About This Document

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

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I. Introduction

Forensic alcohol analysis is defined as the practical application of specialized devices, instruments, and methods by trained laboratory personnel to measure the concentration of ethyl alcohol in samples of blood from persons involved a potential criminal matter.

The method selected for the determination of alcohol content of blood samples utilizes a headspace gas chromatograph to perform a test that is both qualitative and quantitative. The procedure calls for addition of a small aliquot of sample to an internal standard solution. A portion from the headspace of this mixture is injected onto gas chromatographic columns that are capable of separating ethyl alcohol from acetone and the common aliphatic alcohols (i.e., methanol, isopropanol, etc.). Quantitation is accomplished through comparison to calibration curves. Data is captured and calculations are performed by device(s) designed to do so (i.e., integrator, workstation, laboratory automation computer). The method is available to all via SharePoint, where the lab stores controlled copies of documents, forms, etc.

Principle

Aliquots of biological fluids or liquids are mixed with an internal standard solution. The samples are then analyzed by headspace gas chromatography and quantitated using the internal standard technique.

Specimen Requirements

Whole blood-serum-plasma
Liquids and/or beverages (not on the Lab Scope of Analysis)

Chemicals

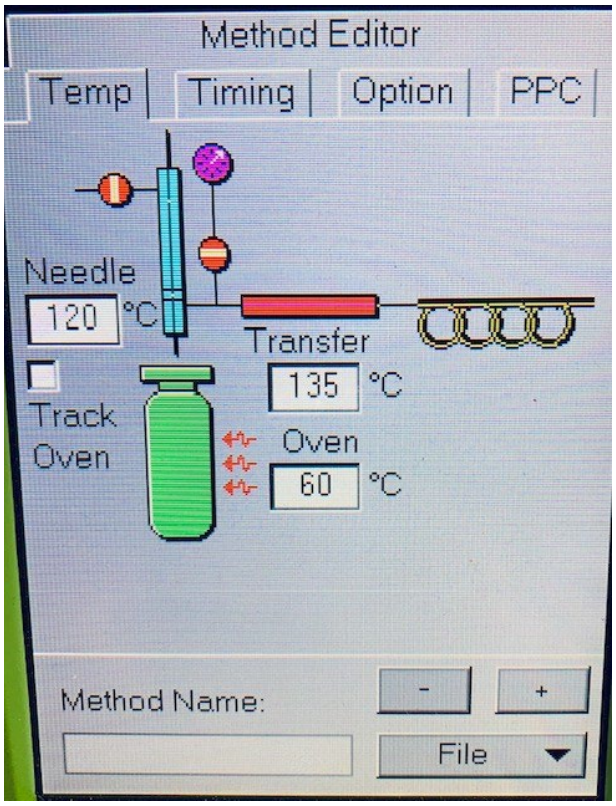
Deionized water, ethanol standards, volatiles standards, whole blood controls, and serum controls

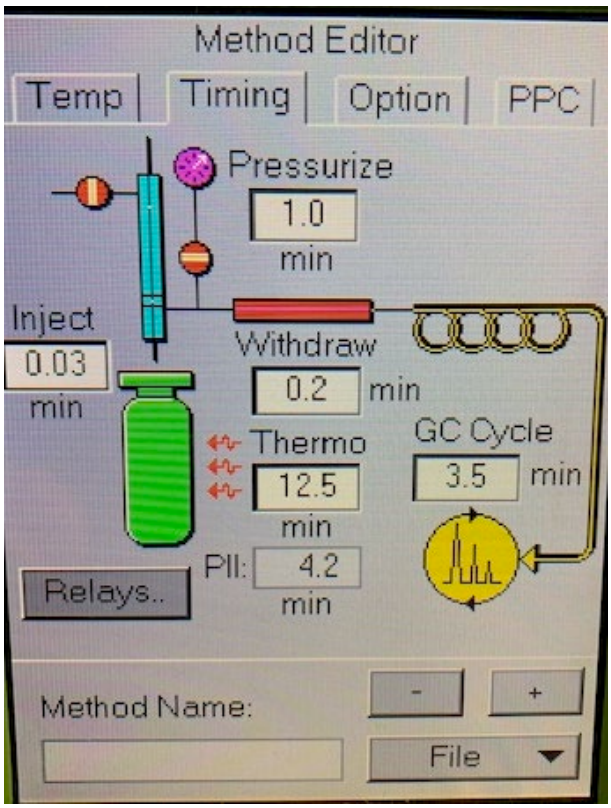
Safety Precautions & PPE

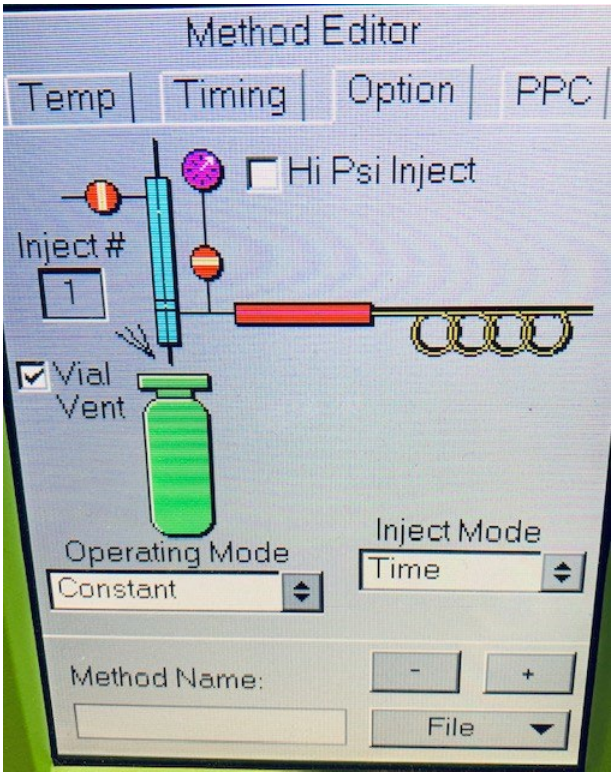
Lab coats, gloves and eye protection will be worn when handling chemicals.
Full-face shield will be worn when handling blood samples.

