

SOP#: 009

Date Issued: 8/03/2021

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TITLE: **Agilent 7890GC/5975 GC-MS**

SCOPE: Research Personnel

RESPONSIBILITY: BORC staff

PURPOSE: To outline the proper procedures for use and maintenance of the LI-COR Odyssey CLx Imager.

1 PURPOSE

This SOP explains how to apply GC/MS in biomedical research such as fatty acid qualification and quantification, metabolomics etc.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

3.1 The principle of gas chromatography

The sample is first introduced into the gas chromatograph (GC), either with a syringe or transferred from an autosampler (Figure 1 (2)) that may also extract the chemical components from solid or liquid sample matrices. The sample is injected into the GC inlet (Figure 1 (3)) through a septum which enables the injection of the sample mixture without losing the mobile phase. Connected to the inlet is the analytical column (Figure 1 (4)), a long (10 – 150 m), narrow (0.1 – 0.53 mm internal diameter) fused silica or metal tube which contains the stationary phase coated on the inside walls. The analytical column is held in the column oven which is heated during the analysis to elute the less volatile components. The outlet of the column is inserted into the detector (Figure 1 (5)) which responds to the chemical components eluting from the column to produce a signal. The signal is recorded by the acquisition software on a computer to produce a chromatogram (Figure 1 (6)).

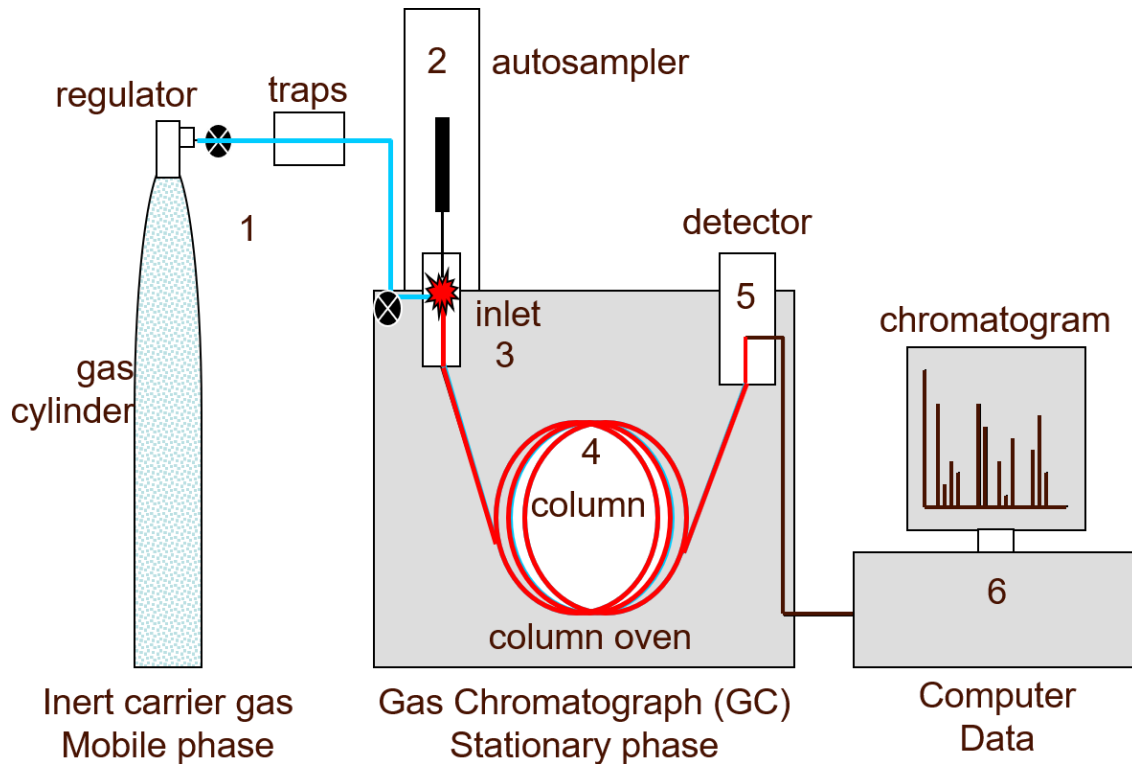


Diagram Credit to Anthias Consulting.

3.2 Overview

Operating the GC involves the following steps:

- 3.2.1 Setting up the GC hardware for an analytical method.
- 3.2.2 Starting up the GC. See ["To Start Up the GC"](#).
- 3.2.3 Preparing the automatic liquid sampler. Install the method- defined syringe; configure solvent and waste bottle usage and syringe size; and prepare and load solvent, waste, and sample vials.
- 3.2.4 Loading the analytical method or sequence into the GC control system.
- 3.2.5 Running the method or sequence.
- 3.2.6 Monitoring sample runs from the GC control panel or the Agilent data system program. See ["About GC Status"](#) or the Agilent data system documentation. • Shutting down the GC. See ["To Shut Down the GC for Less Than a Week"](#) or ["To Shut Down the GC for More Than a Week"](#).

3.3 Instrument Control

The operating panel consists of the display, status lights, and keypad. See "Keypad Operation" and the Advanced User Guide, along with the complete suite of documentation included on the Agilent GC and GC/MS Hardware User Information & Utilities DVD that shipped with your instrument for more detailed information.

