| EDDP (<0.1)                 | Flubromazepam (175)        | Trimipramine (238)           | <b>TRM</b>                       |                            |
| <b>ZOL</b>                  | Prazepam (172)             | Desipramine (206)            | Tramadol (100)                   |                            |
| Zolpidem (100)              | Diclazepam (157)           | Cyclobenzaprine (201)        | O-Desmethyltramadol (34.8)       |                            |
| 4-carboxyzolpidem (47.5)    | Midazolam (116)            | Amitriptyline (190)          | N-Desmethyltramadol (1.39)       |                            |
| <b>THC</b>                  | Flunitrazepam (114)        | Opipramol (167)              | <b>BUP</b>                       |                            |
| 11-nor-9-Carboxy-delta9-THC | Oxazepam (100)             | Promazine (117)              | Norbuprenorphine (100)           |                            |
| <b>FENT</b>                 | Flurazepam (93.4)          | Nortriptyline (100)          | Buprenorphine (16.7)             |                            |
| alpha-methylfentanyl (266)  | Etizolam (28.4)            | Maprotiline (96)             | <b>PCP</b>                       |                            |
| Fentanyl (100)              | Lorazepam (18.4)           | Lofepamine (58)              | Phencyclidine (100)              |                            |
| Acetyl Fentanyl (3.1)       | 7-Aminoflunitrazepam (2.4) | Diphenhydramine (0.1)        | Methylphenidate (<1)             |                            |

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## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Reference Dilution Table:

| 1mL Sample   |        |       | 2mL Sample   |        |        |
|--------------|--------|-------|--------------|--------|--------|
|              | Sample | Water |              | Sample | Water  |
| <b>1:2</b>   | 500uL  | 500uL | <b>1:2</b>   | 1mL    | 1mL    |
| <b>1:4</b>   | 250uL  | 750uL | <b>1:4</b>   | 500uL  | 1.5mL  |
| <b>1:10</b>  | 100uL  | 900uL | <b>1:10</b>  | 200uL  | 1.8mL  |
| <b>1:20</b>  | 50uL   | 950uL | <b>1:20</b>  | 100uL  | 1.9mL  |
| <b>1:50</b>  | 20uL   | 980uL | <b>1:50</b>  | 40uL   | 1.96mL |
| <b>1:100</b> | 10uL   | 990uL | <b>1:100</b> | 20uL   | 1.98mL |
|              |        |       | <b>1:200</b> | 10uL   | 1.99mL |

#### **1:500**

1mL Sample: 10uL Sample + 990uL water, vortex. Take 200uL of mixture + 800uL of water

2mL Sample: 10uL Sample + 990uL water, vortex. Take 400uL of mixture + 1.6mL of water

#### **1:1000**

1mL Sample: 10uL Sample + 990uL water, vortex. Take 100uL of mixture + 900uL of water

2mL Sample: 10uL Sample + 990uL water, vortex. Take 200uL of mixture + 1.8mL of water

#### **1:10,000**

1mL Sample: 10uL Sample + 990uL water, vortex. Take 10uL of mixture + 990uL of water

2mL Sample: 10uL Sample + 990uL water, vortex. Take 20uL of mixture + 1.98mL of water



## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

#### Revision Table:

| Revised By (initials): | Revision # | Revisions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Revision Date |
|------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| EAF                    | 1*         | Revision table added. Updated entire document following optimization and validation of all GC/MS instrument methods.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 1/5/23        |
| EAF                    | 2          | Removed Methylphenidate from urine drug testing menu.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 1/17/23       |
| LN                     | 3          | Evidence Handling and Preservation section updated to include storage of chain of custody forms in Forensic Lab spaces                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 2/14/23       |
| LN                     | 4          | Following the lab move, TOX 1 can only be used to screen for nortriptyline and cannot be used for confirmation. Nortriptyline can only be confirmed on TOX 2.<br>Correction to RT table to remove d3 from cocaethylene compound.<br>Software settings added.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 4/6/23        |
| EAF                    | 5          | Randox Acceptance Criteria: Quality Controls-added information that red=3 std deviations, yellow=2 std deviations.<br>Randox Documentation & Case Documentation Batch Folder: Added QC Summary Report Form.<br>Case Documentation: Each sample folder- added: Photocopy of each batch GCMS Control Review Form used to test the case sample<br>Unextracted acceptance criteria: removed <20% response seen in positive control and added "It is noted that all of the data analysis methods are set to have a upper surrogate % recovery limit of 500%. This upper cap serves two purposes, one allows the data analysis method to calculate percent recovery for all samples in the batch and secondly serves as a indicator to further investigate the response of the UNEX control."<br>Data Analysis Methods: added "It is noted that all of the data analysis methods are set to have a upper surrogate % recovery limit of 500%. This upper cap serves two purposes, one allows the data analysis method to calculate percent recovery for all samples in the batch and secondly serves as a indicator to further investigate the response of the UNEX control."<br>Relative Retention Times: Removed Oxazepam TMS<br>Benzodiazepine Extraction procedure: corrected "run using NARCOTIC instrument method" to "run using Benzodiazepine instrument method"<br>Sample Requirements: added "If a urine sample is received for testing in a non HETL approved urine collection kit/cup it shall be notated on the Urine kit inventory form."<br>Reporting of Confirmatory Drug Testing:<br>QNS Sample: Removed use of the work standard. Now reads "If a urine sample is received by the laboratory with volume not sufficient to perform HETL OUI urine drug panel and only a portion of the confirmation testing menu can be confirmed for, then the following comment shall be included on the COA: "Unable to perform complete HETL OUI urine drug panel due to low sample volume."<br>Inconclusive result/Sample Matrix: Added "If a sample is unable to be tested | 5/12/23       |