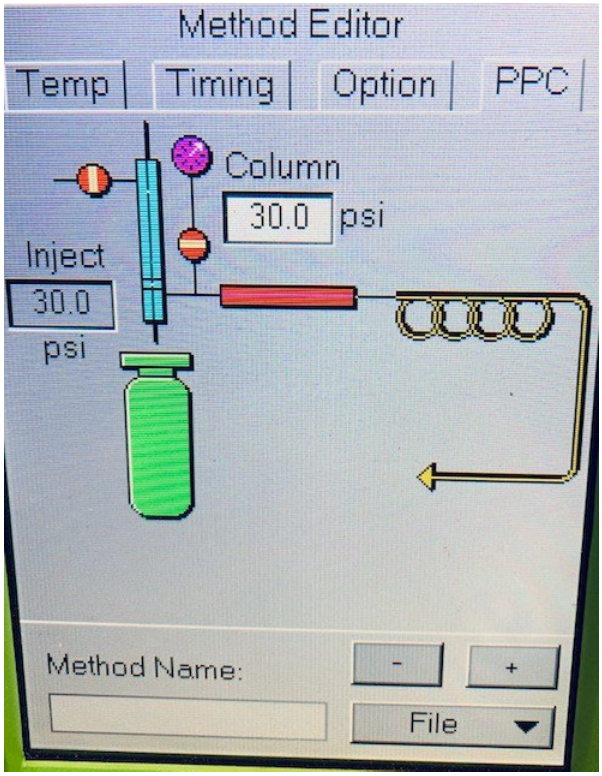
Apparatus

PerkinElmer HeadSspace TurboMatrix 110 Sampler
PerkinElmer Clarus 590 gas chromatograph (GC)
Instrument Name: HS110
TotalChrom 6.3.3.0691 software
Detector –Flame Ionization (FID)
Columns – Elite BAC-1 Advantage 30 meter, 0.32 mmID, 1.8um df; Elite-BAC-2-Advantage 30meter, 0.32mmID, 0.6um df
Carrier Gas – Helium UHP
Detector Gas – Hydrogen
Headspace Parameters – See screen shots below









GC Parameters -See HETLBAC Method Print Out on File with the Validation.

Preparation of Solutions

Internal Standard: 0.02% by volume n-propanol

Dilute 200.0 uL n-propanol to 1.0L with deionized water (scalable as needed).

Ethanol Calibrators and Standards:

- Purchased from an approved vendor (such as Cerilliant / Lipomed, etc)
- Whole Blood Controls from an approved vendor (such as CliniQA Laboratories, etc), only run when blood casework sample is present in batch
- Serum Controls from an approved vendor (such as UTAK), only run when a serum casework sample is present in the batch

Aqueous standards and calibrators are aliquoted directly from a freshly opened ampule and whole blood controls are aliquoted directly from the reagent container. Both are diluted in the same manner as case samples using the diluter. See appropriate reagent sheet for serum control preparation procedures.

Volatiles other than Ethanol: Volatile calibrators shall be purchased from an approved vendor (such as Cerilliant, Lipomed, etc). Controls containing volatiles are part of the whole blood controls from CliniQA Laboratories, and serum controls (when needed) from UTAK. Volatile calibrations will be updated twice a year, preferably prior to proficiency tests containg volatile targets.

QC and Sample Run Scheme

Case samples are run in duplicate. Minimum QC batch should contain a blank (internal standard in 250ul of water), aqueous controls covering at least the minium reporting level standard, a mid-range standard, and a high-range standard, and matrix matched control(s) when appropriate. If a batch contains whole blood sample then both a low concentration whole blood control and a high concentration whole blood control containing volatiles shall be included. If a batch contains a serum samples then a serum control shall be included. If a batch only contains aqueous beverage samples then only aqueous controls are required. The following is an example of how casework samples and QC checks/standards may appear on the batch sheet as appropriate:

Blank
Whole Blood Control – Low BAC (when whole blood samples are included)
Whole Blood Control – High BAC with Volatiles (when whole blood samples are included)
ETOH Standard
3 samples in duplicate

ETOH Standard
3 samples in duplicate
ETOH Standard
3 samples in duplicate
ETOH Standard
3 samples in duplicate
ETOH Standard
3 samples in duplicate
ETOH Standard
2 samples in duplicate
ETOH Standard
Whole Blood Control – Low BAC (when whole blood samples are included)
Whole Blood Control – High BAC with Volatiles (when whole blood samples are included)
Serum Control – 0.080 g/dL EtOH (when serum samples are included)

The form used to record such information can be found on SharePoint.

II. Evidence Handling and Preservation

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

- The work area will be clean and free of any excess debris.
- Countertops will have adequate space for working with samples
- All glassware and tools to be used will be clean
- Test tubes, capillary pipettes, Pasteur pipettes, etc are used only once, then discarded
- To prevent cross contamination of samples, only one case will be opened by the analyst at a time
- Reagents and solvents will be kept in closed containers when not being used in the analysis

Prior to and following testing, the evidence will be properly secured, and during analysis the evidence will be under constant control in the custody of the analyst, as described by the Quality Manual.

Evidence to be analyzed will be removed from evidence refrigerator and the reverse side of the pink Receipt/Request for Examination Contract will be filled out (i.e., internal chain of custody)

The analyst will ensure a proper seal is in place prior to opening the evidence. If the item is found to not be properly sealed, or lacking initials across the seal, a note will be made on the worksheet and communicated to the customer on the case report.

The analyst opening the kit will initial stickers bearing the Lab Identification Number.

If the subject's name is not available at the time of log-in, the analyst will write the subject's name on the label at the time of analysis (if known).

The analyst will verify all identification numbers and names agree with the Receipt/Request for Examination Contract. If the kit, upon opening, is found to contain non HETL approved collection materials this shall be noted on the inventory form and STARLIMS metadata shall be updated appropriately.

If a non-HETL approved collection kit or materials were submitted, the WO# field in STARLIMS folder metadata shall be filled in with N/A.

If the kit, upon opening, is found to contain non HETL approved collection materials this shall be noted on the inventory form and STARLIMS metadata shall be updated appropriately.

The collection kit and all specimens will be labeled with the lab identification number, name of the subject (if known) and the initials of the analyst that opened the kit.

All paperwork contained in the kit will be labeled with the laboratory identification number and initialed by the analyst that opened the kit.

The analyst will verify the case information provided with the kit matches the HETL folder, sample information from the Laboratory Blood Analysis Request form submitted with the sample and all Starlims labels. Any discrepancy shall be documented by the analyst within the case notes, batch sheet(s), and/or with a photograph/photocopy of the evidence. Minor discrepancies shall be communicated with the customer on the case report. Major discrepancies shall require customer communication and correction prior to testing.

The analyst will fill out the Blood Kit Inventory Worksheet and perform a kit inventory, making any necessary notations. For non-approved HETL collection materials, refer to the manufacture's information or the below reference table.