3.3.1 The display

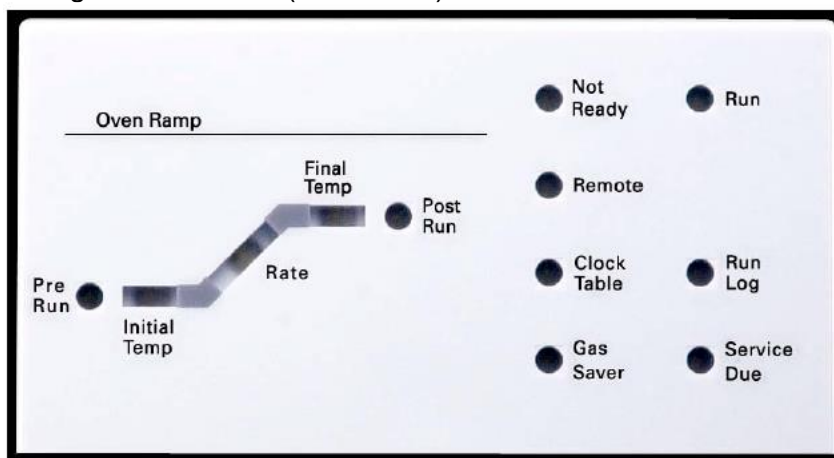
The display shows details of what is currently happening in the Agilent 7890A GC and allows you to make changes to parameters as necessary. Use the scroll keys to view additional lines in the display. A blinking asterisk (*) prompts you to press [Enter] to store a value or [Clear] to abort the entry. You cannot perform any other task until this is done. Refer to “The keypad” and to “Keypad Operation” in this document, or to the Advanced User Guide for more details on how to interact with the displayed information.



3.3.2 Status lights

The status lights provide a basic look at what is currently happening inside the Agilent 7890A GC. A lit LED on the status board indicates:

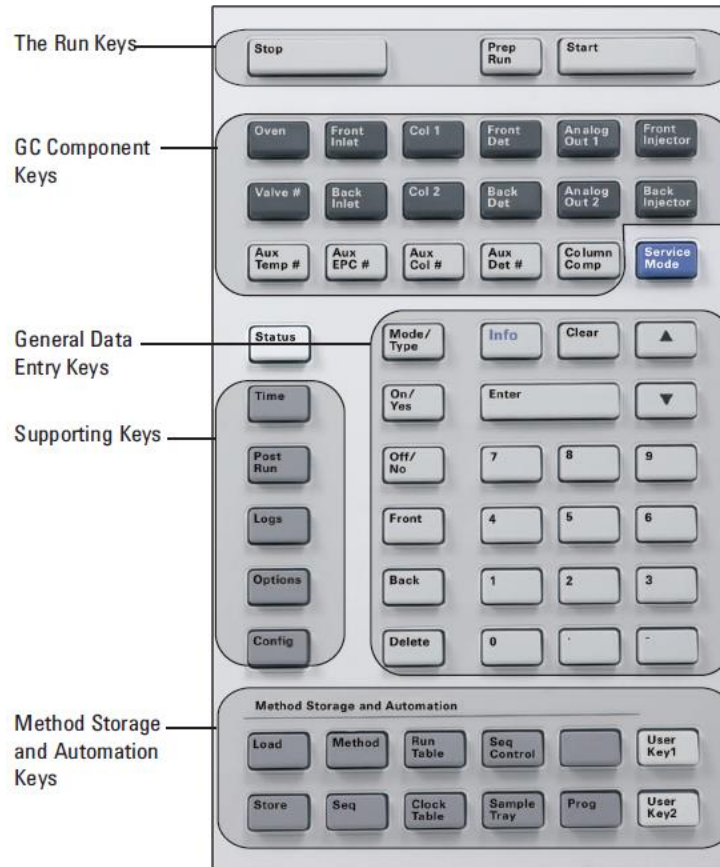
- The current progress of a run (Pre Run, Post Run, and Run).
- Items that may require attention (Rate, Not Ready, Service Due, and Run Log).
- The GC is controlled by an Agilent data system (Remote).
- The GC is programmed for events to occur at specified times (Clock Table).
- The GC is in gas saver mode (Gas Saver).



3.3.3 The keypad

All of the parameters required to operate the Agilent 7890A GC can be entered through the GC’s keypad. Normally, however, **most of these parameters are controlled using an attached data system, such as the Agilent ChemStation.**

When the Agilent ChemStation is controlling your Agilent 7890A GC, it is possible for the ChemStation to disable editing of the GC's current method from the keypad.



4 PROCEDURES

4.1 Check instrument and gather materials needed

This procedure starts with the GC turned off and all hardware previously configured. Items requiring a possible change in configuration to suit a particular method are addressed here. These include the column, gas types used in pneumatic controllers, inlet liners and O-ring, and septums.

4.1.1 Review the Agilent 7890A Gas Chromatograph Safety manual (P/N G3430-90013) and Agilent 7890A Gas Chromatograph Operating manual (P/N G3430-90010) for important safety information and start up details before powering on your instrument.

