GCMSD-Headspace Analysis SOP

Before you start –

- Method and Sequences names are restricted to 50 characters (MSD program will crash otherwise)
- The GC oven and Injection Ports need to be cooled to 50 oC to do the switch over from GC ALS to Headspace
- Read and understand each of the following Sections. Snap shot of key pages are at copied at the end of the written instructions.
- Know how to use a crimper and decapper.
- Know how to integrate and prepare a calibration curve.
- Know how to make precise measurements with the analytical balance to within 0.1 mg.
- Note that there is two method, the same in every instance except the one used for standard set tests has a timed event to turn off the detector during the major solvent elution. See Section 5, MS SIM / Scan Parameters section.

Section 1: Making the Standard Mix – Example Ethyl Acetate (EA)

- Transferring even 1 uL of Ethyl Acetate into a 20 CC vial and injecting only 0.1 mL of the headspace will saturate the detector.
- Take 5 mL of Chloroform (CHCl3), spike with 10 uL of EA. This is the standard mix.
- Keep this vial caped while performing the experiment.
- Make fresh standard mix every time performing a headspace experiment. Hard to keep the component from evaporating over time.
- It is not safe to keep a tightly sealed vial with volatile solvents.
- Take 1, 2, 5, and 10 uL of the standard mix and add to 20 CC vials, one at a time, and quickly cap and crimp. Label the vial before going on the next aliquot.
- If repeat injections are needed. Need to prepare additional vials. Do not inject from the same vial more than once.

Section 2: Testing the Linearity

- Make 0.1 uL injections from each of the 4 standard mix vials prepared in Section 1.
- Integrate the EA signal
- Plot standard mix volume vs. EA signal intensity. This should be linear.
- If not linear, the volume transfer to make the standard set could have been inaccurate, or waited too long to cap the vials, or did not crimp the vials properly.

Section 3: Making the Solid Samples

- This step will require having at least 200 mg of sample.
- Each time the original sample vial is opened, transfer of an aliquot must fast and the vial re-capped immediately.

- Adjust the method according to sample availability.
- Transfer various amounts of the solid that is being tested for residual solvents into 20 CC vials.
- Example – 3, 8, 15, 40 mg
- Prepare one vial at a time and quickly.
- As soon as the solid sample is added to the headspace vial and the weight is measured, cap and crimp the vial before moving on to the next vial.
- Remember, you are measuring for components that are volatile.
- Label vials after they are capped. Write the id and weight of the solid sample on the bottle.

Section 4: Converting GCMSD to Headspace from Liquid Injection mode.

- At the beginning of this process, you should have turned off the oven and the both of the injection ports. Check to see if the temps are at or near 50^{o}C. If not, turn them off now and wait until they are safe to work with.
- Using a few tissues to protect your skin, undo the column from the front injection port and attach it to the rear injection port.
- Check the ferrule to see whether you need to start with a new one.
- Modestly tighten with a wrench, 1/8th of a turn past the point of being finger tightened.
- If you get pressure or flow warning, tighten a little more.
- If the pressure warnings continue, you will have to re-do with a new ferrule.
- At this point, do not run any methods that use GC ALS in the Inlet and Injection Parameters page. Headspace only.

Section 5: Writing the Headspace Method

- Load a default Headspace Method.
- From the top, left, menu bar, select Method, then Edit Entire Method.
- Below is a walkthrough of each page that opens for modification. Leave pages or settings that are not mentioned here the way they appear.
- Inlet and Injection Parameters
    a. Key here is to select Headspace for Injection Source. For liquid injection, this would have been GC ALS.
    b. Headspace is attached to the rear inlet.
    c. Inlet Location should be Rear
    d. MS Connected to Rear Inlet
- Headspace Edit Parameters
    a. Temperatures
        i. Temperatures are set in increasing trend from Oven (this refers to the headspace oven, not the GC oven) to Loop (injection loop) to Transfer Line.

ii. These values need to be adjusted based on thermal stability of the analyte being studied.
iii. 100, 100, and 115 ^{o}C worked well with EA.

b. Times
   i. The higher the selected oven temperature, above, the longer it will take for the vial to come into equilibrium to that temperature.
   ii. Also, some time is needed to build a headspace of the solid sample in the vial.
   iii. Partial heating could result into under-estimation of the residual solvent.
   iv. Standard addition techniques (spiking the sample with known amounts of a standard and testing the recovery) may have to be used to validate the protocol.
   v. For the EA study, Vial Equilibration time of 15 min, injection duration of 0.5 min, and GC Cycle time of 27 min worked well.
   vi. GC Cycle time has to include the time it takes for the GC oven to cool back down to the initial temperature. The lower this temperature, the longer it will take. For a 20 minute GC method, with the initial temperature of 50 ^{o}C, 7 minutes of cooling time had to be allowed.
   vii. If this time is under-estimated the Headspace injections and the GC runs will become out of sync.

c. Vial and Loop
   i. Currently, we only use 20 CC headspace vials.
   ii. Shake vials while in oven feature was used at its lowest setting.
   iii. Solid samples do not require as much as liquid samples.
   iv. Fill mode of Constant Volume should be selected.
   v. Fill volume is set based on the expected concentration of analytes in the test samples and the calibration method.
   vi. For the EA study, 0.1 mL was used.
   vii. Pressure equilibration Time should be adjusted based on the Fill Volume. Higher the volume, the more time is needed.
   viii. Pressure equilibration Time of 0.1 min for a Fill Volume of 0.1 mL is appropriate.
   ix. You can use this ratio to fine tune your method for larger fill volumes.

d. Carrier
   i. There is no options to be selected here.
   ii. The GC method will dictate this part.

e. Advanced Functions
   i. Extraction Mode, use Single Extraction.
   ii. Venting and Purging, check the Vent vial pressure after extraction option and use default settings for Post-injection purge.
   iii. Dynamic Leak Checking, use default Acceptable leak rate.

f. Sequence Actions
   i. Use Skip option if the robot encounters Vial Missing, Wrong Vial Size, and Leak Detected.

ii. On System Not Ready, the GC method and the Headspace Injections could have become out of sync. It will be best to abort the runs at this point.

g. Method Development
    i. This is for more involved headspace experiments.
    ii. Request additional training if you think you need these options.

- GC Edit Parameters
    a. Injector
        i. Select the back injector tab. You do not need to do anything with the front injector.
        ii. Notice that it is all “grayed out”.
        iii. The headspace module takes over this function.
    b. Skip Valve
    c. Inlet
        i. Select Back Inlet
        ii. Note that Heater is at 240 ^{o}C, the max temp of FFAP column
        iii. Pressure is selected as 11.6 psi. This value was selected based on constant flow conditions, taken from liquid injection experiments.
    d. Column
        i. There is only one column installed.
        ii. Use Constant Flow mode.
        iii. Enter 1.0 mL/min
    e. Oven
        i. This is the page where the temperature gradient is programmed.
        ii. Check the oven on option.
        iii. Temperatures below 50 ^{o}C, as starting point, are difficult to maintain.
        iv. Max temperature should be below column max allowed temperature.
        v. For FFAP column, it is 245 ^{o}C.
- MS SIM / Scan Parameters
    a. Do not change the Relative Voltage / EMV Mode as relative.
    b. This is optimized during instrument tuning steps (not covered here).
    c. Acquisition Mode is Scan mode.
    d. Real Time Plot time window is used to set how much of the GC run you want to observe in real time. This does not change the data being collected.
    e. Tune file should be atune.u; this is the only tune file that is optimized.
    f. Scan Parameters button brings up another page that allows you to set the Start and End of the Scan. Typically, air molecules are skipped over. Scan range of 33 to 550 is often used. Max range is 800.
    g. Timed Events button brings up MS Timed Events Table so that the detector could be turned off when very high amounts of any component is eluting from the column. This used during the standard set tests. The solution was made in mostly CHCl3. It was mainly used as a way of diluting EA and it is not needed to be quantitated. Therefore, during the standard set tests, the detector was turned off from 2.75 to 3.25 minutes.

- Note that two methods are needed to run the standard set and solid samples set in the same sequence.
    a. One method has timed events and it is used for all of the standard set tests.
    b. The other method does not have timed events and it is used for all of the solid sample tests.
    c. Based on the nature and the composition of the samples being tested, this step of the method needs to be designed properly.
    d. If unsure, ask for help.