Manufacturers cap/label	Additive(s)	Blood type
Light Blue	Sodium Citrate	Whole Blood (If centrifuged-Plasma)
Gold, Red/Black Mix	Clot activator & separation gel	Serum (*if centrifuged)
Red	None (glass)	Clotted whole blood

	Clot activator (plastic)	(If centrifuged-Serum)
Green	Sodium Heparin Lithium Heparin	Whole Blood (If centrifuged-Plasma)
Light Green, Green/Gray Mix	Lithium Heparin & separation gel	Plasma (*if centrifuged)
Lavender/Pink	K2EDTA	Whole Blood
Gray	Potassium Oxalate & Sodium Fluoride	Whole Blood
*If centrifuged separation gel will travel from the bottom of the tube to the middle of the tube (to between the red blood cells and the serum/plasma).		

For continuity the volumes entered into the blood inventory worksheet and subsequent StarLIMS items received table shall adhere to the following format:

	Volume (mL)
Visibly empty blood tube, no label	0 (leave collection date & time blank)
Visibly empty blood tube, with label	0
Visible droplets of blood in tube (unusable testing volume)	<0.5
Low volume of blood in tube (unusable testing volume)	<0.5
Usable testing volume of blood in tube	Approximate volume

If any packaging items/paperwork submitted are contaminated with biological fluids, the item is difficult to describe, or a more detailed description is necessary, the analyst shall document the items within the case notes, batch sheet(s), and/or with a photograph/photocopy, using a state-controlled camera (not a personal camera or cell phone). If a picture is taken, a ruler shall be included in the photo. The photo shall be printed for the case file and have the following items documented on the printout: HETL case number, item number(s), analyst initials, and date. Paperwork or packaging that has biological contamination, the contaminated packaging/paperwork may be destroyed after proper documentation of the item, updating the chain of custody as necessary.

The analyst will record the lot numbers of the standards, controls, and calibrators on the PE Alcohol Analysis Worksheet.

The blood kit box/container will be labeled with the date and analyst's initials who completed the kit inventory. The blood kit box/container (everything other than blood tubes) will be stored in an appropriately labeled box which is given a unique identification number. The storage box identification number will be recorded on the Chain of Custody form. This box will be retained until filled in the

laboratory. All filled boxes will be placed in long term evidence storage, for a period of at least six months, until being returned to the submitter or destroyed.

The analyst that opened the kits shall ensure the correct test codes are applied to the sample in StarLIMS.

At the time of analysis, the specimen identification number will be added to the PE Alcohol Analysis Worksheet, along with the other samples in that run.

After analysis the remaining blood tubes will be sealed in a plastic tube container, the seal initialed by the analyst, and stored in a tube storage rack in the locked evidence refrigerator. The tube rack and position will be recorded on the Blood Kit Inventory Worksheet. All blood tubes will be held in locked storage for a period of at least six months upon completion of analysis, until being returned to the submitter or destroyed. Beverage samples will be destroyed upon completion of testing.

By request, longer term storage/preservation of samples will be granted. A copy of the request will be attached to the chain of custody, and the original will be in the appropriate case file. The tubes are to be moved to a tube rack in a refrigerator designated for long-term storage, the kit box or other sample packaging will be moved to the box labeled “Sadie” for long-term storage, and the chain of custody will be updated accordingly and moved to a section designated for long-term storage chain of custody forms.

III. Quality Assurance

Equipment Maintenance and Calibration

Perkin Elmer Headspace GC-FID:

Daily (when in use), before running calibration or sample sequence:

- Instrument check sequence consisting of one blank and two standards (aqueous or whole blood)

Monthly (coordinate with generator test):

- Restart Computer
- Back-up data to external hard drive
- Clean Needle

Annually:

- Replace Needle Seal Assembly
- PM by vendor (when possible), includes:
- Replace o-ring seals

As Needed:
- Change Column
- Trim Column
- Bake Out Column

Parker Balston Hydrogen Generator:

As Needed:
- Fill with Deionized Water

Every 6 Months:
- Service A (Refer to Manufacturers Manual)

Every 24 Months:
- Service B (Refer to Manufacturers Manual)

Reagents, Standards, and Quality Control Materials

Refer to Quality Manual and Relevant Reagent Sheets.

Quality Control

Functional checks will be performed to check the performance of equipment and reagents used (either at regular intervals or while testing samples).

Daily instrument checks will be performed, when the instrument is in use, prior to a calibration or sample sequence to check for column leaks. These checks will be reviewed by the analyst for acceptability, initialed for approval and stored in the corresponding batch folder. The check shall consist of sequence containing:
- one blank
- two standards (aqueous or whole blood)

Control checks will be performed during the analysis or testing process.

These checks are used to:
- Determine the performance of the analytical or testing system.
- Quantitate the variability of results from the analysis or test in terms of precision and accuracy.

The frequency of checks will be determined by:

1. Currently accepted practices/standards in the discipline.
2. The number of samples being run in a particular sequence.
    a. After every three case sample sets an aqueous QC check standard shall be run.
    b. Whole Blood Control 1 and Whole Blood Control 2 shall be run at least once per batch when whole blood samples are included in the batch.
    c. One serum control shall be run when serum samples are included in the batch.

Acceptance criteria for both QC checks, calibrations and sample sequences can be found in the Calculations table. Wherever possible, control charts will be set up and used to record results from selected function and control checks. Determination will be made whether the testing or analytical process is out of control and corrective actions taken will be recorded.

Control checks will be performed during the analytical or testing process. These checks are performed either with each analysis or intermittently after a specified number of analyses. These control checks include but are not limited to:

1. Blanks
2. Standard with known or established specifications
3. Matrix matched standards with known concentrations
4. Running samples in duplicate

Calibrators and standards shall be from different sources. Lot numbers and expiration dates will be recorded on worksheets.

Ethanol Calibrators and Standards will be traceable to ISO 17034 compliant suppliers whenever possible. The Forensic Lab Director / Quality Manager will maintain appropriate records of approved vendors, and how vendors are approved.

Frequency of Updating the Calibration Curve

A calibration will be updated at least once every 7 days, when samples are analyzed. This data will be stored with the ethanol control documents. Calibrators used to create the calibration curve will be of the following concentrations (g/100ml): Blank (DI water), 0.010, 0.050, 0.100, 0.200, 0.300, 0.400, 0.500. Three second source aqueous standards will be run with the curve to check accuracy of calibration curve.

Criteria for acceptance will be:

- An r2 value of .998 or greater for both Channels
- All calibrators and second source standards must pass using acceptance critera defined in Calculations and Reporting of Results

Evaluation and Reporting Results Outside the Range of the Calibration Curve

Any sample determined to be greater than the largest calibrator will be reported as '> 0.xxx' (Where xxx denotes the concentration of the largest / highest calibrator).

