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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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 internal standards are added to the urine samples. Compounds of interest and corresponding deuterated internal standards 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.

i. 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. The analyst will verify all identification numbers and names agree with the Receipt/Contract for Examination Form.

k. 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. If a discrepancy is noted, the analyst will reach out to the appropriate individual to clarify information.

l. The sample container from the collection kit will be labeled with the lab identification number and name of the subject.

m. 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.

n. 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.

o. Samples and controls shall be fortified with internal standard(s) and stock(s), respectfully, on the same day as the extraction.

p. 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).

q. Samples to be confirmed by GC/MS will be stored in the refrigerator until analysis is complete.

r. 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.

s. 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 signed by the analyst 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.

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 internal standard, 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 Internal Standard.

Internal Standard(s): Most commonly a compound of interest in the panel matched deuterated equivalent. All quality controls and case samples are fortified with internal standard at a consistent concentration. Internal standard(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 ≥3.3 and a retention time within 0.2 minutes of the positive control.

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.

Autotune: A tuning process that involves adjusting mass spectrometer parameters through the infusion of a tune solution. This tuning process has set temperature parameters (source: 230◦ and quad: 150◦) and sets the ratios for maximum abundance across the entire mass range of the tune.

Tune MSD: A 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 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. This tuning process performs a profile and spectral scan using the set tune parameters.

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 autosample vials shall be performed in a chemical flume hood.

Waste Management:

The most current approved version of the laboratory's RCRA plan, located on Sharepoint, 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 are as follows:

- Urine is not considered a biological hazard and can be disposed of in appropriate chemical waste streams.
- All liquid waste generated by the urine testing methods (residual organic solvent, standards, urine containing solvents and instrument waste) shall be disposed of in the Methylene Chloride/Mixed Flammables hazardous waste stream.
- Used laboratory consumables such as pipet tips, Randox Evidence Investigator Biochips, and extraction tubes that are RCRA empty (no more than 3% by weight of the total capacity of the container remains in the container) shall be disposed of in the red biohazard bins.
- Used autosampler vials shall be disposed of in the GC/MS vial waste stream.

Reagents, Standards, and Quality Control Materials:

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:
Unextracted Control (UNEX)
Internal Standard (IS)
Negative Control (NC)
Positive Control (PC)
Hydrolysis Control (HC) if required by specific analytical procedure
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 Acceptance Criteria sections for quality control acceptance parameters and criteria.

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 with associated methanol blanks.
Urine Drug Screen and GCMS Control Review forms
Randox Template
Urine Extraction form
Urine Drug Testing Benchsheet (for each GC/MS extraction performed)
Instrument Sequence Table
Copy of associated tune report

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
Hard copies of data that support the conclusion of the analyst with associated sample negative controls/blanks with internal standard
Copy of the final Certificate of Analysis
Any preliminary, supplementary or corrected reports
Case Review Form

Urine Collection Kit:

When a shipment of HETL-Forensic Chemistry Urine Collection Kits arrives, one kit from each lot number in the shipment is to be issued at random for testing with the following procedure being followed:

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 Kit QC form shall be submitted to the Quality Manager.
6. The urine collection cup sample must test negative for all taget compounds to be approved.

This process shall be performed prior to the issuing of the HETL-Forensic Chemistry Urine Collection Kits containing the new lot number with acceptable results exhibiting no method interferences caused by manufacturing contaminants or interfering compounds. Records containing the results, acceptability status, and lot number of the urine 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.

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 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.

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. Each calibrator needs to be reconstituted with DI water and is good for 14 days. 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.

Randox Evidence Investigator manufactured controls (high and low controls) are to be used with the Randox Evidence Investigator assay. Each vial must be reconstituted with DI water per the manufactureres instructions, and are good for 14 days. 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 on the Randox Evidence Investigator software, the category fails. If the value is colored yellow 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.

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 µL of assay diluent per well.
3. Pipette 10 µL of calibrator/control/sample per well.
4. Pipette 120 µL 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. 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.
7. 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 Methylene Chloride/Mixed Flammables hazardous waste stream
8. Immediately carry out 2 quick wash cycles. Using wash bottle with diluted wash buffer. add approx. 350 µL 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.

9. 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.
10. 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.
11. Add 250 µL of 1:1 prepared Luminol:Peroxide solution to each biochip well and cover to protect from light.
12. Place the carrier into Evidence Investigator after 2 minutes (± 10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time.
13. Images will be automatically captured after loading chips.

Documentation:
Sample results are printed and stored in respective casefiles.
Calibration curves, positive control, and negative control are printed and stored in the batch folder.
Randox Evidence Investigator Templates are stored in the batch folder.

Screening and/or Confirmation by GC/MS

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.

Quality Control Requirements & Acceptance Criteria:

Internal Standards: Internal standards are used to monitor extraction efficiency, matrix suitability, and instrument performance. An internal standard with similar extraction, derivatization, and chromatographic properties to the analyte(s) of interest will be used in all case samples and controls.

The internal standard(s) must exhibit:

Acceptable chromatography
Recovery at ≥50% of the concurrently run unextracted control
S/N ratio ≥3.3
Retention time within 0.2 minutes of the concurrently run positive control
Exhibit qualifier ion ratios that are +/-20% of the concurrently run positive control

If the internal standard(s) does not meet these criteria, the sample data will be rejected then sample will be re-extracted, sample volume permitting.

If the internal standard(s) fails in a positive, hydrolysis, or negative control, all samples in the extraction batch shall be rejected and 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 preceeding the sample to monitor for potential carryover from the SPE manifold.

All negative controls must meet/exhibit the following:
Recovery of internal standard ≥50%
Internal standard retention time +/-0.20 min of Positive Control & acceptable qualifier ion ratios
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.

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. If a sample negative control does not meet acceptability parameters for the internal standard, the following sample shall be rejected and re-extracted.

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

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:

- Contains all compounds of interest & internal standard
- Compounds of interest exhibit acceptable chromatography
- All compound responses are ≥20% the (extracted) Positive Control
- S/N ≥3.3
- All retention times +/-0.20 min of (extracted) Positive Control
- Internal standard 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)

If a unextracted control does not meet the above parameters, any associated data generated shall be rejected and the batch shall be re-extracted (or reprocessed).

Positive Controls: One positive control per extraction batch will be extracted along with case samples. This control will be split/divided into two autosampler vials with one injected at the begining 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:

- Recovery of internal standard ≥50% of unextracted control
- Contains all compounds of interest
- Compounds of interest exhibit acceptable chromatography
- Compounds of interest S/N ≥3.3
- First Positive control only:
  - All qualifier ion ratios updated (set to 100%+/-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:
  - Compounds of interest exhibit qualifier ion ratios +/-20% of first positive control
  - Compound retention times +/-0.20 minutes of first positive control
  - Contains all compounds of interest of >20% of first positive control

If a 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).