Section 6: Typical Sequence Table

- First, inject from blank, empty, vials to ensure that the system is clean.
- Inject as many times as needed. However, if after two injections, which can be done from the same blank vial, you do not see a clean signal, ask for help.
- Once the system is proven clean, you want to inject the standard set from low to high concentration.
- Inject from a blank vial to ensure there is no carry over.
- Now inject from the solid sample set, starting from low to high amounts.
- For residual solvent experiments, the expected concentrations are very low and therefore very low possibility of carry over. Blanks in-between test samples are most likely not needed.
- When in doubt, always verify your protocol.

Section 7: Calculating the Quantitative Results

- You need to be able to calculate
    a. From the volume of the standard mix added to each of the standard set headspace vials, size of the vials, injection volume, and the density of the analyte being quantitated, to the amount loaded on column.
    b. From signal intensities and amounts loaded on column, you can get the equation for the calibration curve.
    c. From the signal intensities of solid samples and the calibration curve equation (slope and intercept), you can calculated the residual solvent amounts.
    d. Residual solvent amounts as a ratio of the total weight of the solids in each of the vials, reported in ppm, should be within 15%.
    e. Most protocols require this level of accuracy.
    f. For measurement taking more than one day, prepare fresh standards and include a few samples from already measured in the previous day to show the accuracy of day to day measurements.
    g. Repeat injections are dictated by external protocols. If required, adjust the sample prep steps.
- For 1 uL of EA standard mix that was prepared by spiking 10 uL of EA (density 0.902 g/mL) into 5.0 mL of CHCl3, evaporated all into the gas phase by heating in the

headspace oven, and 0.1 mL of the headspace injected on column is equal to 9.0 ng of EA added on column.

- Overall equation → =(F5*(10/5010))/1000*0.902*1000*1000*1000*(0.1/20), where F5 in this case is 0.1 mL.
    a. On column = (volume of std mix*Ratio of EA in std mix)*density of EA*Fraction of injection
    b. Ratio of std mix = (10 uL of EA / 5010 uL total volume)
    c. Std. Mix made by spiking 10 uL of Ethyl Acetate in 5.0 mL of CHCl3 = (10/5010) Ratio
    d. Std. Analyte density is in g/mL. EA = 0.902 g/mL.
    e. Don't forget about unit conversions.
    f. Here is an example table.

Std. Mix Volume (uL)	HS vial Volume (mL)	Injection Volume (mL)	total on column (ng)
1	20	0.1	9.0

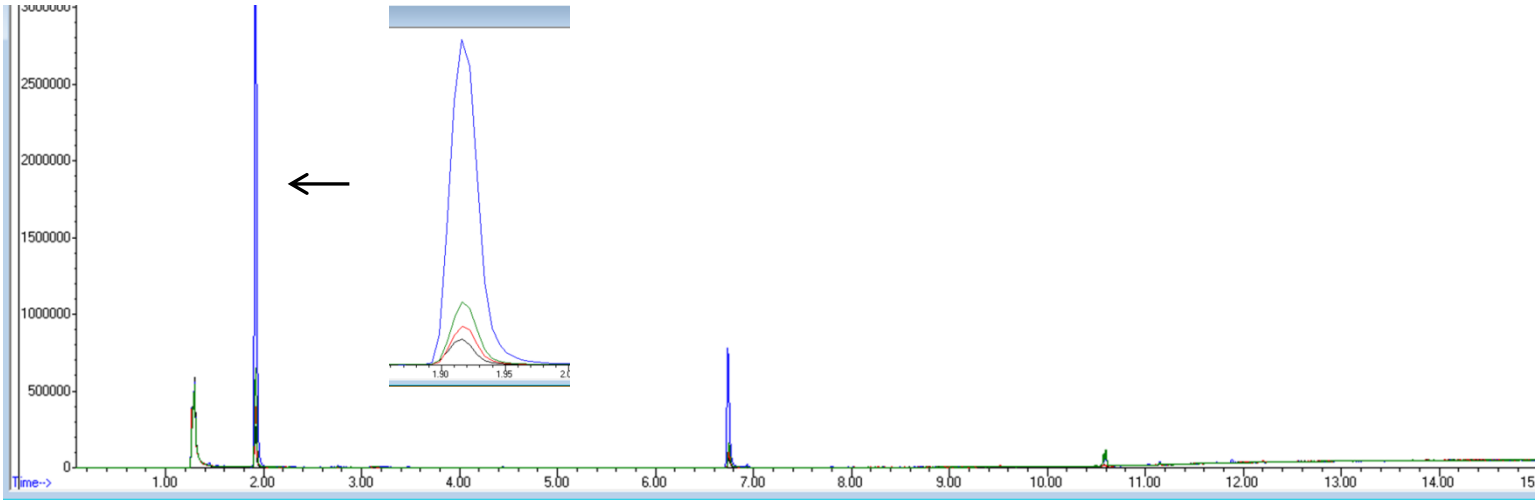
- Below is a table showing that from signal intensity of the analyte peak from each of the standard mix vials and on column calculations, a calibration curve can be plotted. The equation of the Linear fit, i.e. m and b values, can be used to determine the amount on column from the intensity of the analyte signal from solid sample test set.
- Note that in the below table, the Ratios of analytes in the solid samples in ppm is about the same. This is because in the calculations, the ratio is normalized to the size of the sample.
    a. Ratio (ppm) = Amount on column (ng) / Solid Sample (mg)] * 1E6

Vial Loading Volume (uL)	Signal intensity (TIC)	Amount on column (ng)	
1	3.96E+06	9.0	
2	6.75E+06	18.0	
5	1.74E+07	45.0	
Equation	Y = 377878*X + 295530	Average	3.5
	m= 377878	stdev	0.5
	b = 295530	Rel. stdev	14.3 %
Solid Sample (mg)	Signal intensity (TIC)	Amount on Column (ng)	Ratio in Solid (ppm)
2.6	3.81E+06	9.3	3.6
5	5.73E+06	14.4	2.9

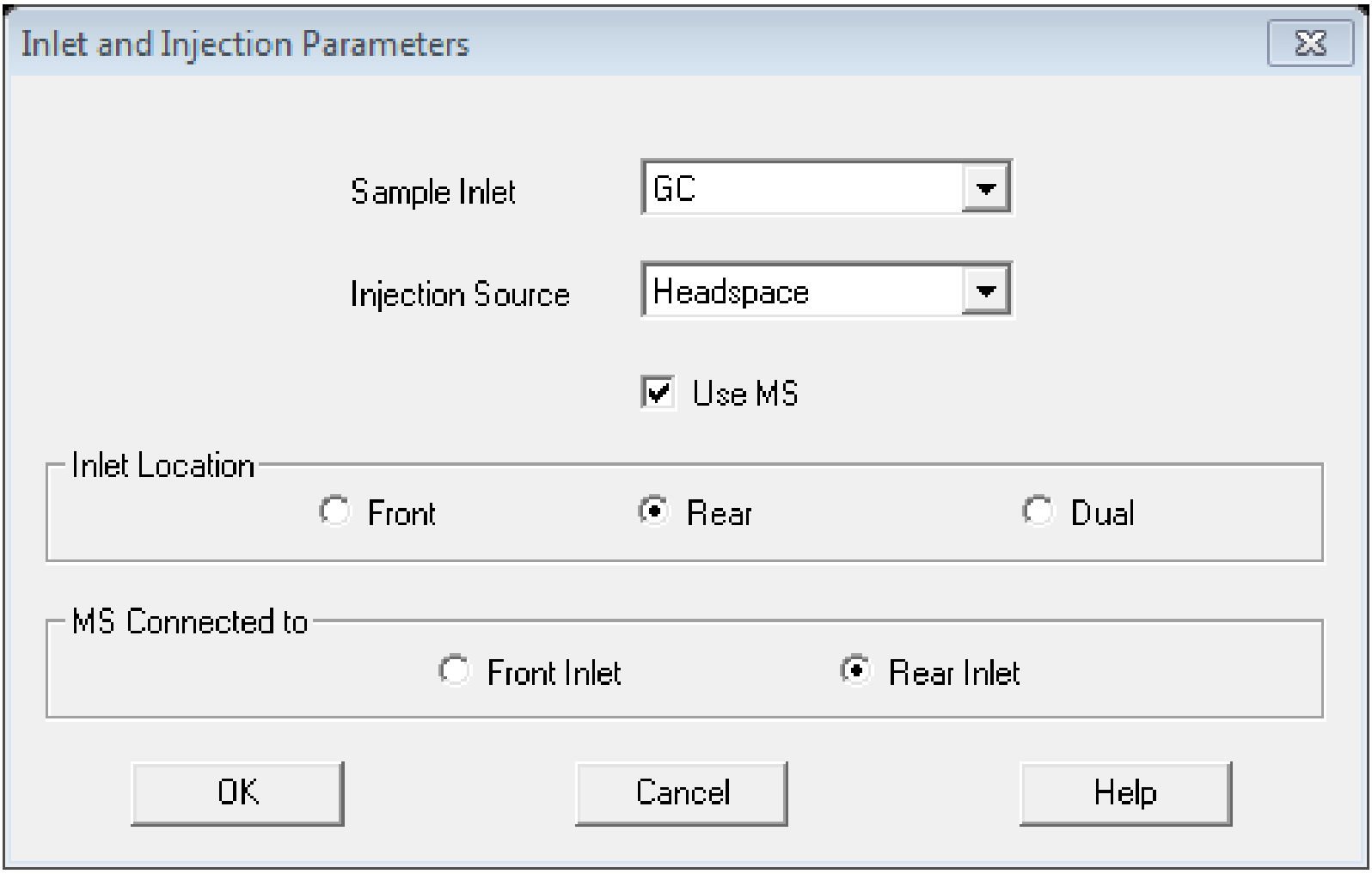
7.1	9.42E+06	24.1	3.4
33.6	5.16E+07	135.9	4.0