QC Check of Blood Kits

1. Record the kit lot, blood tube lot, and PI pad lot numbers on the worksheet.
2. Add 2 mL of DI water and the liquid contents of the PI pad to one tube and vortex.
3. Label two 20mL headspace vials with identification number and suffix (A or B).
4. Using the auto dispenser re-pipetter - pipette 2500 uL of internal standard solution into each vial, and 250ul of above mixture into each vial.
5. Seal the vial by crimping the vial cap.
6. Vortex the vial.
7. Analyze by Headspace GC-FID.
8. Complete the Blood Kid QC Form and submit to the Quality Manager for review and approval.

IV. Sample Preparation

1. Mix the sample thoroughly, allow samples to shake on rocker for at least 10 minutes. Homogenize the sample and break up any clots, where possible. If homogenized, the sample must be placed on a rocker a second time for a minimum of ten minutes.
2. Label two 20mL headspace vials with identification number and suffix (1 or 2).
3. Prime diluter at least 3 times with DI water
4. Prepare all sample A vials at the same time, then repeat for sample B vials. Using the auto dispenser re-pipetter - pipette 2500 uL of internal standard solution into each vial, and 250ul of sample into each vial.
5. Between cases, blanks, and controls rinse the diluter with DI water.
6. Seal the vial by crimping the vial cap.
7. Vortex the vial.

Standards, controls, and blood samples can be prepared and stored in the refrigerator for 6 days before being run on the instrument. If samples are prepared, but not able to be run within 6 days, they must be discarded and prepared again, and a note must be added to the sample worksheet. Serum samples and beverage must be tested on the same day of preparation. Sample preparation

date will be recorded as the start date on the PE Alcohol Analysis Worksheet and control preparation date will be recorded in the column on the PE Alcohol Analysis Worksheet.

Waste

Bleach shall be added to the beaker containing the water that was used to rinse the diluter during analysis. The solution will then require reduction, following the HETL policy, and the solution can be poured down the drain.

GC Vials containing water and aqueous alcohol/volatiles are disposed of in a glass waste box. GC vials containing blood and alcohol/volatiles are disposed of in the mixed dual waste box.

The concentration of any alcohol/volatiles present in the vials is low enough to no longer be considered hazardous.

V. Gas Chromatograph Instrument Setup:

1. Launch TC Navigator Software on computer and log in.
2. Check water level on hydrogen generator.
3. Check carrier pressure is about 30 psi on headspace screen.
4. Check helium and air tanks are on and replace if necessary.
5. Check flames are lit by looking at the mV for each detector flame.
    a. If flames are not lit, follow below procedure.
        i. Turn Flow On and Light Flames:
            1. On TC Navigator Software → Run → Release Control (If not already released)
            2. On GC home screen (blue bar at top of screen should say Method 5)→ Tools → Configuration → A-FIDW → Select Setpoint boxes → Set Air to 450 mL/min; Set H2 to 45 mL/min
            3. On GC home screen → Select A-FIDW → Select Ignite box to light flame (wait for the click of the flame lighting and ensure voltage (mV) increases and stabilizes)
            4. Repeat for B-FIDW
6. On TC Navigator Software → Run → Take Control
7. Turn off H2 and Air when changing tanks and to save gas when instrument is not running:
    a. Turn Flow Off:
        i. On TC Navigator Software → Run → Release Control
        ii. On GC screen (blue bar at top of screen should say Method 5)→ Tools → Configuration → A-FIDW → Select Setpoint boxes for H2 and Air to 0.
        iii. Repeat for B-FIDW

8. Create data file path:
    a. Open file explorer on computer
    b. Navigate to C:/TC DATA/Results
    c. Open most recent casework folder by navigating to the current year folder/casework/month/date
    d. Make a new folder with date and initials (mmddyyXX), subsequent runs on the same date appended with letter after date (mmddyya, mmddyyb, etc…)
9. Build Sequence:
    a. In TC Navigator Software select Build → Sequence
    b. Edit sequence log table:
        i. Under Type field
            1. fill down with 'Sample'
        ii. Under Name field
            1. enter all sample names and QC and standard lot numbers and exp dates
        iii. Under Number field
            1. right click on number column
            2. select smart fill
            3. sample number pattern = ##
            4. uncheck the Synchronize with vial numbers box
            5. enter start & end row numbers
        iv. Under Inst Method field
            1. right click and click folder icon to navigate to correct method
            2. select instrument method "hetlbac" (file path: c:/tc data/methods/hetlbac)
        v. Under Proc Method for Channel A field
            1. right click and select Change Values
            2. click the folder icon to navigate to most recent method/date "hetlproca_mmddyy "
            3. repeat for Channel B and select most recent method/date "hetlprocb_mmddyy"
        vi. Under Calib Method for Channel A field
            1. right click and select Change Values
            2. click the folder icon to navigate to most recent method/date "hetlproca_mmddyy"
            3. repeat for Channel B and select most recent method/date "hetlprocb_mmddyy"
                a. Note – Proc method and Cal method should be the same date
        vii. Rpt FMT leave empty
        viii. Under Raw Data File
            1. right click for Channel A

2. select Path
3. check the "Apply to other channel" box
4. select the folder icon next to "New Path"
5. browse to the correct results (C:\TCDATA\Resutls\Year Casework\Month\Date)

ix. If data file number is not autopopulated – right click for each channel – smart fill
x. Save As and name the same as result folder
xi. Print the sequence.
xii. Load vials on headspace autosampler.
xiii. Sequence Verification Step – As samples and controls after the vials are loaded into the sample tray read off vial labels to a second person while the second person verifies the order listed on the printed sequence. Once the correct order has been verified the second person will signoff on the sequence.
xiv. Enter the vial range on Headspace touch screen
xv. Click Start on Headspace touch screen (method: bac)
xvi. Click Actions in menu bar in TC Navigator software
xvii. Click set up
xviii. Set up instrument window opens

1. Check Sequence Name
2. Check vial numbers
3. For calibration sequences, check mark the box "suppress reports/plots" in the Setup Parameters tab and under Processing
4. Click OK

10. Updating Method Calibration
    a. Run a calibration sequence according to the above section "CREATING A SEQUENCE"
    b. Name the sequence and results folder as mmddyyCAL
    c. Update old calibration with new data.
    d. Select Build Method
    e. Open the last calibration method date for A
        i. In menu bar, select Components, click Calibrate
        ii. Click the folder button and navigate to the results folder of the new calibration
        iii. Select the seven calibration data files for channel A.
        iv. Assign the appropriate concentration levels – make sure replace box is checked
            1. Error message will pop up because only the Ethanol calibrators are being updated.
        v. In menu bar, select Other, click Fit Analysis, save method as hetlproca_mmddyy
            1. A graph will pop up in the window of the methanol calibration.
        vi. In menu bar, select Data, click Next Component
            1. Ethanol graph should now be on the screen.
        vii. Print the graph

viii. Close the window

f. Open the last calibration method date for B
    i. Select the seven calibration data files for channel B.
    ii. Process the new calibration against the old calibration.
g. Select Build Sequence, select Load sequence stored on disk
h. Navigate to Sequences folder
i. Under file type, select .idx with date of sequence
j. Open the .idx file of the newest calibration sequence
k. Save Sequence
l. Under Inst Method for Channel A
    i. right click
    ii. select Change Values
    iii. click the folder icon and navigate to the new calibration date that reads hetlproca
    iv. Repeat for Channel B
m. Under Proc Method for Channel A
    i. right click
    ii. select Change Values
    iii. click the folder icon and navigate to the new calibration date that reads hetlproca
    iv. Repeat for Channel B
n. In menu bar
    i. select Actions
    ii. click Batch
    iii. change the results folder file path under Change File Path to the new calibration date results folder