For a positive control that does not meet the above parameters for one 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, 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.

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:

- Recovery of internal standard ≥50%
- Internal standard retention time +/-0.20 min and qualifier ion ratios +/-20% of positive control
- Compound present with acceptable chromatography
- Compound exhibits qualifier ion ratios +/-20% of positive control

Compound exhibits S/N ≥3.3
Compound retention times +/-0.20 minutes of Positive Control
Compound response ≥20% of Positive control

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

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, sample negative controls shall be extracted in a bracketing formation to monitor for extraction carryover. Methanol blanks shall also be run at the start of each batch and following each case sample to monitor for instrument carryover.

The results of the methanol 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 Masshunter 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. 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 sampl, 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 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 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 diution.

- 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 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, First Positive Control, Hydrolysis Control (as applicable), Batch Negative, Sample Negative, and Second (split) Positive Control) 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 intials and date.

Operation:

Instrument Sequence:
Pre-Run Sequence Check:
Each auto sampler vial position 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 with the date and individuals' initials.

Instrument sequence data file pathways and instrument run sequences shall be created in the following locations:

TOX 1 Data file pathway: C Drive: C/DATA/DATA (Year)
Example: C/DATA/DATA 2022
TOX 1 Instrument run sequence: C Drive: C/SEQUENCES

TOX 2 Data file pathway: D Drive: D/DATA/DATA (Year)
Example: D/DATA/DATA 2022
TOX 2 Instrument run sequence: D Drive: D/SEQUENCES

The following format shall be used for naming:

Data file pathway folder: Date (080422)
As needed the folder format name may be modified (example: 080422 TOX1) as long as the date is included.
Data analysis batches: TestDateInitials (THC080422EAF)

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

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

SPE Manifold Sequence Example #1											
Batch Negative	PC	Sample Negative 1	Sample 1	Sample Negative 2	Sample 2	Sample Negative 3	Sample 3	Sample Negative 4	Sample 4	Sample Negative 5	Sample 5

GC/MS Sequence Example #1			
Sample Name	**Type**	**Data Acquisiton 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*
Methanol 2	Sample	Narcotics.M	
Sampl Neg 1	Sample	Narcotics.M	
Sample 1	Sample	Narcotics.M	
Methanol 3	Sample	Narcotics.M	
Sampl Neg 2	Sample	Narcotics.M	
Sample 2	Sample	Narcotics.M	
Methanol 4	Sample	Narcotics.M	
PC 2	Sample	Narcotics.M	
Methanol 1	Sample	Cocaines.M	
UNEX	Sample	Cocaines.M	1*
Batch Neg	Sample	Cocaines.M	
PC1	Sample	Cocaines.M	2*
Methanol 2	Sample	Cocaines.M	

Sample Neg 1	Sample	Cocaines.M	
Sample 1	Sample	Cocaines.M	
Methanol 3	Sample	Cocaines.M	
PC2	Sample	Cocaines.M	

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

Step by Step GC/MS 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 internal standard 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.

There are compounds in the Narcotics and Benzodiazepine methods (Oxycodone, Oxymorphone, and Temazepam) that have additional ions being collected. Only two qualifier ions print on the Masshunter generated reports. If a sample is being confirmed for Oxycodone, Oxymorphone, or Temazepam and the ions that 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.

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

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 intials.

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 LCMSMS Batch Review form (technical).

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 manor will meet the following:

Compound(s) present with acceptable chromatography and response ≥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 ≥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 internal standard 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 internal standard peak with acceptable chromatography
- A recovery of greater than 50%
- Signal to noise ratio of ≥3.3
- Retention time ± 0.2 minutes
- Qualifier ion ratios within +/-20% of the concurrently run positive control.

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 ≥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 +/-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 ≥3.3 ratio for the primary ion.

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- NONE]"

When confirming the presence of a drug in a urine Sample, 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 standard 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 standard OUI urine drug panel due to low sample volume."

If a sample is to be reported out as inconclusive, the reason shall be documented on the certificate of analysis.

Specific Extraction Procedures:

General Sample Setup

The following sample preparation setup is to be utilized for all GC/MS 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 internal standard
- 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 preceeding 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 methods, see Urine Drug GC/MS Testing Menu in appendix for full list.

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.

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 internal standard (2000 ng/mL). Cover tube and vortex.

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

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

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

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

ID	Target Concentration THC-COOH (or Gluronide)	Urine	Vol THC-COOH Glucuronide Stock (500ng/mL)	Vol THC-COOH Stock (500ng/mL)	Vol THC-COOH-D9 IS (2000 ng/mL)	GC/MS sequence sample type:
	ng/mL	(uL)	(ul)	(uL)	(uL)	
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

THCCOOH in Urine

Hydrolysis:

After all extraction samples have been fortified with appropriate stocks and internal standards:

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.

Cocaine, Cocaethylene & Benzoylecgonine in Urine

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

Cocaine/Benzoylecgonine/Cocaethylene Dilutions		
Dilution Factor	**Volume of Case Sample**	**Volume of Water**
1:2	500uL	500uL
1:4	250uL	750uL
1:10	100uL	900uL

Limitations: Only non-silanized tubes are approved for this method, do no used silanized tubes for this method.

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 150uL Cocaines Stock and 50uL of Cocaethylene-d3 internal standard stock (2000 ng/mL). Cover tube and vortex.

To all Control and Sample Tubes Being Extracted: Using a repeat pipet add 50uL of Cocaethylene-d3 internal standard (2000 ng/mL) to all tubes

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

Positive Control Tube: Add 150uL Cocaines LLOD Stock and 1mL of negative urine. Cover tube and vortex.

ID	Target Concentration	Urine	Cocaines Stock (varied ng/mL)	Cocaethylene-d3 Internal Standard Stock (2000ng/mL)	GC/MS sequence sample type:
	ng/mL	(uL)	(uL)	(uL)	
Unextracted	varied		150	50	Cal, Level 1
Positive Control	varied	1000	150	50	Cal, Level 2
Negative Control	--	1000	--	50	Sample
Sample(s)	--	1000	--	50	Sample

After all extraction samples have been fortified with appropriate stocks and internal standards: Add 2mL 100mM Phosphate Buffer pH 6.0. If needed, centrifuge 10 minutes and transfer sample supernatant into new labeled tubes.

