Common Volatiles Analysis by Headspace GC-MSD/FID

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1 INTRODUCTION

This procedure allows for the screening, identification, confirmation, and quantitation of common volatile chemicals.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation
Matrices	Blood, serum, urine, vitreous fluid and other liquids
Analytes	Ethanol, acetone, isopropanol, methanol (Target Compounds)
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Sample and a diluent containing internal standard are added to a headspace vial using a pipette operating in dilute mode. Samples are qualitatively screened for target compounds by headspace gas chromatography with mass spectrometry (HS-GC/MS). Specimens are quantitatively confirmed through a separate analysis using headspace gas chromatography with flame ionization detection (HS-GC/FID). The headspace technique is based on Henry's gas law, which states that, for dilute solutions at a constant temperature and at equilibrium, a direct relationship exists between the amount of volatile analyte dissolved in a liquid and the amount of the analyte in the vapor above the solution. Determination of the concentration of the volatile analyte in the sampled headspace allows for the calculation of the concentration of that analyte in the original liquid sample.

4 SPECIMEN CRITERIA

0.1 mL of sample is used per replicate analysis.

5 EQUIPMENT

Use of equivalent equipment is allowable.

5.1 Equipment

Electronic Pipettor	Eppendorf Xplorer Plus, single channel, 50-1000µL range
Headspace vial cap crimper	Standard, 10mL
Routine Laboratory Glassware and supplies	Volumetric flasks (50, 100 and 1000 mL), pipettes, disposable tissue grinder
Laboratory Balance	Standard, ≥0.1g resolution. Traceable.

5.2 Consumables

Inlet Liner	Restek 1.0mm Topaz Straight Liner. PN 23333
Pipette Tips	Biotix™ uTIP™ Filter Pipette Tips for Universal Pipettes, Standard. PN M12509FC96
Headspace vials	Gerstel, crimp cap vials, 10 mL, 100 pack. PN 093640-005-00.
Headspace vial caps, magnetic	Gerstel, crimp caps with septum for vials, 100 pack. PN 093640-063-00
Storage tubes	Thermo Matrix 1.0mL ScrewTop tubes PN 3741WHI

5.3 Instruments

GC/MS with Headspace Autosampler	EI ionization, Gerstel autosampler

5.3.1 Columns

GC Columns	Restek RTX-BAC Plus 1: 30m X 0.32mm X 1.8 µm PN 18004 Restek RTX-BAC Plus 2: 30m X 0.32mm X 0.6 µm PN 18006

5.4 Software

Component	**Software**	**Version**
Operating System	Microsoft Windows	10 Pro
GC/MS	Agilent 7890B	B.02.05.2
	MassHunter Workstation	10.0.368
Autosampler	Gerstel Maestro 1	1.5.3.74/3.5
Data Analysis	MassHunter Workstation Quantitative Analysis	10.0

5.5 Chemicals/Reagents

5.5.1 Purchased

Item	**Supplier***	**Description****	**Part Number***
t-butanol	Sigma-Aldrich	ACS/Reagent Grade	360538
Ethanol	Sigma-Aldrich	HPLC grade	E7023
Methanol	Fisher Scientific	HPLC grade	A454
Isopropanol	Fisher Scientific	HPLC grade	A451
Acetone	Fisher Scientific	HPLC grade	A949
Deionized water	Laboratory supplied	18.2 MΩ•cm	N/A
*use of an equivalent product is allowable **listed grade or better			

5.5.2 Prepared

Depending upon the batch size, the absolute amounts may be adjusted so long as the ratios of components are maintained.

5.5.2.1 Stock Sample Diluent (1.0 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 100 mL
2	Add	~90 mL	Deionized water
3	Add	1.0 g	t-butanol
4	QS	100 mL	Deionized water
5	Mix		
6	Transfer		Glass container
7	Storage		Refrigerated or ambient
8	Stability		≥ 6 months
9	Prepares	100 mL	(20 Sample Diluent preparations)

5.5.2.2 Sample Diluent (0.005 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 1000 mL
2	Add	5.0 mL	Stock Sample Diluent
3	QS	1000 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Ambient. Smaller satellite container may also be used.
7	Stability		≥ 6 months
8	Prepares	1000 mL	(1250 analyses)

5.6 Standards/Controls

5.6.1 <u>Purchased</u>

Analyte	Supplier *	Description	Part Number*
Multicomponent Volatiles	Cerilliant	C1-C6 levels containing ethanol, methanol, isopropanol and acetone at 0.010, 0.025, 0.050, 0.100, 0.200 and 0.400 g/100mL in water	A-127
Ethanol	Cerilliant	C7 level containing ethanol at 0.500 g/100mL in water	E-053
Multicomponent Volatiles	Cliniqa	Contain ethanol, methanol, isopropanol, and acetone in whole human blood (varying concentrations)	93221, 93222
*Use of an equivalent product is allowable; be sure to consider additional validation/verification and update Measurement Traceability records if alternate calibrators are used. Store refrigerated. Stability determined by manufacturer. Cerilliant solutions will be used shortly after opening and not reused on subsequent days. Cliniqa controls will be used within 45 days of opening. Refer to 5.5 for additional starting materials.			

5.6.2 Prepared

Depending upon the batch size, the absolute amounts may be adjusted so long as the ratios of components are maintained.

At first opening of each new Cliniqa control vial, the contents of the vial will be portioned out into storage tubes that limit the headspace during storage. The date of opening and portioning will be recorded on each storage vial. Each of these vials should be used on the day of opening and then discarded.

5.6.2.1 TOX200 Stock System Suitability Sample (0.100 g/100mL)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 50 mL
2	Add	~25 mL	Deionized Water
3	Add	0.064 mL	Each of stock ethanol, acetone, isopropanol, methanol
3	QS	50 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Refrigerated.
7	Stability		≥ 12 months
8	Prepares	50 mL	Of stock material

5.6.2.2 TOX200 System Suitability Sample (0.010 g/100mL, S^3)

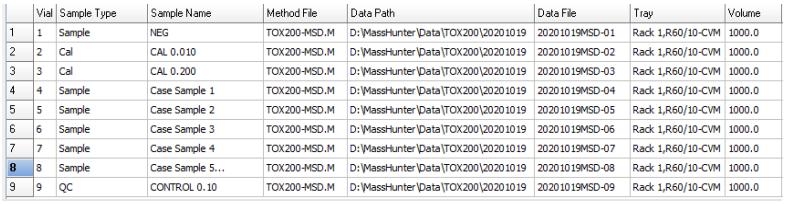
Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 50 mL
2	Add	~25 mL	Deionized Water
3	Add	5 mL	Stock System Suitability Sample
3	QS	50 mL	Deionized water
4	Mix		
5	Transfer		Glass container, tightly sealed
6	Storage		Refrigerated.
7	Stability		≥ 12 months
8	Prepares	50 mL	(500 analyses)

Analysis of an S^3 is used to verify system performance for both FID and MSD methods prior to case analysis.

6 PROCEDURE

6.1 Screening/Identification by HS-GC/MSD

Batch Building: Samples used for screening may be aliquoted in advance up to 15 days prior to analysis (samples and diluent are portioned into a headspace vial, sealed, and placed in secure refrigerated storage). The same lot of Sample Diluent is used throughout a given batch. Any calibrated Xplorer Plus pipette may be used. Batches are coded according to the scheme TOX200.YYYYMMDD-MSD.

<table>
<tr><th>Step</th><th></th><th>Activity</th><th>Note</th><th>Reference/Lot</th></tr>
<tr><td>6.1.1</td><td>☐</td><td>Samples:
Using an Eppendorf Xplorer pipette fitted with a tip, aliquot 800 µL of Sample Diluent and 100 µL of sample into a 10 mL headspace vial. Crimp vial firmly using a magnetic cap. Use a new tip for each sampling.</td><td>See pipette settings in Section 7.1</td><td>S3
Sample Diluent
Xplorer Plus Pipette
3</td></tr>
<tr><td>6.1.2</td><td>☐</td><td>Quality Control Materials:
To start a batch, pipet the following QC materials:
Negative Control (deionized water)
0.010 g% (CRM)
0.200 g% (CRM)
Upon aliquot of the final case sample for the batch, include a closing control:
0.100 g% (CRM)</td><td></td><td>Negative Control
Calibrator C1-C6 Set
2</td></tr>
<tr><td>6.1.3</td><td>☐</td><td colspan="3">Batch Analysis: Input the samples into the instrument sequence using the following order and format:
 