11. Updating One Compound in Calibration
    a. Select the Build tab, and choose Graphic Edit
    b. Select the raw file to be updated
    c. Select the File tab, choose Open, and apply the correct method
    d. Select the Calibration tab, and choose Edit Components
    e. Select the compound/peak of interest to be updated
    f. Mark the box labeled Update Calibration
    g. Select the appropriate concentration Level from the drop-down menu
    h. Select Replace under the Calibration Type
    i. Click Next
    j. Select the return arrow (top left corner of the window)
    k. Select the File tab, and choose New Data File and repeat steps 2-9 for all Channel A files
    l. Save and rename the processing method for Channel A

m. Repeat steps 1-11 for Channel B

NOTE: Make notation on the worksheet of any instrument repair or any issue that prevents the run from going to completion. A QC check shall be performed and pass after any instrument failure, before processing further casework samples. Minimum QC batch should contain a blank (internal standard in 250ul of water), a minimum reporting level standard, a mid-range standard, high-range standard, a whole blood control, and a whole blood volatile control. Recalibration may be necessary depending upon the scope of the repair.

12. Updating Noise/Area Threshold for Data Analysis Method:
    a. Build → Graphic Edit → Select appropriate result file
    b. File → Open → Open appropriate method to update
    c. Process → Noise/Area Threshold… → Outline a section/draw a box on a flat area of baseline
    d. A window will pop up with the suggested and current noise threshold (NT) and area threshold (AT).
    e. Enter the below threshold levels:
        i. Current NT: 10
        ii. Current AT: 25

13. Adding New User in TotalChrome Software
    a. Open TC Navigator
    b. Click Admin in the file menu – click show admin tool bar
    c. Click system configuration
    d. Click users in menu bar – click Add
    e. Enter all information (don't check box)
    f. Click ok
    g. Don't change anything in next window – click ok
    h. Click instrument access – check all instrumnets – click ok
    i. Click password – enter new password – click never expires
    j. Close Software and reopen – type in user name for first time to add to drop down
    k. Enter password

14. Print reports manually from TotalChrom Software not printed at time of analysis
    a. Click Display → TC Publisher reporting
    b. Click "Report Options" in the Preview/Create Reports tab
        i. In the Data box, choose the correct sequence (.idk file)
        ii. In the Report box, choose the correct report C:\TC Data\Methods\HETLBACREPORT.tpm
    c. Click Okay

15. Adding a new printer to TotalChrom Software
    a. Click Admin → System Configuration
    b. In the Printers tab
        i. Delete the old printer
        ii. Add the new printer
    c. In the Users tab
        i. Choose the new printer from the dropdown menu for all users

VI. Calculations and Reporting of Results

Calculations

The acceptable limits of accuracy for the standards, sample replicates during the run are as follows: +/- 0.005 g/dL or +/- 5% relative percent difference (RPD), whichever is greater. RPD is calculated using the following forluma: |(x2 - x1)|/((x2 + x1)/2). (Samples less than 0.010 g/dL will be reported as '< 0.010 g/dL' even if difference in duplicates is greater than .005 g/dL)

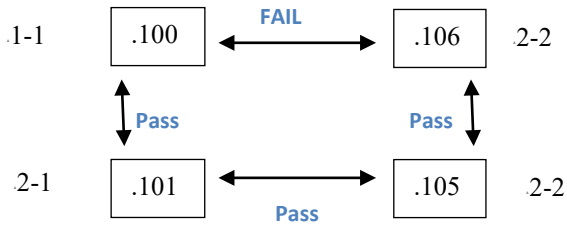
To better define and clarify exactly what is acceptable and what is not:
There are 4 results from each case (duplicate samples examined by dual column FID). Samples will be compared as such:

Vial 1: Result 1 to Result 2:

Vial 2: Result 1 to Result 2:

Vial 1: Result 1 to Vial B: Result 1:

Vial 2: Result 2 to Vial B: Result 2:

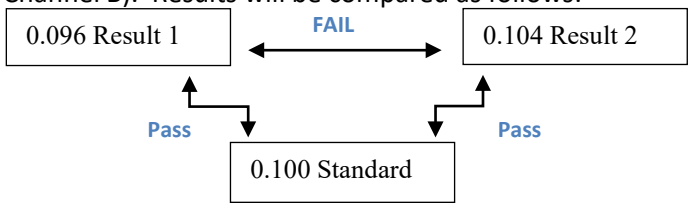


CLARIFICATION FOR STANDARDS: There are 2 results from each QC Check-Standard, one from each channel. (Result 1 – Channel A and Result 2-Channel B). Results will be compared as follows:

Result 1 to Result 2

Result 1 to nominal value of Standard

Result 2 to nominal value of Standard



For both Casework and Standards: Acceptable results are less than or equal to 5.0% RPD, or 0.005, whichever is greater. Meaning, 5.01% = Fail. 0.005 = PASS

If any sample from casework fails, at least 1 (2 if adequate sample exists) new vial(s) will be prepared and tested along with at least 1 blank, 2 aqueous controls, and 1 matrix matched control, depending on the sample matrix. If any QC Check-Standard fails, then all casework samples that bracket the failure will be re-aliquoted and tested, with at least 1 blank, 2 aqueous standards, and 1 matrix matched control, depending on the sample matrix.

Whole Blood Controls and Serum Controls: Whole blood controls (low and high) and the serum control each have 2 results, one from each Channel (Channel A and Channel B) and are compared to each other and to the range provided by the manufacturer. Acceptable results between Channel A and Channel B are 0.005 or 5% RPD, whichever is greater.

For whole blood controls, when compared to the range provided by the manufacturer, both results (Channel A and B) must be within the published range. If the two values are greater than 0.005 or 5% RPD, and/or either result from Channel A or B is outside the published range, then all whole blood samples within the batch will be rejected and re-tested.