Cocaine, Cocaethylene & Benzoylecgonine in Urine

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 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. Rinse columns with 3 mL DI water
6. Rinse columns with 2 mL 1M Acetic Acid
7. Rinse columns with 3 mL methanol
8. Dry columns at full vacuum for 5 minutes.
9. Place new labeled glass tubes in rack
10. Elute with 2 mL DCM/IPA/NH4OH (78:20:2) - Made fresh daily as needed.
11. Evaporate to dryness under nitrogen gas.

Derivatization:

1. Add 50ul ethyl acetate followed by 50ul BSTFA with 1% TMCS, cap and mix/vortex.
    - 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 as follows on GC/MS using COC instrument method

Amines in Urine

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

Amine Dilutions		
Dilution Factor	**Volume of Case Sample**	**Volume of Water**
1:2	500 µL	500 µL
1:20	50µL	950 µL
1:50	20µL	980 µL
1:100	10 µL	990 µL

Limitations: Only non-silanized tubes are approved for this method, do no used silanized tubes for this method.

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 150uL Amine Stock (1000 ng/mL) and 50uL of MDA D5 internal standard (2000 ng/mL). Cover tube and vortex.

All Control and Sample Tubes Being Extracted: Using a repeat pipet add 50uL of MDA-d5 internal standard stock (2000 ng/mL) to all tubes

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

Positive Control Tube: Add 150uL Amine Stock and 1mL of negative urine. Cover tube and vortex.

ID	Target Concentration	Urine	Amine Compounds Stock (1000 ng/mL)	MDA-d5 Internal Standard Stock (2000ng/mL)	GC/MS sequence sample type:
	ng/mL	(uL)	(uL)	(uL)	
Unextracted	150		150	50	Cal, Level 1
Positive Control	150	1000	150	50	Cal, Level 2
Negative Control	--	1000		50	Sample
Samples(s)		1000		50	Sample

After all extraction samples have been fortified with appropriate stocks and internal standards: Add 2mL 100mM Phosphate Buffer pH 6.0. If needed, centrifuge 10 minutes and transfer sample supernatant into new labeled tubes.

Amines in Urine

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 3 mL methanol to each column
2. Add 3 mL DI water to each column
3. Add 3 mL 100 mM phosphate buffer pH 6.0 to each column
4. Load sample with transfer pipette
5. Rinse columns with 3 mL H2O
6. Rinse columns with 3 mL 100 mM Acetic Acid
7. Rinse columns with 3 mL methanol
8. Dry columns at full vacuum for 5 minutes.
9. Place new labeled glass tubes in rack
10. Elute with 3 mL DCM/IPA/NH4OH (78:20:2) - Made fresh daily as needed.
11. Add 100 uL acidified methanol (1%) to each tube **(including unextracted sample).**
12. Evaporate to dryness under nitrogen gas

Derivatization:

1. Add 50ul ethyl acetate followed by 50ul PFAA, cap and mix/vortex.
    a. Apply Nitrogen to the PFAA bottle and cap prior to closing.
2. Incubate at 70◦C for 20 minutes.
3. Cool tubes.
4. Dry under nitrogen gas.
5. Reconstitute with 100 µL ethyl acetate.
6. Transfer samples to GC/MS vials.
7. Run on GC/MS using Amines instrument method.

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 no used 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 either codeine or hydrocodone.
- Oxycodone and Oxymorphone both have additional ions being collected. Only two qualifier ions print on the Masshunter generated reports. If a sample is being confirmed for Oxycodone or Oxymorphone and these additional ions are being used to meet the two qualifier ion ratio critera, the analyst must screen-shot print the additional ions and place the printout in the casefile.
- 1:100 dilutions were evaluated and found to be acceptable for the majority of the compounds, except for oxymorphone di-TMS and oxycodone TMS. These two compounds with a 1:100 dilution may become too dilute and exhibit low responses. If oxycodone TMS or oxymorphone di-TMS are being confirmed it is recommended that a 1:50 or lesser dilution is used prior to performing a 1:100 dilution.

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 Internal Standard 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 Internal Standard Stock (4000 ng/mL) to all tubes.

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 Internal Standard 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 internal standards:
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

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.

Benzodiazepines in Urine

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

Benzodiazepine 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 no used non-silanized tubes for this method.

- **Hydroxy-Midazolam is not included in the Randox Evidence Investigator screen, if this compound is detected the sample must be re-extracted using a second aliquot for confirmation on GC/MS.**
- Temazepam has additional ions being collected as variability was seen in qualifier ion ratios during the initial validation. Only two qualifier ions print on the Masshunter generated reports. If a sample is being confirmed for Temazepam and the ions that are being used to meet the criteria of two qualifier ions with a ratio within 20% of concurrently run positive control aren't visible on the report, then the analyst must screen-shot print the additional ions and place the printout in the casefile.

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 150uL of Benzodiazepine Stock (varied ng/mL) and 50uL of Temazepam-d5 Internal Standard Stock (2000 ng/mL). Cover tube and vortex.

All Control and Sample Tubes Being Extracted: Using a repeat pipet add 50uL of Temazepam-d5 Internal Standard Stock (2000 ng/mL) to all tubes.

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

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

Hydrolysis Control Tube: Add 60uL of Temazepam Glucuronide Stock (5000 ng/mL) and 1mL of negative urine. Cover tube and vortex.

Benzodiazepines in Urine

ID	Target Concentration	Urine	Benzos Stock (varied ng/mL)	Temazepam Glucuronide Stock (5000ng/mL)	Temazepam-d5 Internal Standard Stock (2000ng/mL)	GC/MS sequence sample type:
	ng/mL	(uL)	(uL)		(uL)	
Unextracted	varied		150	--	50	Cal, Level 1
Positive Control	varied	1000	150	--	50	Cal, Level 2
Hydrolysis Control	150	1000	--	60	50	Sample
Negative Control	--	1000	--	--	50	Sample
Sample(s)	--	1000	--	--	50	Sample

Enzyme Hydrolysis:

After all extraction samples have been fortified with appropriate stocks and internal standards:

Add 400 uL of Instant Buffer I to each tube.
Add 100 uL of BG Turbo to each tube.
Cover and Cover tube and vortex.
Let sit at room temperature for 30 minutes, centrifuge for 10 minutes and remove sample supernatant into new labeled tube if needed.