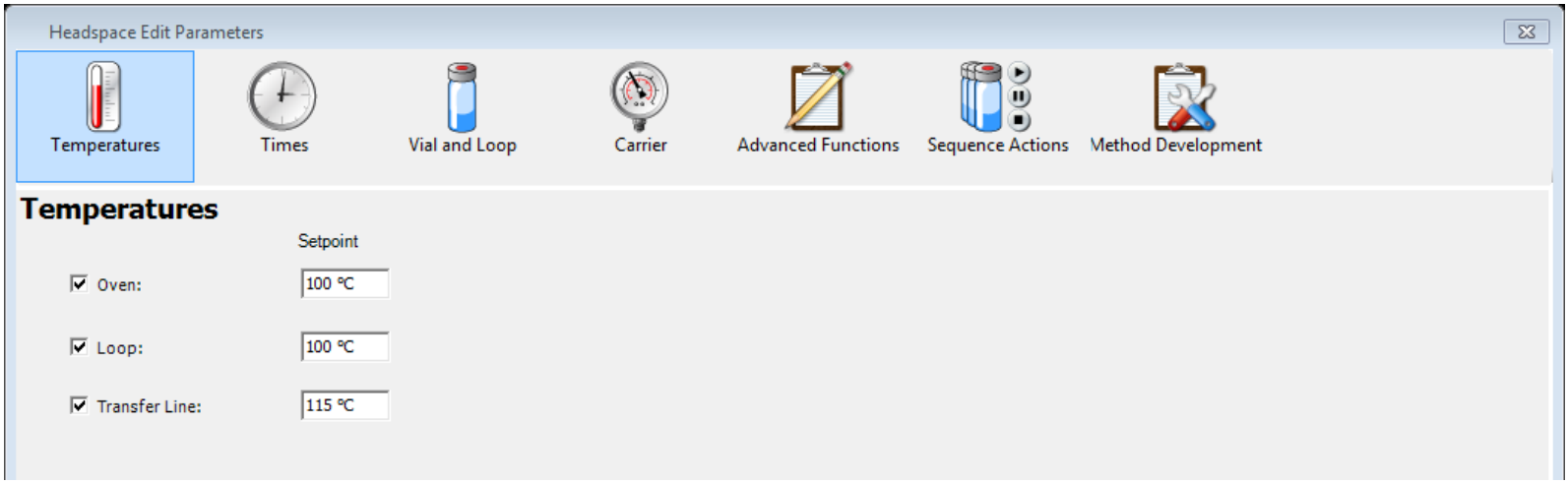
Section 8: Reporting

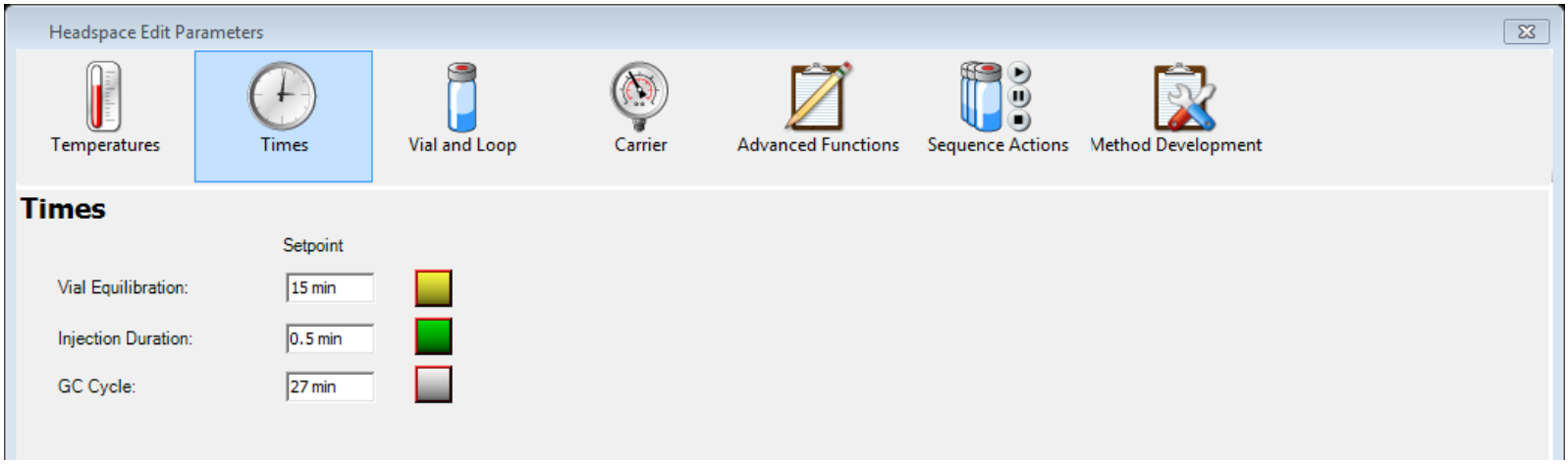
- Reporting needs to be modified based on the requester requirements. Below are a set of suggestions.
    a. Show a table of final results, Solid Sample Weight, Analyte Concentration in ppm.
    b. Show an overlay of chromatograms for each of the solid samples zoomed in on the analyte peak. This will show that, by increasing the solid sample weight, the analyte signal also increases.
    c. Show an overlay of the entire chromatogram.
    d. Identify any other peaks that may not have been quantitated but a library search shows a reliable, high confidence hit. Report these as an FYI and label the peaks as "potential i.d." since the next step would be injection of known standards that may not available and also not required by the requester.