For serum controls, both results (Channel A and B) must be within 5% of the expected concentration or the range established for that lot following the QC procedure outlined in the reagent log, whichever is determined as the passing criteria for that lot during the QC check. If the two values are greater than 0.005 or 5% RPD, and/or or either result from Channel A or B is outside 5% of the expected concentration or accepted range for that lot, then all serum samples within the batch will be rejected and re-tested. The mean values of the controls are being continuously tracked by the Quality Manager.

Blanks: Blanks results must be below 0.010 g/dL to be considered passing. If a blank fails all samples in the batch must be rerun.

Reporting Results

All test results are recorded to three decimal places on worksheet(s).

Individual results from each case are averaged and always rounded down to three decimal places (example: 0.10375 is rounded down to 0.103).

An uncertainty of measurement for each result is calculated based upon the most current expanded uncertainty value. The resulting value is always rounded up to three decimal places, regardless of what the 4th significant figure is (example: 0.01424 is rounded up to 0.015). Any results less than 0.100 g/dL will be reported out with an uncertainty of 0.009 g/dL.

Results are reported in grams of alcohol per 100 mL of blood.

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

Serum/Plasma results will be reported as a serum result with the applied current uncertainty of measurement estimate. The resulting range will be converted to whole blood on a secondary expert opinion letter, using a conversion factor of 1.22:1*. A comment stating “See expert opinion letter for conversion” will be listed on the report. The range of values will be truncated on the low end and rounded up on the high end.

Aqueous beverage sample results will be reported as the raw result with the current uncertainty of measurement estimate. The resulting range will be reported on a secondary expert witness letter, dividing the results by the density of ethanol (0.789) to yield a volume per volume alcohol percentage. The range of values will be truncated on the low end and rounded up on the high end.

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

Any method deviations will be communicated to the customer on the report in the form of a comment. Comments shall also be made on the report for any samples where a full analysis could not be completed, with the reason why noted.

Concentrations of ethanol below 0.010 g/100mL will be reported as "<0.010 g/100mL"

Worksheets and chromatograms for each sample are placed into the appropriate case folder along with a copy of batch sheet used in the calibration, and any other paperwork submitted with the case. Each page will have the case number, and initials of the analyst.

Final reports, worksheets, chromatograms of both samples and standards, and calculations/data transfers are Technically Reviewed, followed by Administrative Review.

The original worksheets, sequence list and chromatograms for calibrators and standards are placed in the Ethanol Controls folder labeled with the run date.

After the administrative review is completed, the analyst will complete the notarization of the report, and it will be sent to the customer. A copy of the consent cards from inside the kit box will also be sent to the customer with the report. The consent card will be labeled with the laboratory identification number, the initials of the analyst that inventoried the kit, and the initials of any analyst that analyzed the sample.

All postmortem samples tested shall have the following comment included in the report: "Postmortem Specimen: (collection site)." The collection site shall be indicated in the comment section of the report, if a submitted sample was obtained from multiple sites then the term "Pooled" shall be used for the collection site.

* Measuring Blood Alcohol Concentration for Clinical and Forensic Purposes, AW Jones and Derrick Pounder, Handbook of Drug Abuse, S Karch, MD, 1998

VII. Notes

Blood alcohol concentrations > 0.08 g/100 ml blood are prima facie evidence in operating under the influence violations.

Many volatile substances can be detected by this procedure. The most common volatiles in body fluids are ethanol, methanol, isopropanol, and acetone. All of these substances can be separated from ethanol on the gas chromatograph. HETL does not quantitate volatiles other than ethanol.

VII. Case Documentation

Case Notes

The minimum information, which must be contained in the case notes are:

Laboratory Identification Number
Collection kit's suspect/police information paperwork
Blood Kit Inventory Worksheet
Run Data
QC Data
Comments/Results

All case notes, chromatograms and other data generated during analysis will bear the initials of the analysts and case number. Addition notes may indicate the stopper color of submitted tubes. Any tubes tested that are not gray top tubes supplied by HETL will have a notation made.

Case Files

The minimal information, which must be contained in the individual case file consists of:

The final report
Any preliminary, supplementary, or corrected reports
Collection kit form (if available)
Worksheet(s)
Evidence receipt
Original chromatograms
Technical and Administrative Review

IX. Uncertainty of Measurement

When estimating the uncertainty of measurement, all uncertainty components which are of importance shall be taken into account using appropriate testing procedures. Bias of Calibrators is also acknowledged and examined as part of the UofM procedure. Documentation, when applicable, will be retained by the Quality Manager.

What is being measured: Ethanol concentration in blood samples.

Traceability of is established by using NIST / Guide 34 / ISO 17034 traceable controls, obtained by an approved vendor, and utilizing equipment calibrated to ISO 17025 standards by an accredited and approved vendor.

The equipment used for determining ethanol concentration:

PerkinElmer Headspace Gas Chromatograph- dual-column

Headspace- TurboMatrix 110 S/N HS110S1805221

Gas Chromatograph- Clarus 590 S/N 590S18053006

Hamilton Diluter – 600 Series

Woody Microlab 600 - S/N: ML600FF8801

Buzz: Microlab 600 – S/N: ML600BF17551

The following components are recognized as potentially contributing to UofM:

Whole Blood control (Reproducibility)

Aqueous Controls (evaluation of bias)

Temperature (liquids and ambient)

Variation of time and room temperature

Uncertainty stated on COA's

Matrix differences

Diluter: samples, controls, calibrators, and internal standard

Stability of controls and calibrators

Staff variability

Headspace variability

Concentration

Internal Standard (n-propanol) stability and concentration

Stability

Instrument parameters

Instrument precision

Calibration model

Integration parameters / processing of data

The following components are considered of significance:

Whole Blood Matrix Control - Type A: (reproducibility of assay)

Matrix - Type B: (5% administrative rule)

Calibrators- Type B from COA

Diluter for Calibrators - Type B from calibration certificate

Diluter for Samples - Type B from calibration certificate

Aqueous Controls - Type B from COA (evaluation of bias)

Data from controls and duplicates are tracked in a Microsoft Excel Spreadsheet. Calibration certificate(s) of the Hamilton diluter, and COA's of respective calibrators, QC standards, and whole blood controls are retained by the Quality Manager. From these spreadsheets, and in particular the WBC's, it can be determined that the Data is/is not of a normal distribution, skewed, and the mean and standard deviation calculated. Additional graphs can also be created as warranted. All values of uncertainty from individual components deemed significant (See E above) are converted to % uncertainty (See ASCLD/LAB Annex D AL-PD-3065 Ver 1.0).