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## HEALTH & ENVIRONMENTAL TESTING LABORATORY

### Forensic Chemistry Section

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|     |   | <p>or completed due to the sample matrix (inconclusive results), then the following comment shall be included on the COA: "Unable to perform HETL OUI testing panel due to sample matrix."</p> <p>Dilutions: Added "Each GCMS extraction method has approved validated dilutions. New dilutions may be performed if the new dilution is within the range of already validated dilutions. If a greater dilution needs to be performed on a sample the dilution may be performed but a deviation form shall be required. Example: Narcotics approved dilutions range from x2 to a x100, therefore a x4 dilution may be performed on a sample without a deviation form but anything greater than a x100 would require a deviation form. "Evidence Handling and Preservation: added "If a sample or urine collection box requires a more detailed description or photographic documentation, the analyst may document the description in the urine inventory form, or by taking a picture using a state controlled camera (not a personal camera or cell phone). If a picture is taken, a ruler shall be included in the photo. The photo shall be printed for the case file and have the following items documented on the printout: HETL case number, item number(s), analyst initials, and date."</p> <p>Entire document: formatted page breaks.</p> <p>Updated Randox QC expiration information to: "good for 21 days when stored at -18°C to -24°C in the original vial."</p> |         |
| EAF | 6 | <p>Dilutions:<br/>Each GCMS extraction method has approved validated dilutions. New dilutions may be performed if the new dilution is within the range of already validated dilutions. If a dilution is need that is greater than the validated dilution options, the non-validated dilution must be run with a validated dilution to compare. Example: Narcotics approved dilutions range from x2 to a x100, therefore a x4 dilution may be performed on a sample without a deviation form, but anything greater than a x100 would require an additional validated dilution to be run concurrently with the non-validated dilution, such as a x10 or a x100 with a x200.</p> <p>Randox Acceptance criteria: combined cal and qc storage information into one initial statement "Randox Evidence Investigator calibration controls are provided with the kit as well as manufactured quality controls (high and low controls). Each calibrator and quality control needs to be reconstituted with DI water and is good for 21 days when stored at -18°C to -24°C in the original vial. "</p> <p>Negative control "If a sample negative control does not meet the above parameters with regards to compounds of interest, the samples bracketing the rejected negative control shall be rejected and the samples shall be re-extracted only if the samples meet positive acceptance criteria for the compound seen in the negative control. See below example: (Table)"</p>     | 6/12/23 |



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|        |   | <p>Randox table, corrected spelling of p-fluorofentanyl</p> <p>Evidence handling section updated to include "The analyst opening the kit will initial stickers bearing the HETL Identification Number."</p> <p>Positive control evaluations updated to accommodate multiple scenarios.</p> <p>Base Extraction: 1:10 dilution option removed as a validated dilution. Typos relating to dilution preparations for 1:10 dilutions on bench sheets and SOP discovered, dilutions not consistently run during validation for this level of dilution. Dilutions higher than 1:4 will follow above updated dilution policy.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |         |
| EAF/LN | 7 | <p>Reformatted:</p> <ul style="list-style-type: none"> <li>Moved/reformatted GC/MS Screening and Confirmation Sample Acceptance Criteria and Reporting of Confirmatory Drug Testing.</li> </ul> <p>Changed (added/subtracted):</p> <ul style="list-style-type: none"> <li>Reagents, Standards, and Quality Control Materials: "An evaluation of all new stocks created shall be performed by running the new stock and old stock unextracted at the same time to compare area counts. These area counts shall be exported into a QC monitoring excel document and be evaluated and the new stock approved prior to the new stock being used for casework. Acceptance criteria for new stocks consist of all expected compounds of interest being present in the new stocks with area counts of <math>\pm 30\%</math> of the old stock."</li> <li>Added the individual performing the pre-sequence check shall also initial the printed instrument sequence following verification</li> </ul> <p>Changes to Quality Control Requirements &amp; Acceptance Criteria</p> <ul style="list-style-type: none"> <li>Removed generic IS section and updated each control with clear IS requirements</li> <li>Positive control: separated the expanding paragraphs of PC1 and PC2. <ul style="list-style-type: none"> <li>Added to PC2: "GCMS method used for screening only: All previously listed acceptance criteria must be met with the exception of qualifier ion ratios. Qualifier ion ratios for the compound(s) of interest of <math>&gt;\pm 20\%</math> of the first positive control will be acceptable for screening."</li> </ul> </li> <li>Negative Control: added "As the THC-COOH method does not have associated sample negative controls, an associated methanol blank shall be run immediately after each sample to monitor for GCMS carryover." and removed methanol blanks from the example cocaine sequences.</li> <li>Removed requirement for instrument methanol blanks for SPE extracted samples. SPE sample negative will serve to monitor for</li> </ul> | 8/15/23 |



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|        |   | <p>carryover on both the manifold and instrument.</p> <p>Case Documentation</p> <ul style="list-style-type: none"> <li>Clarified data for sample negatives and methanol blanks were required as needed</li> </ul> <p>Carryover</p> <ul style="list-style-type: none"> <li>Specified methanol blanks between samples is only required for liquid liquid extraction methods to monitor for carryover</li> </ul> <p>Instrument Sequence</p> <ul style="list-style-type: none"> <li>Added "A methanol blank shall be run after each case sample extracted using a liquid/liquid method." Updated sequence example tables</li> </ul> <p>Diphenhydramine &amp; Acetyl Fentanyl:</p> <ul style="list-style-type: none"> <li>Specific Extraction Methods. Basic Drugs in Urine. Removed Diphenhydramine and Acetyl Fentanyl from the list that Randox does not screen for.</li> <li>Relative retention time table, changed shading on diphenhydramine and Acetyl Fentanyl to indicate that the Randox does screen for them.</li> <li>Randox Cross Reactivity table: removed Perphenazine (17.3) from TCA to make room for Diphenhydramine (0.1). Added Acetyl Fentanyl (3.1) to FENT</li> </ul> <p>Appendix</p> <ul style="list-style-type: none"> <li>Created Reference Dilution table</li> </ul> |         |
| EAF    | 8 | <p>Removed all reference to confirmation testing of Oxycodone, Oxymorphone, Hydrocodone, and Hydromorphone.</p> <p>Kept reference to Hydrocodone in Narcotics Limitations because high levels of Hydrocodone in a sample may impact our ability to detect Codeine.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 9/19/23 |
| EAF/LN | 9 | <p>Evidence Handling: updated COC statement after analysis from signed by analyst to "Chain-of-custody forms will be updated as necessary and retained". Letter "m" updated to include letter designations assigned to each container received.</p> <p>Step by Step SIM analysis: Cocaethylene-D3 added to compounds collecting extra ions</p> <p>Clarified sample negative control acceptance parameters by adding "If a sample negative control does not meet acceptability parameters for the recovery compound, and one or more of the bracketing samples are negative for all compounds of interest AND exhibit acceptable recovery compound then the sample may be reported out as negative." And "If a sample negative control does not meet the above parameters with regards to compounds of interest, the samples bracketing the rejected negative control shall be rejected for those compound(s). The sample(s) shall be re-extracted only if the sample(s) meets positive acceptance criteria for the compound(s) seen in</p>                                                                                                                                                                                                                                                 | 26Oct23 |

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|-----------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
|           |    | <p>the negative control. "</p> <p>Positive Control Acceptance criteria added "when applicable" to statements regarding second positive control.</p> <p>Added to second positive control acceptance criteria "Second Positive Control:</p> <ul style="list-style-type: none"> <li>• The following testing methods do not require a second positive control run at the end of the batch as validation stability studies have been performed for these methods: <ul style="list-style-type: none"> <li>o Amines</li> <li>o Base"</li> </ul> </li> </ul> <p>Added the following to THCCOOH, Cocaines, Narcotics, Benzodiazepines "No validation stability study has been performed for this testing method therefore a second positive control must be run at the end of the batch." Reporting of confirmatory drug testing added "An inventory table will be included on the report to indicate the number and types of containers received, the collection information, the approximate volume of each container, and an indication as to which containers were tested. "</p> <p>Updated dilution section "If a dilution is needed that is greater than the validated dilution options, a validated dilution must be run to compare." And "It is noted that the elevated presence of a compound of interest may impact the recovery compound (such as recovery compound qualifier ion ratios outside acceptance parameters) in a sample and even in a validated dilution. In these cases, all validated dilutions/the highest validated dilution must be evaluated prior to, or concurrently with, running a non-validated dilution"</p> <p>Spelling throughout</p> <p>Removed reference to LCMSMS for batch review form</p> <p>Added requirements for expert letter.</p> |            |
| LN/EAF/AH | 10 | <ul style="list-style-type: none"> <li>- Clarified metadata kit information for HETL approved and non-HETL approved collection kits, and how to record various submissions on inventory form and COA</li> <li>- Clarified only positive control stocks are run and checked before use in casework</li> <li>- Specified Randox positive screening criteria</li> <li>- Added preparation of luminol solution for Randox procedure</li> <li>- Specified Stock B used for THC procedure</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 01/19/2024 |
| LN        | 11 | <ul style="list-style-type: none"> <li>- Changed stock QC check to only fail if compound was -30% of previous stock</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |            |
| EAF       | 12 | <ul style="list-style-type: none"> <li>- Clarified discrepancies in submitted information with table.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3/13/24    |
| LN        | 13 | <ul style="list-style-type: none"> <li>- Updated waste section</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 3/28/24    |



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|-----|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| EAF | 14 | - Added photography section                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 4/9/24     |
| EAF | 15 | <ul style="list-style-type: none"> <li>- Added to stock evaluation: In the event that the old stock is not available to use for comparison (broken bottle/not enough volume) an acceptable alternative to evaluate the new stock is to, prior to any casework being performed using this new stock, to run a batch consisting of UNEX, PC, NC, HC (when applicable), and a Reporting Limit. All acceptance criteria must be met for controls and the compounds in the reporting limit must meet all positive acceptance criteria for the new stock to be deemed acceptable to be used for casework."</li> <li>- Added step by step guidance on how to use the Randox computer.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 5/3/24     |
| EAF | 16 | <ul style="list-style-type: none"> <li>- Updated wording to in Appendix "A tune report is generated daily (when in use), prior to running casework and/or following maintenance. If a batch of casework runs overnight, into the next day the run does not need to be stopped and the instrument tuned, but the instrument must be tuned prior to a new batch of samples being put on the instrument to run."</li> <li>- Case Documentation: reformatted document list for batch files for ease. Added to casefile documentation: "It is noted that photocopies of the Urine Drug Screen Form, GCMS Control Review Forms, and associated methanol/sample negatives may be included in applicable casefiles. The Urine Drug Screen Form and GCMS Control Review Forms must be photocopied and added to the casefile once the associated batch has completed technical and administrative review, this is to ensure that all appropriate changes and documentation are captured."</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                    | 8/12/24    |
| EAF | 17 | <p>Case Documentation: Clarified existing language: "It is noted that photocopies of the Urine Drug Screen Form, GCMS Control Review Forms, and associated methanol/sample negatives may be included in applicable casefiles as a convenience. If these forms are photocopied and added to the casefile, the photocopying shall be performed once the associated batch has completed technical and administrative review, this is to ensure that all appropriate changes and documentation are captured. "</p> <p>Dilutions: Added: "If a case sample screens high or is found to contain high levels of a compound of interest or if the sample matrix is impacting the ability to confirm, a dilution may be performed to confirm presence without running a concurrent undiluted sample. "</p> <p>Narcotics Limitations: Added "Hydrocodone is not currently included in the Urine Drug Testing Menu but does still have the ability to interfere with the detection of codeine as described above. If a sample exhibits the aforementioned attributes for codeine it shall be re-extracted with a dilution for confirmation."</p> <p>Amines Limitations: Added "A stability study has been performed for this method, extracted samples are stable at room temperature for 3 days and in freezer storage for 2 weeks. "</p> <p>Base Limitations: Added "A stability study has been performed for this</p> | 12/20/2024 |