<table>
<tr><th></th><th>Vial</th><th>Sample Type</th><th>Sample Name</th><th>Method File</th><th>Data Path</th><th>Data File</th><th>Tray</th><th>Volume</th></tr>
<tr><td>1</td><td>1</td><td>Sample</td><td>NEG</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-01</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>2</td><td>2</td><td>Cal</td><td>CAL 0.010</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-02</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>3</td><td>3</td><td>Cal</td><td>CAL 0.200</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-03</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>4</td><td>4</td><td>Sample</td><td>Case Sample 1</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-04</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>5</td><td>5</td><td>Sample</td><td>Case Sample 2</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-05</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>6</td><td>6</td><td>Sample</td><td>Case Sample 3</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-06</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>7</td><td>7</td><td>Sample</td><td>Case Sample 4</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-07</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>8</td><td>8</td><td>Sample</td><td>Case Sample 5...</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-08</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
<tr><td>9</td><td>9</td><td>QC</td><td>CONTROL 0.10</td><td>TOX200-MSD.M</td><td>D:\MassHunter\Data\TOX200\20201019</td><td>20201019MSD-09</td><td>Rack 1,R60/10-CVM</td><td>1000.0</td></tr>
</table>
A maximum of 116 samples may be analyzed in one batch. In batches that contain 20 or more samples, an additional “mid-run” positive control will be added.</td></tr>
</table>

6.2 Confirmation/Quantitation by HS-GC/FID

Batch Building: Allow specimens and quality control samples to stand at room temperature for at least 15 minutes. Samples used for confirmation/quantitation are aliquoted from the original item or an intermediary container into a headspace vial and sealed. The same lot of Sample Diluent is used throughout a given batch. Any calibrated Xplorer Plus pipette may be used. Batches are coded according to the scheme TOX200.YYYYMMDD-FID.

Step		Activity	Note	Reference/Lot
6.2.1	☐	**Samples:** Using an Eppendorf Xplorer pipette fitted with a filter tip, aliquot 800 µL of Sample Diluent and 100 µL of sample into a 10 mL headspace vial. Crimp vial firmly using a magnetic cap. Perform in duplicate. Use a new tip for each sampling.	See pipette settings in Section 7.1	S[3] Sample Diluent Xplorer Plus Pipette 3
6.2.2	☐	**Quality Control Materials:** Use the following QC materials for each batch: • Negative Control (deionized water) • CAL1-CAL6, CAL7 (CRM) • Cliniqa Controls (Two Levels)		Negative Control Calibrator C1-C6 Set Calibrator C7 Cliniqa Level 1 Cliniqa Level 2 5
6.2.3	☐	**Batch Analysis:** Input the samples into the instrument sequence using the following order and format:		

	Vial	Sample Type	Sample Name	Method File	Data Path	Data File	Tray	Volume
1	1	Sample	Negative QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-01	Rack 1,R60/10-CVM	1000.0
2	2	Cal	CAL1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-02	Rack 1,R60/10-CVM	1000.0
3	3	Cal	CAL2	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-03	Rack 1,R60/10-CVM	1000.0
4	4	Cal	CAL3	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-04	Rack 1,R60/10-CVM	1000.0
5	5	Cal	CAL4	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-05	Rack 1,R60/10-CVM	1000.0
6	6	Cal	CAL5	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-06	Rack 1,R60/10-CVM	1000.0
7	7	Cal	CAL6	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-07	Rack 1,R60/10-CVM	1000.0
8	8	Cal	CAL7	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-08	Rack 1,R60/10-CVM	1000.0
9	9	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-09	Rack 1,R60/10-CVM	1000.0
10	10	QC	Low QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-10	Rack 1,R60/10-CVM	1000.0
11	11	QC	High QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-11	Rack 1,R60/10-CVM	1000.0
12	12	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-12	Rack 1,R60/10-CVM	1000.0
13	13	Sample	Case 1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-13	Rack 1,R60/10-CVM	1000.0
14	14	Sample	Case 1	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-14	Rack 1,R60/10-CVM	1000.0
15	15	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-15	Rack 1,R60/10-CVM	1000.0
16	16	Sample	Case 2	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-16	Rack 1,R60/10-CVM	1000.0
17	17	Sample	Case 2...	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-17	Rack 1,R60/10-CVM	1000.0
18	18	Sample	blank	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-18	Rack 1,R60/10-CVM	1000.0
19	19	QC	Low QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-19	Rack 1,R60/10-CVM	1000.0
20	20	QC	High QC	TOX200-FID.M	D:\MassHunter\Data\TOX200\20201019	20201019FID-20	Rack 1,R60/10-CVM	1000.0

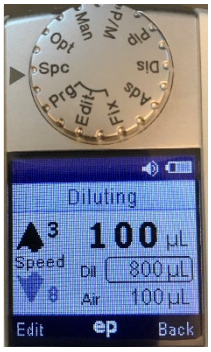
A maximum of 35 samples may be analyzed in one batch. In batches that contain 20 or more samples, an additional “mid-run” positive control will be added.

6.3 Screening or Confirmation for Nonstandard Samples

A sample is considered nonstandard if it cannot be rendered homogenous through mixing/vortexing, which are the preferred methods. If a case sample is clotted and cannot be pipetted accurately, it may be homogenized with a clot grinder before pipetting. If the entire specimen or a portion of the specimen is homogenized, the details of the homogenization are recorded. For additional guidance, refer to TOX-100.