The following calculations are performed: the standard uncertainties of the six sources identified in

section E, are individually squared, and totaled. The square root is determined of the resulting sum, and this value is equal to the combined uncertainty or k. The expanded uncertainty (K2) is calculated by doubling the uncertainty (Kx2). (The method is identical to ASCLD/LAB Annex D AL-PD-3065 Ver 1.0).

$$\sqrt{(u1)^2 + (u2)^2 + (u3)^2 + (u4)^2 + (u5)^2 + (u6)^2} = K$$

K(2) = (K*2) = reported UofM at 95.45% confidence interval

For reporting purposes, the lab will round up the K2 value for ease of use and understanding of case reports by our customers.

The schedule to review the measurement uncertainty will be conducted annually or upon the addition or replacement of laboratory equipment, staff or other factors considered of significance. The Quality Manager will retain calculations, verifications of spreadsheets, graphs, etc.

X. References:

ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – Annex D. (ASCLD/LAB document: AL-PD-3065 Ver 1.0 22 May 2013)

Moffat, A. C., Osselton, M. D., Widdop, B., & Watts, J. (2011). Clarke's analysis of drugs and poisons: In pharmaceuticals, body fluids and postmortem material. London: Pharmaceutical Press.

ASB Standard for the Minimum Content Requirements of Forensic Toxicology Procedures, ANSI/ASB Standard 152, First Edition 2021

XI. Revision Table:

REVISED BY	REV#	DATE	Revisions
LN	1*	6/8/21	Evidence Handling and Preservation: Updated documentation of discrepancies to include the option of a photograph of evidence. Added procedure for handling packaging/paperwork which is contaminated with biological fluids. Revisoins: Added Revision table to document.
LN	2	07/29/21	Section III F was updated to remove the transfer of the liquid to the second tube in the kit. The second tube will be reserved for blood drug testing.
LN	3	01/06/22	Section V Updating Method Calibration: added “save sequence” following letter d Section VI: Serum control passing range updated to include a laboratory determined range based on QC data. Rerun section updated to include serum matrix.
LN	4	04/12/22	Section I F added guidance to see reagent records for preparation instructions. Section II added guidance to evidence control while testing and ensuring a proper seal is in place prior to testing. Clarified the analyst opening the kit should be the one initialing the stickers. Included a section on dealing with minor discrepancies. Added that photographs shall be taken using a state controlled device. Added steps for the analyst opening the kits to add the case number to the blood kit box excel sheet and ensure all test codes are assigned in LIMS. Section V added instructions for updating one compound in calibration curve Section VI added deviaitons shall be communicated to customer in the form of a comment on the report, and consent cards will be sent along with the report to the customers. Section IX added second diluter
EAF, LN, & MS	5	6/30/22	Added postmortem sample reporting to VI. Reporting Criteria. V. Gas Chromatogram Instrument Setup section rewritten and added Added Whole Blood Controls to section I Preparations of Solutions. Updating Noise/Area Threshold for Data Analysis Method. Added small grammatical and formatting changes throughout document.
LN	6	08/17/22	Updated section I, III, and VI to only require matrix matched controls when those specific matricies are included in the batch. Batches with only aqueous samples do not require a whole blood control or serum control.

LN	7	11/08/22	Updated Section II to note that beverage samples are destroyed upon completion of testing. Updated Section IV to clarify that only blood samples may be prepared and stored for up to six days before analysis. Updated section VI and VII to note that any tubes received for analysis other than HETL approved gray top tubes will be noted on the report.
LN	8	11/17/22	Updated following addendum verification to adjust the AT and NT in the method. Fixed typo for instrument model. Added the software model used to section I.
LN	9	06/30/23	Appendix added: instrument processing method Section II: added procedure for documenting evidence with a camera. Section IV 1.: added "Homogenize the sample and break up any clots, where possible. If homogenized, sample must be placed on a rocker a second time for a minimum of ten minutes" Section IV: Waste section added
LN	10	10/26/23	Section II – updated the box location to the COC instead of worksheet. Removed reference to excel sheet for tracking boxes. Section IV changed vial naming scheme from vial A and vial B to vial 1 and vial 2 Waste – updated to include bleach reduction prior to disposal. Section V – added step 9 xviii 3, 14, and 15 Section VI – changed vial naming scheme from vial A and vial B to vial 1 and vial 2 to prevent confusion with blood kit inventory sheet. Added section discussing blood kit inventory table that will be included in reports. Updated process for reporting of serum and beverage samples. Removed comment requirements for items included on report. Updated spelling throughout. Added requirements for expert letter.

EAF	11	01/18/24	Added to Evidence Handling and Preservation section"For continuity the volumes entered into the blood inventory worksheet and subsequent StarLIMS items received table shall adhere to the following format: (table containing guidance with different volumes)" Added non-HETL approved collection material reference table. Added to evidence handling: If a non-HETL approved collection kit or materials were submitted, the WO# field in STARLIMS folder metadata shall be filled in with N/A. If the kit, upon opening, is found to contain non HETL approved collection materials this shall be noted on the inventory form and STARLIMS metadata shall be updated appropriately.

*For previous revisions please see version history within SharePoint.

Appendix: Instrument Processing Method

TotalChrom Method File C:\TC DATA\Methods\HETLPROCA_111522_3.mth

Printed by	: MSMOKER on: 11/23/2022 3:29:56 PM
Created by	: MSMOKER on: 11/16/2022 2:13:12 PM
Edited by	: MSMOKER on: 11/16/2022 2:13:12 PM
Number of Times Edited	: 0
Number of Times Calibrated	: 309
Description:	

Instrument Conditions

Instrument Control Method
Instrument Name : Clarus 590
Instrument Type : PE AutoSystem GC

Channel Parameters
Data will be collected from both channels
Delay Time : 0.00 min
Run Time : 4.00 min
Sampling Rate : 12.5000 pts/s

	Channel A	Channel B
Signal Source	DetA	DetB
Analog Output	INT	INT
Attenuation	0	0
Offset	5.0 mV	5.0 mV

Carriers Parameters
Carrier A control : Press - He
Column A length : 30.00 m
Vacuum Compensation : OFF
Split Flow : 20.0 mL/min
Initial Setpoint : 25.0 PSIG

Diameter : 320 µm

Initial Hold : 999.00 min

Valve configuration and settings
Valve 1 : SPLIT On
Valve 2 : NONE
Valve 3 : NONE
Valve 4 : NONE
Valve 5 : NONE
Valve 6 : NONE

11/23/2022 3:29:56 PM Method: C:\TC DATA\Methods\HETLPROCA_111522_3.mth

Detector Parameters

	Detector A	Detector B
Detector	FID	FID
Range	1	1
Time Constant	200	200
Autozero	ON	ON
Polarity		