4.1.2 Gather the following:

- Evaluation column, HP- 5 30 m x 0.32 mm x 0.25 μ m (19091J- 413)
- FID performance evaluation (checkout) sample (PN 5188- 5372)

- Chromatographic- grade iso-octane
 - 4- mL solvent and waste bottles or equivalent for autoinjector
 - 2- mL sample vials or equivalent for sample
- 4.1.3 Verify the following:
- Capillary column jet installed. If not, select and install a capillary column jet.
 - Capillary column adapter installed (adaptable FID only). If not, install it.
 - Chromatographic- grade gases plumbed and configured: helium as carrier gas, nitrogen, hydrogen, and air.
 - Empty waste vials loaded in sample turret.
 - 4-mL solvent vial with diffusion cap filled with iso-octane and inserted in Solvent A injector position.
- 4.1.4 Verify that the split/splitless (S/SL) inlet septum, liner, and O-ring are clean, properly installed, and in good condition. See the procedure in the 7890A Maintenance manual (G3430-90010).
- 4.1.5 Install a conditioned (HP- 5 30 m x 320 μ m x 0.25 μ m) column in the GC. Attach the column inlet to the S/SL inlet and its outlet to the FID. See the procedure in the 7890A Maintenance manual (G3430-90010).
- 4.1.6 Verify that the He carrier gas line valve is open and the carrier gas is attached to the GC inlet flow module.
- 4.1.7 Verify that the H₂, makeup gas, and air gas line valves are open and these lines are attached to the GC FID module.
- 4.1.8 Verify that the GC is connected to a grounded power source.

4.2 To Start Up the GC and specify configurations

- 4.2.1 Turn on the GC power. Wait for Power on successful to be displayed.
- 4.2.2 Specify the column configuration. From the GC keypad press [Config][Col 1] (or [Config][Col 2]). The column dimensions are located on a metal tag located on the column basket.
- 1) Scroll to the Length line, type the column length, in meters, followed by [Enter].
 - 2) Scroll to Diameter, type the column inside diameter in microns, followed by [Enter].
 - 3) Scroll to Film thickness, type the film thickness in microns, followed by [Enter]. The column is now defined.
 - 4) Scroll to Inlet. Press [Mode/Type] to select a gas pressure control device for this end of the column. Select the front or back inlet location with the split/splitless inlet and press [Enter].
 - 5) Scroll to Outlet. Press [Mode/Type] to select a gas pressure control device for this end of the column. Select the front or back detector location with the FID and press [Enter].
 - 6) Scroll to Thermal zone. Press [Mode/Type] to see the available choices. Select the GC Oven and press [Enter].
- 4.2.3 Specify carrier gas flow to the column. From the GC keypad, press [**Col 1**] (or [**Col 2**]).
- 1) Scroll to Mode. Press [Mode/Type], select Constant Flow and press [Enter].

- 2) Scroll to Velocity and enter 30 to set the column velocity to 30 cm/s. The Pressure and Flow values are calculated and displayed by the GC.
- 4.2.4 Bake out the flow path
- 1) Purge the column with carrier flow for at least 20 minutes before heating the column. The HP-5 column in our example has a maximum temperature of 325 °C. Do not exceed the maximum temperature noted on your column.
 - 2) To set up the inlet temperature and flow:
 - a From the GC keypad, press [Front Inlet] (or [Back Inlet] if the Split/Splitless is located there) and with Temperature selected press [On/Yes] and enter 300 °C or 25 °C above the normal operating temperature, to turn on the inlet heater and set its temperature.
 - b Press [Up Arrow] to select Mode and press [Mode/Type] to select Split mode and press [Enter].
 - c Press [Down Arrow] to select Pressure and press [On/Yes] to open the carrier gas control valve.
 - d Press [Down Arrow] twice to select Split flow, enter 200 mL/min and press [Enter]. Split ratio will be calculated for you.
 - 3) To set the column temperature from the GC keypad, press [Oven] and with Temperature selected press [On/Yes] and enter 225 °C, or 25 °C above the GC method final oven temperature. This turns on the oven heater and set its temperature. Do not exceed the column manufacturer's maximum temperature limit.
 - 4) To set up the FID:
 - a From the GC keypad, press [Front Det] (or [Back Det] if the FID is located there) and with Temperature selected press [On/Yes] and enter 325 for the detector Temperature to turn on the detector heater and set its temperature to 325 °C.
 - b Press [Down Arrow] to select H2 Flow and press [On/Yes] and enter 30 mL/min.
 - c Press [Down Arrow] to select Air Flow and press [On/Yes] and enter 400 mL/min.
 - d Press [Down Arrow] to select Makeup and press [On/Yes] and enter 25 mL/min.
 - e Press [Down Arrow] to select Flame and press [On/Yes] to ignite the flame.
 - f To check the FID baseline output, press [Down Arrow] to view the Output. It should be between 5 pA and 20 pA and relatively stable. (If using a gas generator or ultra pure gas, the signal may stabilize below 5 pA.) If the output is outside this range or unstable, resolve this problem before continuing.
 - g If the output is too low:
 - Check that the electrometer is on.
 - Check that the flame is lit.
 - 5) Bake out the flow path for a minimum of 30 minutes.

4.3 Create method through keypad (optional)

A method is the group of setpoints needed to run a single sample on the GC, such as oven temperature programs, pressure programs, inlet temperatures, sampler

parameters, etc. A method is created by saving a group of setpoints as a numbered method using the **[Store]** key. At least 10 methods can be stored. Setpoint parameters are saved when the GC is turned off and loaded when you turn the instrument back on. However, if the hardware was changed while the instrument was turned off, it may not be possible to restore all setpoints in the method.

