

## **Varian 450 GC and MS Workstation Guide**

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Updated 18 Dec 2013 by Pat Megonigal

This document is arranged with increasing detail from front to back. It begins with tables of key features of the GC, then some quick-start guides for people who are familiar with the instrument, and ends with detailed guides required to troubleshoot, maintain or alter the configuration of the GC.

### **Brief History**

The GC and autosampler were purchased from Varian as a *greenhouse gas* package with detectors for CH<sub>4</sub> (FID), CO<sub>2</sub> (TCD) and N<sub>2</sub>O (ECD). It was shipped to Custom Solutions to be custom plumbed in a way suitable for analyzing these gases. Questions about how to set flows or alter the plumbing should be directed to *Custom Solutions*. However, I have found Matt Stevens of CS to also be extremely responsive and helpful for general GC troubleshooting, much more so than Joe Kane.

### **Technical Support**

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### **General Setup**

The Varian 450 has two largely independent plumbing systems, one that operates the TCD→FID series and the other the ECD. The two systems are connected by a single tube through which the sample is loaded.

**Table 1.** Cross reference guide to the locations and identity of the different detectors, which varies across screens in the GC interface, MS workstation software, and physical components.

<b>Characteristic</b>	<b>Detector Type</b>		
	<b>TCD</b>	<b>FID</b>	<b>ECD</b>
Analyte	CO <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub> O
Carrier	He	He	N <sub>2</sub>
Make-up	None	He	N <sub>2</sub>
Valve	V1	V1	V2
~Peak time (oven at 50°C)	2.589	1.695	2.318
Position in flow diagram	Front	Middle	Rear
Position on pressure screen	Middle tab (left tab empty)		Right tab
Position on detector screen	Rear		Middle
Position in MS Workstation	Rear		Middle
Position of manual flow knob	Labelled "middle"		Labelled "rear"

**Table 2.** Guide to approximate flow and pressure settings for Varian GC 450.

<b>Characteristic</b>	<b>TCD &amp; FID</b>	<b>ECD</b>
~EFC pressure	48.5 psi	53.0 psi
Carrier flow V1=(+)	15 mL/minute	15 mL/minute
Carrier flow V1=(-)	15 mL/minute	15 mL/minute
Makeup flow	15 mL/minute	15 mL/minute
Needle valve flow	15 mL/minute	15 mL/minute
Hydrogen flow	30 mL/minute	None
Air flow	300 mL/minute	None
Carrier gas tank pressure	80 psi	60 psi
Hydrogen tank pressure	50 psi	None
Air tank pressure	100 psi	None
Sample Loop Volume	250 µL	1000 µL

### Common Start-Up Problems:

A common problem when running the GC is the carrier and flame gases are low or there is a problem with the regulator setting. Be aware that when the gas pressure gets low, the GC will automatically protect itself and electronics by activating a safety method.

## **TO PREPARE SAMPLES FOR USE IN AUTOSAMPLER:**

### **Needles and Syringe:**

The best way needle and syringe combinations required for prepping vials and preparing samples for running samples in the GC is as indicated below. Ensure you are as consistent as possible between all vials in each step, as this will minimize errors in your data.

1. If using 7 mL vials with gray septa:
  - a) Evacuate 30 mL from the vial using a 30 mL syringe and 26G3/8 (brown) needle.
  - b) Inject 10 mL of sample or standard using a 10 mL syringe and 25G1½ (blue) needle.
2. If using 12 mL exetainers:
  - a) Evacuate 30 mL from the vial using a 30 mL syringe and 26G3/8 needle.  
Alternatively, you may choose to evacuate a greater volume, such as 60 mL.
  - b) Inject 20 mL of sample or standard using a 20 mL syringe and 25G1½ needle.

When possible, use custom-made needles (from Hamilton) with Hamilton syringe for injecting sample into vials – 10 mL RN syringe with 26 Gauge/0.6” Length/ Point Style 2 needles. Point Style 2 helps reduce septa coring while maintaining optimal ease of penetration. Fine needle size minimizes size of the hole resulting from septa penetration. 0.6” length eliminates long needle lengths unnecessary for gas sampling while increasing needle integrity at such a fine needle size, and thus increasing life of needle tip.

### **Vials and Septa:**

**Option 1 – Use gray septa with crimp tops on 7 mL vials.** Out of all septa tested, the gray (and orange) are competitively better than all others, producing more consistent results and higher maintenance of sample integrity. However, the orange get stuck on the syringe, so do not use them unless you physically hold the vials down.

**Option 2 – Use 12 mL Exetainers with custom Exetainer tray.** These vials test very well, with good sample retention and re-use capabilities, and allowing for larger samples to be collected (although the same volume will still be analyzed by the machine). Depending on the type of sampling being completed, these may be the better option.

### **Procedure for Preparing Samples for GC Use:**

1. Collect samples.
2. Evacuate vials.
3. Inject sample into vials.
4. Place samples in appropriate places in sample tray, corresponding to the locations each vial is to be listed in the sample run list.

### **Procedure for Switching Trays:**

1. Unlock CombiPAL by clicking the “Unlock CPAL” button in the CPAL. 24 window in MS Workstation. The screen on CombiPAL’s handheld device should no longer say “Remote Control”. This allows you to manually change settings for CombiPAL.
2. Click the “Menu” button (F1).
3. Scroll down to find “Setup” using the dial, and select this by clicking the centre button on the dial.
4. Next, scroll and select “Objects”. CombiPAL considers everything it uses as an “object”, including trays, injectors, etc.
5. Scroll and select “Trays”, then select a tray number you would like to change. We generally use Tray 1, unless multiple trays are being used.
6. Once Tray 1 is selected, it will highlight the tray type. We use one of two types of trays:
  - a) VT32-10 – This is the regular GC tray used with the 7 mL vials. It is assumed that the “-10” extension on the tray name refers to the regular 10 mL vial settings used for our 7 mL vials. When using this tray, ensure that the small risers (flat septa) are placed underneath the vial or else the vial will not be high enough to be recognized by CombiPAL. Ideally, use a lid on the tray to prevent vials from being picked up by CombiPAL. Also, if a lid for the tray is not available, ensure you use small pieces of tube to place between the vial and the vial hole in the tray to ensure the vials are secured in place.
  - b) VT-21 – This is the Exetainer tray. Ensure the lid is attached securely using springs on both the front and back of the tray.
7. Scroll through the tray types and select the desired tray (VT32-10 or VT-21).
8. Click “Home” (F4) to take you back to the original CombiPAL screen.
9. Lock CombiPAL by clicking on “Lock CPAL” in the CPAL. 24 window. The handheld device should now say “Remote Control”, indicating the software is now controlling CombiPAL.
10. Proceed to loading and running samples.

### **STARTING WITH MACHINE OFF:**

1. Turn gases ON first. All 4 gases (N, He, H, Air) will need to be turned on.
2. Turn GC On/Off switch to ON. This switch is located at the top of the machine in the back left-of-center area.
3. Turn on CombiPAL using switch at the back of the CombiPAL box on the counter (NOTE: ensure this is turned on before opening computer software, to make sure software recognizes both GC and CombiPAL).
4. Allow 15 minutes for gases to stabilize and to allow O<sub>2</sub> out of the system before raising temperatures and changing to usual settings.

5. Now you can start heating up columns, etc. manually or preferably using software (see Step 7). Note: TCD can take up to several hours for temperature to stabilize, so plan accordingly.
6. Turn on computer and open MS Workstation software.

Computer Login: pat

Password: Mond@y123

7. Go to File – Activate Method – Find and click on Gases.mth – Open. This will activate the method that will be used to run samples and will send this method over to the GC to stabilize.
8. **To Bake Out GC** (If machine has not been on or used in a while, let bake out overnight):
  - a) Instead of using Gases.mth, go to File – Activate Method – Open Sleep.mth.
  - b) Once GC stabilizes under Sleep.mth, raise column/oven temperatures to 200°C to burn off anything on the columns.
  - c) Leave overnight.
  - d) After baking out, see Step 7 to activate regular method.
9. Allow machine to stabilize. Once stabilized, machine should have green light showing “Ready” above the front touch screen. The software will also show all components as green and “Ready”. NOTE: Ensure no red/orange lights are showing on GC or in software prior to running samples.

#### **STARTING WITH MACHINE ON (IN SLEEP MODE):**

1. Make sure all gases are turned ON. If GC is already on and running under Sleep.mth, only the H and Air (two gases on the right) will need to be turned on.
2. CombiPAL should already be on; however, if it is not, turn on according to Step 3 in the above section.
3. Ensure computer is on and MS Workstation software is open. It should be showing the active method as Sleep.mth.
4. Go to File – Activate Method – Find and click on Gases.mth – Open.
5. Allow machine to stabilize, and ensure all lights on machine and software show as green and “Ready” prior to running samples.

## MS WORKSTATION PRIMARY WINDOWS:

- A) **System Control – Bruker GC/MS #1:** Shows menus that can be used, and the method the machine is currently set to.
- B) **450-GC. 44:** Shows a detailed status of the GC, including detector signals, temperatures, etc.
- C) **Instrument 1 Status:** Shows status, current method, all files run since the program has been opened. Can use this as a quick link to access chromatograms and detector reports after runs are completed.
- D) **CPAL. 24:** Shows the status of the autosampler, CombiPAL. Vial tray diagram shows status of vials (i.e. blue filled = vial has been run, etc.).
- E) **Message Log:** Shows messages including any errors, machine connections that occur, stages of sample runs that have been completed, etc.



Figure 1: Screen shot of 5 primary MS Workstation windows (circled in yellow), when MS Workstation is opened.



**Figure 2: Maximized view of 450-GC.44 MS Workstation window, shown minimized at the bottom of Figure 1.**

### **MS WORKSTATION BASICS:**

1. MS Workstation toolbar will show up at the top of the screen after logging in. Put cursor over each “button” in the toolbar to reveal its name and a detailed description of the button’s functions.
2. Click the button on the far left of the toolbar that shows a computer monitor to open “System Control/Automation”.
3. This will open the 5 primary MS Workstation windows (listed in the above section and shown in Figures 1 and 2).

4. If the Message Log window does not show up immediately, go to the “Windows” menu and make sure there is a check-mark next to Message Log. If not, click on Message Log and it will open the window.
5. The different windows can be minimized or maximized, and you are able to change the size and shape of the window in the same manner you would a regular window.
6. In the 450-GC. 44 window, you can change which status for the GC (e.g. detectors, oven, etc.) shows using the drop-down menus below the status details (located above the yellow output signal graphs).
7. The views of the signal outputs can be changed as follows:
  - a) Left-click and drag across the desired part of the graph to create a box to zoom in on a particular part of the graph.
  - b) Use the scroll icon on the right side of the graph to change the y-axis scale of the graph (i.e. to make peaks more visible, etc.).
  - c) Click on the icon above the graphs showing 4 arrows pointing outwards to return to originally views (i.e. non-zoomed in) of the graphs.
8. You can change which signals/detectors show using the drop-down menus above the graphs (e.g. View F, M, R Horz to view all, or choose another option).
9. The method bar in the System Control window shows the method that is currently activated (i.e. “Sleep.mth” or “Gases.mth”).
10. Left-click on the method bar to “View/Edit Method” or “Re-Activate Method”.

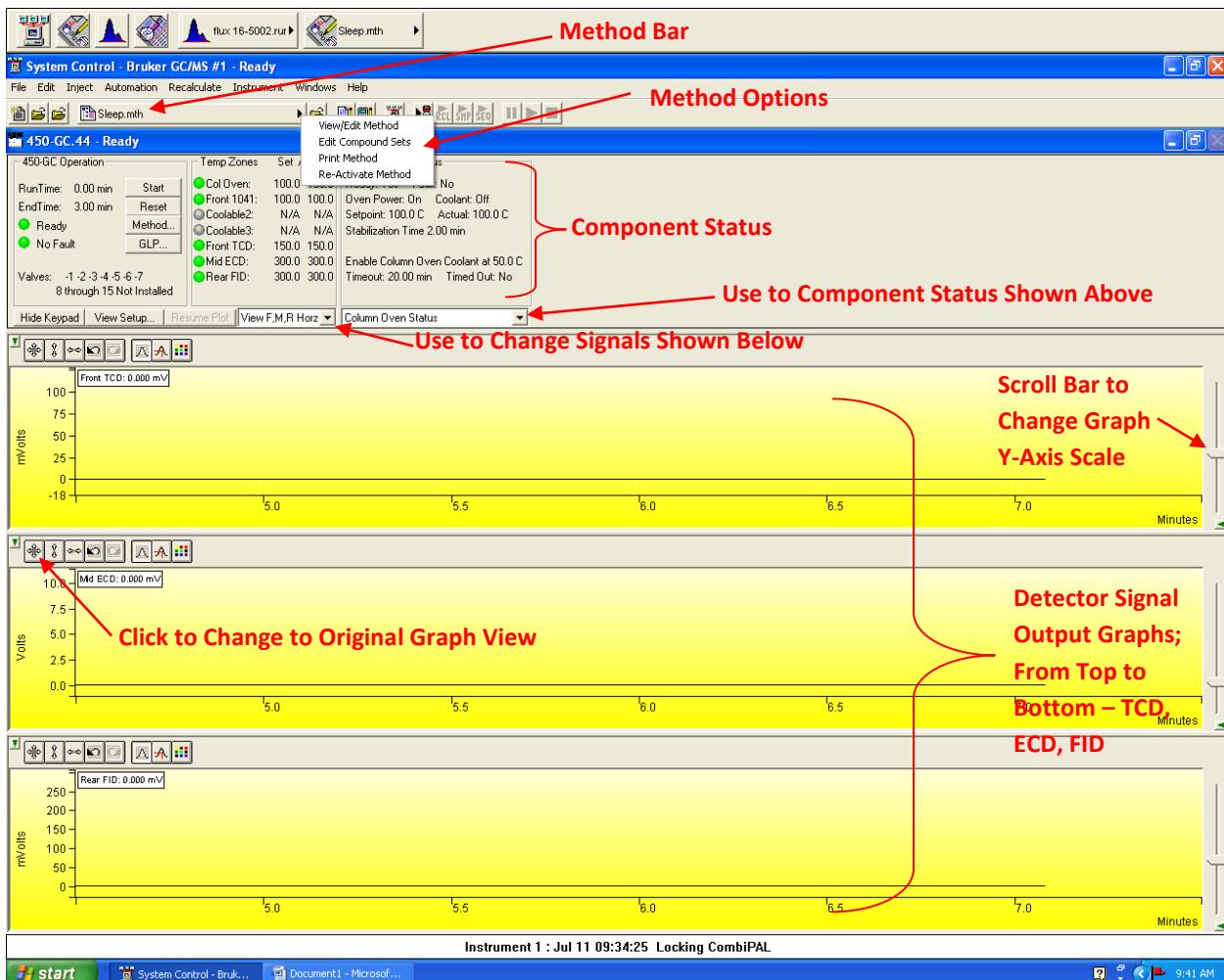


Figure 3: Components of the primary System Control and 450-GC.44 windows. Refer to Steps 5 -9.

11. A different method can be activated by clicking “File” then “Activate Method”. In the “Activate a System Control Method File”, choose the desired method. Or, you can open the file folder icon on the right side of the method bar and choosing a method. There are 4 different methods currently programmed in:
  - a) Gases.mth – Use to run samples on TCD, ECD and FID.
  - b) CO<sub>2</sub>, CH<sub>4</sub>.mth – Use to run samples on TCD and FID. ECD is disabled.
  - c) Sleep.mth – Use to put machine in sleep mode.
  - d) Shutdown.mth – Use to easily set machine to cool and prepare for being shut down completely.