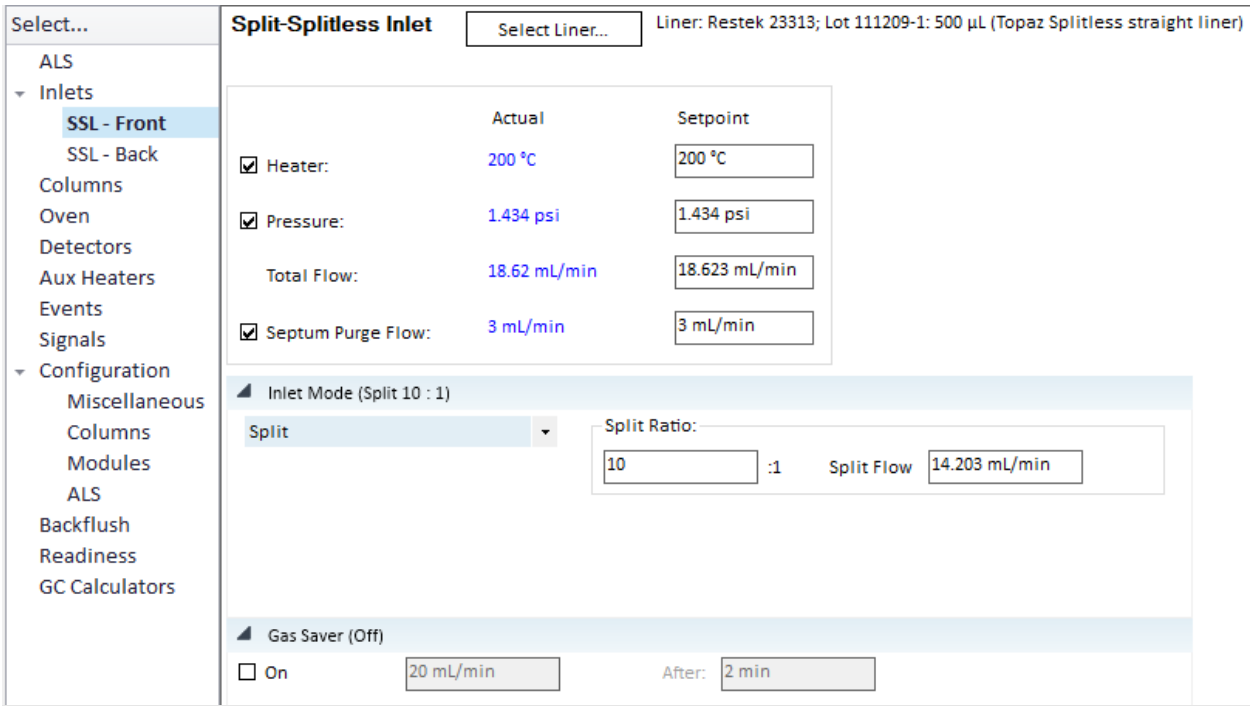
7 ANALYTICAL PARAMETERS

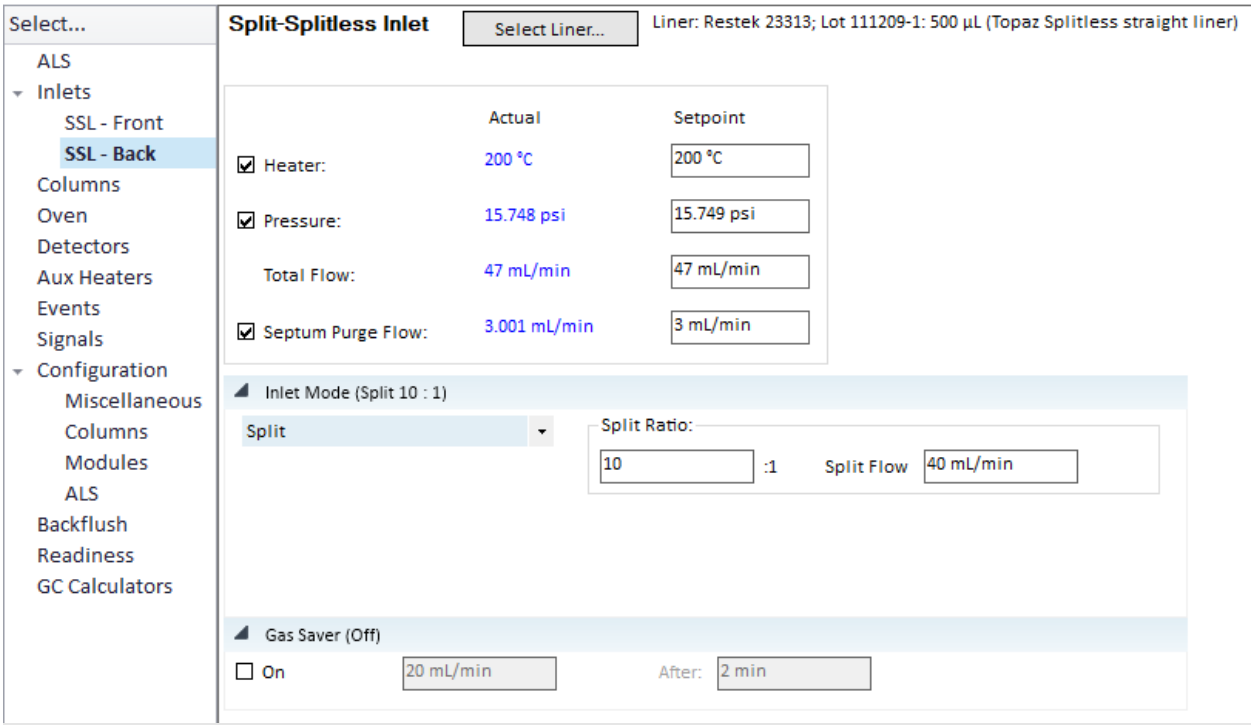
7.1 Pipettor Settings (MSD and FID)



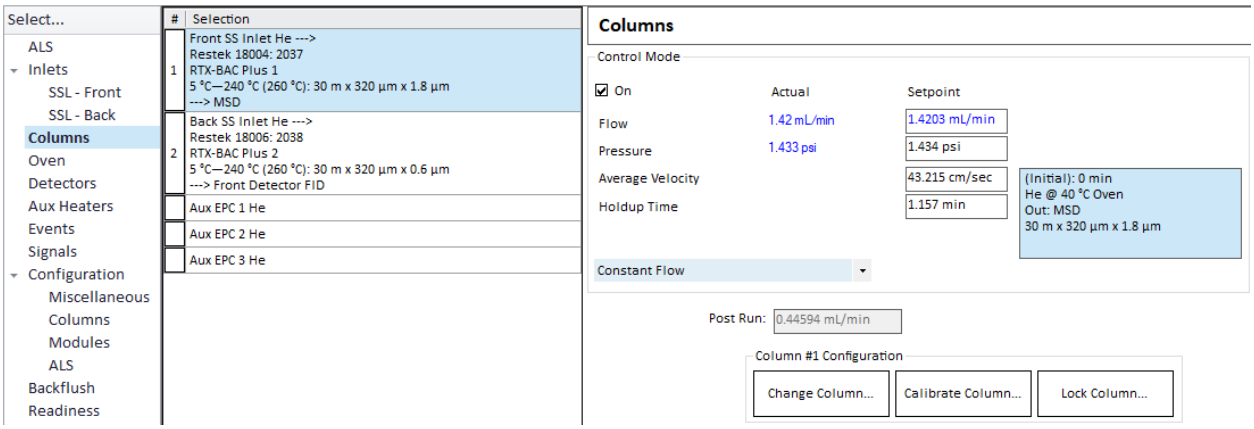
7.2 Mass Spectrometry Method (Screening)

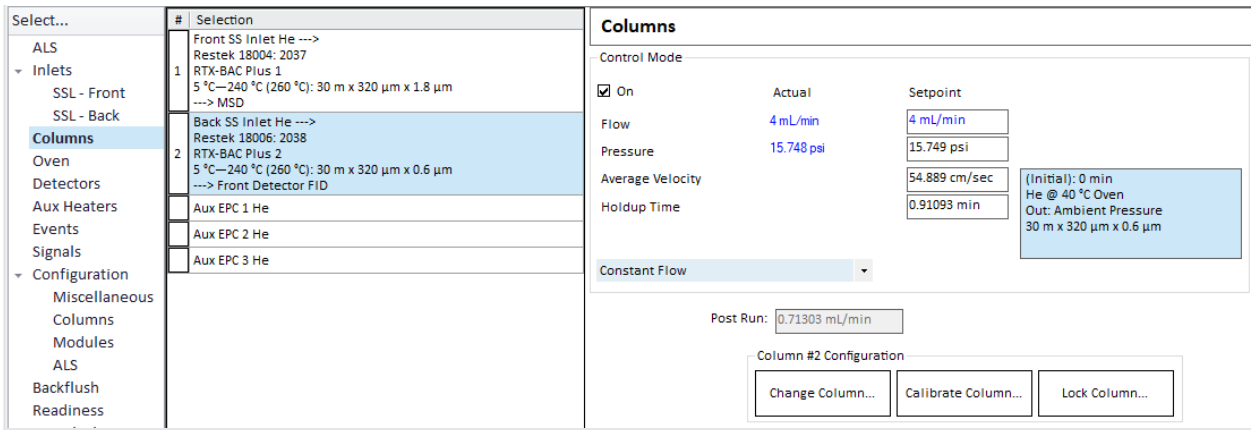
7.2.1 Inlets



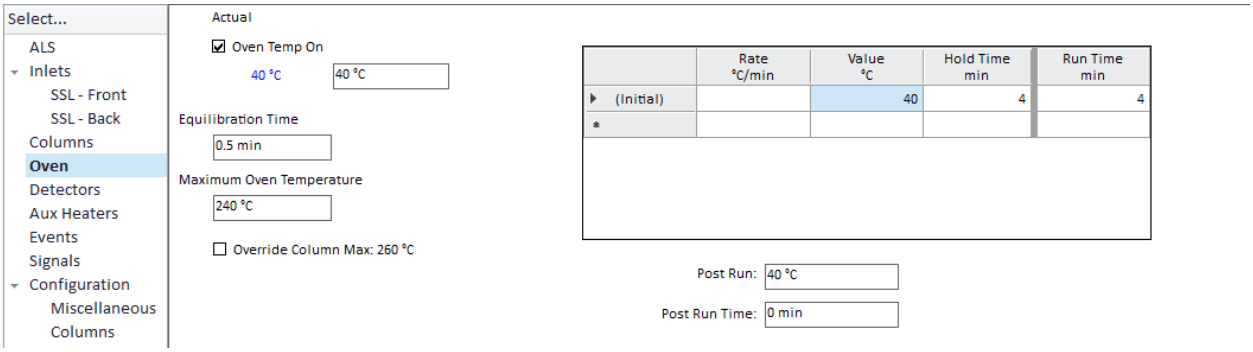

Select...
ALS
Inlets
SSL - Front
SSL - Back
Columns
Oven
Detectors
Aux Heaters
Events
Signals
Configuration
Miscellaneous
Columns
Modules
ALS
Backflush
Readiness
GC Calculators
Split-Splitless Inlet
Select Liner...
Liner: Restek 23313; Lot 111209-1: 500 µL (Topaz Splitless straight liner)
Actual
Setpoint
Heater:
200 °C
200 °C
Pressure:
15.748 psi
15.749 psi
Total Flow:
47 mL/min
47 mL/min
Septum Purge Flow:
3.001 mL/min
3 mL/min
Inlet Mode (Split 10 : 1)
Split
Split Ratio:
10
:1
Split Flow
40 mL/min
Gas Saver (Off)
On
20 mL/min
After:
2 min