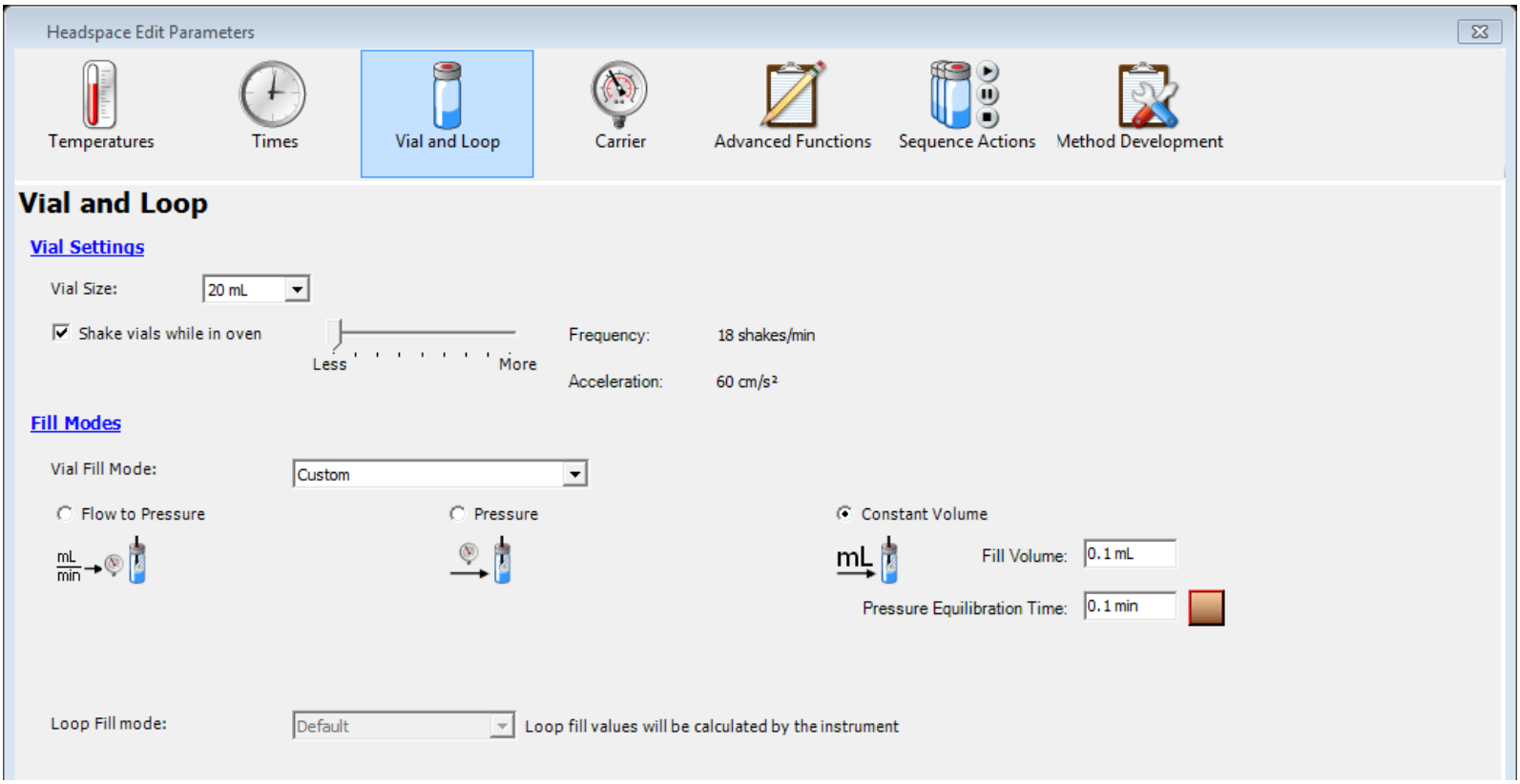


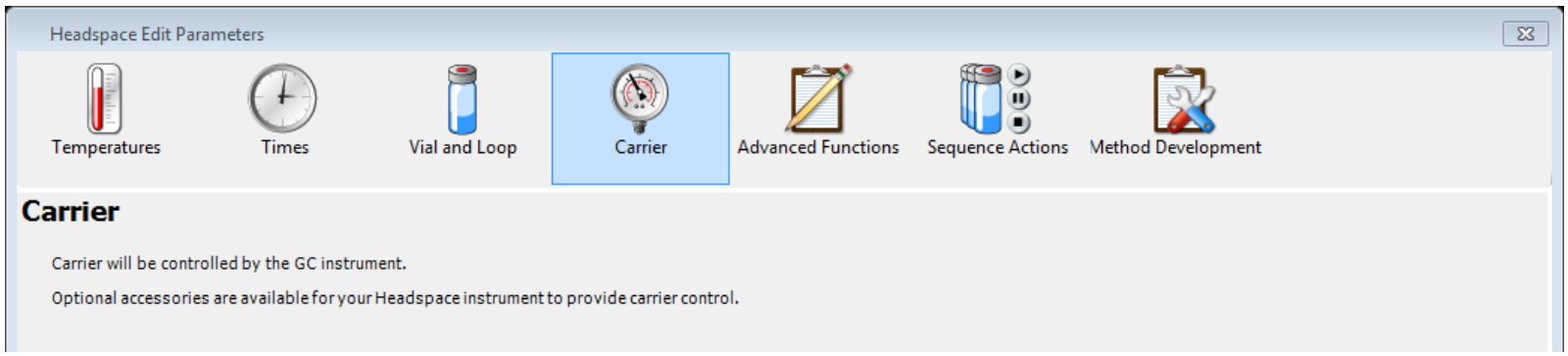
Snap Shots of Method Pages……

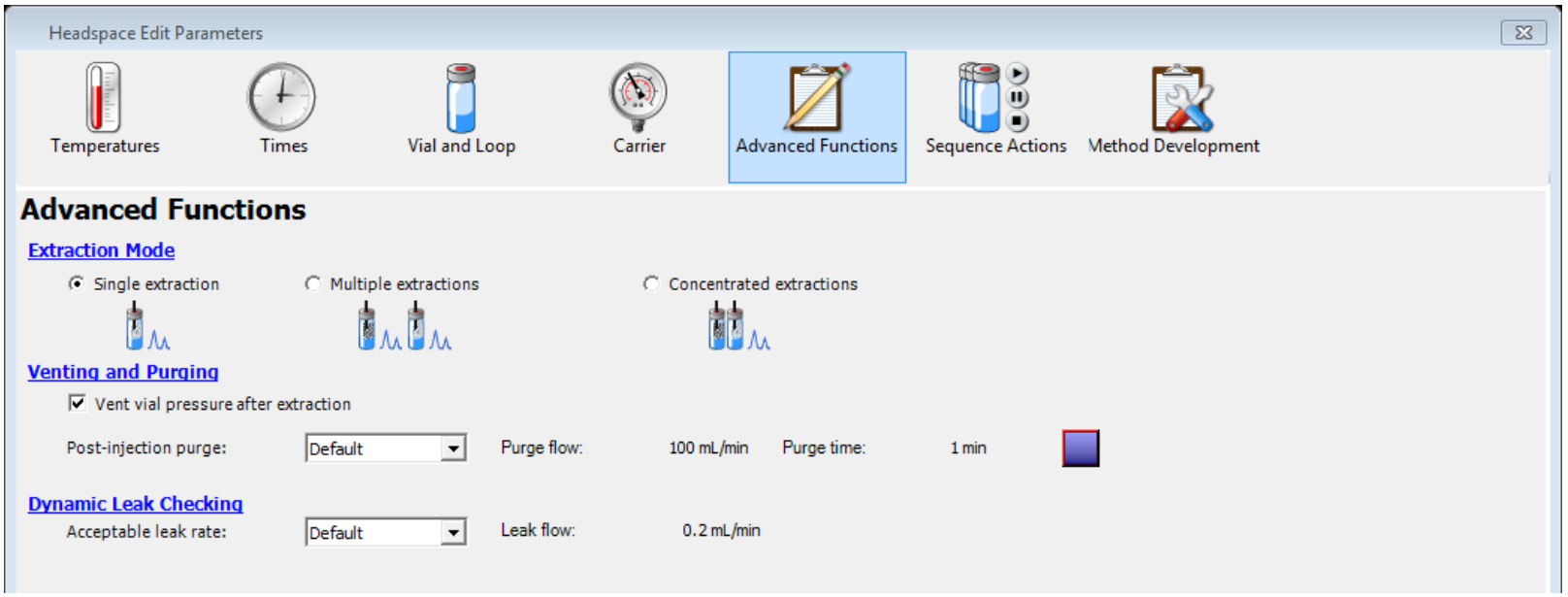


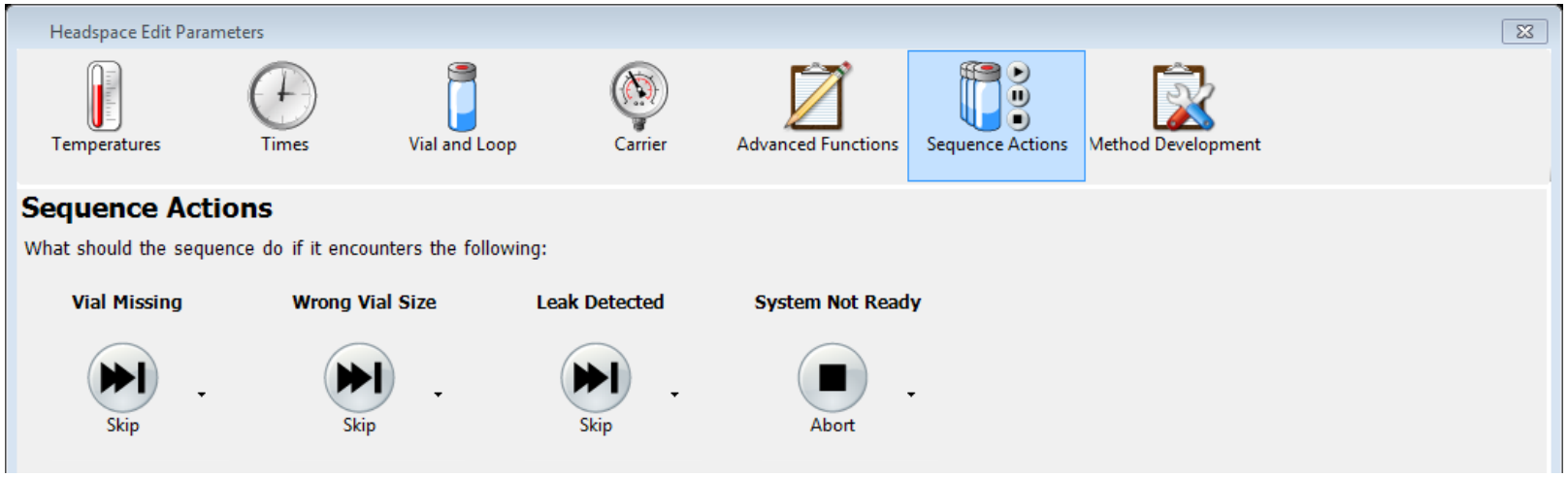


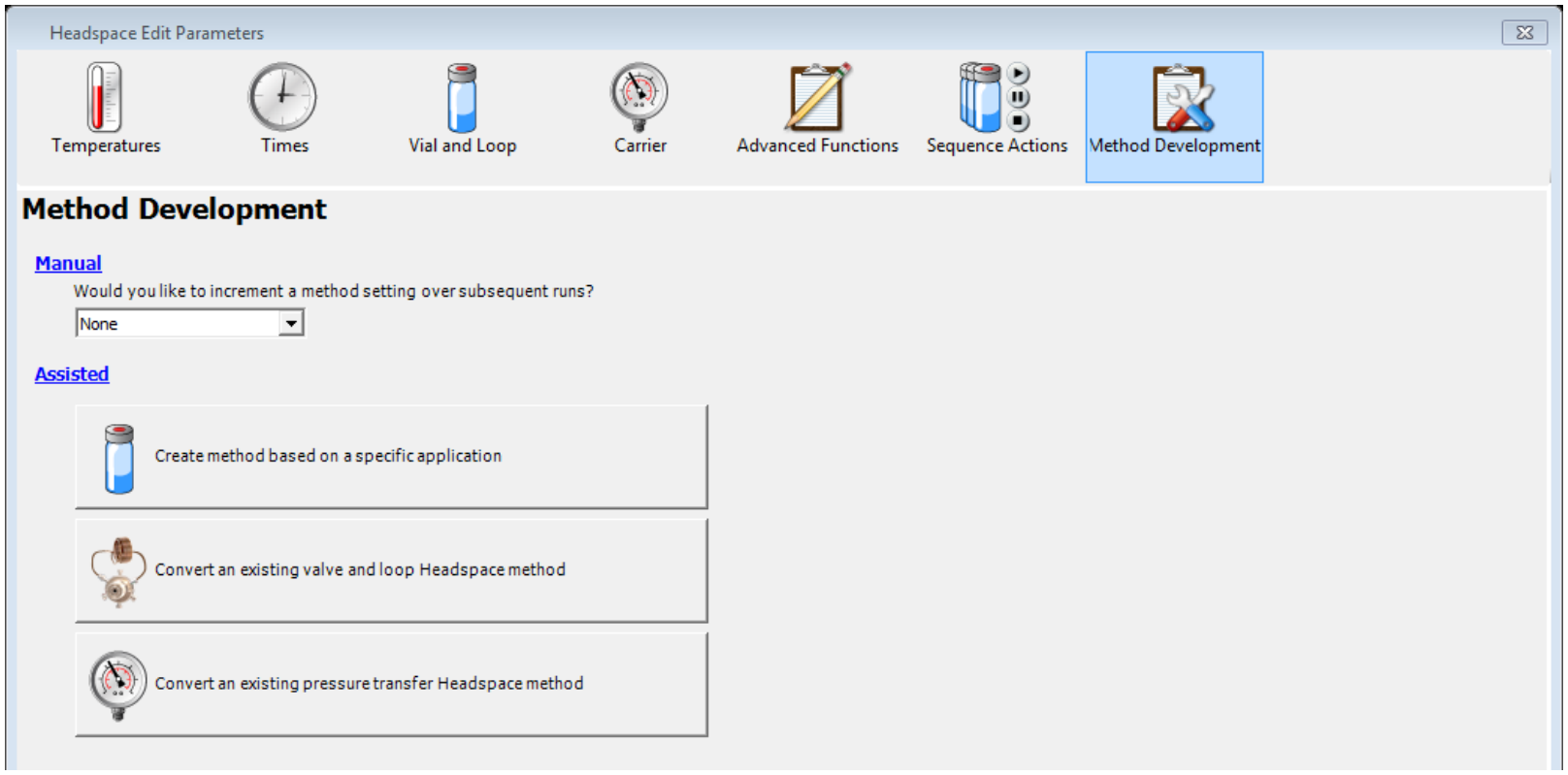

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Times
Setpoint
Vial Equilibration:
15 min
Injection Duration:
0.5 min
GC Cycle:
27 min

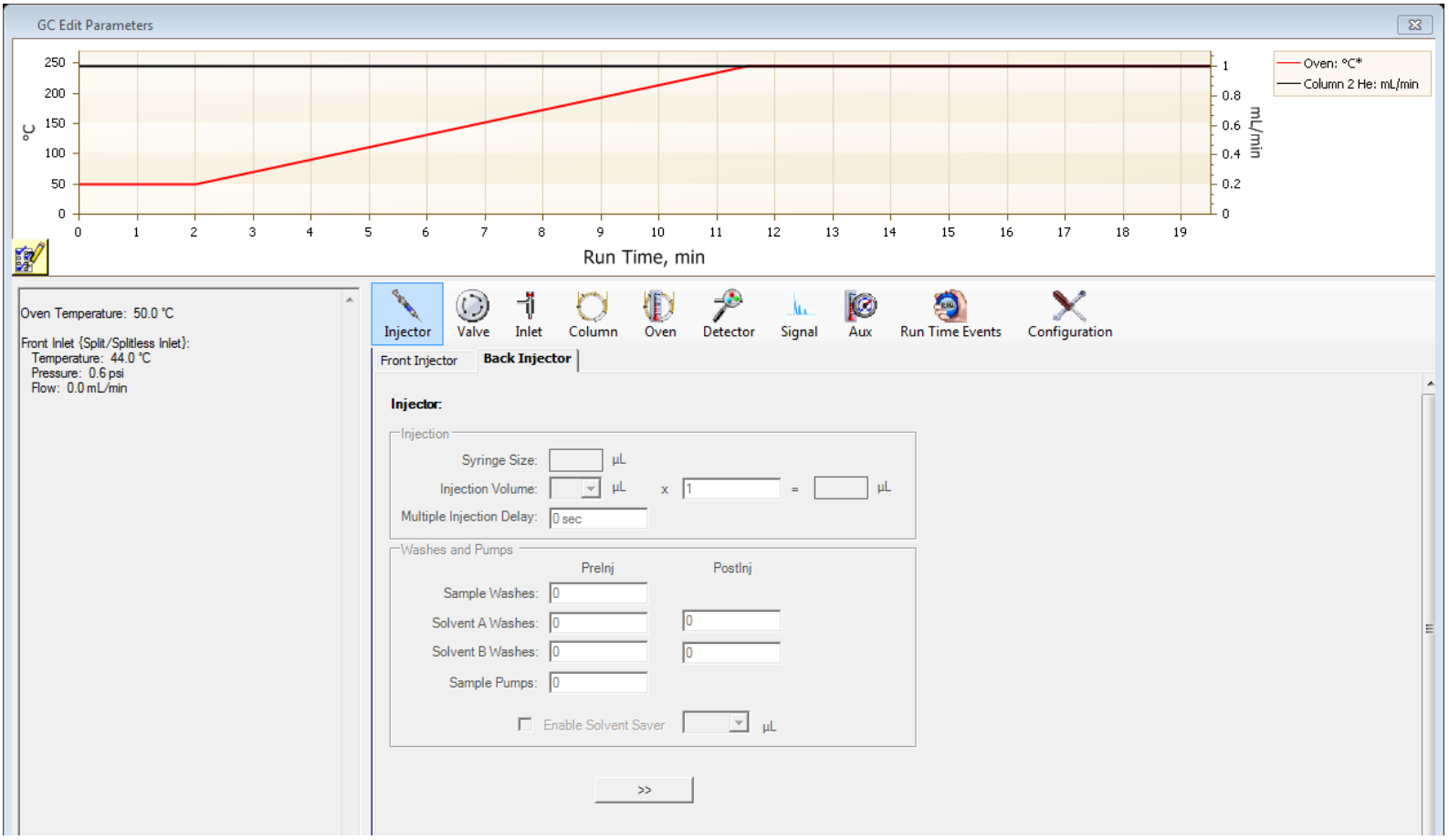

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Vial and Loop
Vial Settings
Vial Size:
20 mL
Shake vials while in oven
Less
More
Frequency:
18 shakes/min
Acceleration:
60 cm/s²
Fill Modes
Vial Fill Mode:
Custom
Flow to Pressure
Pressure
Constant Volume
Fill Volume:
0.1 mL
Pressure Equilibration Time:
0.1 min
Loop Fill mode:
Default
Loop fill values will be calculated by the instrument