Table 17 Setpoint parameter components

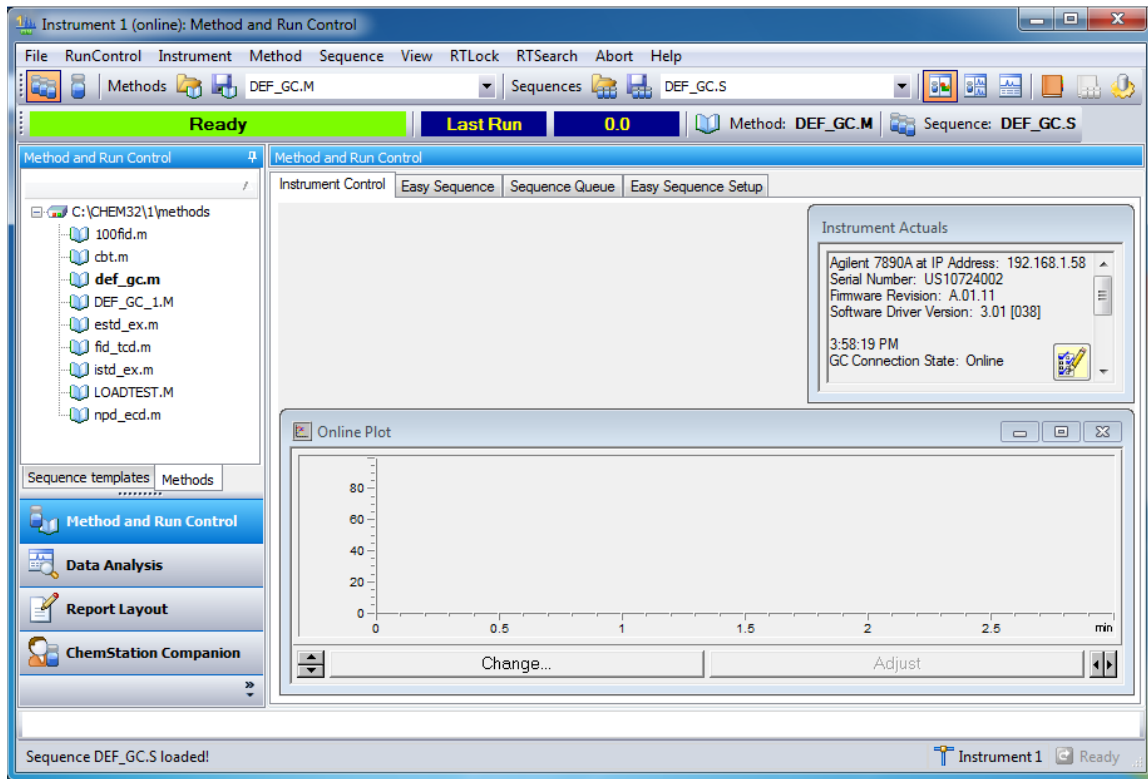
Component	Component
Oven	Aux temp
Valve 1–8	Aux EPC
Front and back inlet	Aux column
Columns 1 to 6	Aux detector 1 and 2
Front and back detector	Post run
Analog 1 and 2	Run table
Front and back injector (see Table 18)	Sample tray

- 4.3.1 Individually select each component for which setpoint parameters are appropriate for your method. (See [Table 17](#).)
- 4.3.2 Examine the current setpoints and modify as desired. Repeat for each component as appropriate.
- 4.3.3 Examine the current setpoints for the ALS, if appropriate, and modify as desired. (See [“To program the ALS”](#) on page 117.)
- 4.3.4 Save the setpoints as a stored method. (See [“To save a method”](#) on page 119.)
- 4.3.5 Press [Load][Method]. Supply the method number and press [Enter]. The specified method will replace the current active method.

4.4 Create method using Agilent ChemStation

From the Windows start icon, click All Programs, click Agilent ChemStation, then click the on-line ChemStation program for your instrument.

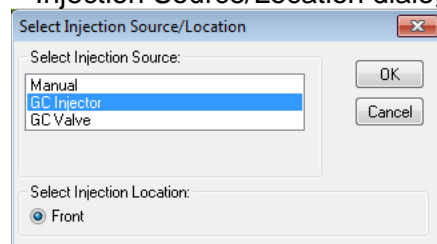
The ChemStation user interface is displayed. Click the Method and Run Control button located in the left pane at the bottom, to display the ChemStation Method and Run Control view, if that view is not displayed.



4.41 Edit Entire Method

With on-line session connected to the 7890A, upload the current method from the GC and Save with a new Method Name such as default.m or first connect.m.

- 1) Click the Method menu then click Edit Entire Method..., to create the method.
The Edit Method dialog box opens.
- 2) Mark the Method Information and Instrument/Acquisition checkboxes only.
Clear the Data Analysis and Run Time Checklist checkbox.
The Data Analysis part of the method will be updated after the run.
- 3) Select OK to close the Edit Method dialog box. If Method Information was selected, the Method Information dialog box opens.
- 4) In the Method Comments field, enter a description of this method.
- 5) Select OK to close the Method Information dialog box and display the Select Injection Source/Location dialog box.



- 6) From the Select Injection Source dropdown list, select your source. Only configured injector sources are listed.

- If you are injecting from the GC using the Automatic Liquid Sampler (ALS), select GC Injector.
 - If you are manually injecting select Manual.
 - If using a valve select GC valve. The GC used for this example does not have valves installed.
- 7) In the Select Injection Location area, select an injection location of Front, Back, or Dual.
 - 8) Select OK to close the Select Injection Source/Location dialog box and display the Setup Method window.