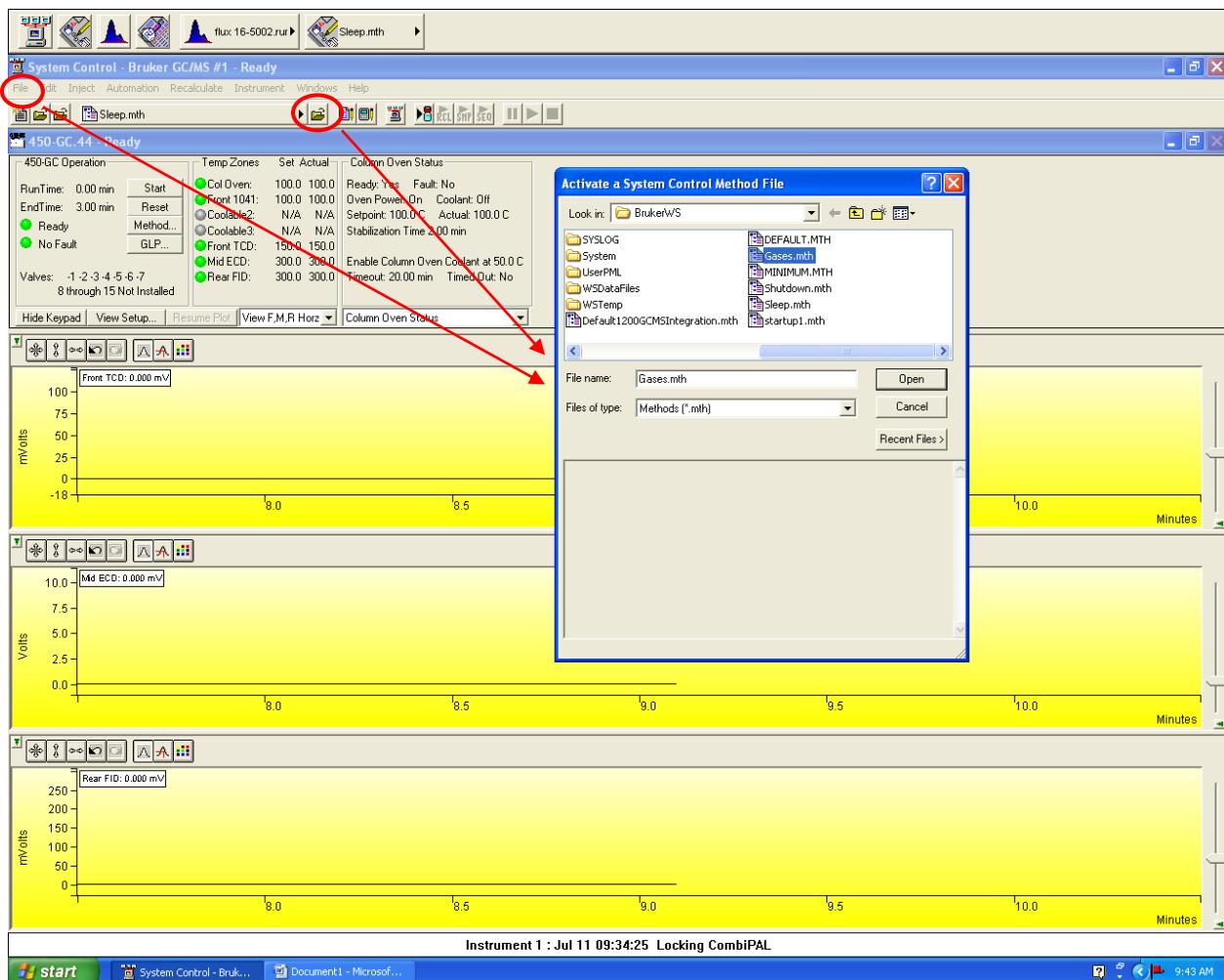
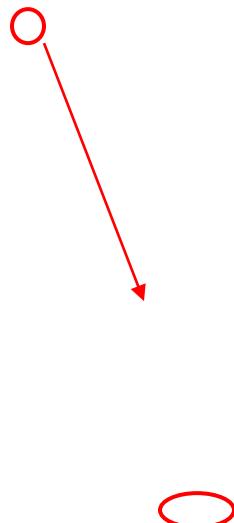


Figure 4: Two options are available to Open and Activate a Method. Refer to Step 10.

### **SINGLE SAMPLE RUN:**

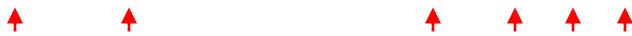
1. A quick, single sample run can be done using the icon on the right side of the method showing a triangle (like a “play” button symbol) pointing at a vial. Click this button to start this option.
  
2. The first window to pop up will be “Instrument 1 Parameters”. Do not change anything. Click OK.



**Figure 5: To Open a Single Sample Run, click the button circled above, then click OK. Refer to Steps 1 and 2.**

3. Under the “Inject Single Sample” window, enter in the following:
  - a) Sample Name – Enter desired name.
  - b) Sample Type – Choose from either Analysis or Calibration.
  - c) Injection Mode – Choose either Automatic (i.e. autosampler CombiPAL) or Manual Injection (i.e. you will manually inject into the injection port on the GC).

- d) Tray – Generally Tray 1, unless running samples using additional trays in positions 2 or 3.
- e) Vial – Enter in the vial position for each sample to be run (i.e. 1, 2, 3, etc.).
- f) Injection Volume – ALWAYS use 5000.0 (units are  $\mu\text{L}$ ).
- g) Leave the remaining columns as they are.
- h) Inject the Sampling Using the Method: C:\BrukerWS\Gases.mth.
- i) Click “Inject”.



**Figure 6:** Fill in all columns indicated by arrows, as outlined in Step 3.

## RUN SAMPLES USING A NEW SAMPLE LIST:

1. Go to File – New SampleList. In the “Create a New System Control SampleList File” window, enter in desired name for the list. Do not change the file location, unless necessary.

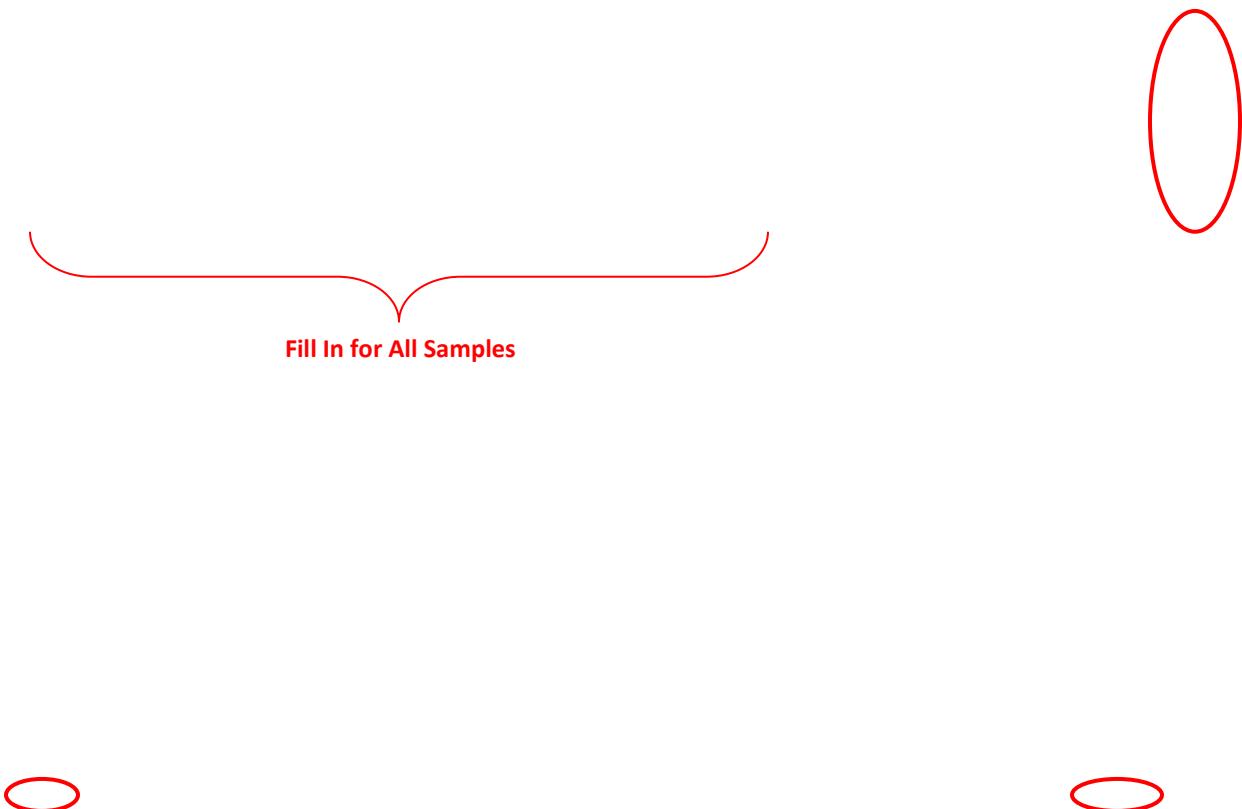
**Figure 7: Create a new sample list by clicking on File – New SampleList, then enter in a name. Refer to Step 1.**

2. Enter in the following:
  - a) Sample Name – Enter desired name.
  - b) Sample Type – Choose from either Analysis or Calibration.
  - c) Injection Mode – Choose either Automatic (i.e. CombiPAL autosampler) or Manual Injection (i.e. you will manually inject into the injection port on the GC).
  - d) Tray – Generally Tray 1, unless running samples using additional trays in positions 2 or 3.
  - e) Vial – Enter in the vial position for each sample to be run (i.e. 1, 2, 3, etc.).

f) Injection Volume – ALWAYS use 5000.0 (units are  $\mu\text{L}$ ).

g) Leave the remaining columns as they are.

NOTE: You can use the “Fill Down” button on the right side of the table to fill in the blocks above and below by highlighting the block with the info you want and dragging down (or up) across the other blocks to highlight the ones you want to fill. You can also add (will add more lines to bottom of list), insert (will add above highlighted line), or delete (highlighted lines) sample lines as needed.



**Figure 8:** Enter in information for all samples to be run, as shown above. Refer to Step 2.

3. Change the file location that the run files will be saved to by clicking on “Data Files...” in the bottom right corner and selecting the folder you would like to save the data files in.
4. Load tray with samples. Make sure samples are placed in the appropriate order in the correct tray, as listed in the SampleList. Use the numbers written on the tray as a guide for placing vials.

5. Click “Begin” at the bottom of the sample list window to start the list.
6. Instrument 1 Parameters window will pop up. Do not change anything, just click OK.
7. Begin Sample List window will pop up. Make sure it shows the C\B brukerWS\Gases.mth method, then click OK. This will begin the sample run.
8. Let samples run.

## **RUN SAMPLES USING AN EXISTING SAMPLE LIST:**

1. Go to File – Open SampleList. Choose desired sample list file name from the folder.
2. Double-check the parameters entered in the list to ensure all are correct (see step 2 in the “Run Samples Using a New Sample List” section above).
3. Make sure samples are placed in the appropriate order in the correct tray.
4. Click “Begin” at the bottom of the sample list window to start the list.
5. Instrument 1 Parameters window will pop up. Do not change anything, just click OK.
6. Begin Sample List window will pop up. Make sure it shows the C\BrukerWS\Gases.mth method, then click OK. This will begin the sample run.
7. Let samples run.

## **RUN SAMPLES USING MANUAL INJECTION:**

1. Follow procedures to run samples either using a single sample run, by creating a new sample list, or by opening an existing sample list as outlined in the above sections.
2. Instead of selecting “Automatic” under the Injection Mode column, select “Manual”. This will allow you to manually inject a sample directly into the injection port instead of using the CombiPAL autosampler.
3. Click “Begin”.
4. A window will pop up that says: “Please manually inject sample “Name” into “Front” or click cancel”
5. Inject the sample directly into the injection port and let it run. Or click cancel if manual injection is no longer desired.

## **VIEWING RESULTS AFTER A SAMPLE RUN IS COMPLETE:**

1. After a sample run is complete, the file will show up in the “Instrument 1 Status” window. All samples run since the program was last opened will show here.
2. Click on the desired run file in the white box and the run name will show in the gray box with arrow directly above the white box.
3. Click on this gray box, and several options will be given:  
View/Edit Chromatogram –
  - a) Will ask you to “Select Data File Channel”. Click on desired channel (i.e. TCD, ECD or FID) and click OK.
  - b) The signal output chromatogram for the selected channel will open.
  - c) Right click on the chromatogram to view additional options:
    - “View Results Only” will open a file that shows the calculated peak results and additional information on the run. This will initially show the TCD results – change the channel in the channel dropdown menu at the top to view results for the ECD or FID.
    - “View Standard Report” will open a file that has two windows: one showing the chromatogram and one showing the results. The channel results shown can be changed using the channel dropdown menu.
  - d) Chromatogram views can be changed by left-clicking and dragging a box across the desired area; or by using the scroll bar on the right side of the graph to change the scale of the graph.

### View Results Only –

- e) Same as in 3.c) above.

### View Standard Report –

- f) Same as in 3.c) above.

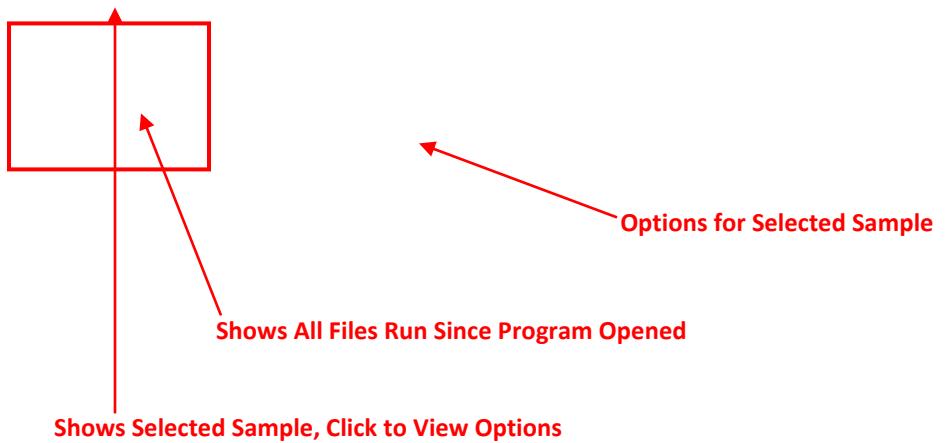


Figure 9: Shortcuts to view results after a sample run is completed.

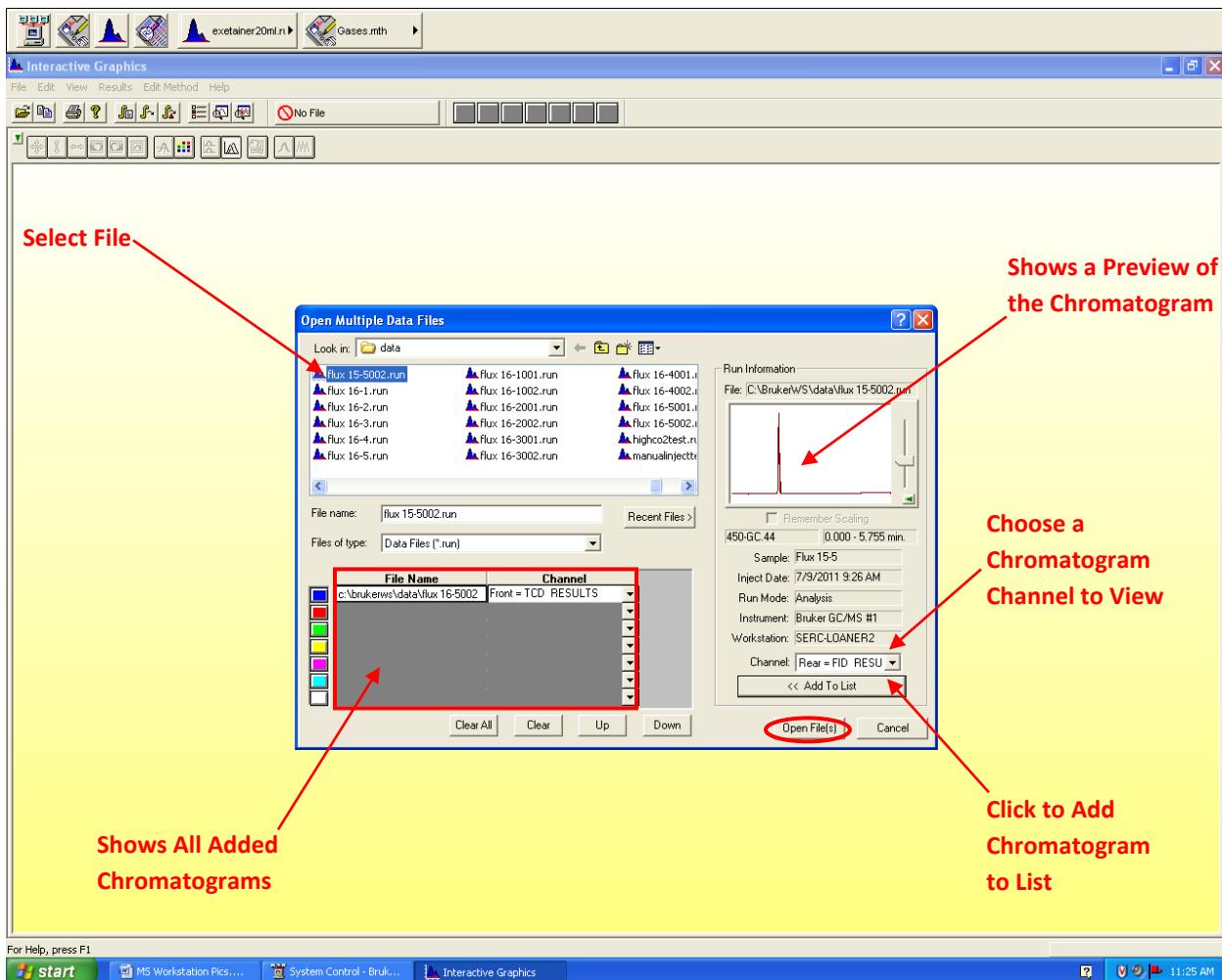
**Figure 10:** Viewing a chromatogram after selecting on “View/Edit Chromatogram”.

**Figure 11:** Viewing a results report after selecting “View Results Only”.

**Figure 12:** View a standard, comprehensive report showing chromatogram and results, after selecting “View Standard Report”.

## **HOW TO REVIEW A CHROMATOGRAM:**

1. Click on the Chromatogram button in the top MS Workstation toolbar (3<sup>rd</sup> from the left, showing a picture of a blue chromatogram).
2. In the “Open Multiple Data Files” window, choose the desired file from the list.
3. In the bottom right corner, choose the “Channel” output signal you want to view the chromatogram for.
4. To view a single chromatogram, next click “Open File(s)”.
5. To view more than one chromatogram, click “<< Add to List”. Then proceed to select additional chromatogram file(s) and/or channel(s) in the same way as in step 2. Click “<< Add to List” for each additional chromatogram. Then click “Open File(s)” when all desired selections have been added.



**Figure 13: How to view or add additional chromatograms. Refer to Steps 1-5.**

6. Once the chromatogram(s) is open, you can do the following:
  - a) Right click on the chromatogram to view additional options:
    - “View Results Only” will open a file that shows the calculated peak results and additional information on the run. This will initially show the TCD results – change the channel in the channel dropdown menu at the top to view results for the ECD or FID.
    - “View Standard Report” will open a file that has two windows: one showing the chromatogram and one showing the results. The channel results shown can be changed using the channel dropdown menu.
  - b) Change chromatogram views by left-clicking and dragging a box across the desired area; or by using the scroll bar on the right side of the graph to change the scale of the graph.

Lists All Chromatograms Added

Open File(s) When Ready

Add to List

## **PUTTING MACHINE IN SLEEP MODE:**

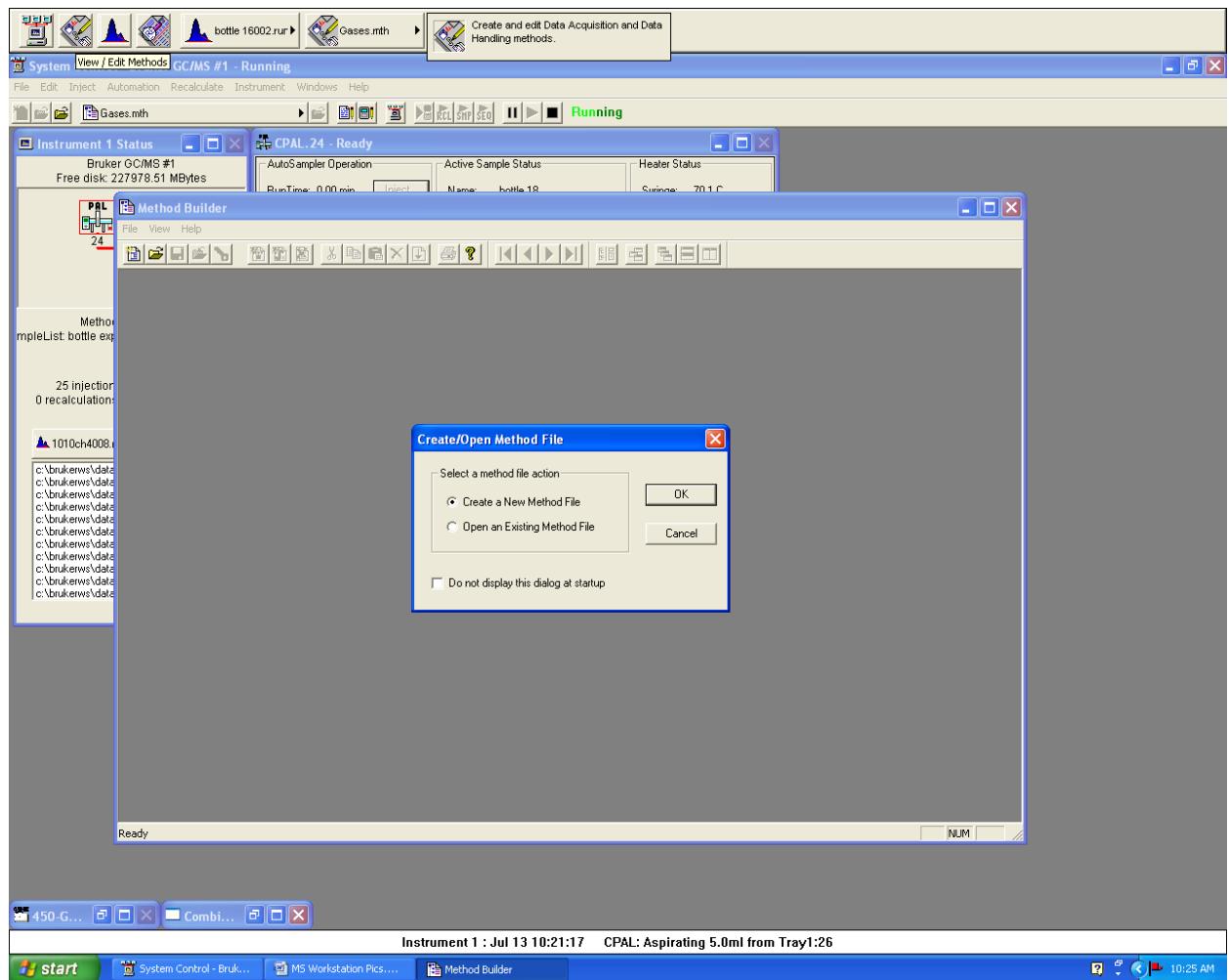
1. Go to File – Activate Method – Find and click on Sleep.mth – Open.
2. Allow machine to stabilize, and ensure all lights on machine and software show as green and “Ready”.
3. Turn H<sub>2</sub> and Air gases OFF. NOTE: Make sure you leave N<sub>2</sub> and He off, as these are the carrier gases that go through the columns and detectors.
4. Leave GC ON
5. Leave CombiPAL and the computer ON. You may close MS Workstation if you want.

## **TURNING THE MACHINE OFF COMPLETELY:**

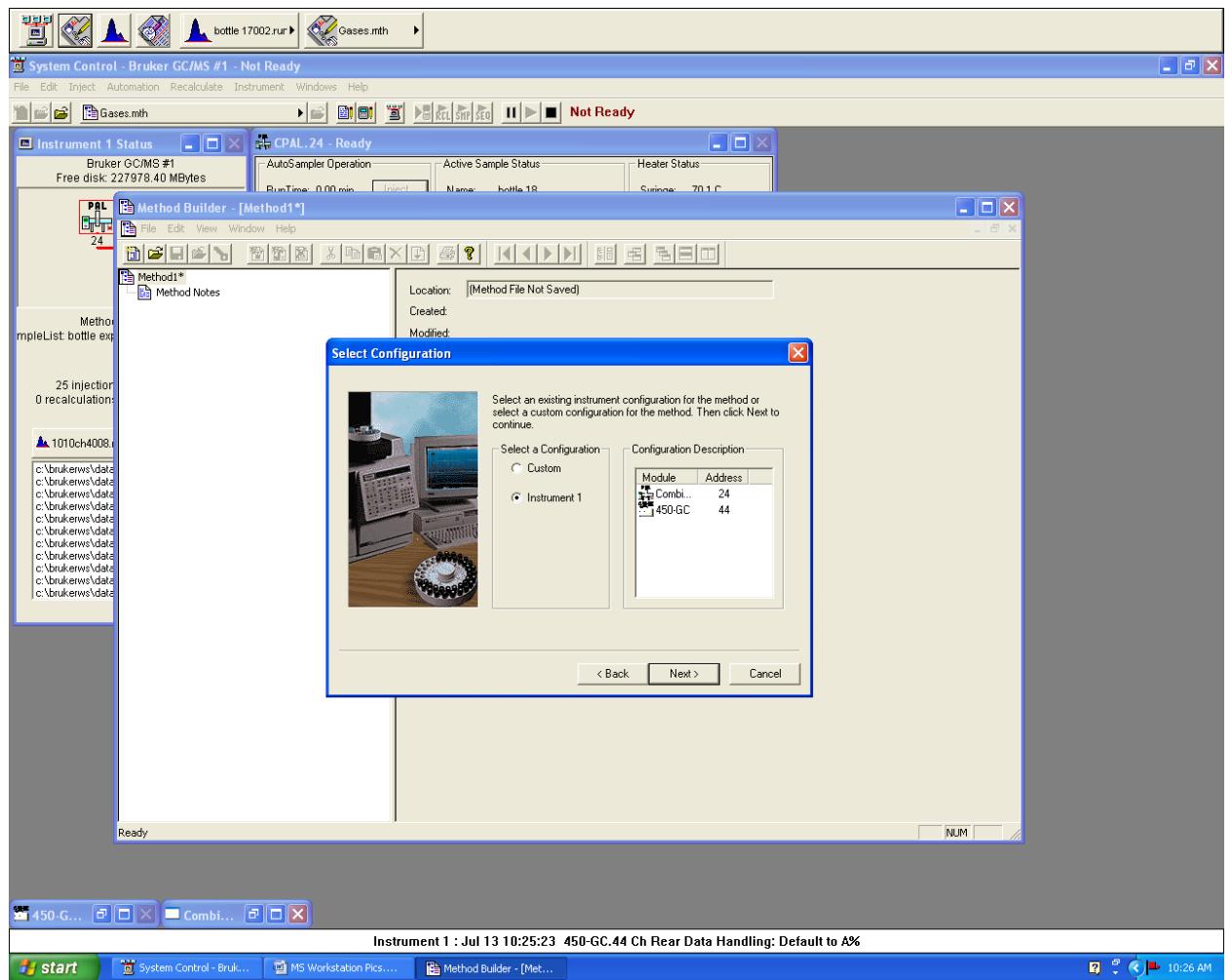
1. Go to File – Activate Method – Find and click on Shutdown.mth – Open.
2. Allow machine to stabilize, and ensure all lights on machine and software show as green and “Ready” prior to shutting machine off. This will take several hours, as the TCD takes a long time to cool. Once all detector temperatures have achieved 50°C or lower, everything can be turned off.
3. Turn CombiPAL On/Off switch to OFF.
4. Turn GC On/Off switch to OFF.
5. Turn gases OFF. All 4 gases (N<sub>2</sub>, He, H<sub>2</sub>, Air) will need to be turned OFF.

## BUILDING A NEW METHOD:

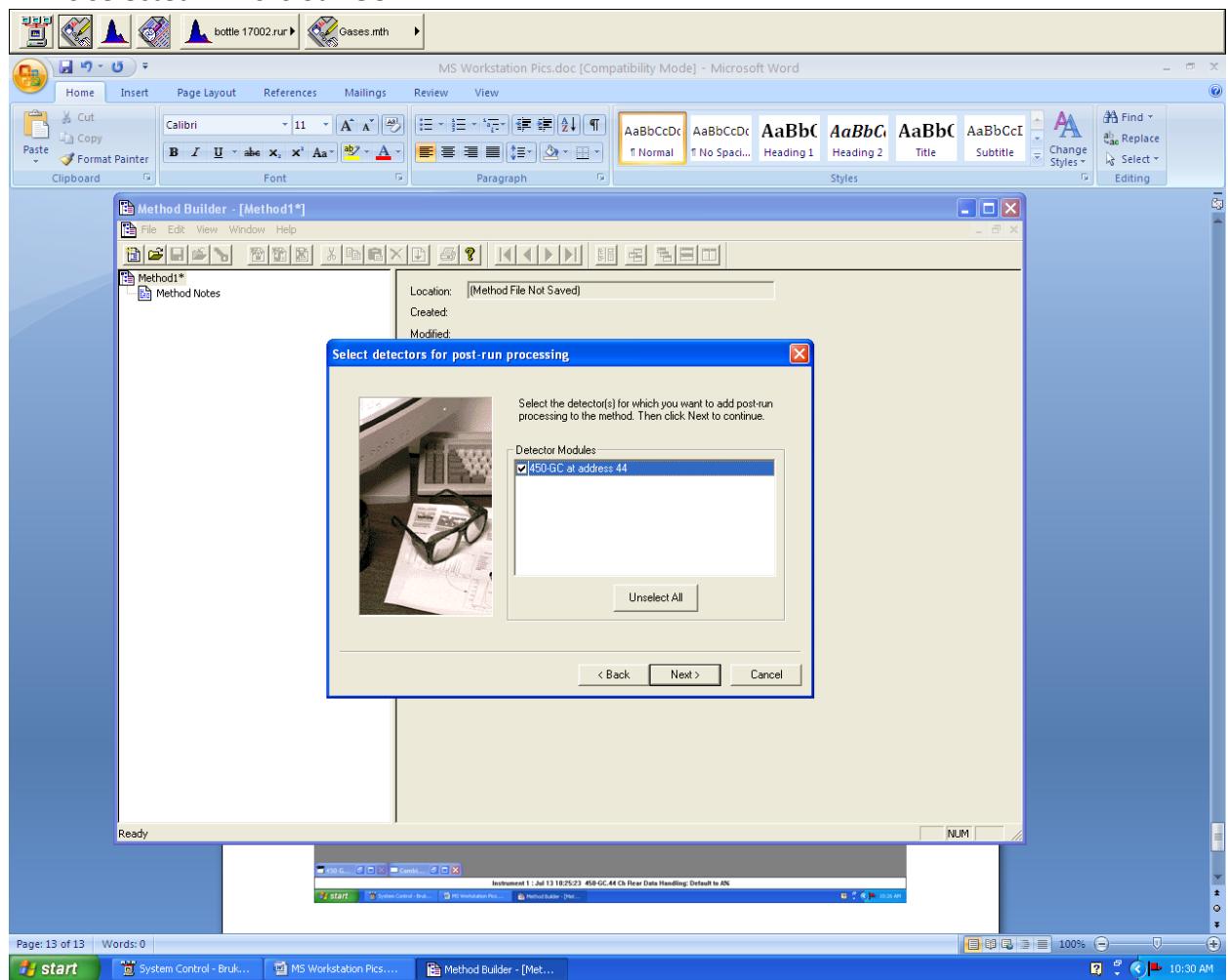
1. On the MS Workstation toolbar, click the second button from the left. This will start the process to create a new method.
2. In the “Configuring Method” window, click “Next >”.



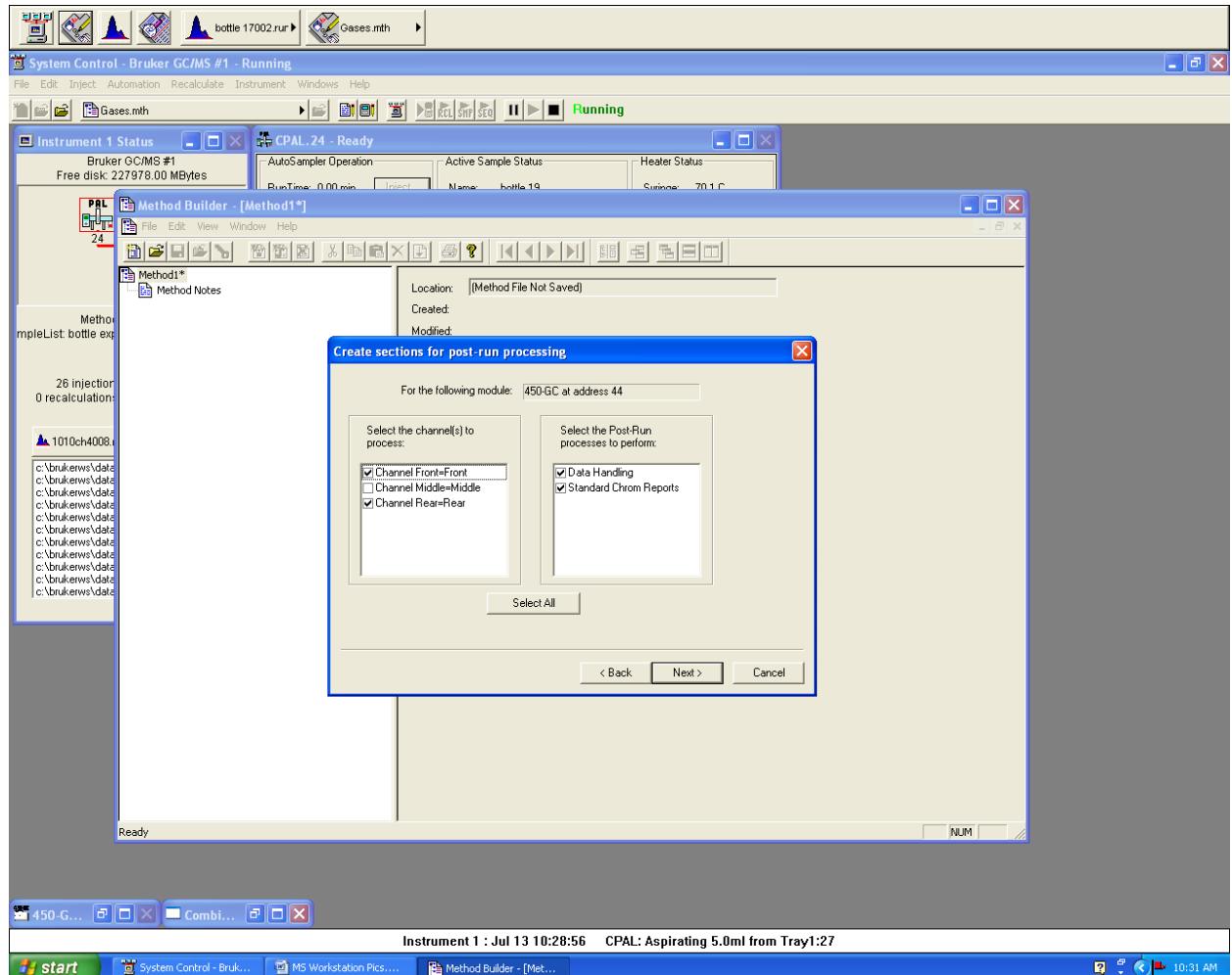
3. In the “Select Configuration” window, select “Instrument 1” then click “Next >”.



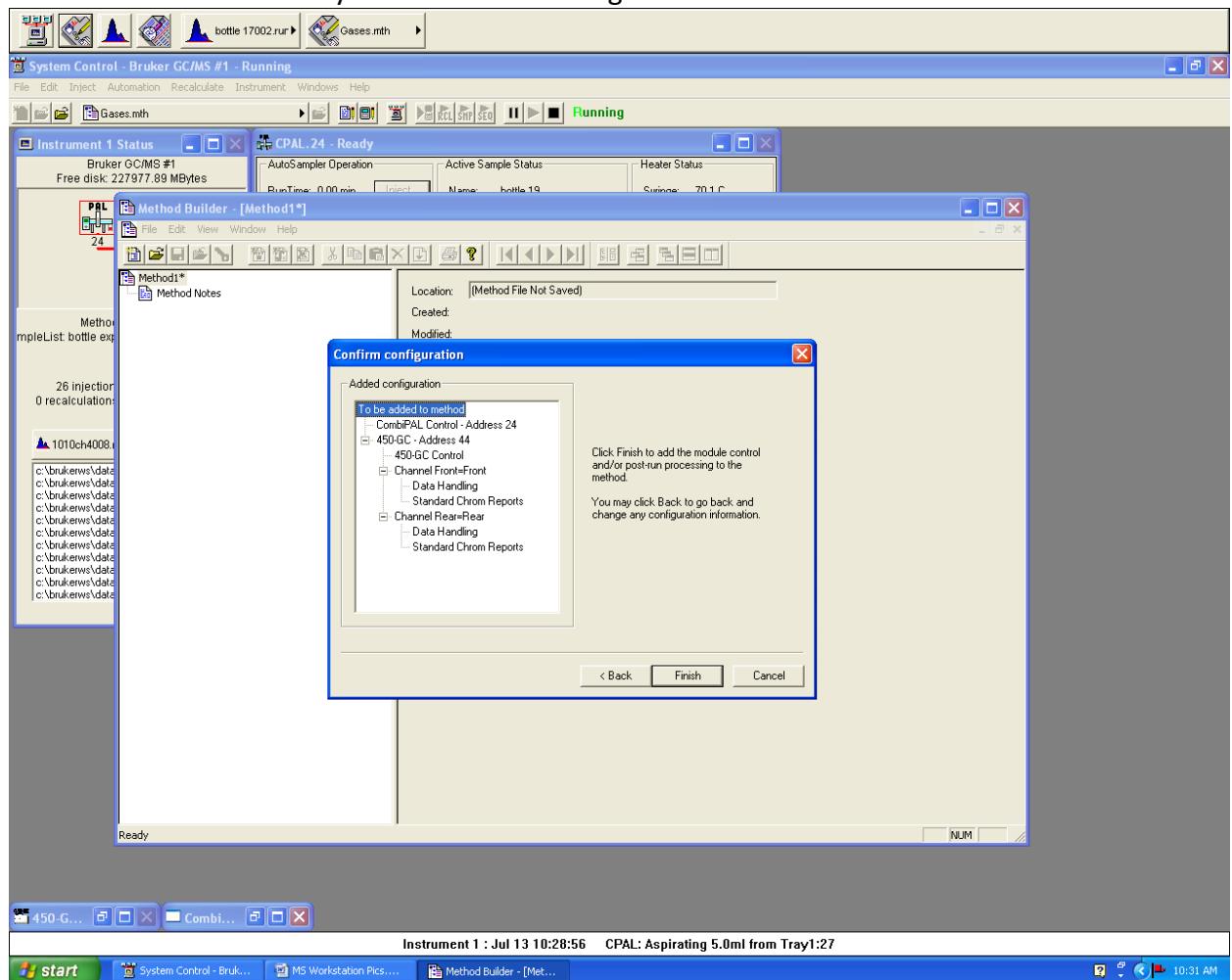
4. In the “Select detectors for post-run processing” window, make sure “450-GC at address 44” is selected. This is our GC.



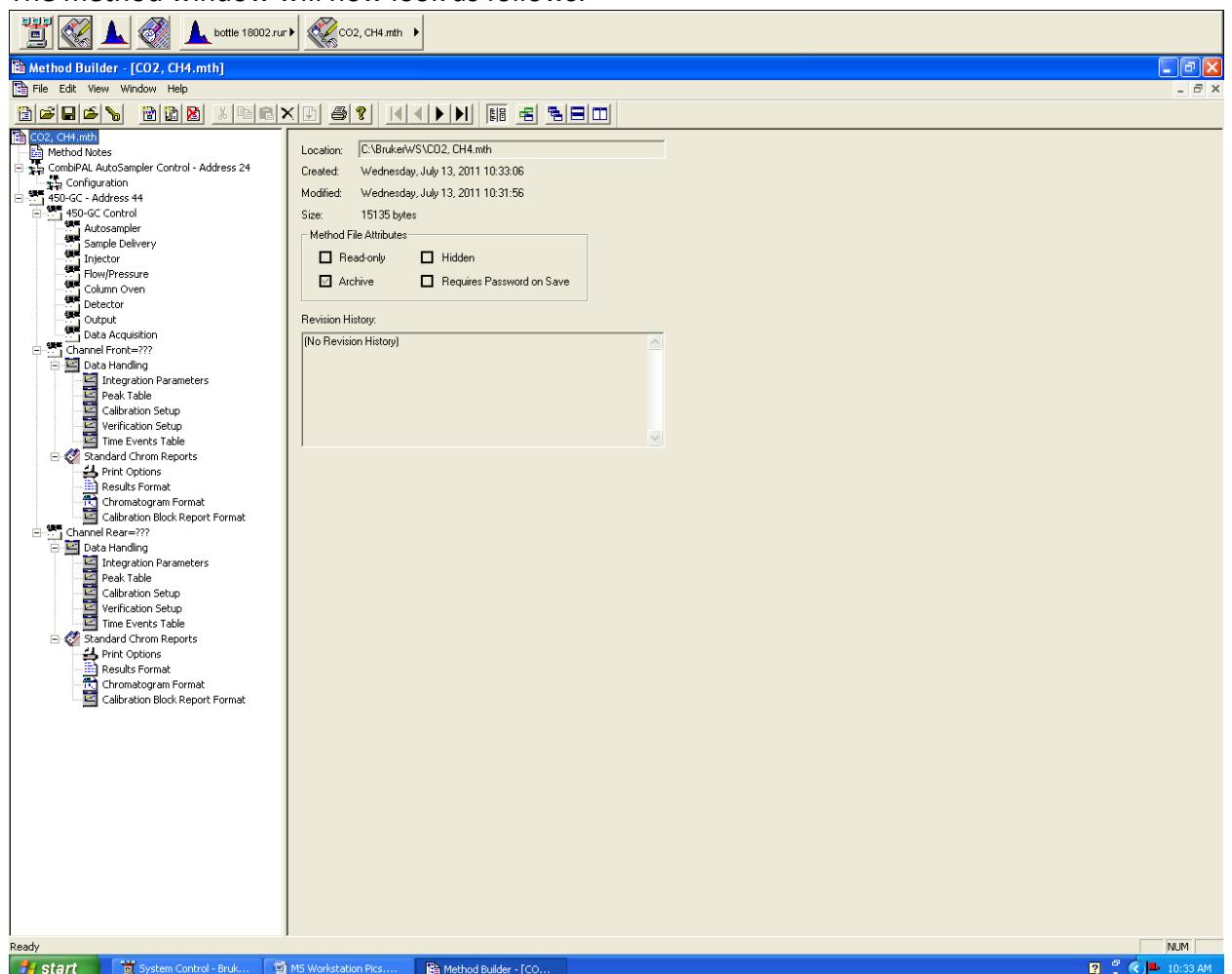
5. The “Create sections for post-run processing” window allows you to choose which detectors and what kind of post-run processing you would like the software to use. Make the desired selections and click “Next >”.



6. The final window will ask you to “Confirm configuration”. Click “Finish”.

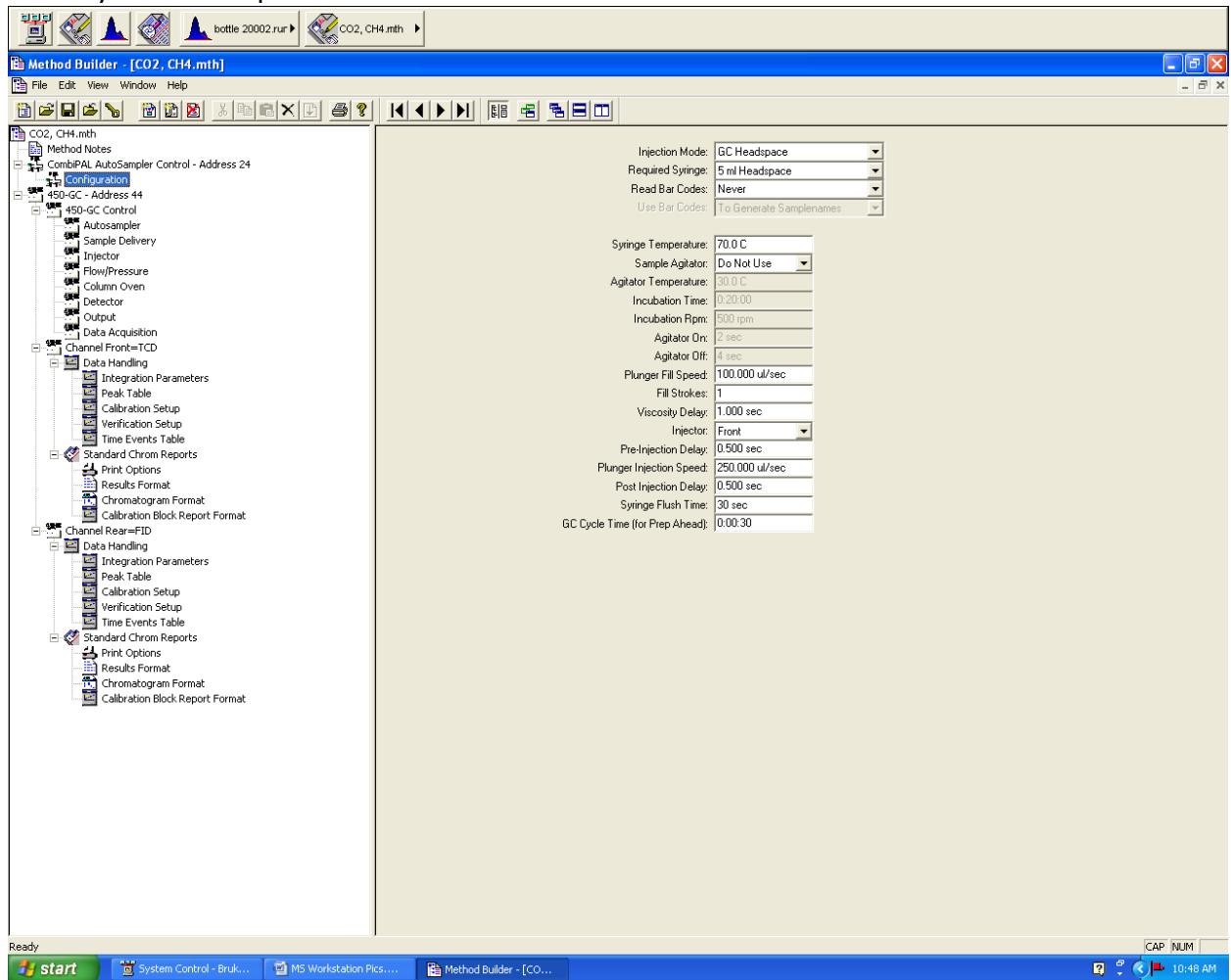


7. The method window will now look as follows:



The sections on the left show the various parts of the method that can be changed. If you click on a section, it will show you more details and settings that can be entered in and/or changed in the right-hand section of the screen.

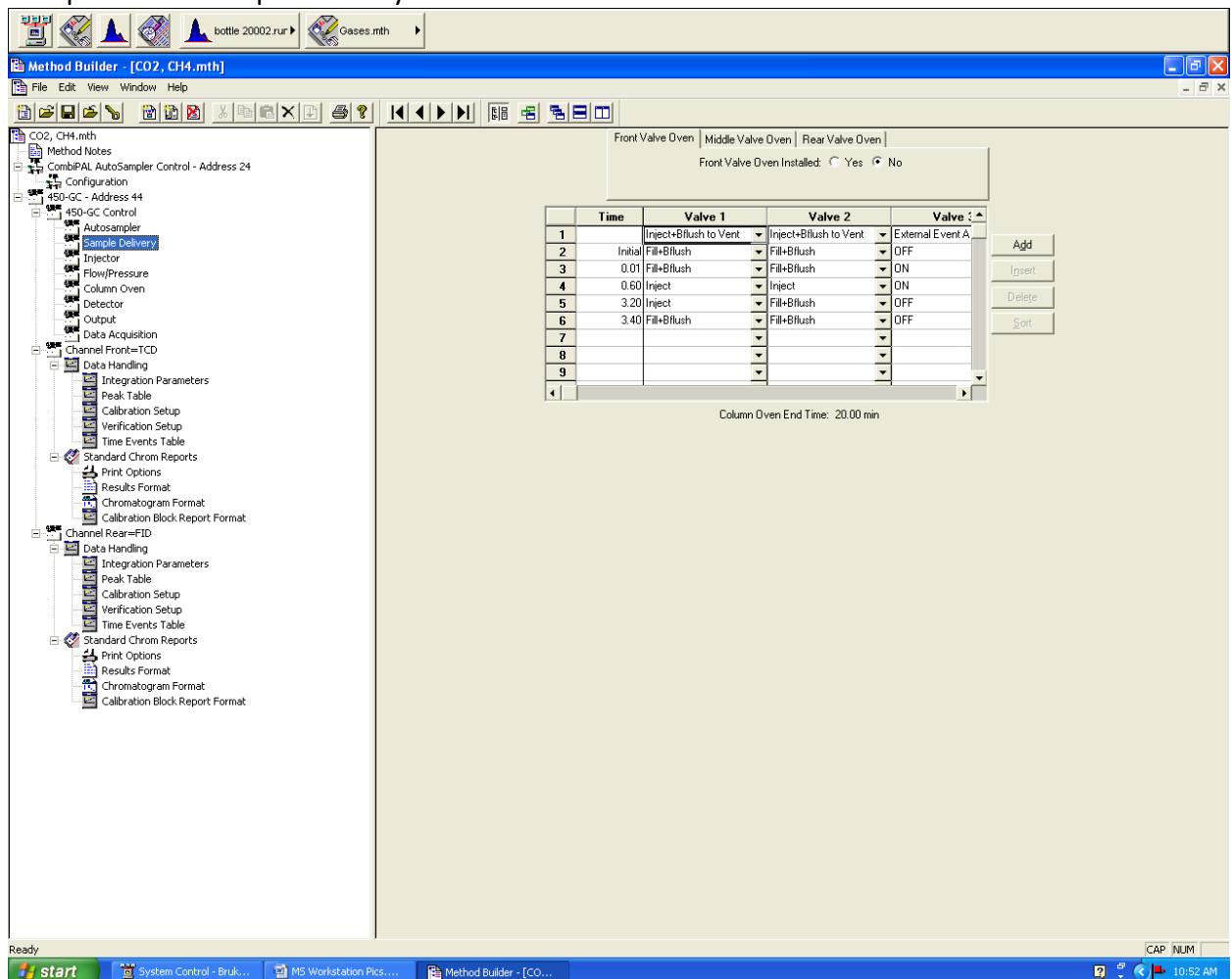
8. The “Configuration” section under “CombiPAL Autosampler Control – Address 24” is where you enter in parameters for CombiPAL.



9. “450-GC – Address 44” is the section where all GC and detector post-run processing parameters are entered.

10. Do not enter anything in the “Autosampler”, “Output” or “Data Acquisition” sections.

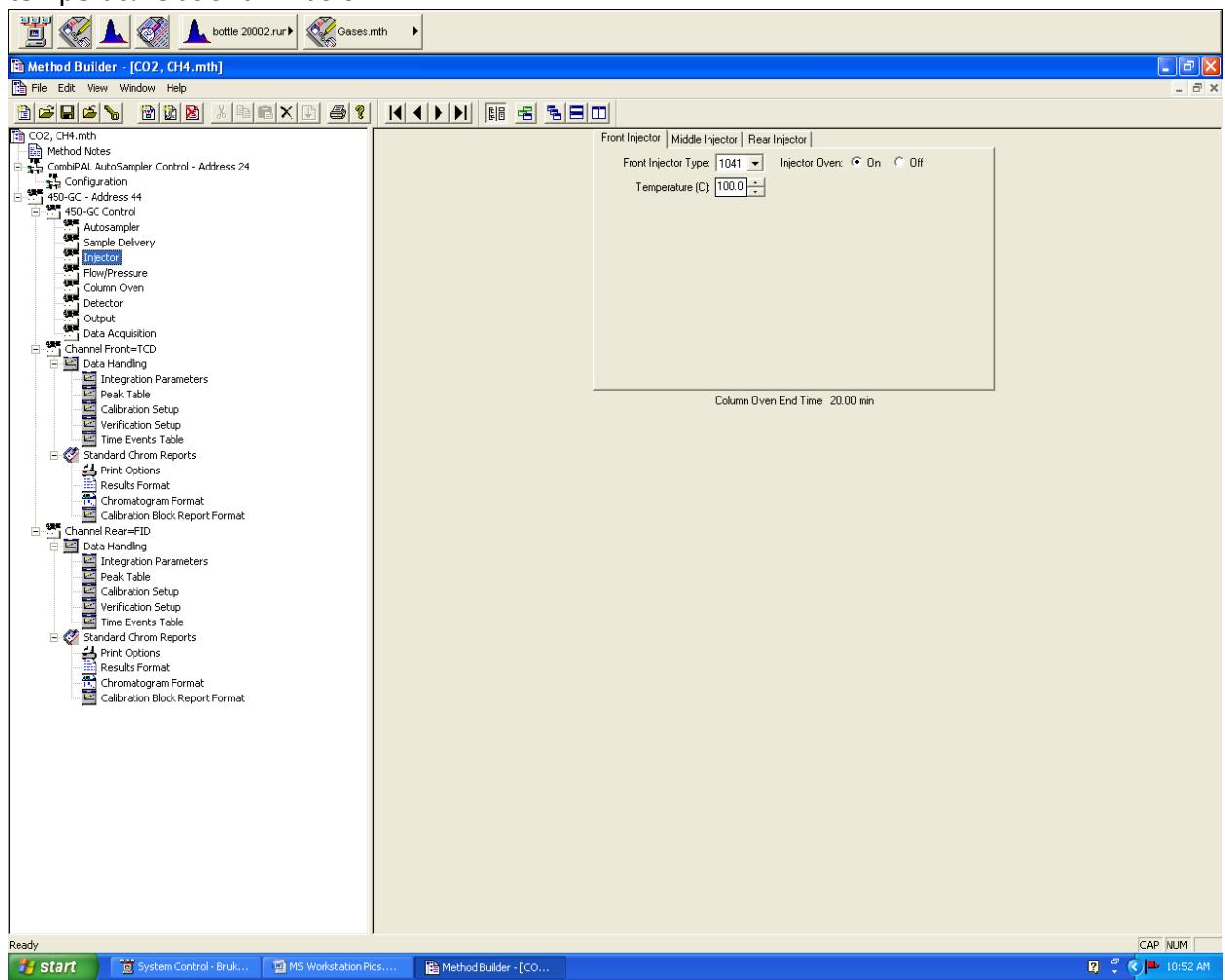
11. Complete the “Sample Delivery” section as follows:



Ready      CAP NUM      start      System Control - Bru...      MS Workstation Pics....      Method Builder - [CO...      10:52 AM

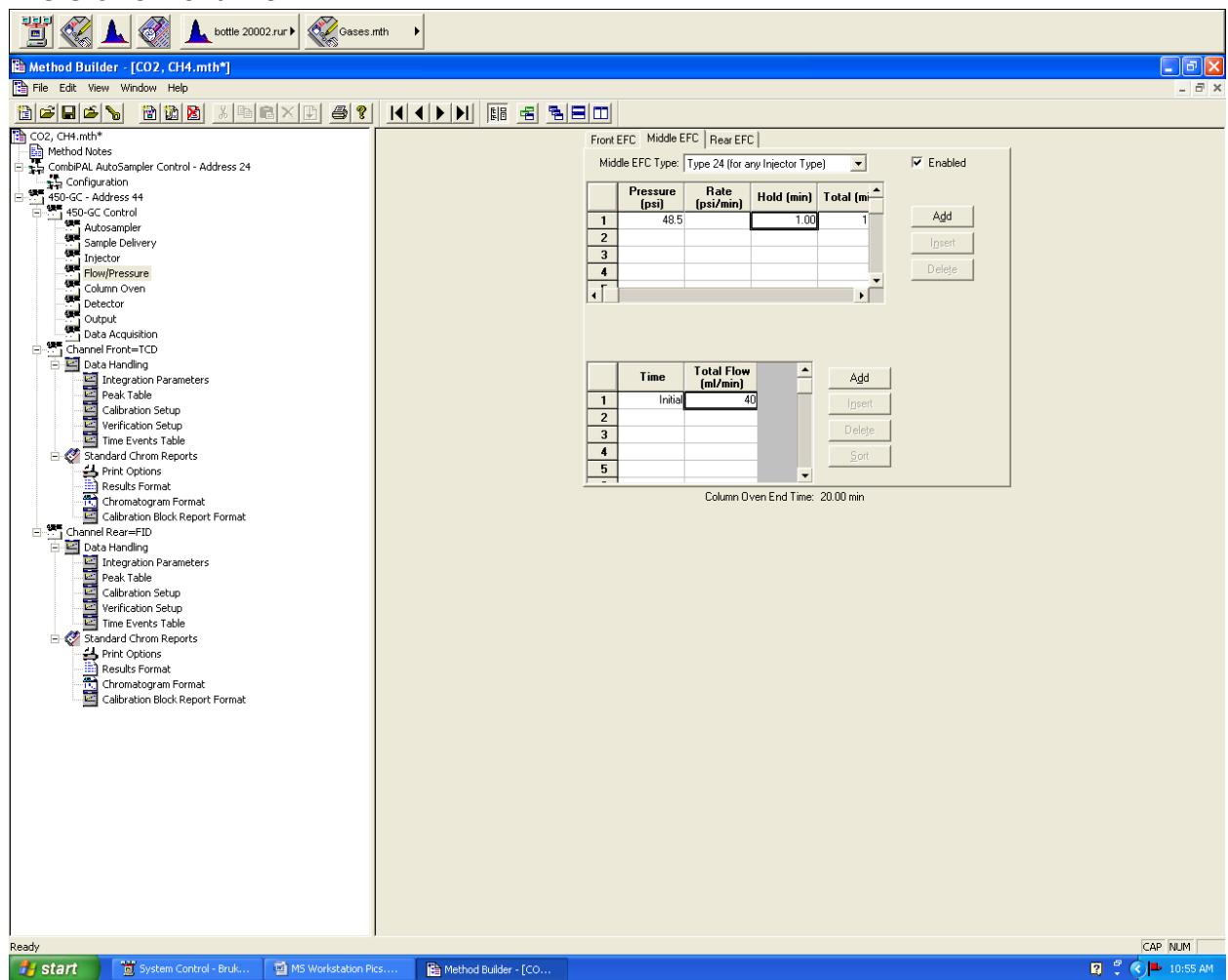
NOTE: Once the Front Valve Oven parameters are entered, it will automatically fill in the Middle and Rear Valve Oven parameters.

12. To complete the Injector section, turn the “Injector Oven” to On and set the temperature as shown below:



NOTE: There is only the Front 1041 in our GC.

13. For the “Flow/Pressure” section, ensure to fill in both the Middle and Rear EFC sections.  
There is no Front EFC.



Method Builder - [CO2, CH4.mth\*]

File Edit View Window Help

Front EFC Middle EFC Rear EFC

Rear EFC Type: Type 24 (for any Injector Type)  Enabled

	Pressure (psi)	Rate (psi/min)	Hold (min)	Total (m)
1	53.0	1.00		1
2				
3				
4				
5				

Add Insert Delete Sort

Column Oven End Time: 20.00 min

Method Notes

CombiPAL AutoSampler Control - Address 24

450-GC - Address 44

450-GC Control

Autosampler

Sample Delivery

Injector

Flow/Pressure

Column Oven

Detector

Output

Data Acquisition

Channel Front=TCD

Data Handling

Integration Parameters

Peak Table

Calibration Setup

Verification Setup

Time Events Table

Standard Chrom Reports

Print Options

Results Format

Chromatogram Format

Calibration Block Report Format

Channel Rear=FD

Data Handling

Integration Parameters

Peak Table

Calibration Setup

Verification Setup

Time Events Table

Standard Chrom Reports

Print Options

Results Format

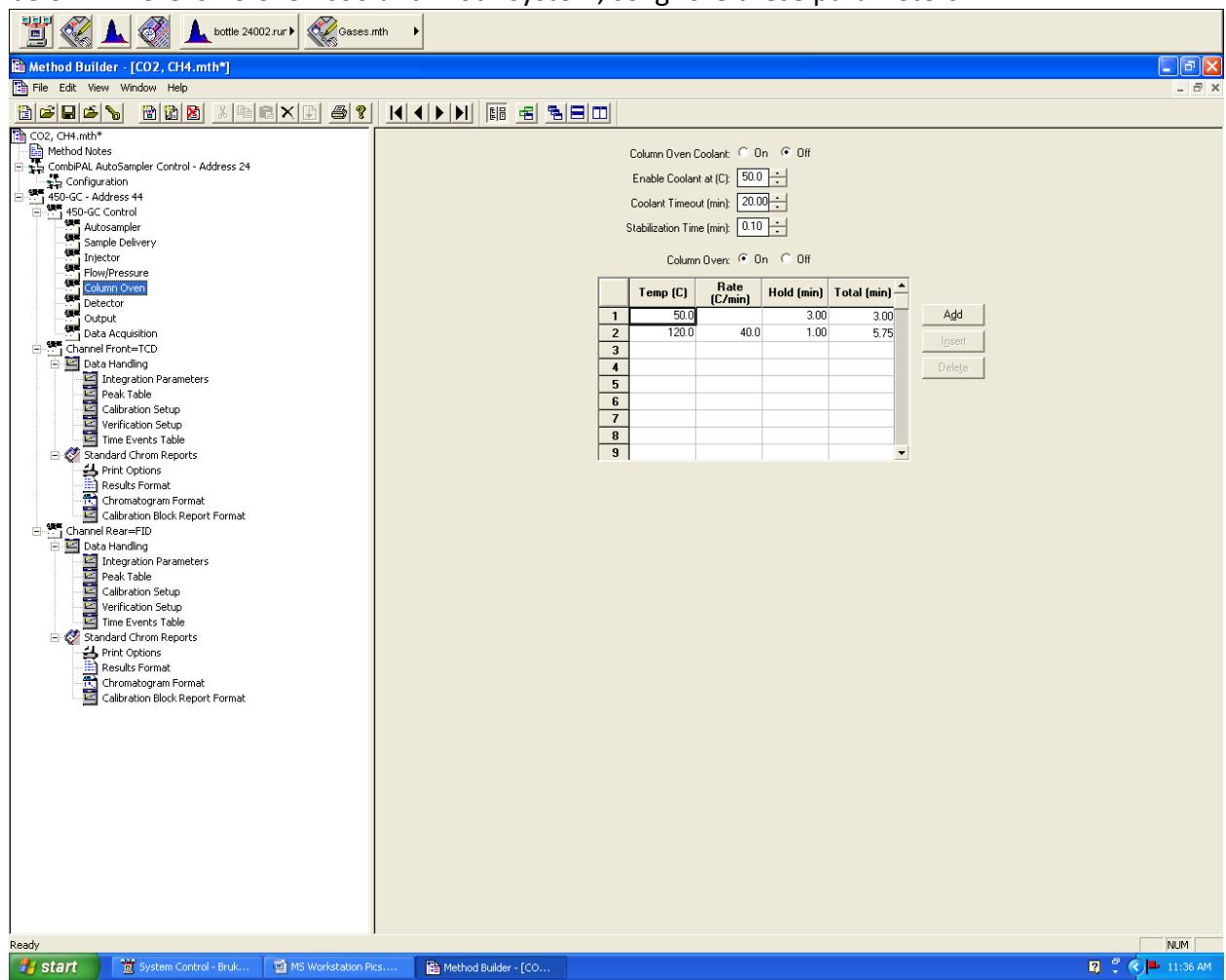
Chromatogram Format

Calibration Block Report Format

Ready

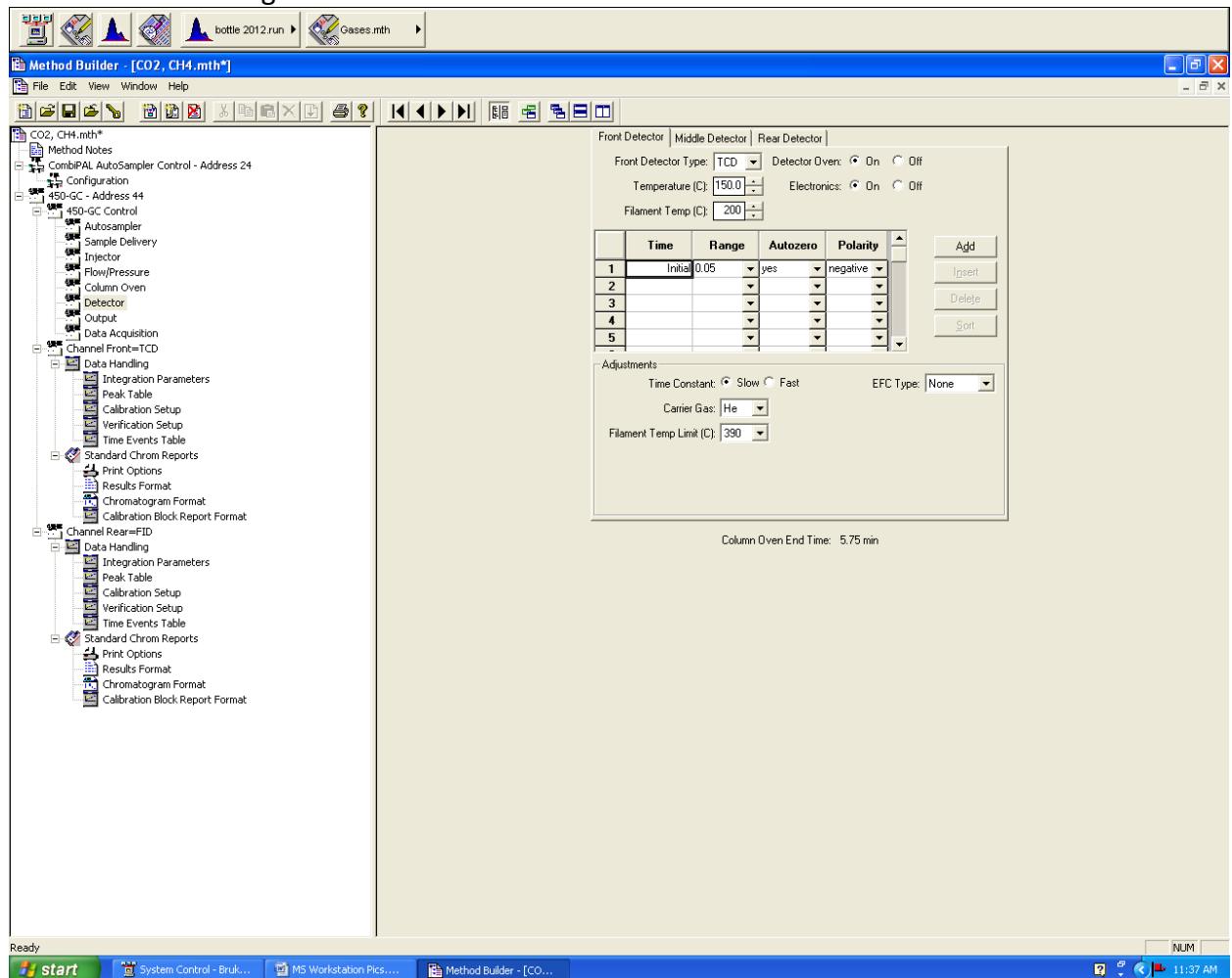
start System Control - Bruk... MS Workstation Pics.... Method Builder - [CO... CAP NUM 10:55 AM

14. Fill in the “Column Oven” heating settings and temperature program, as indicated below. There is no oven coolant in our system, so ignore these parameters.

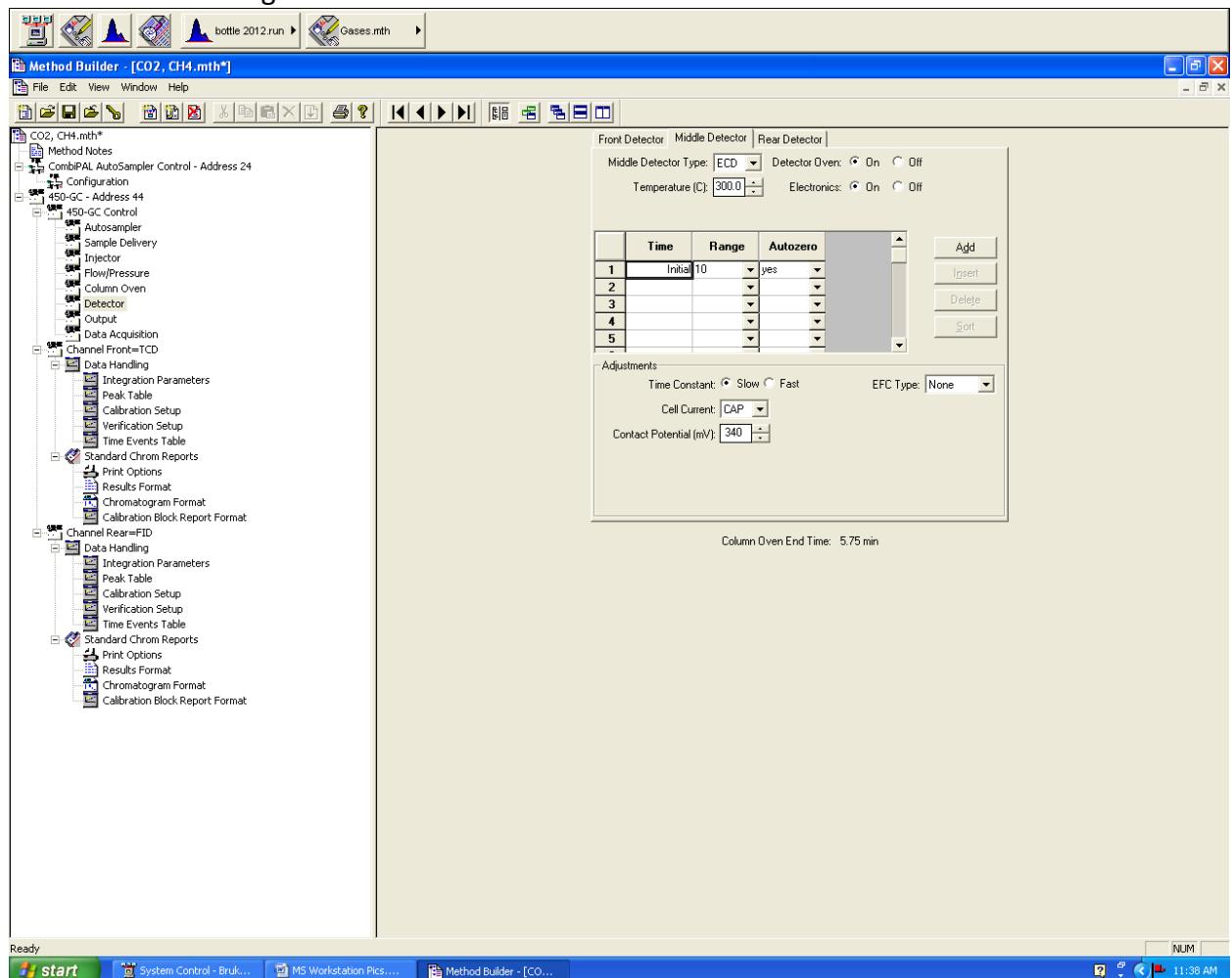


15. In the “Detector” section, there are individual sections for the TCD, ECD and FID. To enable the detectors for use in running samples, make sure the “Detector Oven” and “Electronics” parameters are set to On. All detectors should have appropriate temperatures set, and the “Time Constant” parameter should be set to Slow. Please review the below three screenshots for the normal operating settings for each detector.

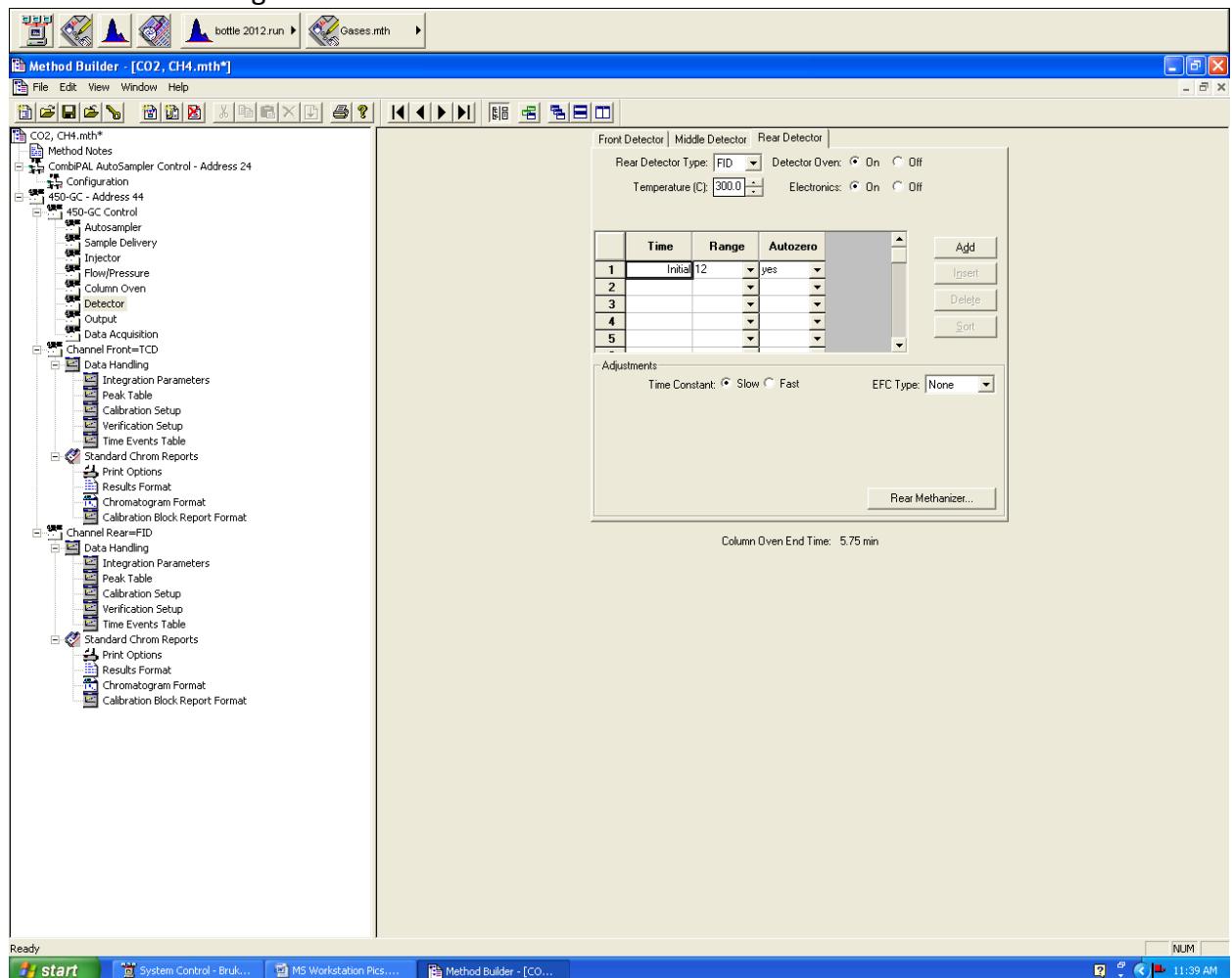
#### TCD Detector Settings:



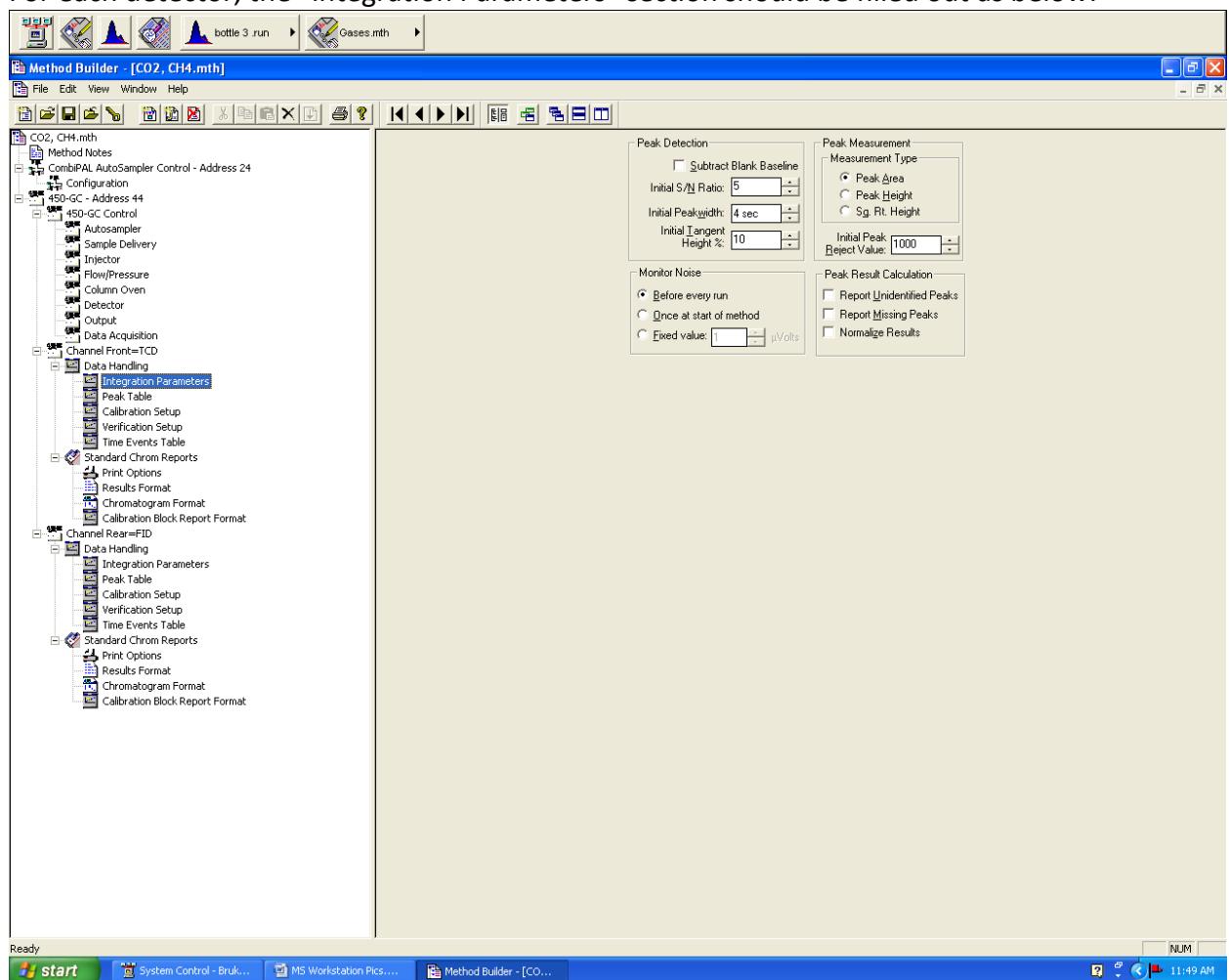
## ECD Detector Settings:



## FID Detector Settings:



16. For each detector, the “Integration Parameters” section should be filled out as below:



17. The “Peak Table” needs to be filled out for each detector. The Retention Time and Peak Name should be filled in, as shown in the example for CO<sub>2</sub> below:

The screenshot shows the Method Builder software interface for a method named "CO2, CH4.mth". The left pane displays the method structure, including sections for Method Notes, CombiPAL AutoSampler Control - Address 24, 450-GC - Address 44, Channel Front=TCD, and Channel Rear=PID. The right pane shows the "Peak Table" configuration, which is a grid with columns for Retention Time, Peak Name, Ref, Std, RRT, Standard Peak Name, Group, Level 1 Amount, Level 2 Amount, and Level 3 Amount. The first row is populated with data for CO<sub>2</sub>, while other rows are empty. A toolbar at the top provides options for Add, Insert, Delete, Fill Down, and Sort.

	Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount
1	2.560	CO2					0	1	1	1
2										
3										
4										
5										
6										
7										
8										
9										
10										

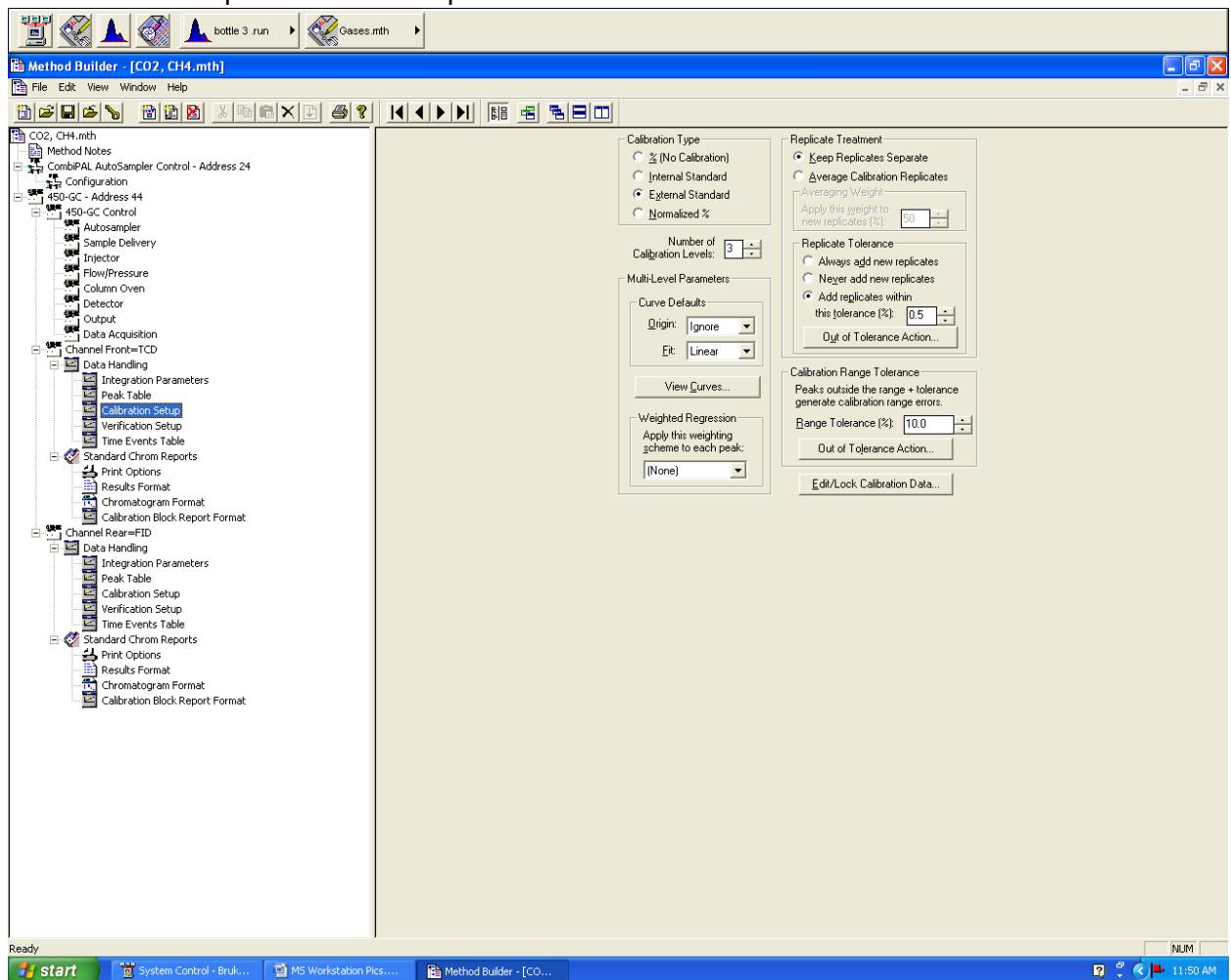
Ready start System Control - Bruk... MS Workstation Pics... Method Builder - [CO...

CO<sub>2</sub> Retention Time – 2.560

N<sub>2</sub>O Retention Time – 2.450

CH<sub>4</sub> Retention Time – 1.680

18. "Calibration Setup" should be completed in the same manner for all three detectors:



19. Under "Print Options", make sure all parameters are NOT checked off. The computer with the MS Workstation software is not connected to a printer.

20. It is not necessary to make any changes to the following sections:

Verification Setup

Time Events Table

Chromatogram Format

Calibration Block Report Format

21. And the last step to creating a new method is to MAKE SURE YOU SAVE IT!!!

## TROUBLESHOOTING AND MAINTENANCE:

### GENERAL GC TROUBLESHOOTING:

**GC Error Codes and instructions for troubleshooting can be found in APPENDIX A on page 483 at the end of the Varian 450-GC User Manual, or in the black information binder.** In order to troubleshoot, match the error code showing in the GC's Log (accessed through the "Home" view on the GC's front touch screen) to one in APPENDIX A. The potential causes of the error and possible actions that may be taken to fix it are listed.

For problems with chromatogram curves, such as peak tailing, change in size, no peaks when expected, etc. refer to the black information binder where further troubleshooting resources have been added.

### ORDERING REPLACEMENT GAS TANKS:

Replacement gas tanks should be ordered as follows:

- a) When using the machine to steadily run samples, order the N and He when the gas tanks reach approximately 750 psi left. The Air should be ordered around 1000 psi left (since high volumes are used); and H at about <500 psi (since H is used up very slowly).
- b) When the machine is spending most time in sleep mode, order N and He at around <500 psi left. The Air and H are not being used in sleep mode, so these will only need to be ordered when running low from running samples (see above).

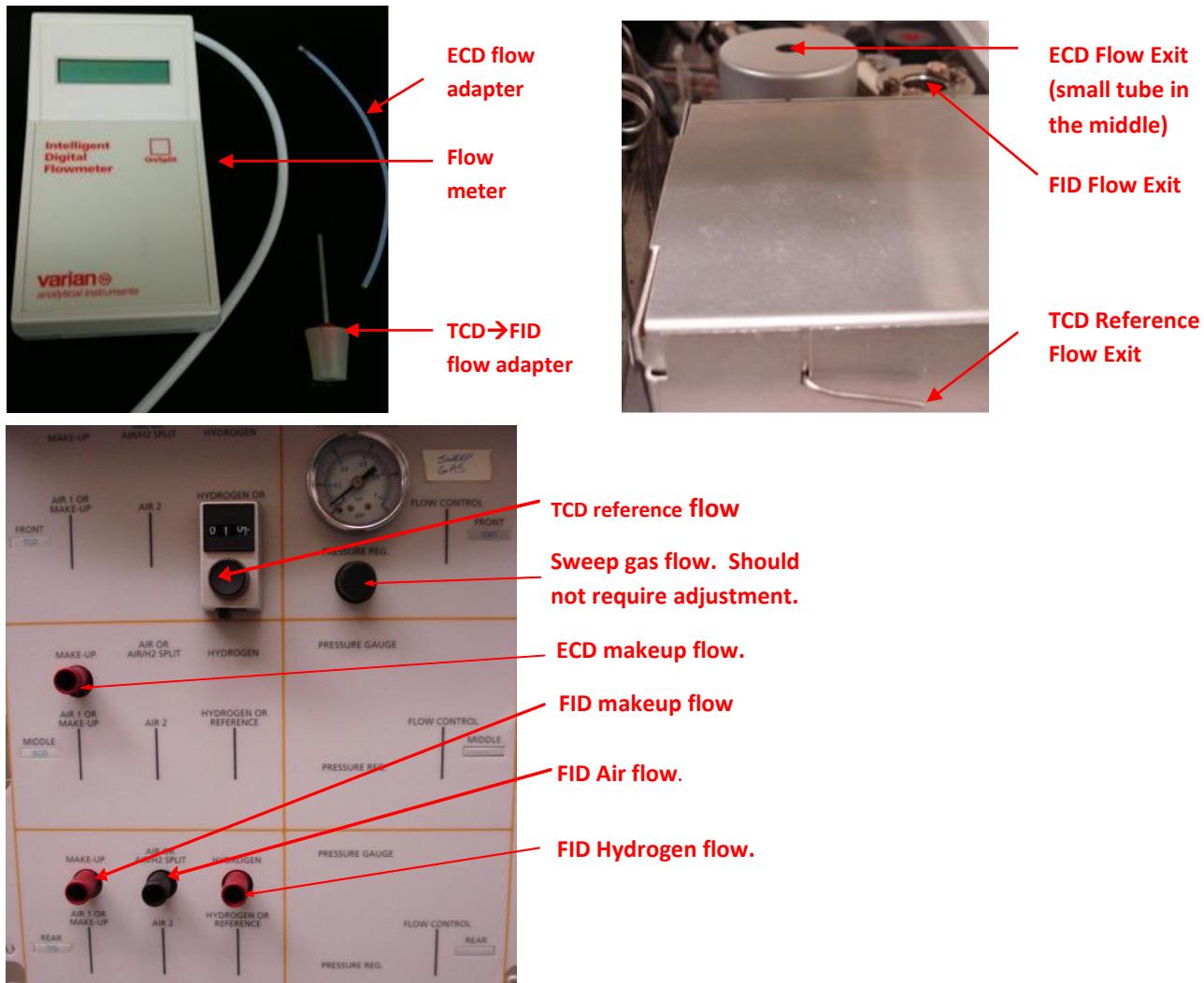


## SETTING AND BALANCING FLOWS:

The Varian 450 has two largely independent plumbing systems, one that operates the TCD→FID series and the other the ECD. Flows through the two systems are balanced separately. In each case, flows are controlled by a combination of EFCs (electronic flow controllers) and manual valves with knobs. Manual flows should be checked periodically.

### Things to Know:

- Flows are set with the oven at operating temperature.
- Turn off the electronic to all detectors.
- Flows are measured with a digital flow meter. For the ECD→FID series, the meter tubing is attached to a large stopper, which is plugged into the top of the FID tower. For the ECD the stopper is replaced with a small tube (shown below).
- Some flows are set electronically with EFCs (electronic flow controllers). Others are set manually using the red and black knobs under the front left panel of the GC (next page).
- Changing pressure at the tank regulators will affect flows.



### Balancing Carrier Gas Flows on TCD→FID series:

1. Turn off electronics.
2. Check the settings of the pressure regulators on the tanks.
3. Turn off all manual FID gases (makeup, air, H<sub>2</sub>); this leaves helium carrier gas flowing. Also turn the air off at the tank because the manual GC valve leaks slightly.
4. Check to see that pressures at the tank regulator are set properly.
5. At the “valve table” of the GC interface, set valve 1 to (+) or INJECT (note: valve is labelled IBV1 on GC). He carrier is now flowing through both the guard and separation columns.
6. Attach the flow meter with the TCD→FID flow adapter to the top of the FID tower.
7. Helium is now passing through the EFC24 on the middle-tab of the GC interface. Use the GC interface to set the pressure to a target flow rate of 15 mL/min. Record both this flow rate and the pressure setting. NOTE: IGNORE THE FLOW RATE INFORMATION ON THIS SCREEN; IT MEANS ABSOLUTELY NOTHING.
8. Set valve 1 to (-) which is the LOAD position. He carrier flow is not passing only through the guard column. Manually adjust the flow from a big black knob on top of the GC and behind the FID. It is the one labelled “middle”. Adjust the flow until it is within 0.1 of the flow rate you recorded in step 7 (close to 15 mL/min).
9. Leaving valve 1 at (-), remove the flow meter from the FID tower. Replace the stopper attachment from the meter hose and replace it with the ECD attachment. Put this over the metal tubing coming from NV1 (needle valve 1), which sits right behind the oven door. Gently turn the needle valve until the flow is close to the value recorded in step 7 (close to 15 mL/min). You are finished balancing carrier gas flows.

### Adjusting Make-up and Flame Flow:

1. Reattach the flow meter to the FID tower.
2. Turn on the TCD→FID makeup gas by twisting the red “FID makeup gas” knob counter-clockwise (left). The outer knob on this type of valve should always be turned all the way until it stops because it is meant to be on/off; it is not for adjustment. DO NOT force the knob!
3. The flow should now be 30 mL/min (15 carrier + 15 makeup). If not, flows can be adjusted by inserting a small screw driver into the center of the valve and turning flow up (counter-clockwise) or down (clockwise) as needed.
4. Turn on the hydrogen flow to the FID flame with the right-most red knob in the FID section. Now the flow should be 60 mL/min (15 carrier + 15 makeup + 30 hydrogen). If not adjust the screw in the center of the knob as above.
5. Turn on the air at the tank regulator, then open the black air knob on the GC. Total FID flow (column + makeup + hydrogen + air) should total 360 mL/min. If not, adjust with the center screw as above.
6. Edit the MS Workstation method to the new EFC pressure recorded in step 6.

Balancing Carrier Gas Flows on ECD:

1. Turn off the ECD electronics.
2. Make sure the carrier gas pressure is set correctly at the tank regulator.
3. Remember there should always be carrier gas flowing through the ECD when it is warm. Drop temperature of ECD to 150 °C for setting flows.
4. Attach the flow meter by sliding the ECD attachment (small tube) over a small metal tube in the center top of the ECD.
5. Turn off the ECD makeup gas by twisting the red knob in the ECD section of the front panel clockwise. Do not force the knob. The only flow through the ECD now is N<sub>2</sub> carrier gas (i.e. no makeup gas).
6. Use the valve table screen to set valve 2 to (+) or INJECT (note: valve 2 is labelled IBV2 in GC interface). Now flow is through both the guard and separation columns.
7. Using the GC interface, electronically adjust the pressure of the EFC24 in the right-most tab of the GC interface until the flow is very close to 15 mL/min. Record both the flow rate and the pressure.
8. Set valve 2 to (-), which is the LOAD position. He carrier flow is now passing only through the guard column. Manually adjust the flow from a big black knob on top of the GC and behind the FID. It is the one labelled "rear". Adjust the flow until it is within 0.1 of the rate you recorded in step 6 (close to 15 mL/min).
9. Turn on the ECD makeup gas by turning the red knob in the ECD section completely counter clockwise. Do not force the knob. The flow should now be about 30 mL/min (15 carrier + 15 makeup). If not, adjust the small screw needle valve in the center of the red knob.
10. Remove the flow meter from the ECD and attach the same tube to NV2, which is just behind the oven door. Adjust the value until it is about the same as the value recorded in step 7 (about 15 mL/min).
11. Check flow on TCD Reference exit tube. Adjust value to match flow recorded in step 7 (this is not usually necessary).
12. Edit the MS Workstation method to the new EFC pressure recorded in step 7.

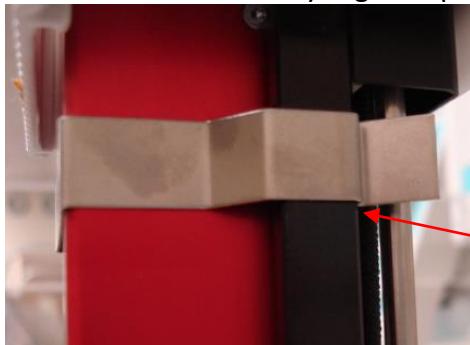
## CHANGING THE COMBIPAL INJECTOR SYRINGE:

### Removing the Syringe

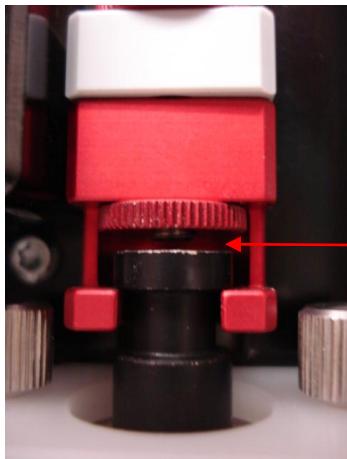
1. Turn off CombiPAL, using the switch on the back of the box on the counter. Once off, the “tower” holding the syringe should slide down so that the Injection Unit rests on the surface of the GC.
2. Gently slide the tower forward (towards you) and to the left (where Tray 1 and 2 positions are) as indicated below, making sure you don’t let the Injection Unit fall down too hard against the GC surface.



3. Pull the Injection Unit down enough so that the silver metal bar across the front of the red “syringe adapter” is exposed. This metal bar was added on at a later date to prevent the syringe adapter from coming out of the tower when running samples.
4. Carefully remove the metal bar by unhooking it from behind the syringe adapter on both sides. This allows the syringe adapter to be removed.



5. Pull the syringe adapter upwards high enough to be able to easily access the area around the top of the plunger.
6. Loosen the red plunger retaining screw by twisting it to the right. Move the black plunger slightly out of the red plunger holder. CAUTION: BE CAREFUL! The syringe will still be hot if CombiPAL was just recently turned off. Once unscrewed, it should look like this:



There will be a gap here  
when unscrewed.

7. Pull the syringe adapter out and then carefully upwards to remove the syringe adapter with the syringe from the Injection Unit. The idea is to pull it completely clear of the window.



8. Unscrew the white plastic retainer.
9. Place the gray plastic tube (located in the 5.0 mL CombiPAL syringe kit in the drawer below the GC) over the needle.



10. Using the gray plastic tube, push the syringe through the syringe adapter and pull it out.

### **Installing a New Syringe After Removing the Old One**

**NOTE:** If the suspected reason for syringe replacement is a broken syringe caused by poor alignment, please perform a position check for the Injection Unit with respect to both tray/vial locations and the GC injector port prior to replacing the syringe and syringe adapter. This can be done as indicated in the “Check Object Positions” section below. This will ensure that the syringe will not be broken just after being replaced.

1. Carefully place the new syringe into the syringe adapter, making sure to avoid bending the needle. Make sure the small red elastic is placed at the base (near the top of the plunger) of the syringe.
2. Pull the black plunger out to approximately 20% of its length.
3. Once the syringe is in place, screw on the white plastic retainer.
4. Move the syringe installed in the syringe adapter partially into the Injection Unit. First, guide the needle into the upper needle guide and then carefully lower it into the lower needle guide. NOTE: For proper placement, the needle should not come lower than the lower needle guide.
5. Place the top of the black plunger into the red plunger holder.
6. Press the syringe adapter inwards so it sits against the back of the syringe carrier/Injection Unit. You will hear/feel it lightly click into place against the magnets used to hold it in.
7. Hook the metal bar back around the syringe adapter.
8. Once in proper position, tighten the red plunger holder and ensure all components of the syringe adapter are sitting firmly in place.
9. Carefully lift the tower and Injection Unit up and move near to its normal resting position on the elevated Tray 3 area on top of the GC.
10. Turn on CombiPAL. It will move back into normal position and can now be used.

### **CHECK OBJECT POSITIONS:**

#### **Injector Alignment**

1. Unlock CombiPAL in the CPAL. 24 window.
2. Click on “Menu” (F1).

3. Select "Setup", then "Objects".
4. Under Objects, select "Front Injector" to check alignment of CombiPAL's injection needle with the GC injector port. We only have this one injector, so ignore the others.
5. You can now check the position of the needle by clicking on the "Check Pos" (F1) and "Mov to Zero" (F3) selections.
  - a) Selecting F1 will move the needle to the injector port position for X, Y and Z axes.
  - b) F3 will move the needle back to the original CombiPAL position at the top of the machine.
6. You can also check each axis individually by selecting (using the button in the middle of the scroll) the axis. This will highlight the axis coordinates and CombiPAL will then move to the position shown for that axis.
7. If absolutely necessary, such as if the needle is out of alignment, the axis values can be changed to re-align the needle. This is done while the axis is selected and highlighted by scrolling up or down to increase or decrease the axis coordinates.
8. After checking (and, if necessary, changing) the alignment, click "Home" (F4) to return to the original CombiPAL screen and lock CombiPAL. Then proceed as normal.

### **Tray Position/Alignment**

1. Unlock CombiPAL in the CPAL. 24 window.
2. Click on "Menu" (F1).
3. Select "Setup", then "Objects".
4. Under Objects, select "Trays" to check alignment of CombiPAL's injection needle with the sample tray. It will check the position for only the tray that is currently selected for use (i.e. V232-10 OR VT-21, not both).
5. You can now check the position of the needle by clicking on the position # (F1) and "Mov to Zero" (F3) selections.
  - c) Selecting F1 once will move the needle to the 001 position; twice will move to the 008 position; and three times to the 032 position. This allows you to check 3 of the 4 corners of the tray to see how they are aligned.

- d) F3 will move the needle back to the original CombiPAL position at the top of the machine.
6. After checking (and, if necessary, changing) the alignment, click "Home" (F4) to return to the original CombiPAL screen and lock CombiPAL. Then proceed as normal.

#### **ECD CONTACT POTENTIAL ADJUSTMENT:**

**NOTE:** Follow this procedure if having problems with lack of sensitivity of the ECD detector. ECD should be able to detect very small amounts of N<sub>2</sub>O, and if not, then the optimum sensitivity will need to be re-adjusted using this procedure. This is performed directly in the ECD section on the GC's touch screen.

- a) Carrier flow must be flowing through the ECD cell.
- b) Set the ECD range to 10.
- c) Turn the Auto Zero to "OFF".
- d) Turn the cell current to "ZERO".
- e) Clear Auto Zero.
- f) Set the contact potential to -750 mV. This will turn off the pulser circuit. Signal should be close to -127 mV. This is your target value. Write this value down.
- g) Set the Contact Potential to a high positive value such as 750 mV. Allow the signal time to respond.
- h) Lower the Contact Potential by 50 mV at a time until the signal becomes the target value.
- i) Go back to the previous 50 mV value and reduce the Contact Potential by 10's until the signal is 5 mV greater than the target value. This is the Contact Potential value you should use for best results.
- j) It may be necessary to adjust the contact potential several times until the ECD is "settled" in.

#### **TO THERMALLY CLEAN THE ECD DETECTOR:**

**NOTE:** This should be used as a last resort to clean the ECD, using N<sub>2</sub> as make-up gas, to increase sensitivity. Only do this if adjusting the ECD contact potential does not fix a sensitivity problem.

- a) Turn heat down on detector to 50°C before handling. Disable column oven by un-checking the "Enable" box in Column Oven screen. Leave all gases flowing normal under Sleep.mth or GASES.mth (doesn't matter as long as gases are flowing through the components).  
**\*IMPORTANT\*** Wait until detector and oven have cooled.
- b) Remove column from the detector, but leave attached to the carrier. Do not cap the end of the column – leave open so carrier can flow through.

- c) Cap the detector inlet using appropriate no-hole (high temperature) ferrule and column nut. These are in an appropriately labeled small bag in the drawer below the GC.
- d) Confirm makeup gas is flowing out of the ECD vent tube – flow should be at least 30 mL/min. This can be done by attaching the appropriate ECD flow connector (found in the black ECD box in the cabinet above the GC) to the flow meter, and measuring at the top of the ECD where the vent tube exits. If flow is not 30 mL/min, adjust accordingly by inserting a screw driver into the ECD's red manual flow control under the front left panel of the GC. Turn counter-clockwise to increase and clockwise to decrease makeup flow into the ECD.
- e) Increase the detector temperature to 400°C. **DO NOT HEAT ABOVE 400°C – This is the absolute maximum the radioactive foil can handle!**
- f) Monitor the output signal while the detector heats to its maximum temperature. The signal will increase initially, and will then gradually decrease as chemical contaminants vaporize from the detector surfaces. This cycle may repeat itself several times.
- g) Allow the detector temperature to remain at 400°C for several hours, or until the signal reaches a relatively stable level. A stable signal indicates that the foil has been cleaned as much as it can be by this method.
- h) Set the detector to normal operating temperatures (300°C) and flow carrier gas through the detector overnight.
- i) Cool detector to 50°C and disable oven prior to handling.
- j) Remove no-hole ferrule/column nut from detector inlet.
- k) Carefully place column back into the detector inlet and tighten the nut enough to ensure the column remains firmly in place. **NOTE: Avoid scratching, etc. the column or it will weaken the coating and potentially cause the column to break. See next section for instructions if this happens.**
- l) Once column is firmly replaced into the detector, regular Sleep.mth or GASES.mth settings may once again be used.
- m) Check and re-adjust the Contact Potential setting if necessary.
- n) If the performance of the detector is not restored by these cleaning methods, the detector will need to be sent back to be refurbished.

#### **IF THE ECD CAPILLARY COLUMN BREAKS:**

- a) Don't panic!
- b) The capillary column serves two purposes: 1) it acts as a particle trap to catch anything that may come out of the column, and 2) it allows for proper length of insertion into the ECD, whereas metal column cannot. Because of this, the column CAN be cut shorter if it happens to break, with minimal interference to sample analysis (however, a very slight retention time change could occur).

- c) Make sure ECD inlet remains sealed using a no-hole ferrule and nut, so that oxygen doesn't get in.
- d) Next step is to "cut" the capillary column shorter to eliminate the broken end and be able to reposition the ferrule and nut.
- e) Place nut and ferrule onto end of column with nut opening facing away from the column coil and towards the broken end. Ensure the nut and ferrule are pulled far enough down to be out of the way for cutting.
- f) Using a proper Capillary Column Cutter (i.e. Alltech Fused Silica Scriber, etc.), cut the column as follows:
  - 1) Place capillary column against finger and draw an edge of the column cutter across the column using a single, light stroke to scratch the coating – DO NOT PRESS TOO HARD.
  - 2) Bend the column away from you on the side opposite of the score.
  - 3) Inspect using a magnifying glass or other magnifier to ensure a clean, square cut has been made.
  - 4) There cannot be any rough edges or an angled cut, as this may negatively affect the chromatograms obtained during analysis (i.e. cause peak tailing, disappearance, etc).
- g) Once cut properly, place nut and ferrule appropriately on the column for the proper insertion depth required for the ECD (see diagram below). This should be 10.5 cm from the end of the column to the bottom of the nut/ferrule.

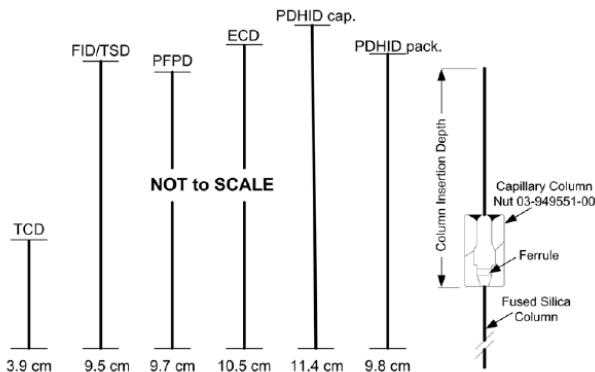


Figure 2: Column Insertion Depths for Detectors

- h) Once nut and ferrule are positioned correctly, insert column back into the detector inlet and tighten just enough to ensure a good seal and the column will be held in place.

#### **ADDITIONAL GENERAL GC NOTES:**

##### Detector Maximum Temperatures

TCD: Filament temp. limit is set to 390°C, which means the max. allowed is 450°C.

ECD: 400°C because of the radioactive foil.

FID: 400°C

#### Additional Flow Notes

Total flow in the ECD is flow through the detector as a whole, including extraneous flows not moving through the column but that are used by the ECD to function correctly.

We have a transfer line TCD, which means there is one line (reference).

To measure TCD column flow, look at the FID flow. Reference flow should match column flow through TCD (that is, through FID).

When the EFC is ON, it gives pressure to the TCD/FID.

Valve table for changing gas flows should be set as follows:

Time	1 IBV	2 IBV	3 EA
Initial	+	-	-