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 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. Rinse columns with 2 mL DI water
6. Rinse columns with 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6) (made daily)
7. Dry columns at full vacuum for 5 minutes.
8. Rinse columns with 2 mL hexanes.
9. Place new labeled glass tubes in rack.
10. Elute with 5 mL (if using single-dispense pipette, aliquot 2.5ml twice) ethyl acetate containing 4% ammonium hydroxide - Made fresh daily as needed
11. Evaporate to dryness under nitrogen gas.

Benzodiazepines in Urine

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.

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
1:10	10 uL	990 uL

Limitations: Only silanized tubes are approved for this method, do no used 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
- Diphenhydramine
- Sertraline
- Citalopram
- Acetyl Fentanyl
- 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.

Sample preparation

Unextracted Control: Add 150uL of Base Stock (varied ng/mL) and 50uL of Internal Standard Stock (2000 ng/mL). Cover tube and vortex.

To all controls and sample tubes being extracted: Using repeat pipet add 50uL of Internal Standard Stock (2000 ng/mL) to all tubes

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

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

Basic Drugs in Urine

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

After all extraction samples have been fortified with appropriate stocks and internal standards:

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.

Reconsitution:

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

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 asterick on the Masshunter Urine Report.

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

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.

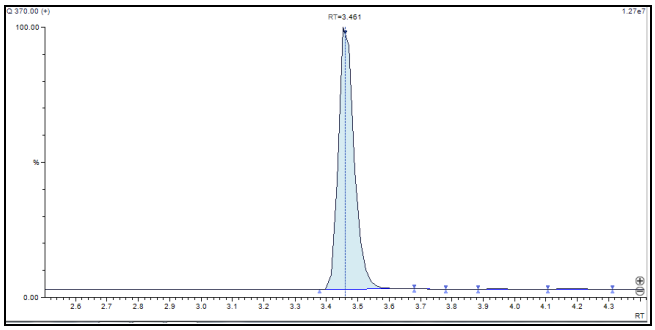
All compound ions within a sample shall be, when possible, integrated in a comparable manner.

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.



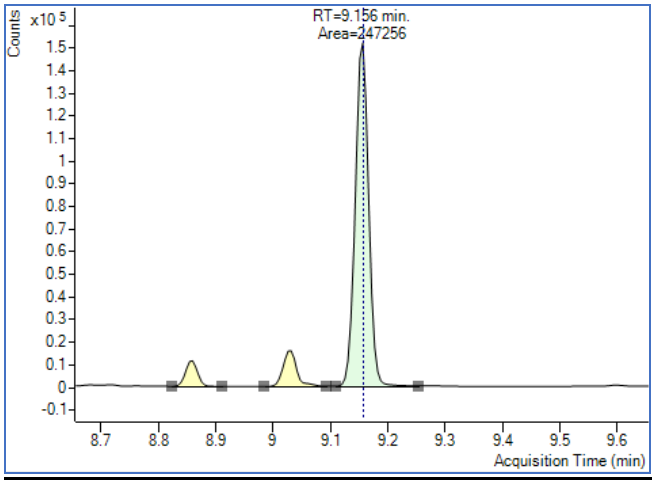


Figure 2: Properly integrated coeluting peak.

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.

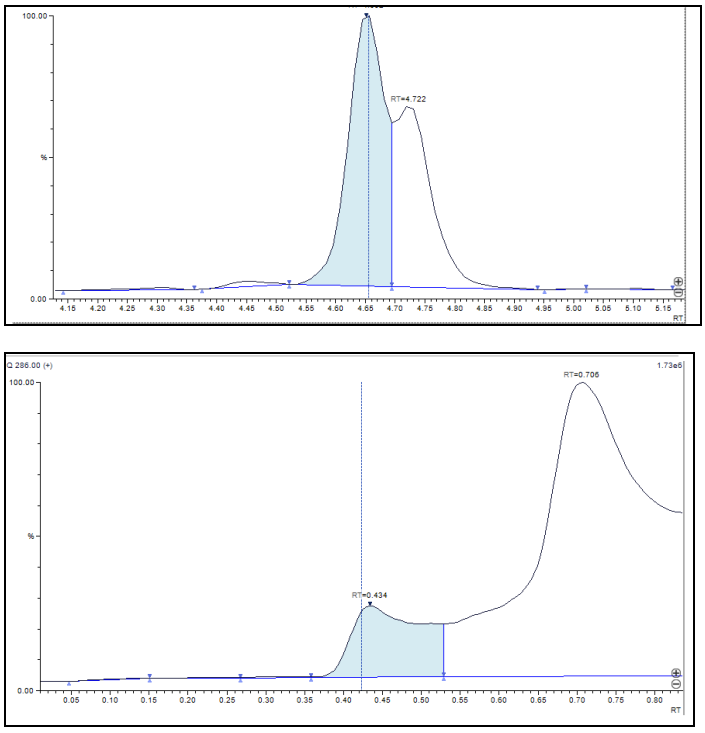


Figure 2A: Properly integrated co-eluting peak with a rising baseline.

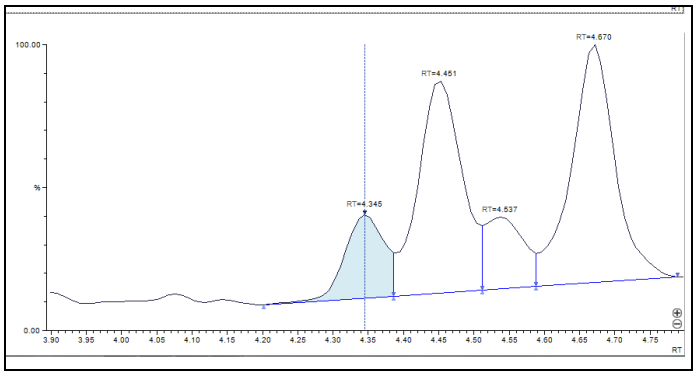


Figure 3: Peak Plateauing

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.

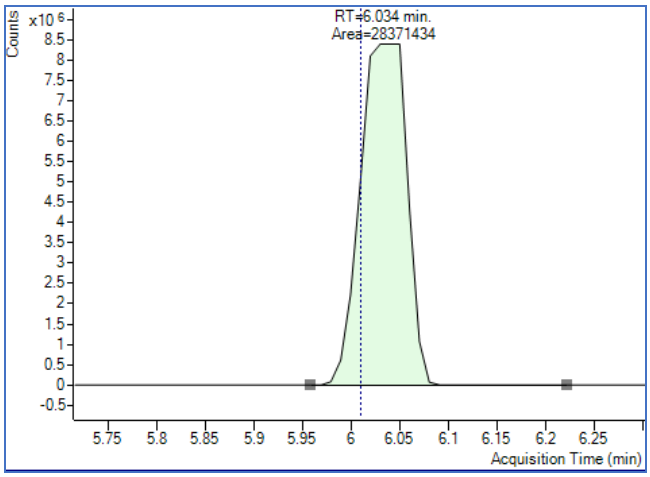


Figure 4: Baseline Noise Example.