4.4.2 Set GC configurations

- 1) Select the Configuration button, to specify pressure units, configure a column, and specify gas types for pressure and flow controllers associated with detectors, inlets, and auxiliary devices.
- 2) With the Miscellaneous tab selected, select the Pressure Units type. Under Valve Configuration set up valves if installed or set all Valve Types to Not Installed. Review the online help for an explanation of Slow fan and Thermal Aux Type.
- 3) Select the Columns tab to display the column configuration parameters. The HP-5 checkout column is listed in the Column position 1.
- 4) If the HP-5 is not listed under Column, or another column will be used, click the Inventory button and add it to inventory before listing it here. See "Add a column to ChemStation local inventory " on page 36.
- 5) Use the up and down arrow keys to move the HP-5 column into one of the six column positions. The column is generally installed in the 1 position.
- 6) For the Inlet pressure for this column, select the location of the Split/Splitless inlet from the dropdown.
- 7) For the column Outlet pressure select the FID location.
- 8) For the column Heated By select Oven from the dropdown.
- 9) Click the Apply button and select the Modules tab.
- 10) Select He or H2 as the carrier gas from the dropdown for the inlet connected to column 1.
- 11) Select He or N2 as the Makeup gas from the dropdown for the Front Detector.
- 12) Click the Apply button and select the ALS tab.
- 13) Enter a Syringe Size of 10 μ L.
- 14) Select a Solvent Wash Mode of A, B. The other Solvent Wash Mode options, A-A2, B-B2 and A-A3, B-B3, are used with sequences.
- 15) Click the Apply button to download any edits to the GC.

4.4.3 Set the GC readiness state

The 7890 GC readiness state is reached when all GC parameters specified here are within their method specified setpoints. This Readiness requirement can also be set from the 7890A GC keypad.

- 1 Select the Readiness button, . The Readiness parameters are displayed.
- 2 Select the Oven, Front Inlet and Front Detector (both attached to column 1). These selections require the GC to wait until all setpoints related to the oven, inlet, and detector are held at a steady value before allowing a run to begin.

3 Click Apply to download these selections to the GC.

4.4.4 Set the GC oven parameters

- 1 Select the Oven button, The Oven parameters are displayed.
- 2 Mark the Oven Temp On checkbox.
- 3 In the Equilibration Time field, enter 0.5 min to shorten the default time of 3 min.
- 4 In the Maximum Oven Temperature field, enter 325 °C. This is the maximum temperature for the HP-5 column.
- 5 Clear the Override Column Max. 325 °C checkbox.
- 6 In the Oven Ramp table, enter the settings shown in Table 3.
- 7 In the Post Run field, enter 220 °C.
- 8 In the Post Run Time field, enter 2 min to hold the 220 °C oven temperature for 2 minutes after the run is finished.
- 9 Select Apply to download these settings to the GC.

4.4.5 Set the GC column parameters

- 1 Select the Columns button, . The Column parameters are displayed
- 2 Check the Column information in the Selection list.
 - Column: 19091J-413 (HP -5 30 m x 320 µm x 0.25 µm)
 - In: front or back (split/splitless inlet position)
 - Out: FID
- 3 Mark the Control Mode checkbox.
- 4 Select Flow in the Control Mode area.
- 5 In the Flow Setpoint field, enter 6.5 mL/min. The Pressure, Average Velocity, and Holdup Time Setpoints are calculated and displayed in the corresponding fields.
- 6 Select Apply to download these settings to the GC.

4.4.6 Set the GC inlet parameters

- 1 Select the Inlets button, . The Inlet parameters are displayed.
- 2 Select the front or back tab, depending on your hardware configuration.
- 3 Mark the Heater checkbox and enter 250 °C in the corresponding Setpoint field.
- 4 Mark the Pressure checkbox. The psi in the corresponding Setpoint field is automatically set when the column flow rate is set.
- 5 Mark the Septum Purge Flow checkbox and enter 3 mL/min in the corresponding Setpoint field.
- 6 From the Septum Purge Flow Mode drop down list, select Standard. Gas Saver reduces the carrier gas flow rate from the split vent after a sample is within the column. The program maintains column head pressure and flow rate while purge and split vent flow rates are decreased. See online help for details.

In general, start Gas Saver after inlet purge time for splitless injection or 2 minutes after a split injection. Reduced flow rates continue until the next run.

To use Gas Saver, select it and enter:

- The reduced split vent flow rate you want (in mL/min)

- The time, in minutes, when flow rate is to decrease after a run has started
Keep Gas Saver 15 mL/min above the maximum column flow rate during the run.

- 7 In the Mode area select Splitless.
- 8 In the Purge Flow to Split vent area:
 - a In the field, enter 40 mL/min.
 - b In the at field, enter 0.5.
- 9 Select Apply.

4.4.7 Set the GC injector parameters

If you are not using the autosampler, skip this section.

- 1 Select the ALS button, .
- 2 Select the Front Injector, Back Injector, or Dual tab, depending on your hardware configuration.
- 3 In the Injection area:
 - a Verify that the 10 μ L Syringe Size matches your hardware configuration.
 - b In the Injection Volume field, enter 1.
- 4 In the Washes and Pumps area:
 - a For Solvent A Washes, enter 5 in the PostInj field.
 - b For Sample Washes, enter 3 in the PreInj field.
 - c For Sample Pumps, enter 5 in the PreInj field.
- 5 Select the Advanced button, . Additional options are displayed in the window.
- 6 In the Plunger Speed area, select Fast.
- 7 Select Apply.

4.4.8 Set the GC detector

- 1 Select the Detectors button, .
- 2 Select the Heater checkbox and enter 300 for the heater setpoint.
- 3 Select the H2 Flow checkbox and enter 30 mL/min for the H2 Flow setpoint.
- 4 Select the Air Flow checkbox and enter 400 mL/min for the Air Flow setpoint.
- 5 Select the Makeup Flow checkbox and enter 25 mL/min for the Makeup Flow setpoint.
- 6 Select the Flame checkbox to ignite the flame. When igniting the flame, the detector will attempt ignition several times.
- 7 Select Apply.