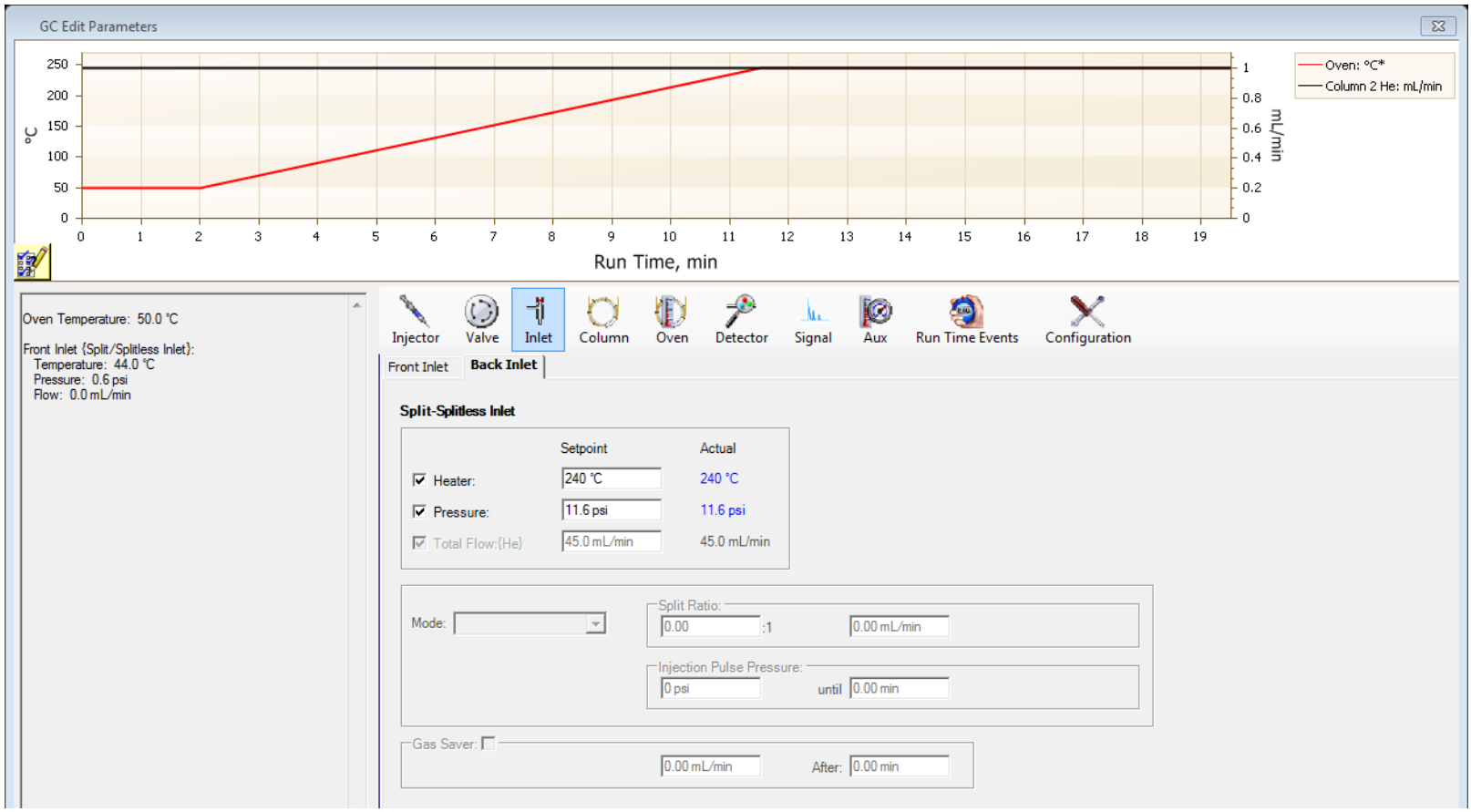

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Carrier
Carrier will be controlled by the GC instrument.
Optional accessories are available for your Headspace instrument to provide carrier control.

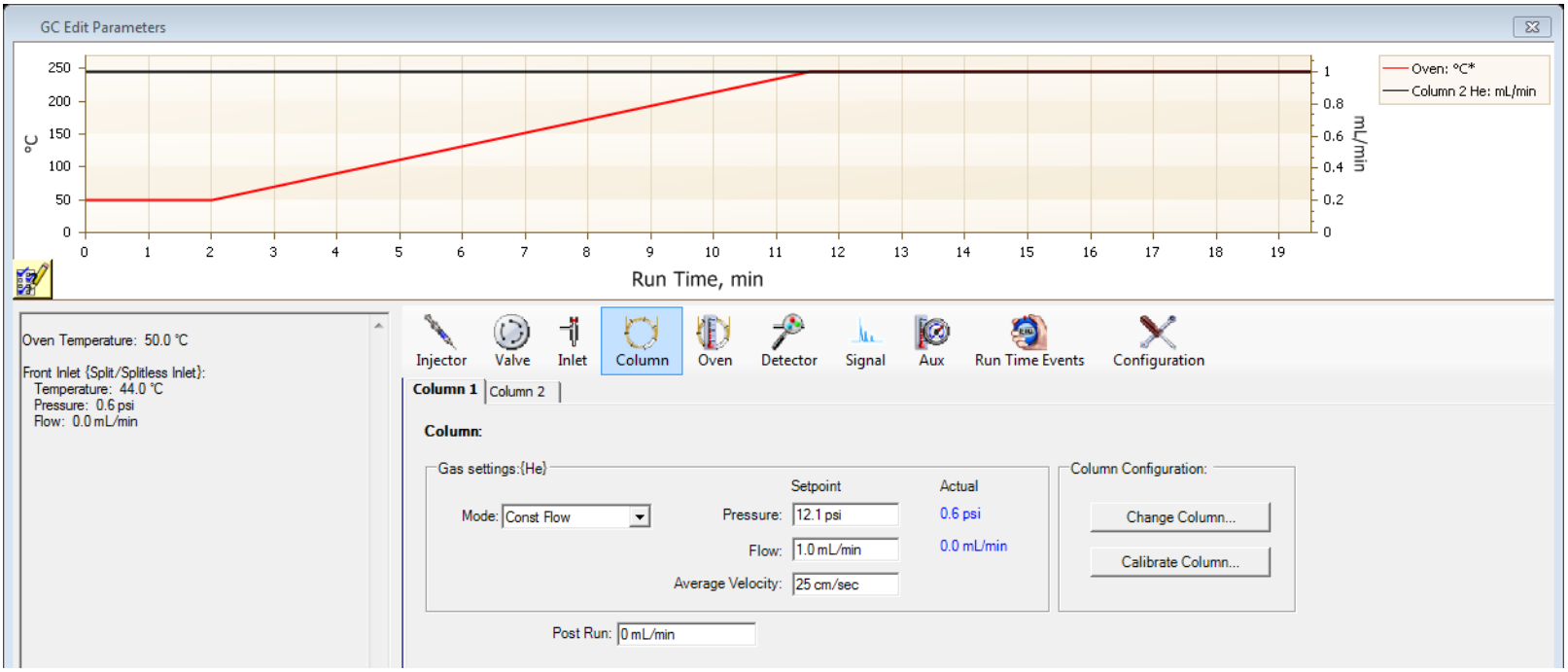

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Advanced Functions
Extraction Mode
Single extraction
Multiple extractions
Concentrated extractions
Venting and Purging
Vent vial pressure after extraction
Post-injection purge:
Default
Purge flow:
100 mL/min
Purge time:
1 min
Dynamic Leak Checking
Acceptable leak rate:
Default
Leak flow:
0.2 mL/min