7.2.2 *Columns*

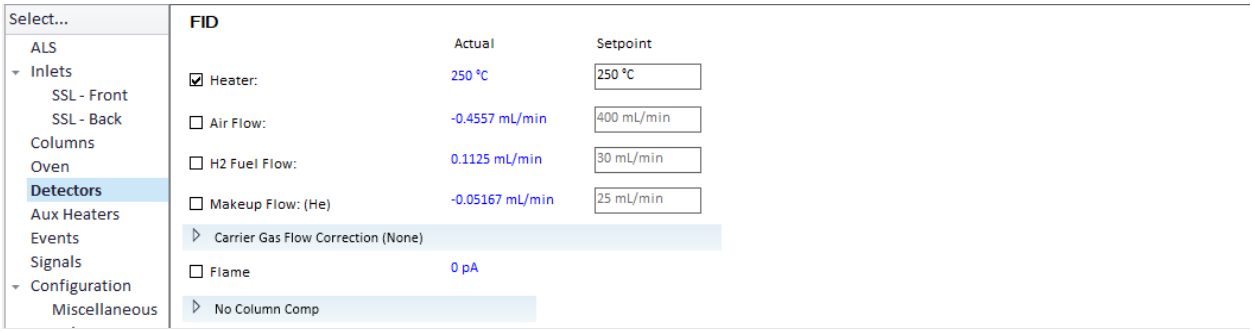

Select...
ALS
Inlets
SSL - Front
SSL - Back
Columns
Oven
Detectors
Aux Heaters
Events
Signals
Configuration
Miscellaneous
Columns
Modules
ALS
Backflush
Readiness
#
Selection
1
Front SS Inlet He --->
Restek 18004: 2037
RTX-BAC Plus 1
5 °C—240 °C (260 °C): 30 m x 320 µm x 1.8 µm
---> MSD
2
Back SS Inlet He --->
Restek 18006: 2038
RTX-BAC Plus 2
5 °C—240 °C (260 °C): 30 m x 320 µm x 0.6 µm
---> Front Detector FID
Aux EPC 1 He
Aux EPC 2 He
Aux EPC 3 He
Columns
Control Mode
On
Actual
Setpoint
Flow
1.42 mL/min
1.4203 mL/min
Pressure
1.433 psi
1.434 psi
Average Velocity
43.215 cm/sec
Holdup Time
1.157 min
(Initial): 0 min
He @ 40 °C Oven
Out: MSD
30 m x 320 µm x 1.8 µm
Constant Flow
Post Run:
0.44594 mL/min
Column #1 Configuration
Change Column...
Calibrate Column...
Lock Column...


Select...
ALS
Inlets
SSL - Front
SSL - Back
Columns
Oven
Detectors
Aux Heaters
Events
Signals
Configuration
Miscellaneous
Columns
Modules
ALS
Backflush
Readiness
#
Selection
1
Front SS Inlet He --->
Restek 18004: 2037
RTX-BAC Plus 1
5 °C—240 °C (260 °C): 30 m x 320 µm x 1.8 µm
---> MSD
2
Back SS Inlet He --->
Restek 18006: 2038
RTX-BAC Plus 2
5 °C—240 °C (260 °C): 30 m x 320 µm x 0.6 µm
---> Front Detector FID
Aux EPC 1 He
Aux EPC 2 He
Aux EPC 3 He
Columns
Control Mode
On
Actual
Setpoint
Flow
4 mL/min
4 mL/min
Pressure
15.748 psi
15.749 psi
Average Velocity
54.889 cm/sec
Holdup Time
0.91093 min
(Initial): 0 min
He @ 40 °C Oven
Out: Ambient Pressure
30 m x 320 µm x 0.6 µm
Constant Flow
Post Run:
0.71303 mL/min
Column #2 Configuration
Change Column...
Calibrate Column...
Lock Column...