4.4.9 Set the GC signals parameters

- 1 Select the Signals button.
- 2 From the Signal Source dropdown list, select Front (or Back) Signal (FID) for the signal source. When using multiple detectors you may assign specific signals to both the Front Signals and the Back Signals. See the online help for configuration instructions.
- 3 For the Data Rate / Min peak Width select 20 Hz/.01 min from the dropdown menu.
- 4 In the Signal Source dropdown list, select None for the other signal sources.

4.4.10 Save the method

- 1 From the Method menu select Save Method As to display the Save Method As dialog.
- 2 Enter checkout.M in the Method File field.
- 3 Click OK to save the current ChemStation method as checkout.m method.
If the Method Audit Trail is enabled you are prompted to enter an Audit Trail comment. Enter a comment and click OK to close the dialog.

4.5 Run a Single Sample

4.5.1 Prepare the Sample

- 1 Fill a sample vial with the contents of the 0.5 mL ampule, Agilent sample p/n 5188-5372, and cap the vial. If you are not using an ALS skip the remaining steps.
- 2 Place the sample vial into position 1 of the GC sample tray.
- 3 For solvent wash mode A, B:
 - Fill a solvent wash vial with iso-Octane and place it in injector turret location A.
 - Place a waste vial in injector turret location W.

4.5.2 Load the Method

- 1 To start the GC ChemStation, from the Windows start icon, click All Programs, click Agilent ChemStation, then click the online ChemStation program for your instrument.
- 2 Select the Load Method button, to open the Load Method window. Navigate to and select checkout.M.

4.5.3 Complete the Run Time Checklist

On the Run Time Checklist dialog, you will mark the check box for each section of a method that you want to be run.

- 1 In the Method and Run Control view in the Method menu click Run Time Checklist to display the Run time Checklist dialog.
- 2 In the Check Method Selections to Run area, for this example:
 - a Mark the Data Acquisition check box.
 - b Mark the Standard Data Analysis check box.
 - c Clear the other check boxes.
- 3 You can optionally mark the Save Method with Data check box.
- 4 Select OK to set the sections of the method to run and close the dialog box.

4.5.4 Run the Method

- 1 Open the RunControl menu and choose Sample Info.
- 2 In the Operator Name field, enter your name.
- 3 In the Data File area:
 - a For the Path accept the default shown.
 - b Enter CHECKOUT as a subdirectory name.
 - c Select Manual and enter checkout.D in the Signal 1 field.
- 4 In the Sample Parameters area:
 - a Select Front or Back to specify the location of the ALS.
 - b In the Sample Name field, enter a name for your sample (optional).
 - c In the Location field, enter the tray location where the vial is located. Here we entered 10 and the system changes our entry to Vial 10. Vial position numbers are labeled on the trays. If you are making a blank run do not locate a vial at the specified position.
 - d If not using a tray the vial locations are, 101 for front and 201 for back.

- e The Sample Amount field and the ISTD Amount fields are not required for Qualitative calculations.
- f For the Dilution and Multiplier fields keep the default value of 1.
- g In the Comment field, enter a comment (optional).
- 5 Click Run Method to close the dialog box and start the run. The ready state indicator changes to Run. The data acquired during this run is used later starting in "Setup Data Analysis Options"

4.5 Correcting Problems

- 4.5.1 A series of warning beeps sound if the GC encounters a problem. For example, a series of beeps sound if the front inlet gas flow cannot reach setpoint. The message **Front inlet flow shutdown** is briefly displayed. The flow shuts down after 2 minutes. Press **[Clear]** to stop the beeping. A continuous beep sounds if a hydrogen flow is shut down or a thermal shutdown occurs. Press **[Clear]** to stop the beep. A single beep means that a problem exists, but the problem will not prevent the GC from executing the run. The GC will emit one beep and display a message. The GC can start the run and the warning disappears when a run starts. Fault messages indicate hardware problems that require user intervention. Depending on the type of error, the GC will beep once or not at all. If a gas flow, multiposition valve, or the oven is shut down by the system, **Off** or **On/Off** will blink on the appropriate line of the components parameter listing.
- 4.5.2 If the GC stops operation because of a fault, for example a flow module shutdown after running out of carrier gas, do the following:
- 1) Use the keyboard or data system to stop the alert tone. Click [Clear] on the keyboard or turn off the offending component in the data system.
 - 2) Resolve the problem, for example, by changing gas cylinders or fixing the leak. See the [Troubleshooting Guide](#) for details.
 - 3) Once the problem is fixed, you may need to either power cycle the instrument, or use the software keyboard or data system to turn the problem component off, then on again. For shutdown errors, you will need to do both.

4.6 Qualitative Data Analysis

4.6.1 Setup Data Analysis Options

- 1 From the Windows Start button select All Programs>Agilent ChemStation and click the name of your Offline Instrument. The ChemStation is displayed in offline mode.
- 2 Click the Data Analysis button in the ChemStation Explorer window to view the Data Analysis window. The Integration tab is selected by default.
- 3 Click the View Preferences icon to display the Preferences dialog. When we load a data file we can set an option to automatically integrate the file after it is loaded.
- 4 Under the Signal/Review Options tab, in the Load Signal Options Area, select Integrate after load.
- 5 In the Data Review Options area, for the Method used for Single Run Data select Current Method. This is the master method.