Detector A Gas Flows
Air : 450.0 mL/min
H2 : 30.0 mL/min

Detector B Gas Flows
Air : 450.0 mL/min
H2 : 30.0 mL/min

Heated Zones
Injector A: CAP
Setpoint : 200 °C

Injector B: NONE
Setpoint : OFF

Detector A : 220°C
Detector B : 220°C
Auxiliary (NONE) : 0°C

Oven Program
Cryogenics : Off
Initial Temp : 40°C
Initial Hold : 4.00 min

Total Run Time : 4.00 min
Maximum Temp : 260°C
Equilibration Time : 0.0 min

Timed Events
There are no timed events in the method

Real Time Plot Parameters

	Pages	Offset (mV)	Scale (mV)
Channel A	1	0.000	1000.000
Channel B	1	0.000	1000.000

11/23/2022 3:29:56 PM Method: C:\TC DATA\Methods\HETLPROCA_111522_3.mth

Processing Parameters
Bunch Factor : 12 points
Noise Threshold : 10 µV
Area Threshold : 25.000 µV

Peak Separation Criteria
Width Ratio : 0.200
Valley-to-Peak Ratio : 0.010

Exponential Skim Criteria
Peak Height Ratio : 5.000
Adjusted Height Ratio : 4.000
Valley Height Ratio : 3.000

Baseline Timed Events
Event #1 - Set Noise Threshold 2.000 at 0.000
Event #2 - Set Bunching Factor 8.000 at 0.000

Optional Reports
Keep temporary files
No report format files given

Optional Report Plot Parameters

Plot Number	1	2	3	4
Generate this plot	Yes	No	No	No
Start plot at end of delay time	Yes	Yes	Yes	Yes
Start Time				
End Time				
Scale Type	Vertical Scaling	Vertical Scaling	Vertical Scaling	Vertical Scaling
Scale Factor	1.000	1.000	1.000	1.000
Full Scale				
Offset				

Plot Number	5
Generate this plot	No
Start plot at end of delay time	Yes
Start Time	
End Time	
Scale Type	Vertical Scaling
Scale Factor	1.000
Full Scale	
Offset	

11/23/2022 3:29:56 PM Method: C:\TC DATA\Methods\HETLPROCA_111522_3.mth

Annotated Replot Parameters

Offset & Scale determined automatically
Draw baselines
Include timed event annotations
Automatically set plot start and end times to data limits

Scale Factor	: 1.000000
Number of Pages	: 1
Plot Title	: Chromatogram
X-Axis Label	: Time [min]
Y-Axis Label	: Response [mV]
Orientation	: Landscape
Retention Labels	: Top of Plot
Component Labels	: Actual Time

User Programs

No user programs will be executed

TotalChrom Method File C:\TC DATA\Methods\HETLPROCB_111522_3.mth

Printed by	: MSMOKER on: 11/23/2022 3:30:34 PM
Created by	: MSMOKER on: 11/16/2022 2:14:02 PM
Edited by	: MSMOKER on: 11/16/2022 2:14:02 PM
Number of Times Edited	: 0
Number of Times Calibrated	: 261
Description:	

Instrument Conditions

Instrument Control Method

Instrument Name : Clarus 590
Instrument Type : PE AutoSystem GC

Channel Parameters

Data will be collected from both channels
Delay Time : 0.00 min
Run Time : 4.00 min
Sampling Rate : 12.5000 pts/s

	Channel A	Channel B
Signal Source	DetA	DetB
Analog Output	INT	INT
Attenuation	0	0
Offset	5.0 mV	5.0 mV

Carriers Parameters

Carrier A control : Press - He
Column A length : 30.00 m
Vacuum Compensation : OFF
Split Flow : 20.0 mL/min
Initial Setpoint : 25.0 PSIG

Diameter : 320 µm

Initial Hold : 999.00 min

Valve configuration and settings

Valve 1 : SPLIT On
Valve 2 : NONE
Valve 3 : NONE
Valve 4 : NONE
Valve 5 : NONE
Valve 6 : NONE

11/23/2022 3:30:34 PM Method: C:\TC DATA\Methods\HETLPROCB_111522_3.mth

Detector Parameters

	Detector A	Detector B
Detector	FID	FID
Range	1	1
Time Constant	200	200
Autozero	ON	ON
Polarity		

Detector A Gas Flows
Air : 450.0 mL/min
H2 : 30.0 mL/min

Detector B Gas Flows
Air : 450.0 mL/min
H2 : 30.0 mL/min

Heated Zones
Injector A: CAP
Setpoint : 200 °C

Injector B: NONE
Setpoint : OFF

Detector A : 220°C
Detector B : 220°C
Auxiliary (NONE) : 0°C

Oven Program
Cryogenics : Off
Initial Temp : 40°C
Initial Hold : 4.00 min

Total Run Time : 4.00 min
Maximum Temp : 260°C
Equilibration Time : 0.0 min

Timed Events
There are no timed events in the method

Real Time Plot Parameters

	Pages	Offset (mV)	Scale (mV)
Channel A	1	0.000	1000.000
Channel B	1	0.000	1000.000

11/23/2022 3:30:34 PM Method: C:\TC DATA\Methods\HETLPROCB_111522_3.mth

Processing Parameters
Bunch Factor : 8 points
Noise Threshold : 10 µV
Area Threshold : 25.000 µV

Peak Separation Criteria
Width Ratio : 0.200
Valley-to-Peak Ratio : 0.010

Exponential Skim Criteria
Peak Height Ratio : 5.000
Adjusted Height Ratio : 4.000
Valley Height Ratio : 3.000

Baseline Timed Events
Event #1 - Set Noise Threshold 2.000 at 0.000
Event #2 - Set Bunching Factor 8.000 at 0.000

Optional Reports
Report Format File #1 : C:\TC DATA\Methods\HETLBACREPORT.tpm

Keep temporary files

Optional Report Plot Parameters

Plot Number	1	2	3	4
Generate this plot	Yes	No	No	No
Start plot at end of delay time	Yes	Yes	Yes	Yes
Start Time				
End Time				
Scale Type	Vertical Scaling	Vertical Scaling	Vertical Scaling	Vertical Scaling
Scale Factor	1.000	1.000	1.000	1.000
Full Scale				
Offset				

Plot Number	5
Generate this plot	No
Start plot at end of delay time	Yes
Start Time	
End Time	
Scale Type	Vertical Scaling
Scale Factor	1.000
Full Scale	
Offset	

11/23/2022 3:30:34 PM Method: C:\TC DATA\Methods\HETLPROCB_111522_3.mth

Annotated Replot Parameters

Offset & Scale determined automatically
Draw baselines
Include timed event annotations
Automatically set plot start and end times to data limits

Scale Factor	:	1.000000
Number of Pages	:	1
Plot Title	:	Chromatogram
X-Axis Label	:	Time [min]
Y-Axis Label	:	Response [mV]
Orientation	:	Landscape
Retention Labels	:	Top of Plot
Component Labels	:	Actual Time

User Programs

No user programs will be executed