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.

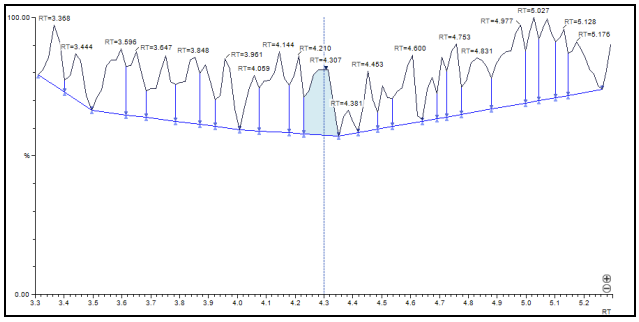


Figure 5: Peak Fronting

This is usually caused by an overloading of the column.

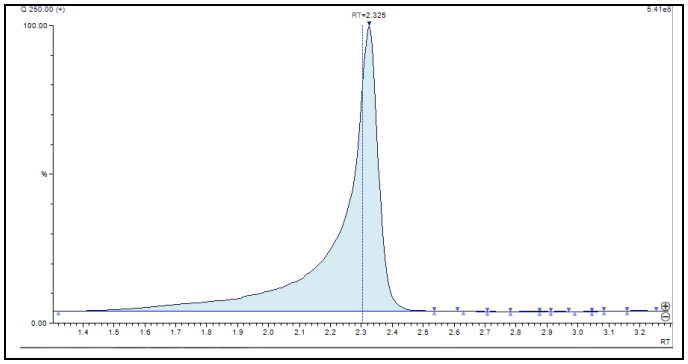


Figure 6: Peak Tailing

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.

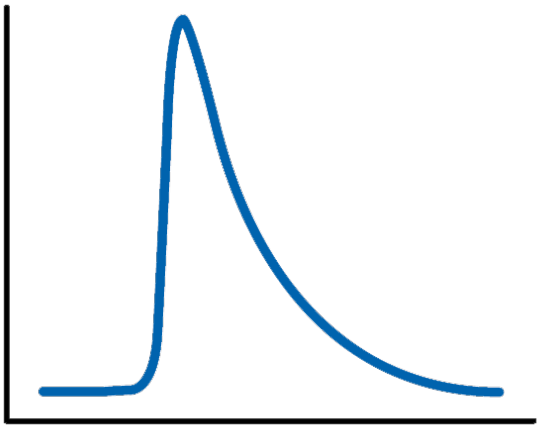


Figure 7: Improper Peak Shaving

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 tailing edges of the peak.

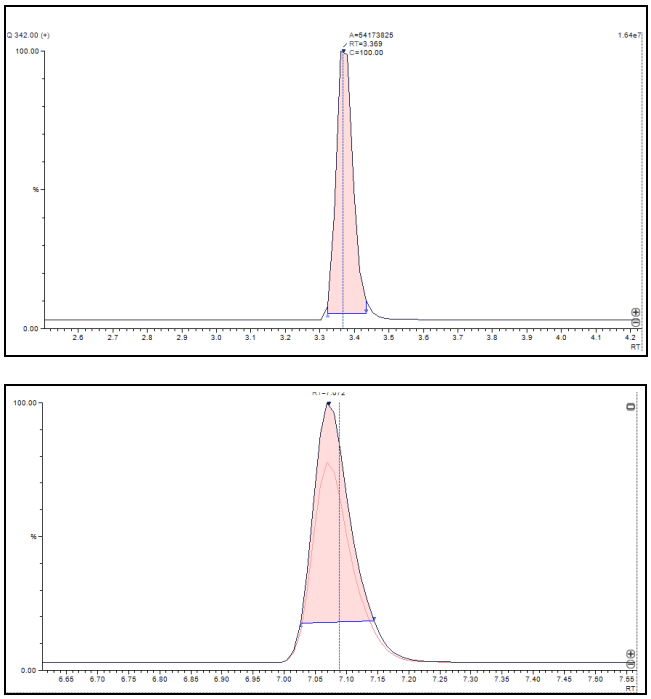
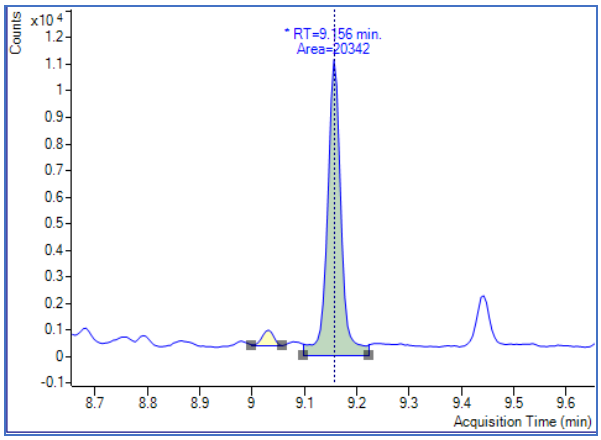


Figure 8: Improper Peak Enhancing

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.



GC/MS Operation and procedures

Tuning

A tune report is generated daily (when in use) and/or following maintenance. 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 (H2O, “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.

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
Replace traps and filters

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.
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:
    a. Open the analyzer chamber door enough to ensure that the end of the GC/MS interface is visible.

b. Slide the column into the GC/MS interface. The tapered end of the ferrule must point towards the nut.

c. 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.

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.

5. 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.

6. Check the GC oven to be sure that the column does not touch the oven walls.
7. 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.
8. Close the analyzer chamber door and vent valve.
9. 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.

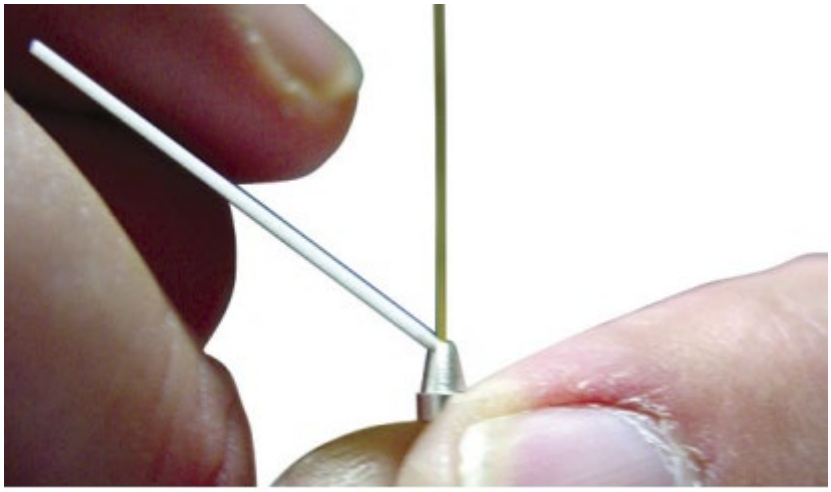
Always wear safety glasses when handling capillary columns. Use care to avoid puncturing your skin with the end of the column.

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

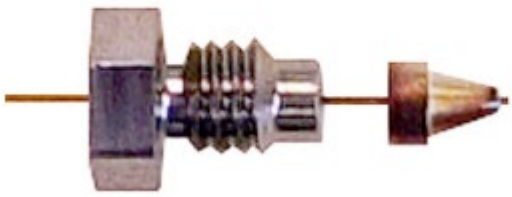
Tip: Recommend that this task is performed with a secondary individual for assistance, as one person needs to weave the column guard onto the holder and the second person holds the remainder of the column guard and ensuring it does not tangle.

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.



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. Repeate 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.

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.

GC/MS Data Acquisition and Data Analysis Methods:

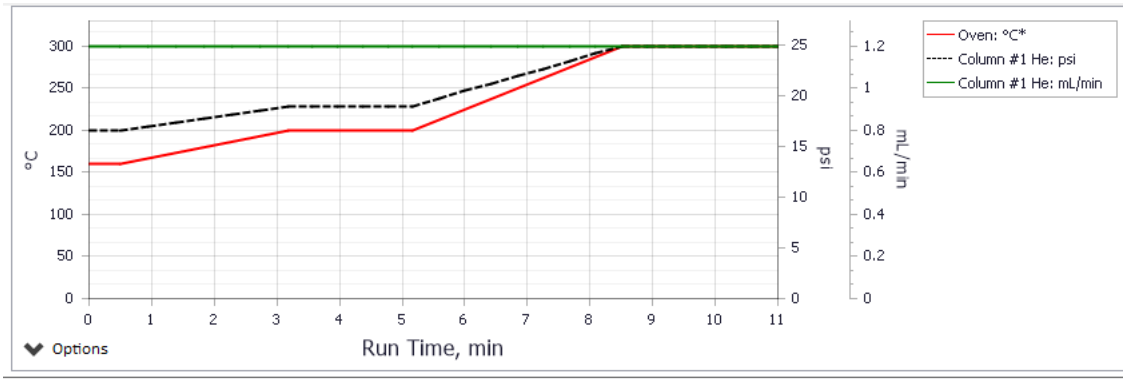
All GC/MS instrument shall use the following data acquisition parameters:

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^{o}C
7. Quadrupole temperature of 180^{o}C
8. Dwell time of 20 msec for each ion.
9. Injection volume of 2uL

The following are specific GC/MS 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:



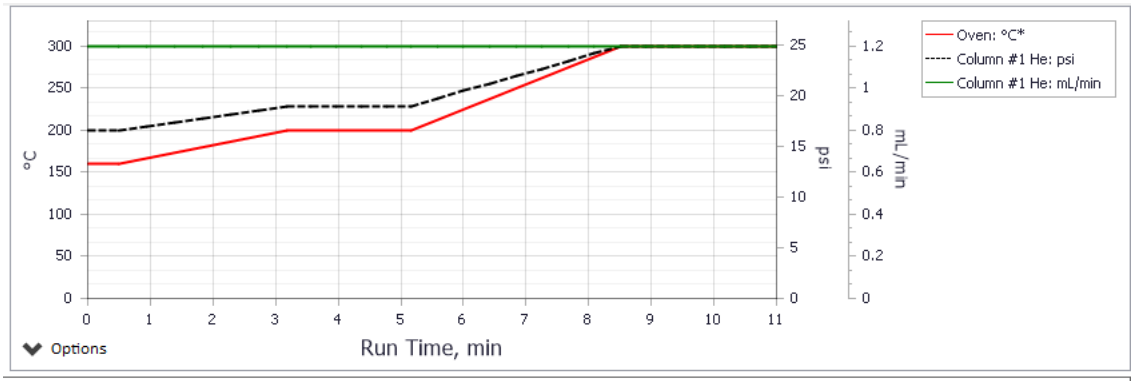
Options

	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		160	0.5	0.5
Ramp 1	15	200	2	5.1667
Ramp 2	30	300	2.5	11
*				

Compound	Ions		
THC-COOH	371	473	488
THC-COOH-D9	380	479	497

Cocaine

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



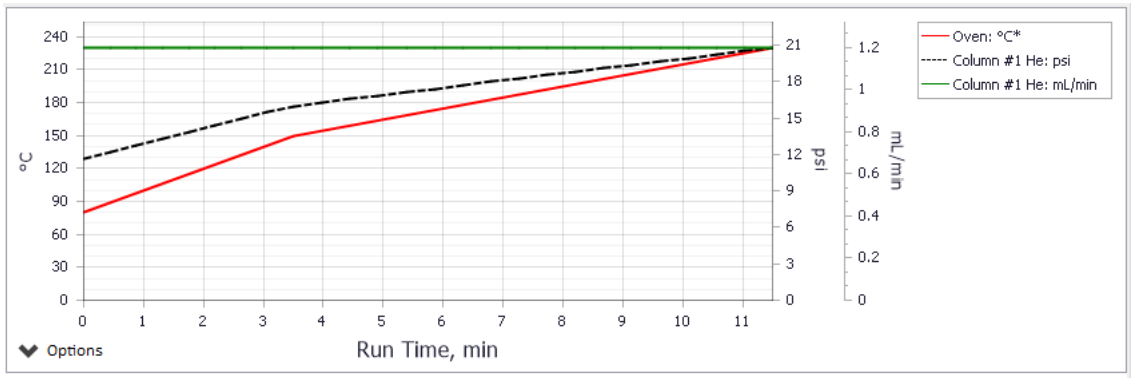
Options

	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		160	0.5	0.5
Ramp 1	15	200	2	5.1667
Ramp 2	30	300	2.5	11
*				

Compound	Ions			
Benzoylecgonine	240	346	361	
Cocaethylene	196	212	317	
Cocaethylene-d3	199	275	320	215
Cocaine	182	198	303	

Amines

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



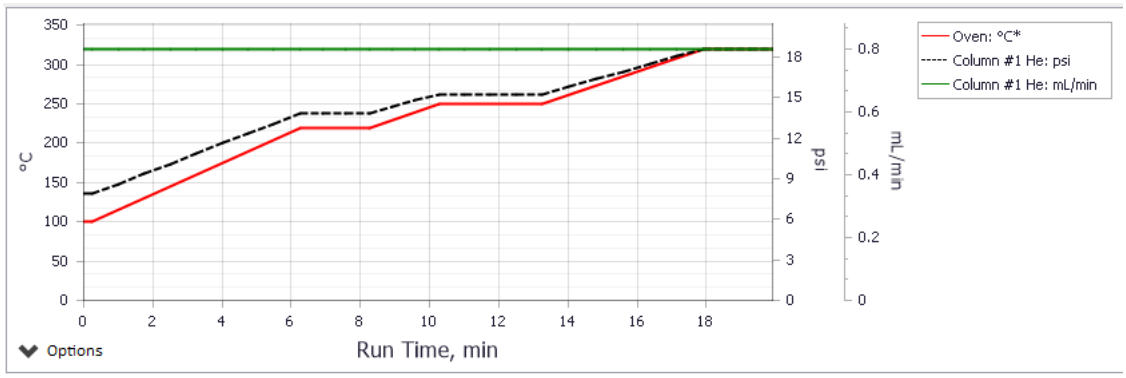
Options

	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		80	0	0
Ramp 1	20	150	0	3.5
Ramp 2	10	230	0	11.5
*				

COMPOUND	IONS		
Amphetamine	117	118	190
Methamphetamine	118	160	204
MDA	135	162	325
MDMA	162	204	339
MDEA	190	218	353
MDA D5	136	167	330

Narcotics

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



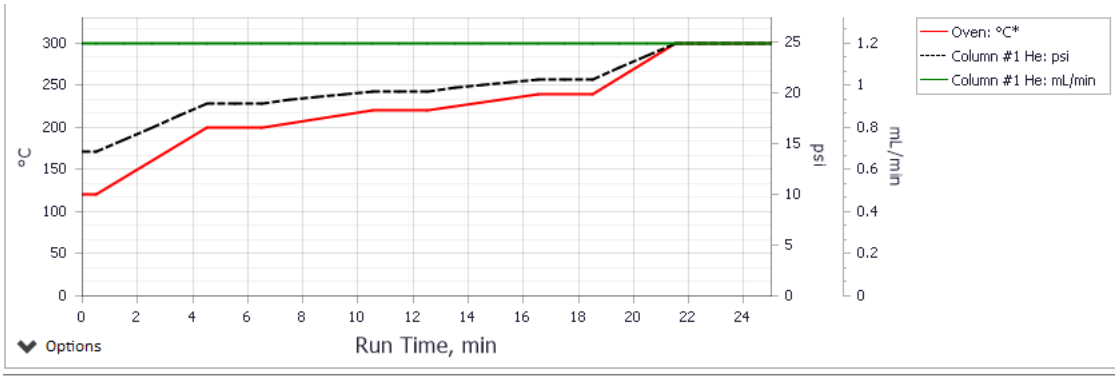
Options

	Rate °C/min	Value °C	Hold Time min	Run Time 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
*				

Compound	Ions			
6-MAM-d6	405	343	290	
6-MAM	399	340	287	
Codeine TMS	371	196	178	
Dihydrocodeine	373	236	178	
Hydrocodone TMS	371	356	234	
Hydromorphone TMS	357	300	243	
Morphine di-TMS	429	236	146	
Oxycodone TMS**	387	372	388	330
Oxymorphone di-TMS**	445	430	446	431

Benzodiazepines

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



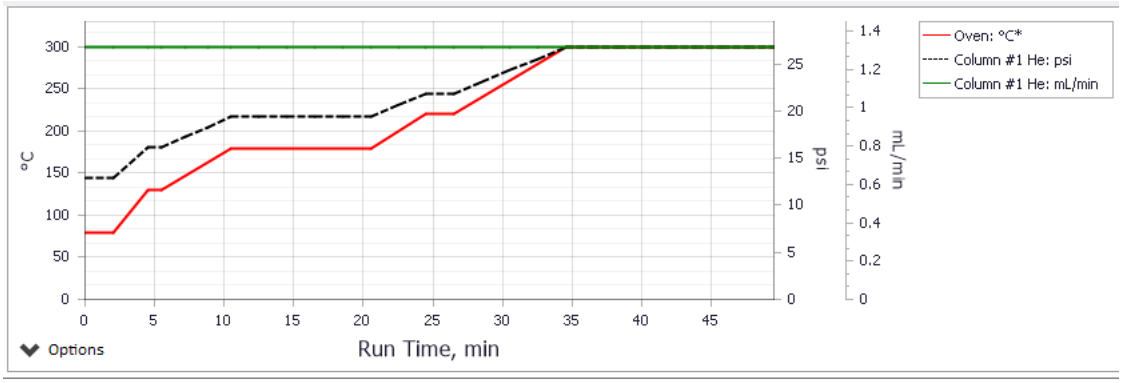
Options

	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		120	0.5	0.5
Ramp 1	20	200	2	6.5
Ramp 2	5	220	2	12.5
Ramp 3	5	240	2	18.5
Ramp 4	20	300	3.5	25
*				

COMPOUND	IONS			
Diazepam	256	284	283	
Midazolam	310	297	325	
Temazepam	343	283	256	357
Hydroxyalprazolam	381	396	383	
Hydroxymidazolam	310	398	413	
Temazepam D5	348	262	288	

Base

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



Options

	Rate °C/min	Value °C	Hold Time min	Run Time 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 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	
Fluorofentanyl	263	164	220	

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	

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, internal standards 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% internal standard concentration, Surrogate Conc Limit High-500, Surrogate Concentration-concentration of internal standard.

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 (example: oxycodone TMS shall always have an earlier retention time that oxymorphone di-TMS). 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.