- 6 Click OK to close the dialog.
- 7 Select Graphics>Signal Options, to display the Signal Options dialog to set the Autoscale feature.
- 8 In the Ranges area select Autoscale and click OK to close the dialog. The algorithm for auto scale does not work by selecting peaks. It splits the data points into “buckets”, then looks at the max and min in each bucket, throws out the largest, and uses the second largest to base the scaling on. This is not necessarily the second largest peak (it depends on the size of the data point “buckets”).
- 9 Double click the ChemStation Explorer tree CHECKOUT subdirectory to load all single run data files into the Navigation Table. Data files are organized into Single runs and Sequences.
- 10 Navigate to the CHECKOUT.D. data file in the Navigation Table. This is the acquisition data file created from our sample run.
- 11 Double-click the CHECKOUT.D. data file line in the table. The data file is loaded and its chromatogram is displayed according to its method settings. The data file is automatically integrated and the chromatogram is automatically scaled to fit the in the window.
If the chromatogram displays only a single large peak, you may not have set the Auto scale feature, as described in Step 7

4.6.2 Edit Integration Events

Display integration events

When the data analysis part of your method is run, the chromatogram is integrated. Most chromatograms can be successfully integrated using the ChemStation default auto integration parameters. However, you may edit these auto integration event values and add additional integration events for your specific method.

- 1 Click the Auto Integrate icon to calculate suitable initial integration event parameters for the loaded signal.
- 2 Select the Edit/Set Integration Events Table icon. The Integration Event Table displays next to the chromatogram.

Set area reject

To demonstrate how Integration Events are used to filter peaks, we will remove the peak located at time 4.554 using the Area Reject event and then remove the peaks located at times 0.637 and 1.387 by turning the integrator off before it reaches these peaks and then turning the integrator back on after these peaks are processed.

To remove a peak using the Area Reject event:

- 1 Select FID1 A Specific from the Specific Events for Signal drop down menu. In this example this is the front detector signal shown in the chromatogram. These events apply to this signal only.
- 2 Click on the Area Reject icon , mouse over the chromatogram, and observe the vertical line that moves with the cursor.

- 3 Move the vertical line to the 2.3 minute time and click the left-mouse button. The Area Reject event is added to the Integration Events table at this time and given a Value of 0.
- 4 Set the Area Reject Value to 3.0 in the Integration Events table and click to complete this edit. This peak at 4.554 minutes is filtered out and removed from the table.

Set integration off and on

To remove peaks using the Integration Off/On event:

- 1 Click on the Integration icon , mouse over the chromatogram, and observe the vertical line that moves with the cursor.
- 2 Move the vertical line to the 0.2 minute mark and click the left-mouse button. The Integration OFF event is added to the Integration Events table at this time.
- 3 Move the vertical line to the 2.0 minute mark and click the left-mouse button. The Integration ON event is added to the Integration Events table at this time.
- 4 Click on to complete this edit.

4.6.3 Manually Integrate Peaks

Manual peak integration allows interactive modification of peak integration for a data file. A set of graphic tools allows modification of the chromatogram directly. These changes can be saved with the data file (available since ChemStation revision B.04.01) or saved with the method. This section examines manual integration saved with data files.

- 1 Load a signal data file by selecting File > Load Signal from the menu to display the Load Signal dialog. You can also load the file from the navigation table, which is read only.
- 2 In the right pane select the CHECKOUT folder where the data file is located then, from the left panel, select the CHECKOUT.D data file to be integrated and click OK.
- 3 Click the Integrate icon to integrate the signal with the specified method's integration events.
- 4 Select the Zoom In icon then, click and drag the left mouse button to zoom in on the peak of interest at 2.92 minutes in the chromatogram.
- 5 Select the Manual integration: Remove integrated Peak(s) from the Integration Results icon , then click the peak at 2.92 minutes to delete its peak. The peak table located below the chromatogram no longer contains this peak.
- 6 Select the Manual integration: Draw Peak baseline(s) and integrate icon , then click and drag the right mouse button to draw an integration baseline on the peak. When you release the button, the peak will be integrated and added to the peak table located below the chromatogram.
- 7 Other tools available include:
 - The Undo icon to undo the last manual event edit
 - The Zoom out icon
 - The Draw Negative Peak Baseline icon
 - The Tangent Skim One Peak icon
 - The Split Manually Integrated Peaks icon

- 8 Click the Save icon to save the current manual integration events to this data file. This is the recommended method for saving a manual integration. When a manual integration events file is saved with the data file, the Manual Events column of the Navigation Table is marked with the symbol.
- 9 The Delete icon is used to delete all manual events from the data file. The manual integration file remains with the data file until this delete action is saved. Click the Save icon to permanently delete the current manual events file from this data file.

4.6.4 Modify the Method to Print a Report

- 1 Click the Specify Report Calculation and Print Style icon to display the Specify Report dialog. You can also select this dialog from the menu by selecting Report > Specify Report.
- 2 To create an area percent report, in the Quantitative Results area, select Percent for Calculate, Area for Based On and Signal for Sort By.
- 3 For destination select Printer and Screen.
- 4 Click the Identify Peaks, calculate and preview results icon to view the Report. To Print the Report, from the main menu select Print > Report.
- 5 Click the Identify Peaks, calculate Results and Print Reports icon to send a copy of the report to the printer.
- 6 Click the Save Method icon to save the report specification to the method or select Method > Save Method As to create a method with a new name.

4.7 Create and Run a Sequence (optional)

A sequence is a list of samples to be analyzed and a designated method to be used for each analysis. Once defined, the sequence may run unattended, automatically processing the samples defined in the sequence.

When an ALS is installed, the entire analysis, from injection of the sample through reporting of results, can be automated to save you time.