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|     |    | method, extracted samples are stable at room temperature for 3 days and in freezer storage for 3 days. "<br>Cocaine Dilutions: Added "Once one of the compounds of interest in this method have been confirmed, further testing/dilutions to confirm the remaining is not required. "                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |          |
| EAF | 18 | Removed Fluorofentanyl from the testing menu and Urine Drug SOP.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1/27/25  |
| EAF | 19 | Updated Benzodiazepine extraction following performance check of modifying dry down times.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3/25/25  |
| EAF | 20 | Added additional requirement for instrument sequence check: "Each auto sampler vial position and the performance of a daily tune shall be verified by a secondary individual in comparison to the instrument sequence, prior to starting your run."                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 7/16/25  |
| EAF | 21 | <p>Benzodiazepine Extraction: Enzyme hydrolysis, removed duplicate word.</p> <p>Dilutions: The urine drug testing menu contains many compounds that are drugs and their associated metabolite (Examples: Cocaine &amp; Benzoyllecgonine or Methadone &amp; EDDP), it is acceptable to only report out a drug or its associated metabolite instead of performing numerous dilutions to report out a drug and its associated metabolite. Care must be taken as there are some drugs and their associated metabolite in which the associated metabolite is also a drug (Example: Methamphetamine metabolizes to Amphetamine, but Amphetamine is also a drug in its own right), in these cases, sample volume permitting, appropriate dilutions must be found to report out these drugs.</p> <p>Methanol Blanks: Methanol blanks shall be run at the start of each batch and following each case sample extracted by a liquid-liquid extraction method to monitor for instrument carryover.</p> <p>All methanol blanks must meet/exhibit the following:</p> <p>Each compound of interest and recovery compound must meet one or more of the following to be considered negative:</p> <p>No peak at expected RT</p> <p>Peak response is below threshold (20% of positive control)</p> <p>Unacceptable qualifier ion ratios</p> <p>S/N &lt;3.3</p> <p>If a methanol blank does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted.</p> <p>Dilution Appendix: <b>1:500</b></p> <p>1mL Sample: 10uL Sample + 990uL water, vortex. Take 200uL of mixture + 800uL of water</p> <p>2mL Sample: 10uL Sample + 990uL water, vortex. Take 400uL of mixture + 1.6mL of water</p> | 11/17/25 |
| EAF | 22 | Reporting of Confirmatory Drug Testing: Changed NONE to No Drugs Detected.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3/9/26   |
| EAF | 23 | Changed evaluation of new positive control stocks created: "shall be performed by, prior to any casework being performed, using this new stock and/or this new stock and the old stock (if available)" by a creating a batch                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 4/2/26   |

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|     |    | consisting of UNEX, PC, NC, HC (if applicable), and RL. Updated waste disposal to accommodate change of HETLs safety document name, added "Once analysis has been completed for a urine sample, the evidence shall be destroyed no earlier than six months from the last analysis end date unless claimed by the officer or a responsible official. " to accommodate elimination of SOP manual.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |         |
| EAF | 24 | <ul style="list-style-type: none"> <li>Evidence handling and preservation: Changed bullet points to consistent lower case letters. And added: Sample disposal shall be performed in accordance with HETL hazardous waste disposal policies. The casefile shall be updated to indicate the sample has been destroyed.</li> <li>Reformatted Urine Collection Kits Quality Assurance section</li> <li>Randox Evidence Investigator Cross Reactivity Table: removed shading on MAMP: MDMA</li> <li>Screening by Immunoassay-Randox Evidence Investigator: remove specific reagent information and just to refer electronic information provided with each kit.</li> <li>Added Immunoassay decision point cutoffs to reporting limit table.</li> <li>Added missing DMP to Randox cross reactivity table.</li> <li>Highlighted Flualprazolam in the relative retention time table.</li> </ul> | 5/28/26 |
| EAF | 25 | <ul style="list-style-type: none"> <li>Added clarifying language pertaining to qualifier ion ratio acceptance criteria.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 7/23/26 |
| EAF | 26 | <ul style="list-style-type: none"> <li>Full update following Qualitative A LCMSMS method validation.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 8/12/26 |
| EAF | 27 | <ul style="list-style-type: none"> <li>Correction made to Urine Drug GC/MS Testing Menu, Reporting Limits, &amp; Positive Control Levels table.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 8/17/26 |
| EAF | 28 | <ul style="list-style-type: none"> <li>Corrected Qualitative A dilution table title, added 1:10 &amp; 1:100 dilutions to table.</li> <li>Added "If sequence stops due to instrument error, an additional sequence and tune check is required the following day by a secondary individual to ensure sequence is acceptable and tune has been performed." from 2025 CAR #6.</li> <li>Separated LC-MS/MS screening and confirmation acceptance criteria.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                        | 8/31/26 |

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