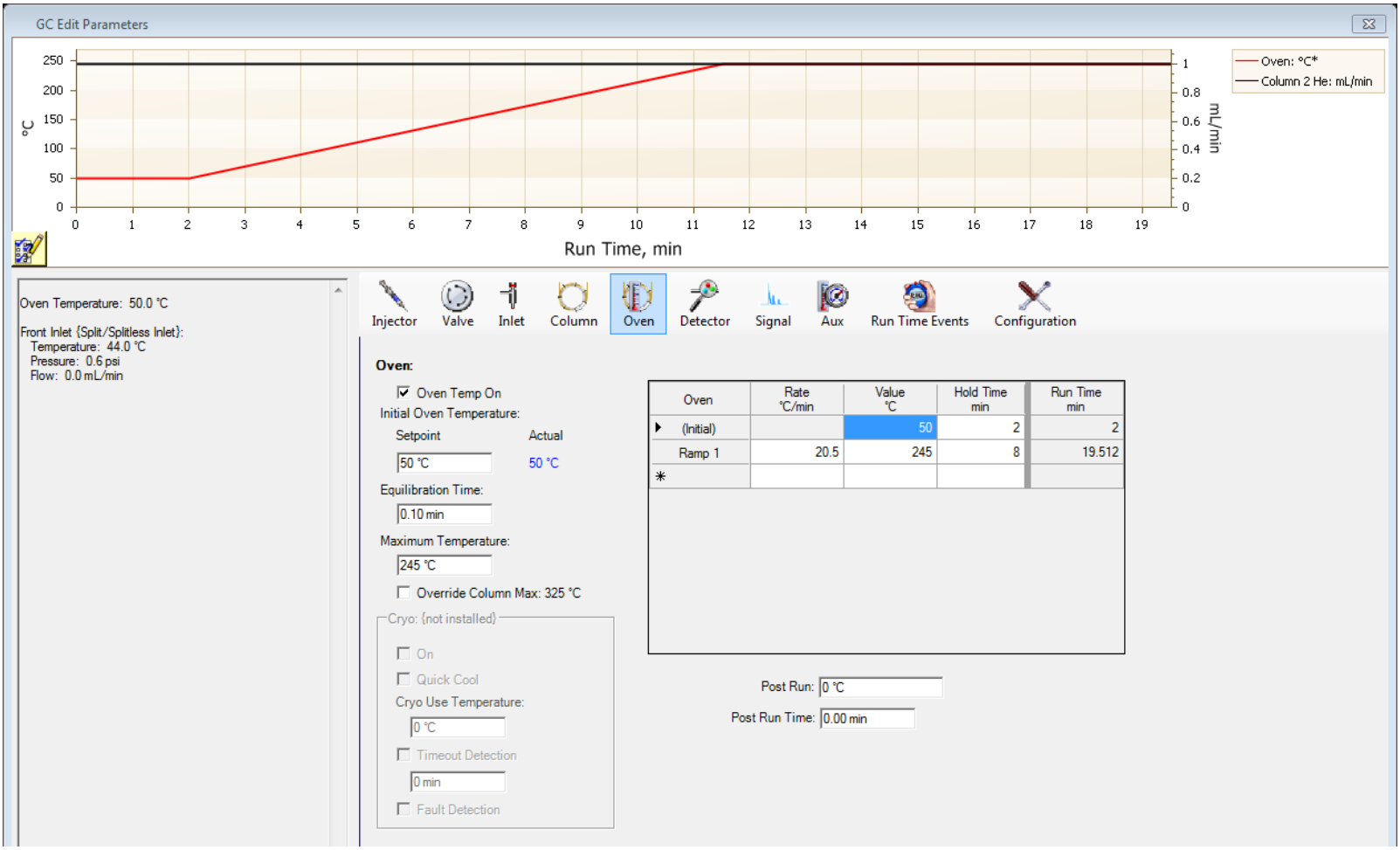

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Sequence Actions
What should the sequence do if it encounters the following:
Vial Missing
Wrong Vial Size
Leak Detected
System Not Ready
Skip
Skip
Skip
Abort

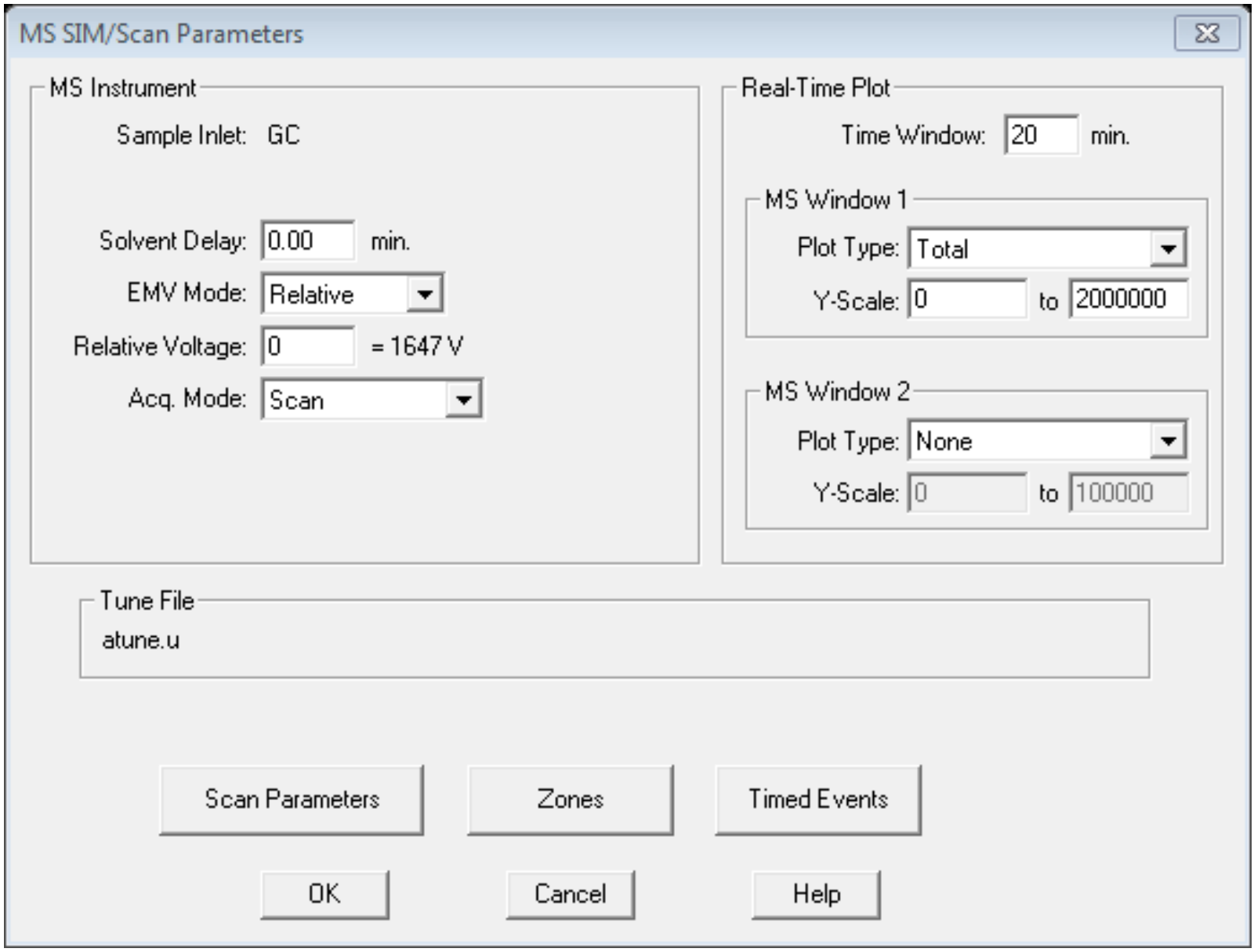

Headspace Edit Parameters
Temperatures
Times
Vial and Loop
Carrier
Advanced Functions
Sequence Actions
Method Development
Method Development
Manual
Would you like to increment a method setting over subsequent runs?
None
Assisted
Create method based on a specific application
Convert an existing valve and loop Headspace method
Convert an existing pressure transfer Headspace method