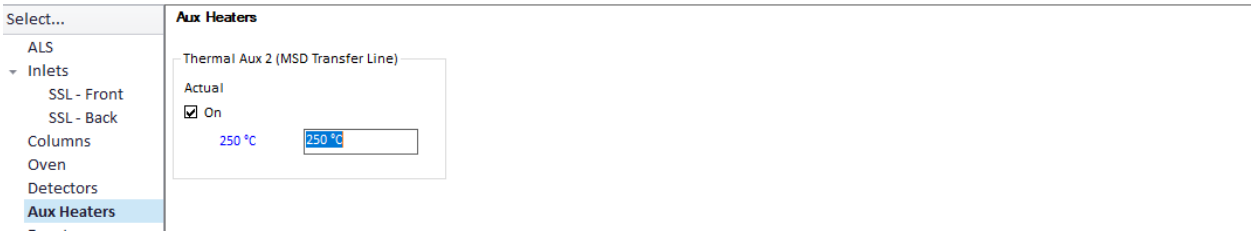
7.2.3 *Oven*



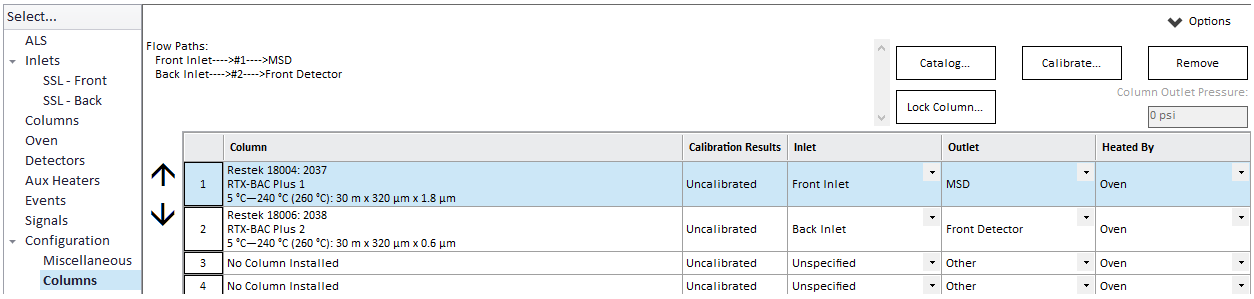
7.2.4 *Detector*



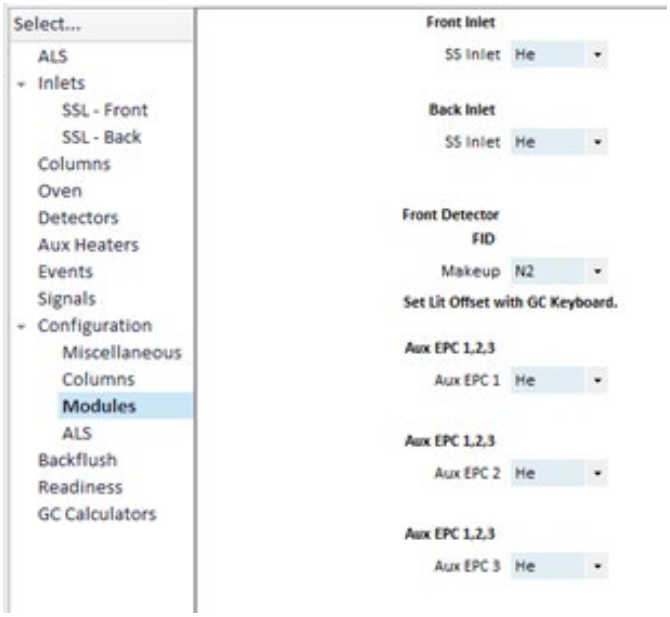
7.2.5 *Aux Heaters*



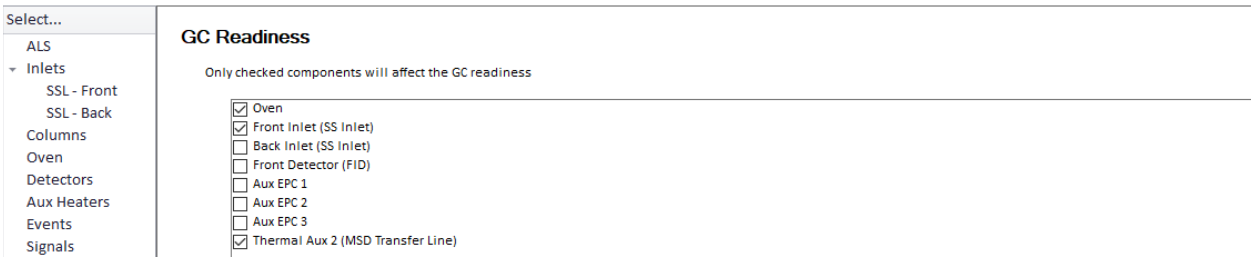
7.2.6 *Column Configuration*



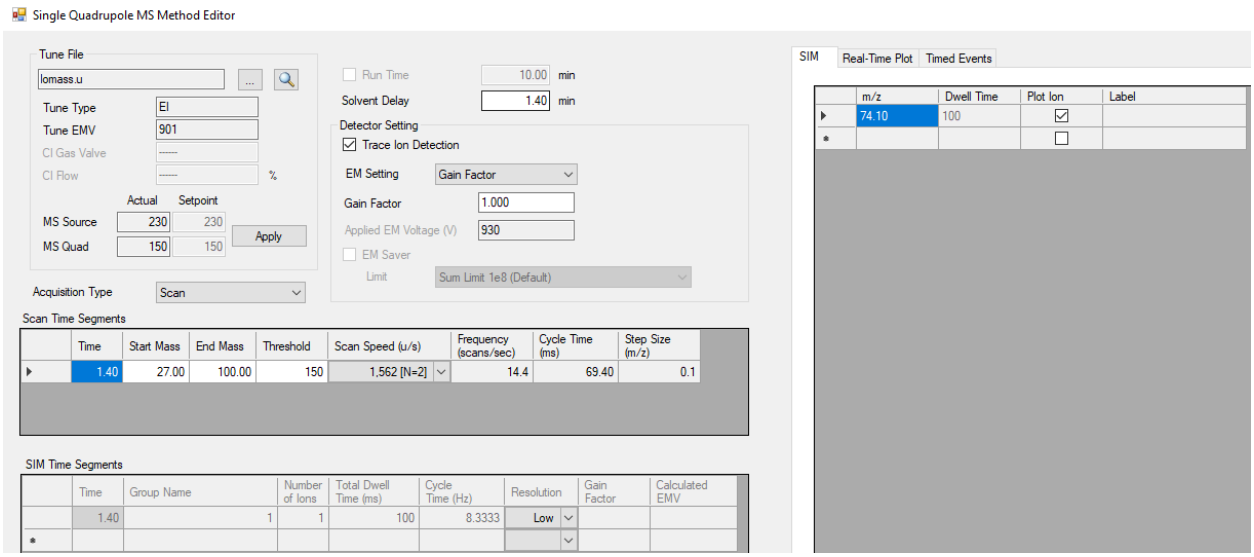
7.2.7 *Module Configuration*



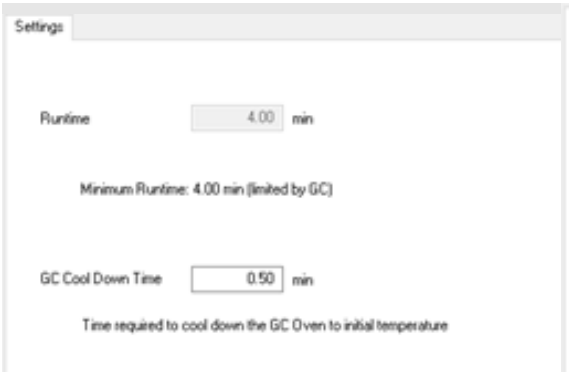
7.2.8 *GC Readiness*



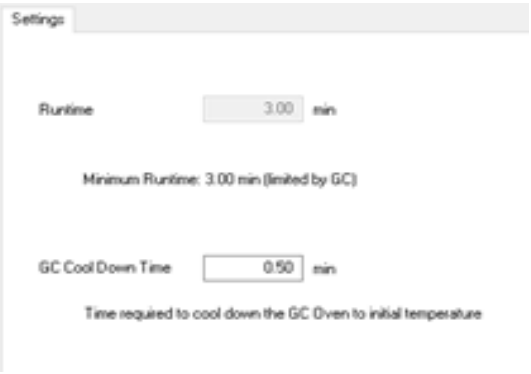
7.2.9 *Quadrupole Settings*



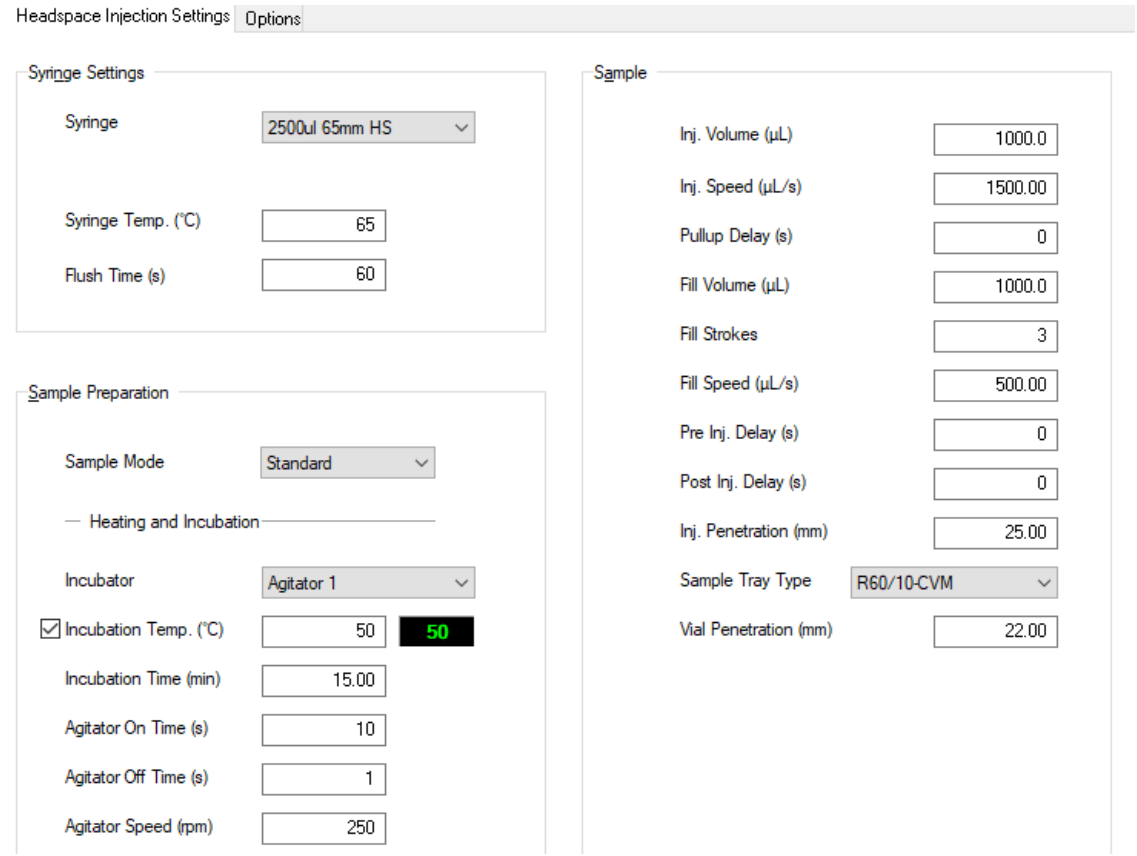
7.3 Gerstel AutoSampler Settings (FID and MSD)

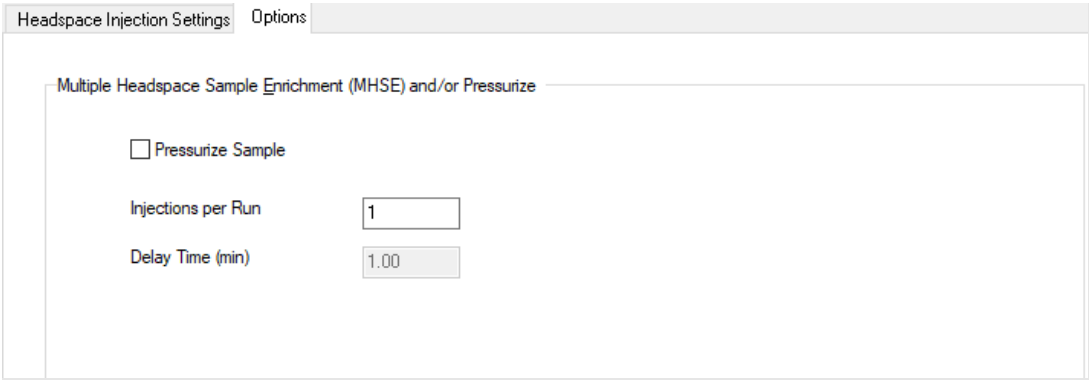


MSD (Screening)



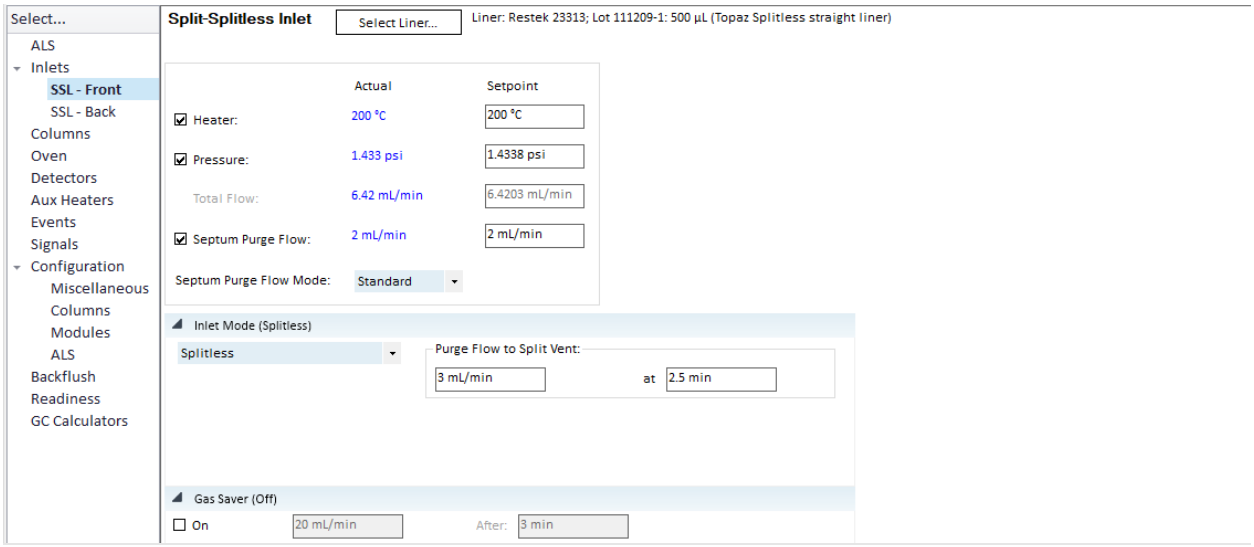
FID (Confirmation)

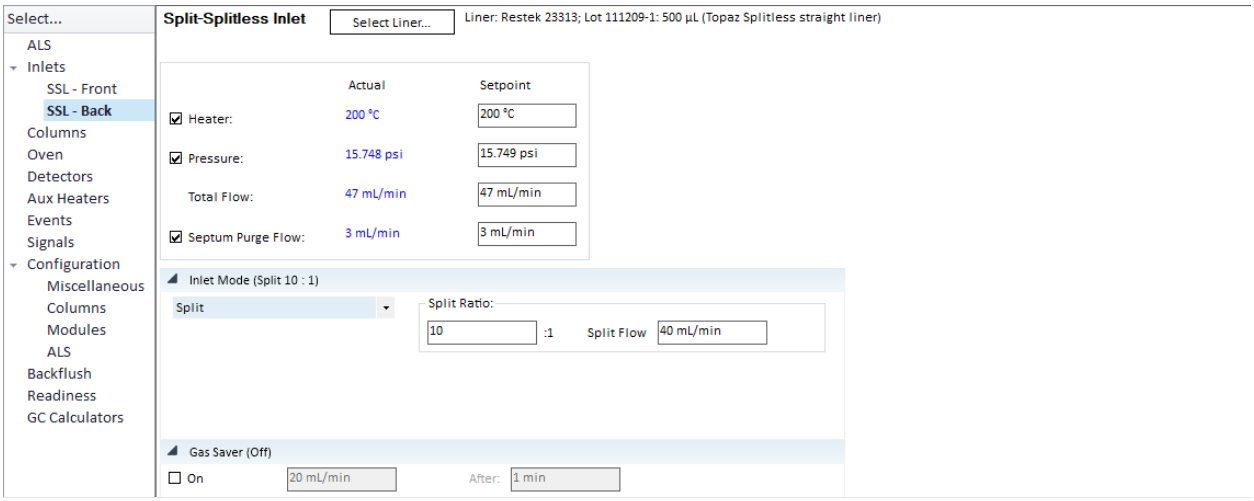




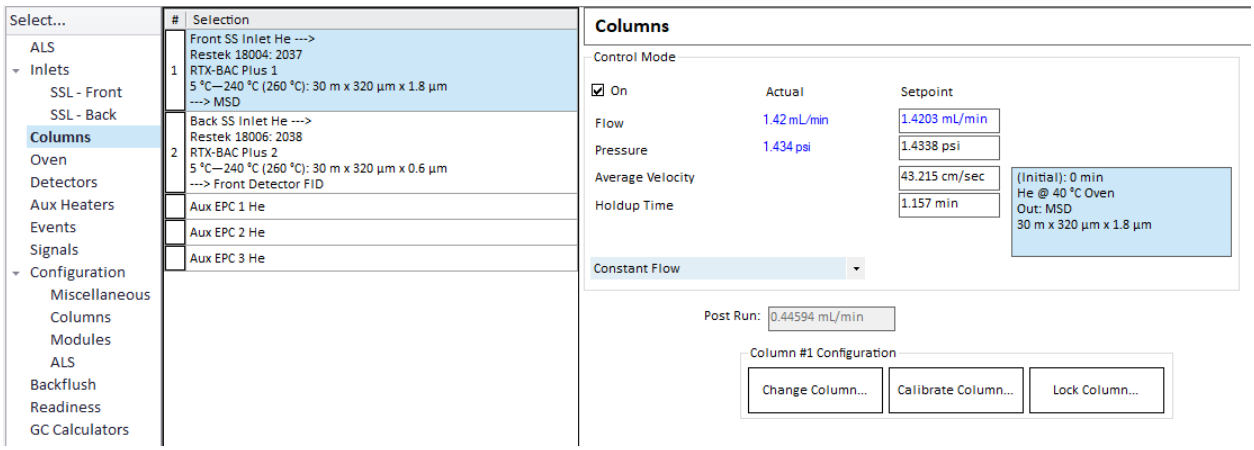
7.4 Flame Ionization Method (Confirmation)

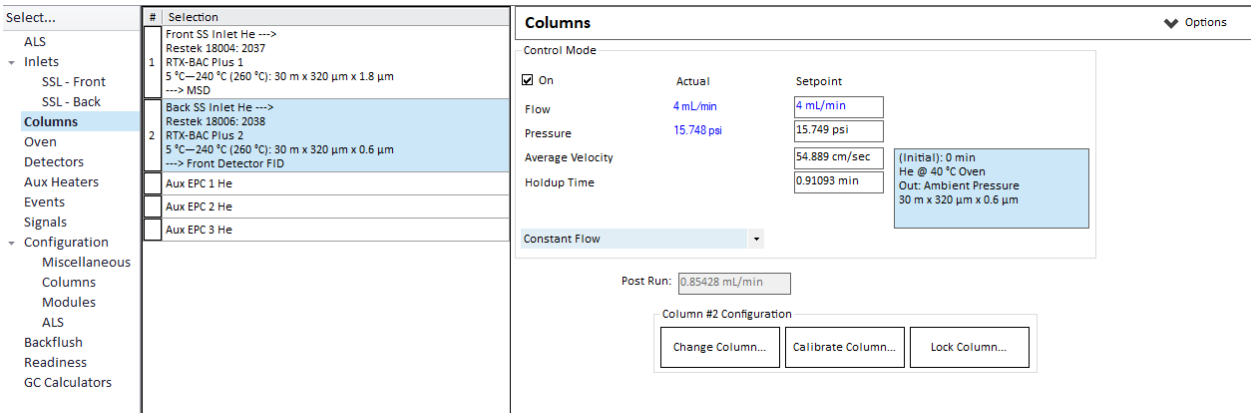
7.4.1 Inlets



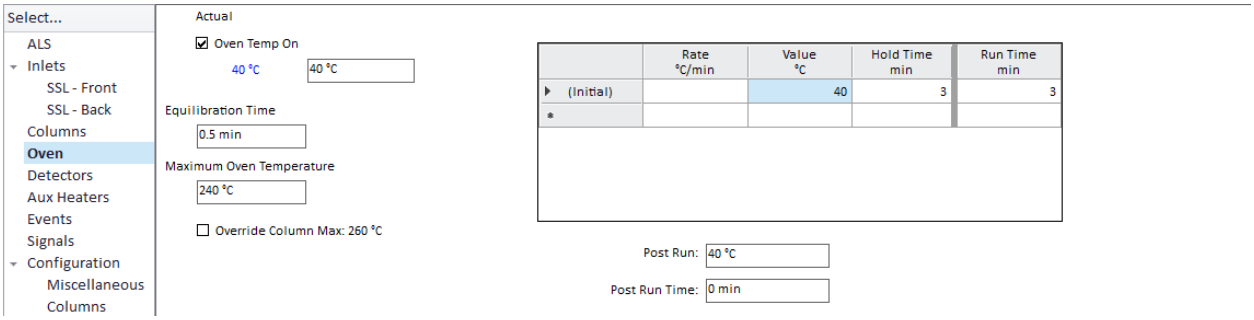


7.4.2 Columns

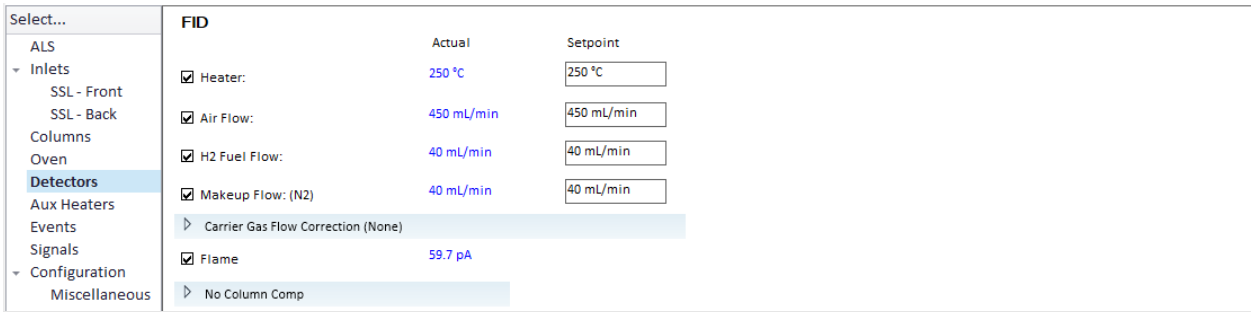




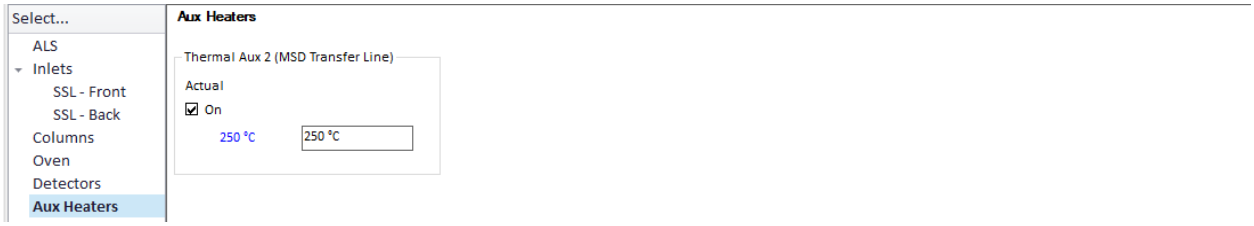
7.4.3 *Oven*



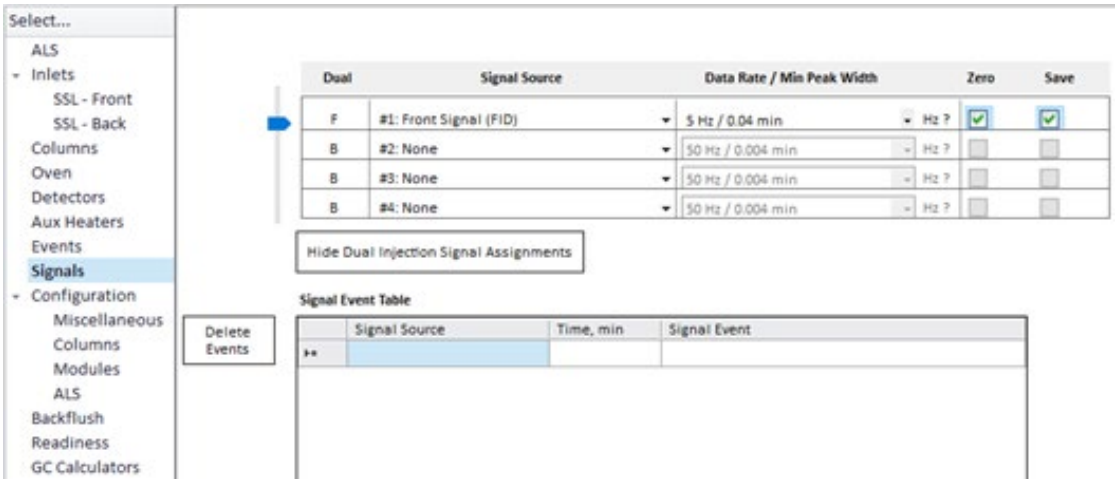
7.4.4 *Detector*



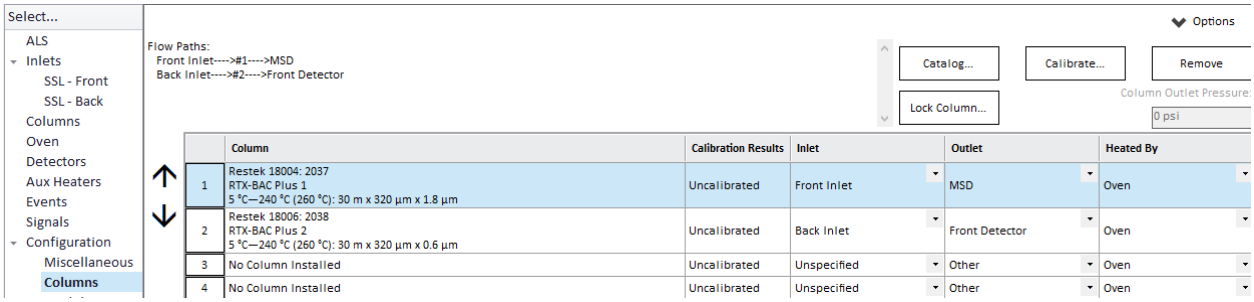
7.4.5 *Aux Heater*



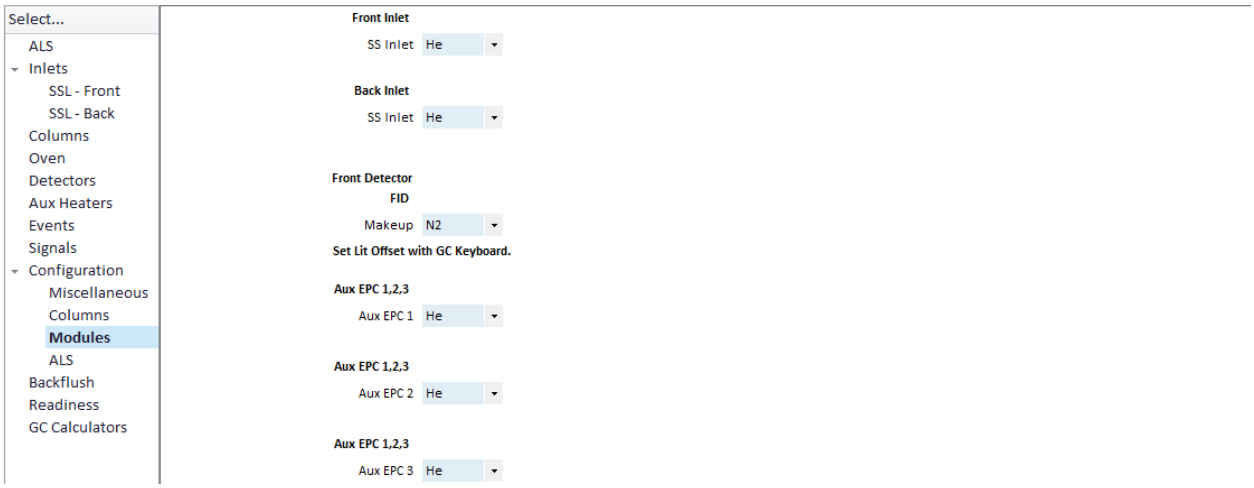
7.4.6 *Signals*



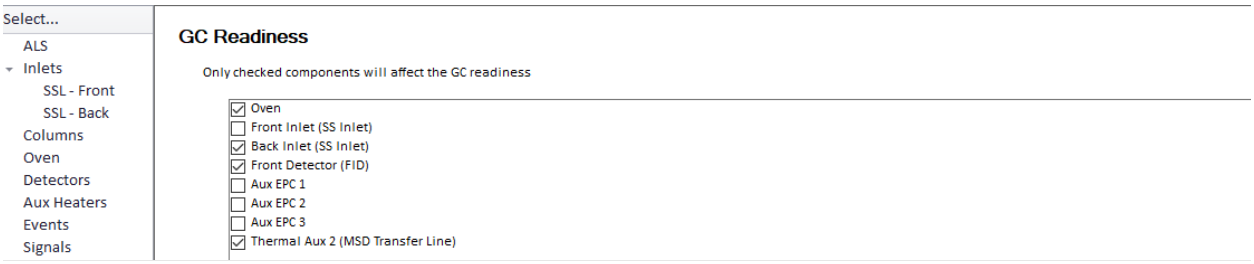
7.4.7 *Column Configuration*



7.4.8 *Module Configuration*



7.4.9 GC Readiness



8 DATA ANALYSIS

8.1 Decision Criteria

The following criteria are applied through automated data analysis via Agilent MassHunter software. Integration parameters may be adjusted to effect proper integration. See TOX-101 for further guidance.