Base	**RT**		**Narcotics**	**RT**		**Amines**	**RT**
PCP-d5	18.405		6-MAM-D6	15.66		MDA-D5	7.84
Fentanyl-d5	33.639		Dihydrocodeine	13.87		Amphetamine	4.9
Norketamine	16.156		Codeine TMS	14.613		Methamphetamine	5.84
Ketamine	17.132		Hydrocodone TMS	14.747		MDA	7.87
Diphenhydramine	17.628		Morphine di-TMS	15.08		MDMA	9.049
PCP	18.504		Hydromorphone TMS	15.19		MDEA	9.411
Tramadol	20.887		6-MAM	15.725			
N-desmethyl tramadol	22.232		Oxycodone TMS	15.736		**Benzodiazepines**	**RT**
EDDP	23.259		Oxymorphone di-TMS	15.958		Temazepam-D5	20.39
Methadone	25.530					Oxazepam TMS	16.47
Dextromethorphan	25.646		**THCCOOH**	**RT**		Diazepam	18.03
Amitriptyline	26.933		COOH-d9	10.551		Midazolam	20.4
Nortriptyline	27.367		COOH	10.592		Temazepam TMS	20.4
Cyclobenzaprine	27.931					Hydroxy-Midazolam	21.67
Sertraline	29.834		**Cocaines**	**RT**		Hydroxy-Alprazolam	24.12
Citalopram	30.462		Cocaethylene-d3	9.11			
Acetyl Fentanyl	33.191		Cocaine	8.688			
Fluorofentanyl	33.352		Cocaethylene-d3	9.11			
Fentanyl	33.657		Benzoylecgonine TMS	9.16			
Zolpidem	34.339						
Flualprazolam	35.075						
Estazolam	35.327						
Alprazolam	35.686						
Etizolam	36.565						

Highlighted compounds are not screened for on the Randox Evidence Investigator, for confirmation a second aliquot shall be extracted and run.

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

Compound	Lower Reporting Limit (ng/mL)	Positive Control Level (ng/mL)
THC-COOH	5	15
Benzoylecgonine	50	150
Cocaethylene	20	60
Cocaine	20	60
Amphetamine	50	150
Methamphetamine	50	150
MDA	50	150
MDMA	50	150
MDEA	50	150
6-MAM	25	75
Codeine	50	150
Dihydrocodeine	50	150
Hydrocodone	50	150
Hydromorphone	50	150
Morphine	50	150
Oxycodone	300	900
Oxymorphone	300	900
Diazepam	50	150
Midazolam	50	150
Temazepam	50	150
Hydroxyalprazolam	50	150
Hydroxymidazolam	50	150
Norketamine	100	300
Ketamine	100	300
Diphenhydramine	50	150
PCP	10	30
Tramadol	50	150
N-Desmethyl Tramadol	100	300
EDDP	50	150
Methadone	50	150
Dextromethorphan	100	300
Amitriptyline	100	300
Nortriptyline	100	300
Cyclobenzaprine	10	30
Sertraline	25	75
Citalopram	100	300
Acetyl Fentanyl	10	30
Fluorofentanyl	2.5	7.5
Fentanyl	10	30
Zolpidem	50	150
Flualprazolam	25	75
Estazolam	50	150
Alprazolam	50	150
Etizolam	100	300

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)	Dextrorphan tartrate salt (32)
Noroxycodone (29)	**AMPH**	Codeine (287)	Heroin (353.6)	Nordextromethorphan (20.4)
MBP	MDA (323.3)	6-Acetyl-Codeine(166.8)	Desomorphine (159.9)	**MAMP**
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)	Desomorphine (41.5)	Ethyl Morphine (66.5)	+/- Methamphetamine (69.8)
	Phentermine (25.4)	Morphine Glucuronide(35.1)	Hydromorphone (50.8)	
	R-Amphetamine (16.6)	Heroin (29.5)	Hydrocodone (38.4)	
	MDEA (4)	Morphine (26.3)		
		6-MAM (21.2)		

BARB	BENZ 1	BENZ 2	BENZ 3
Secobarbital (371)	Temazepam (382)	Lorazepam (100)	Desmethylflunitrazepam (128)
Butabarbital (166)	Flubromazolam (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)
Phenobarbital (100)	Nimetazepam (266)	Desalkylylflurazepam (27.1)	Nitrazepam (38.9)
Cyclopentobarbital (70.1)	Alprazolam (258)	Flubromazepam (25.9)	Phenazepam (29.7)
p-OH-phenobarbital (64)	Diazepam (256)	Lorazepam Glucuronide (24.8)	N-desmethyl clotiazepam (15.3)
Butalbital (51.1)	Estazolam (253)	Oxazepam (13)	**BZG**
Amobarbital (44)	Clobazam (204)	Meclonazepam (12.8)	Cocaine (103.8)
Barbital (33.3)	Nitrazepam (194)	**PCP**	Benzoylecgonine (100)
MDONE	Brotizolam (191)	Phencyclidine (100)	m-hydroxybenzoylecgonine (95.6)
Methadone (100)	2-OH-Ethylflurazepam (188)	**TCA**	Cocaethylene (54.4)
EDDP (<0.1)	Flubromazepam (175)	Imipramine N Oxide (1127)	**TRM**
ZOL	Prazepam (172)	Imipramine (294)	Tramadol (100)
Zolpidem (100)	Diclazepam (157)	Trimipramine (238)	O-Desmethyltramadol (34.8)
4-carboxyzolpidem (47.5)	Midazolam (116)	Desipramine (206)	N-Desmethyltramadol (1.39)
THC	Desalkylflurazepam (115)	Cyclobenzaprine (201)	**BUP**
11-nor-9-Carboxy-delta9-THC	Pyrazolam (115)	Amitriptyline (190)	Norbuprenorphine (100)
FENT	Flunitrazepam (114)	Opipramol (167)	Buprenorphine (16.7)
alpha-methylfentanyl (266)	Oxazepam (100)	Promazine (117)	Norbuprenorphine Glucuronide (15.0)
p-fluorofentanil (194)	Flurazepam (93.4)	Nortriptyline (100)	
Fentanyl (100)	Delorazepam (77)	Maprotiline (96)	
Benzylfentanyl (57.1)	Phenazepam (61.2)	Doxepin (95)	
Butyrylfentanyl HCl (54)	Lormetazepam (50.2)	Clomipramine (76)	
Norfentanyl (27)	Chlordiazepoxide (46.8)	Protryptiline (67)	
w-Hydroxy fentanyl (15.2)	Meclonazepam (40.7)	Cyproheptadine (61)	
	Triazolam (29.6)	Lofepramine (58)	
	Etizolam (28.4)	Dothiepin (50)	
	N-Desmethylflunitrazepam (23.6)	Chlorpromazine (24.3)	
	Bromazepam (21.6)	2-Hydroxyimipramine (19.5)	
	Alpha-OH-Etizolam (19.0)	Nordoxepin (19.4)	
	Lorazepam (18.4)	Perphenazine (17.3)	

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

*for previous revisions please see version history in SharePoint