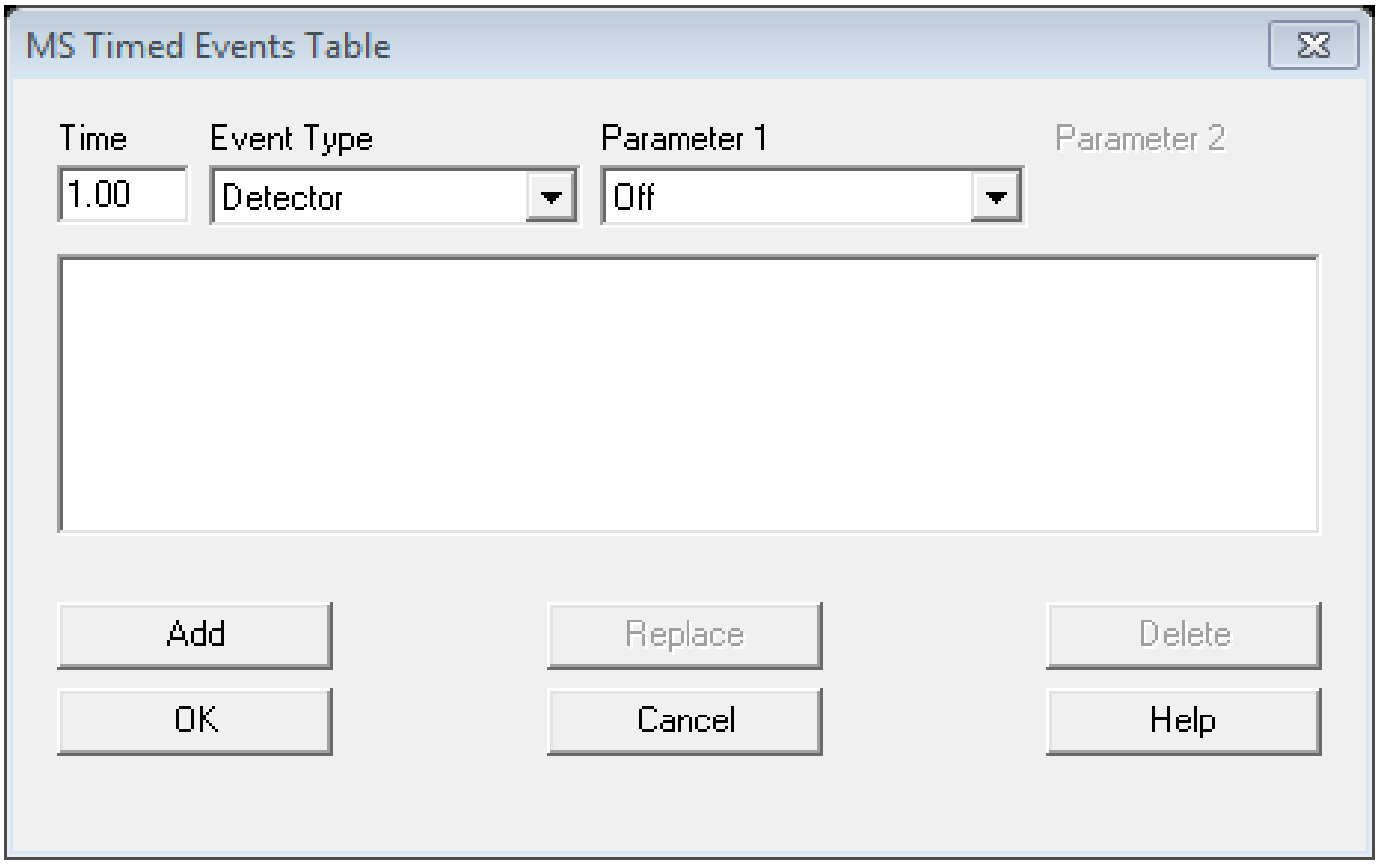

GC Edit Parameters
Oven: °C*
Column 2 He: mL/min
°C
mL/min
Run Time, min
Oven Temperature: 50.0 °C
Front Inlet {Split/Splitless Inlet}:
Temperature: 44.0 °C
Pressure: 0.6 psi
Flow: 0.0 mL/min
Injector
Valve
Inlet
Column
Oven
Detector
Signal
Aux
Run Time Events
Configuration
Front Injector
Back Injector
Injector:
Injection
Syringe Size:
µL
Injection Volume:
µL
x
1
=
µL
Multiple Injection Delay:
0 sec
Washes and Pumps
PreInj
PostInj
Sample Washes:
Solvent A Washes:
Solvent B Washes:
Sample Pumps:
Enable Solvent Saver
µL
>>


GC Edit Parameters
Oven: °C*
Column 2 He: mL/min
°C
mL/min
Run Time, min
Oven Temperature: 50.0 °C
Front Inlet {Split/Splitless Inlet}:
Temperature: 44.0 °C
Pressure: 0.6 psi
Flow: 0.0 mL/min
Injector
Valve
Inlet
Column
Oven
Detector
Signal
Aux
Run Time Events
Configuration
Front Inlet
Back Inlet
Split-Splitless Inlet
Setpoint
Actual
Heater:
240 °C
240 °C
Pressure:
11.6 psi
11.6 psi
Total Flow:{He}
45.0 mL/min
45.0 mL/min
Mode:
Split Ratio:
0.00
:1
0.00 mL/min
Injection Pulse Pressure:
0 psi
until
0.00 min
Gas Saver:
0.00 mL/min
After:
0.00 min


GC Edit Parameters
Oven: °C*
Column 2 He: mL/min
°C
mL/min
Run Time, min
Oven Temperature: 50.0 °C
Front Inlet {Split/Splitless Inlet}:
Temperature: 44.0 °C
Pressure: 0.6 psi
Flow: 0.0 mL/min
Injector
Valve
Inlet
Column
Oven
Detector
Signal
Aux
Run Time Events
Configuration
Column 1
Column 2
Column:
Gas settings:{He}
Mode: Const Flow
Setpoint
Actual
Pressure: 12.1 psi
0.6 psi
Flow: 1.0 mL/min
0.0 mL/min
Average Velocity: 25 cm/sec
Column Configuration:
Change Column...
Calibrate Column...
Post Run: 0 mL/min


GC Edit Parameters
Oven: °C*
Column 2 He: mL/min
°C
mL/min
Run Time, min
Oven Temperature: 50.0 °C
Front Inlet {Split/Splitless Inlet}:
Temperature: 44.0 °C
Pressure: 0.6 psi
Flow: 0.0 mL/min
Injector
Valve
Inlet
Column
Oven
Detector
Signal
Aux
Run Time Events
Configuration
Oven:
Oven Temp On
Initial Oven Temperature:
Setpoint
Actual
50 °C
50 °C
Equilibration Time:
0.10 min
Maximum Temperature:
245 °C
Override Column Max: 325 °C
Cryo: {not installed}
On
Quick Cool
Cryo Use Temperature:
0 °C
Timeout Detection
0 min
Fault Detection
Oven
Rate °C/min
Value °C
Hold Time min
Run Time min
(Initial)
50
2
2
Ramp 1
20.5
245
8
19.512
Post Run: 0 °C
Post Run Time: 0.00 min


MS SIM/Scan Parameters
MS Instrument
Sample Inlet: GC
Solvent Delay: 0.00 min.
EMV Mode: Relative
Relative Voltage: 0 = 1647 V
Acq. Mode: Scan
Real-Time Plot
Time Window: 20 min.
MS Window 1
Plot Type: Total
Y-Scale: 0 to 2000000
MS Window 2
Plot Type: None
Y-Scale: 0 to 100000
Tune File
atune.u
Scan Parameters
Zones
Timed Events
OK
Cancel
Help


MS Timed Events Table
Time
Event Type
Parameter 1
Parameter 2
1.00
Detector
Off
Add
Replace
Delete
OK
Cancel
Help

Edit Scan Parameters

Scanning Mass Range | Threshold and Sampling Rates | Plotting

	Start Time (minutes)	Start at Mass... (amu)	End at Mass... (amu)
Scan Group 1 ☑	0.00	33.00	550.00
Scan Group 2 ☐			
Scan Group 3 ☐			

Summary of Settings

Group	Start Time	Low Mass	High Mass	Threshold	Samples	S
1	0.00	33.00	550.00	150	2	2.

Low to High mass range must be in ascending order from 1.60 - 800.00.

Close | Help