8.1.1 FID Method

8.1.1.1 Integration Criteria

Analyte	RT	%RT	Criteria	Integrator	Peak Filter
Methanol	1.227	10	Close RT	Agile2	Area ≥ 3000 counts
Ethanol	1.477	10	Close RT	Agile2	Area ≥ 3000 counts
Acetone	1.593	10	Close RT	Agile2	Area ≥ 50000 counts
Isopropanol	1.683	10	Close RT	Agile2	Area ≥ 3000 counts
T-butanol	1.857	10	Close RT	Agile2	Area ≥ 3000 counts

8.1.1.2 Calibration Criteria

Analyte	Curve Fit	Origin	Weight	Accuracy (+/-)	Levels (g/100mL)
Methanol	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400
Ethanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400, 0.500
Acetone	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400
Isopropanol	Linear	Ignore	$1/x^2$	15%	0.010, 0.025, 0.050, 0.100, 0.200, 0.400

8.1.1.3 Control Criteria

Analyte	Accuracy (+/-)	Levels
Methanol	15%	Cliniqa 1, 2
Ethanol	10%	Cliniqa 1, 2
Acetone	15%	Cliniqa 1, 2
Isopropanol	15%	Cliniqa 1, 2

8.1.1.4 Reporting Criteria

Analyte	Calculated Limit of Detection	Limit of Quantitation	Reporting Limit (Administratively Set)
Methanol	0.0019	0.010	0.005
Ethanol	0.0021	0.010	0.005
Acetone	0.0008	0.010	0.005
Isopropanol	0.0016	0.010	0.005

8.1.2 MSD Method

8.1.2.1 Integration Criteria

Analyte	RT	%RT	Criteria	Integrator	Quantifier Ion (m/z)	Peak Filter
Methanol	1.592	2	Close RT with Qualifiers	Agile2	31	Area ≥ 3000 counts
Ethanol	2.010	2	Close RT with Qualifiers	Agile2	31	S/N ≥ 10
Isopropanol	2.456	2	Close RT with Qualifiers	Agile2	45	S/N ≥ 10
Acetone	2.668	2	Close RT with Qualifiers	Agile2	43	S/N ≥ 10
T-butanol	2.909	2	Close RT with Qualifiers	Agile2	59	S/N ≥ 10

8.1.2.2 Qualifier Ion Criteria

Analyte	Qualifier Ion (m/z)	Relative Response	Criteria (+/-)
Methanol	29	72.0	24%
	32	79.0	37%
Ethanol	46	31.0	15%
	45	76.0	15%
Isopropanol	43	25.0	28%
Acetone	42	7.7	15%
	58	39.9	15%
t-butanol	57	10.0	15%
	41	23.0	30%

8.1.2.3 Calibration Criteria (Semi-Quantitative)

Analyte	Curve Fit	Origin	Weight	Accuracy (+/-)	Levels (g/100mL)
Methanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Ethanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Acetone	Linear	Ignore	$1/x^2$	10%	0.010, 0.200
Isopropanol	Linear	Ignore	$1/x^2$	10%	0.010, 0.200

8.1.2.4 Control Criteria

Analyte	Accuracy (+/-)	Level (g/100mL)
Methanol	10%	0.010
Ethanol	10%	0.010
Acetone	10%	0.010
Isopropanol	10%	0.010

8.1.3 Batch Acceptance

8.1.3.1 Control Criteria

Target analyte(s) shall not be detected in the Negative Control. Positive Control(s) shall have all target analytes identified. The software will automatically flag any control values that fail to meet the conditions in Section 8.

8.1.3.2 Internal Standard

The internal standard shall be recovered for all samples. The software will automatically flag any samples that exceed 10% variation in response of the calculated mean of the calibrators for that batch.

8.2 Calculations

8.2.1 MSD Screening

Calibration is linear with $1/x^2$ weighting. A two point semi-quantitative curve provides an estimated analyte concentration. For additional guidance, refer to Section 8.1.2.3 and TOX-101.

8.2.2 FID Confirmation

Calibration is linear with $1/x^2$ weighting. A six or seven point calibration curve is used to provide quantitative results (analyte dependent). Case samples are analyzed in duplicate and the values are averaged. For additional guidance, refer to Sections 8.1.1.2 and TOX-101.

8.2.3 Characterization of Whole Blood Controls

For commercial volatiles controls, each newly acquired lot of control will be analyzed at least 20 times in a minimum of four batches. The initial target value for the new control will be the average of these 20 values. Should the initial calculated value of the control exceed ± 5% of the nominal value for ethanol (or ± 0.005 g/100mL, whichever is greater), or ±10% of the nominal value for any of the other volatiles, troubleshooting will occur before the control will be used in casework.

At least every six months, the accepted target value will be recalculated as the average value from all runs to date, excluding any failed analytical runs. Should the initial or recalculated target value of the control ever exceed ± 5% of the nominal value for ethanol (or ± 0.005 g/100mL, whichever is greater), or ±10% of the nominal value for any of the other volatiles, the control may be degrading and a new lot should be purchased and characterized. The Technical Leader will ensure that a database of the lot performance of each lot of volatiles control is maintained.

9 REPORTING

9.1 Measurement Uncertainty

Refer to CHEM-100 and TOX-101.

9.2 MSD Screening

Analytes that are identified above the estimated 0.010 g/100mL reporting limit are confirmed by FID quantitative analysis prior to reporting. If no analytes are identified, then the results are reported as not detected.

9.3 FID Confirmation

Analytes are reported according to the following scheme:

Scenario	
Quantitated ≥ 0.010 g/100mL	[analyte]: [concentration] [expanded measurement uncertainty]
Quantitated ≥0.005 < 0.010 g/100mL	[analyte]: less than 0.010 g/100mL
Quantitated <0.005 g/100mL	[analyte]: not detected
Quantitated > highest calibrator	[analyte]: > [highest calibrator] g/100mL

9.4 Quantitative Values

Replicate values are averaged. This average value is truncated to three decimal places. The method's expanded uncertainty value is rounded up to the third decimal place. The current *k* value and a truncated coverage probability of 99.7% are also expressed.

Example:
Ethanol identified: 0.051 +/- 0.006; Acetone identified: 0.097 +/- 0.012; Methanol: not detected; Isopropanol: not detected; reported units g/100mL (grams per 100 milliliters). Measurement uncertainty provided at the 99.7% confidence level, k=3.041

10 Corrective Measures

If any criteria listed in Section 8 are not met, some or all of the following action steps may be appropriate (refer to TOX-101 for additional potential responses to QC failure(s)):

- Not reporting results from the batch and/or affected case samples
- Reaccession and reanalysis of the batch and/or affected case samples
- Performing instrument maintenance
- Remaking or using new reagents, calibrators, or control materials
- Notifying the Technical Leader who will ensure the root cause is determined and appropriate actions taken to address the issue(s)

11 Performance Characteristics

11.1 LOD (MSD and FID)

Analyte	FID LOD Calculated (g/100mL)	MSD Reporting Limit (g/100mL)
Ethanol	0.0021	0.010
Methanol	0.0019	0.010
Acetone	0.0008	0.010
Isopropanol	0.0016	0.010

11.2 LOQ (FID)

Analyte	Calculated (g/100mL)	Quantitation Reporting Limit (g/100mL)
Ethanol	0.0065	0.0100
Methanol	0.0057	0.0100
Acetone	0.0025	0.0100
Isopropanol	0.0049	0.0100

11.3 Linearity (FID)

Analyte	(g/100mL)
Ethanol	0.010 – 0.500
Methanol	0.010 – 0.400
Acetone	0.010 – 0.400
Isopropanol	0.010 – 0.400

11.4 Precision (FID)

Analyte	Low (%)	High (%)
Ethanol	1.83	1.60
Methanol	1.92	1.81
Acetone	5.83	5.13
Isopropanol	2.15	1.70

11.5 Carryover

There was no significant carryover for the MSD or FID methods at 0.400 g/100mL (methanol, acetone, isopropanol) or 0.500 g/100mL (ethanol).

11.6 Processed Sample Stability

When secured in unanalyzed, sealed headspace vials, samples are stable for at least 15 days in refrigerated conditions. Once the septum on a vial is punctured, the analyte response will decrease, becoming less stable after 24 hours. Samples may be reanalyzed for up to 24 hours after the initial analysis for screening purposes (GC-MSD analysis). Quantitative analyses (GC-FID) will not be reanalyzed.

12 LIMITATIONS

12.1 Interferences

No interferences have been identified for this method.

12.2 Interpretation

Ethanol is normally present in the human body at low levels (<0.001 g/100mL) due to bacterial fermentation in the intestines. Ethanol can also be produced because of putrefactive processes,

attributed to post-mortem processes and/or sample storage conditions. Consequently, caution should be exercised in the interpretation of low ethanol results (<0.04 g/100mL) in post-mortem cases.

13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 REVISION HISTORY

Revision	Issued	Changes
18	06/15/2022	Document reformat. Additional performance characteristics described in Section 11.
19	02/01/2023	Updated storage conditions 5.6.1. Updated when to add an additional positive control 6.1.3 and 6.2.3.
20	03/06/2023	Clarified language in 3. Added storage tubes to 5.2. Clarified calibrator usage in 5.6.1. Added control storage guidance in 5.6.2. Clarified batch naming convention in 6.1. Updated 6.1.1 to be consistent with 6.2.1. Clarified batch naming convention and added optional intermediary container to 6.2. Added additional guidance for homogenizing samples and removed the option to dilute samples in 6.3. Updated Module Configuration screen capture in 7.2.7. Updated Gerstel AutoSampler Settings (FID and MSD) screen capture in 7.3. Updated Signals screen capture in 7.4.6. Updated qualifier ion criteria in 8.1.2.2. Clarified language used in 8.2.3.