4.7.1 Set the sequence preference

- 1 Start a ChemStation online session.
- 2 From the main menu select View > Preferences to display the Preferences dialog.
- 3 Under the Data Storage area select Unique Folder Creation ON. This option specifies that each time a sequence is run, the system creates a unique directory where it stores all files related to the completed sequence run including its methods, logs, batch files, and data files.
- 4 In the Name Pattern area, optionally change the default name pattern for the sequence container (storage directory). This default name pattern is made using the sequence name with a time stamp appended to assure its uniqueness. See the online help for other naming options.
- 5 Click OK to close the dialog.

4.7.2 Prepare the Samples

- 1 Fill a sample vial with the contents of the 0.5 mL ampule, Agilent sample p/n 5188-5372 and cap the vial. Mark this vial as the 23.6 mg/μL calibration standard.

- 2 Fill a sample vial with the contents of the 0.5 mL ampule, Agilent sample p/n 5188-5372. Add an equal amount iso-Octane to the vial for a 1:2 serial dilution and mix the vial contents.
- 3 Remove 0.5 mL from this vial, place it in a new vial, and cap the original vial. Mark this capped vial as the 11.8 mg/μL calibration standard.
- 4 Take the uncapped vial from the previous step and add 0.5 mL of iso-Octane to the vial for a 1:2 serial dilution and cap the vial. Mark this vial as the 5.9 mg/μL calibration standard.
- 5 Make an unknown concentration sample in a vial by diluting a 23.6ug/mL solution. Cap and mark this vial unknown sample.
- 6 If you are not using an ALS skip the remaining steps.
- 7 If you are using an ALS:
 - a Place the sample calibration vials into positions 20 through 22 of the GC sample tray. Place the lowest concentration level in position 20, and the other two in order of increasing concentration.
 - b Place the unknown sample vial in position 23.
 - c Fill a solvent wash vial with iso-Octane and place it in injector turret location A for solvent wash mode A, B.
 - d Place a waste vial in turret location W specified for solvent wash mode A, B.

4.7.3 Set the Sequence Parameters

- 1 With the ChemStation running in online mode, click the Method and Run Control button to display the Method and Run Control view with the Instrument Control tab selected.
- 2 Load the CHECKOUT.M method.
- 3 From the Sequence menu, select New Sequence Template to clear any entries from the current sequence table.
- 4 From the Sequence menu, select Sequence Parameters to display the Sequence Parameters dialog.
- 5 With the Sequence Parameters tab selected, select a Data File Path from the drop down. If the required path is not shown, access the Preferences dialog to add it.
- 6 In the Part of method to run area, select According to Runtime Checklist.
- 7 Enter your name in the Operator Name field.
- 8 Click OK to close the dialog.

4.7.4 Create and Run a Sequence

- 1 From the Sequence menu, select Sequence Table to display an empty Sequence Table dialog.
- 2 Click the Append Line button twice to add two lines to the table.
- 3 Click the Insert/Filldown Wizard button to display the Filldown Wizard dialog.
- 4 In the Action area select Fill down and In the List of detected ranges, select both lines.
- 5 In the Location assignments area, set the Starting location to 20 and the Increment by value to 1. This specifies that the first vial is located in position 20 and the other vials are in locations 21 and 22 in the ALS tray.

- 6 In the Fields area, click the Clear All Fields button and do not select Omit other sample types.
- 7 Select the CHECKOUT method from the Method name drop down list and select calibration from the Sample type drop down list.
- 8 In the Sample name field enter CAL\C1\. The \C1\ parameter creates samples names CAL1, CAL2, and CAL3. For other applicable configurations click the Help button.
- 9 Click OK to close the dialog and fill in the required columns in the Sequence Table.
- 10 In the Sequence Table Cal Level column, for samples 2 and 3, change the entries to 2 and 3.
- 11 Click the Append Line button to append a line to the table.
- 12 Enter a Vial number of 23 and a Sample Name of unknown01.
- 13 Click OK to close the dialog.
- 14 From the main menu select Sequence > Save Sequence As to open the Save Sequence as dialog and enter CHECKOUT as the sequence name. For the location use the chem32/instrumentNumber/sequence directory. This is the directory where the sequence templates are stored. This sequence becomes the current default sequence.
- 15 From the main menu select Sequence > Run Sequence to run the CHECKOUT.s sequence or click the Start Sequence button on the sampling diagram.

5 MAINTENANCE

5.1 Shut Down the GC for Less Than a Week

- 5.1.1 Wait for the current run to finish.
- 5.1.2 If the active method has been modified, save the changes.
- 5.1.3 Turn off all gases, except the carrier gas, at their sources. (Leave the carrier gas on to protect the column from atmospheric contamination.)
- 5.1.4 If you are using cryogenic cooling, turn off the cryo coolant at the gas source.
- 5.1.5 Reduce detector, inlet, and column temperatures to between 150 and 200 °C. If desired, the detector can be turned off. See the following table to determine if it is advantageous to shut down the detector for a short time period. The time required to return the detector to a stable condition is a factor.

5.2 Shut Down the GC for More Than a Week

- 5.2.1 Load a GC maintenance method and wait for the GC to become ready. For more information about creating maintenance methods, see the Maintaining Your GC manual. (If a maintenance method is not available, set all heated zones to 40 °C.)
- 5.2.2 Turn off the main power switch.
- 5.2.3 Shut off all gas valves at the gas source. 4 If you are using cryogenic cooling, shut off the cryo coolant valve at the source.

5.2.5 When the GC is cool, remove the column from the oven and cap both ends to keep out contaminants.

5.2.6 Cap the inlet and detector column fittings and all GC external fittings.

6 REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT