Title: Operation of the Agilent Technologies 1100 HPLC / 1200 RRHPLC Using OpenLAB Chromatographic Data System (CDS) ChemStation Edition

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Approval/Date:

Approval/Date:

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1.0 Purpose

This procedure describes the basic operation and maintenance of the Agilent Technologies 1100 HPLC / 1200 RRHPLC Systems using OpenLAB CDS ChemStation Edition software for Windows.

2.0 Scope

The 1100 HPLC/1200 RRHPLC are multi-component, analytical liquid chromatography systems capable of performing a wide variety of analytical analysis such as reverse-phase, size exclusion, and ion-exchange chromatography. Trained Process Analytics personnel will use these instruments.

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC personnel are responsible for the maintenance of these systems and the implementation of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Equipment

4.1 Quaternary Pump, Agilent #G1311A, or equivalent (and/or Binary Pump, Agilent # G4220A, or equivalent).

4.2 Thermostated Autosampler, Agilent #G1367A or #G1329A, or equivalent.

4.3 Diode Array Detector, Agilent #G1315A, or equivalent (and/or Fluoresce Detector, Agilent #G1321B, or equivalent).

4.4 Column Compartment, Agilent #G1316A, or equivalent.

4.5 Solvent Degasser, Agilent #G1322A, or equivalent.

4.6 Dell Computer or compatible, with OpenLAB CDS ChemStation Edition software.

5.0 Materials

5.1 HPLC-grade water, Sartorius Arium Pro Water, or equivalent.

5.2 Methanol, BPD PN 10115.

5.3 Acetonitrile, BPD PN 30075.

5.4 Trifluoroacetic Acid (TFA), BPD PN 30333.

5.5 Isopropanol, BDP PN 10178.

6.0 Maintenance

6.1 Priming and Purging the System

Priming the system with a syringe is recommended, when the vacuum degasser or connected tubing are used for the first time, vacuum tubes are empty, or changing to solvents that are immiscible with the solvent currently in the vacuum tubes.

Priming the system by using the pump at high flow rate (3-5 mL/min.) is recommended when the system was turned off for a length of time (for example, overnight), if volatile solvent mixtures are used, or solvents have been changed.

6.1.1 Priming with a Syringe

6.1.1.1 Before using a new degasser or new tubing, prime all tubing with at least 30 mL of isopropanol no matter whether the channels will be used with organic mobile phase or with water.

6.1.1.2 Before changing to a solvent that is immiscible with the solvent currently used in the tubing:

6.1.1.2.1 Replace the current solvent with isopropanol, ethanol or methanol, if the current solvent is organic. Use water, if the current solvent is an inorganic buffer or contains salt.

6.1.1.2.2 Disconnect the solvent outlet tube of the channel that is supposed to be primed from the pump.

6.1.1.2.3 Connect the syringe adaptor to the solvent outlet tube.

6.1.1.2.4 Push the syringe adaptor onto the syringe.

6.1.1.2.5 Pull the syringe plunger to draw at least 30 mL of solvent through the degasser and tubing.

6.1.1.2.6 Replace the priming solvent with the new solvent.

6.1.1.2.7 Pull the syringe plunger to draw at least 30 mL of solvent through degasser and tubing.

6.1.1.2.8 Disconnect the syringe adaptor from the solvent tube.

6.1.1.2.9 Connect the solvent tube to the appropriate channel of the MCGV (multi-channel gradient valve).

6.1.1.2.10 Repeat these steps for the other solvent channels.

6.1.2 Priming with the Pump

6.1.2.1 Open the purge valve of the pump by turning it counter-clockwise and set the flow rate to 3-5 mL/minute.

6.1.2.2 Flush the vacuum degasser and all the tubes with at least 30 mL of solvent.

6.1.2.3 Repeat steps 1-4 for other solvent channels.

6.1.2.4 Set flow to required value of your application and close the purge valve.

6.2 Optimizing the Performance of the Pump

6.2.1 Always place the solvent cabinet with the solvent bottles on top of the pump component (or at a higher level).

6.2.2 When using salt solutions, regularly flush all MCGV with water. This will remove any salt deposits in the valve ports. Before operating the pump, flush the vacuum degasser with at least two volumes (30 mL), especially when the pump has been turned off for a certain length of time or volatile solvent mixtures were used in the channels.

6.2.3 Check for blockage of the solvent inlet filters by removing the tubing at the end of the inlet port of the vacuum degasser. If the filter is in good condition, the solvent will drip freely out of the solvent tube as a result of hydrostatic pressure. If the solvent filter is partially blocked, no solvent or very little solvent will drip out of the solvent tube. If the filter is blocked, remove it from the bottle-head assembly and place it in a beaker containing 35% nitric acid for one hour. Thoroughly flush the filter with distilled water before replacing it.

6.2.4 Check the purge valve frit and column frit regularly. A blocked purge valve frit is identified by black or yellow layers on its surface or a pressure greater than 10 Bar, by pumping distilled water at a rate of 5 mL/minute, with an open purge valve.

6.2.5 When using buffered solutions, flush the system with water before switching it off.

7.0 Procedure

7.1 Turn on the computer used to control the 1100 HPLC/1200 RRHPLC.

7.2 Log on to the network using the appropriate username and password as specified by the IT department within the Biopharmaceutical Development Program (BDP). This will be used primarily for printing purposes.

7.3 Click on Start → Programs →OpenLAB →OpenLAB Control Panel→Log in/ Password/Domain (NIH) →Launch available Instruments Online and/or Offline (Figures 1-2)

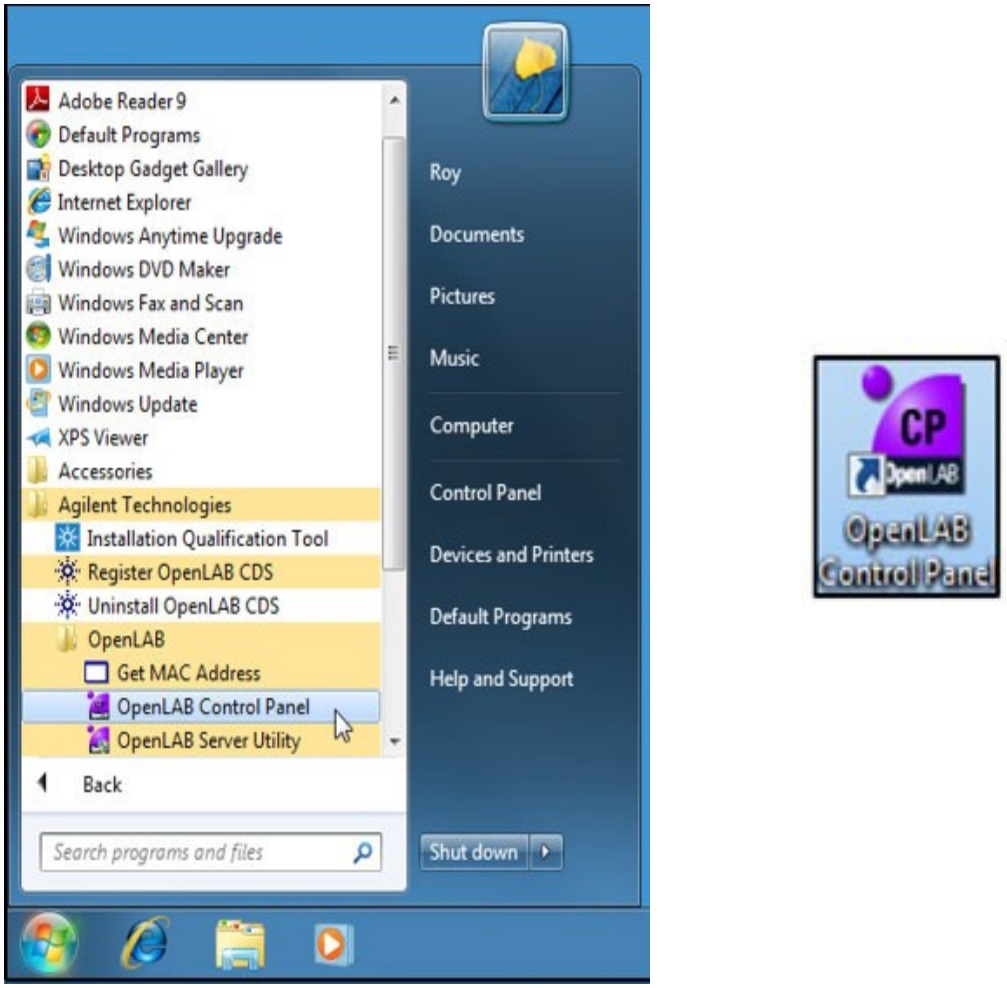


Figure 1: Using OpenLAB Control Panel

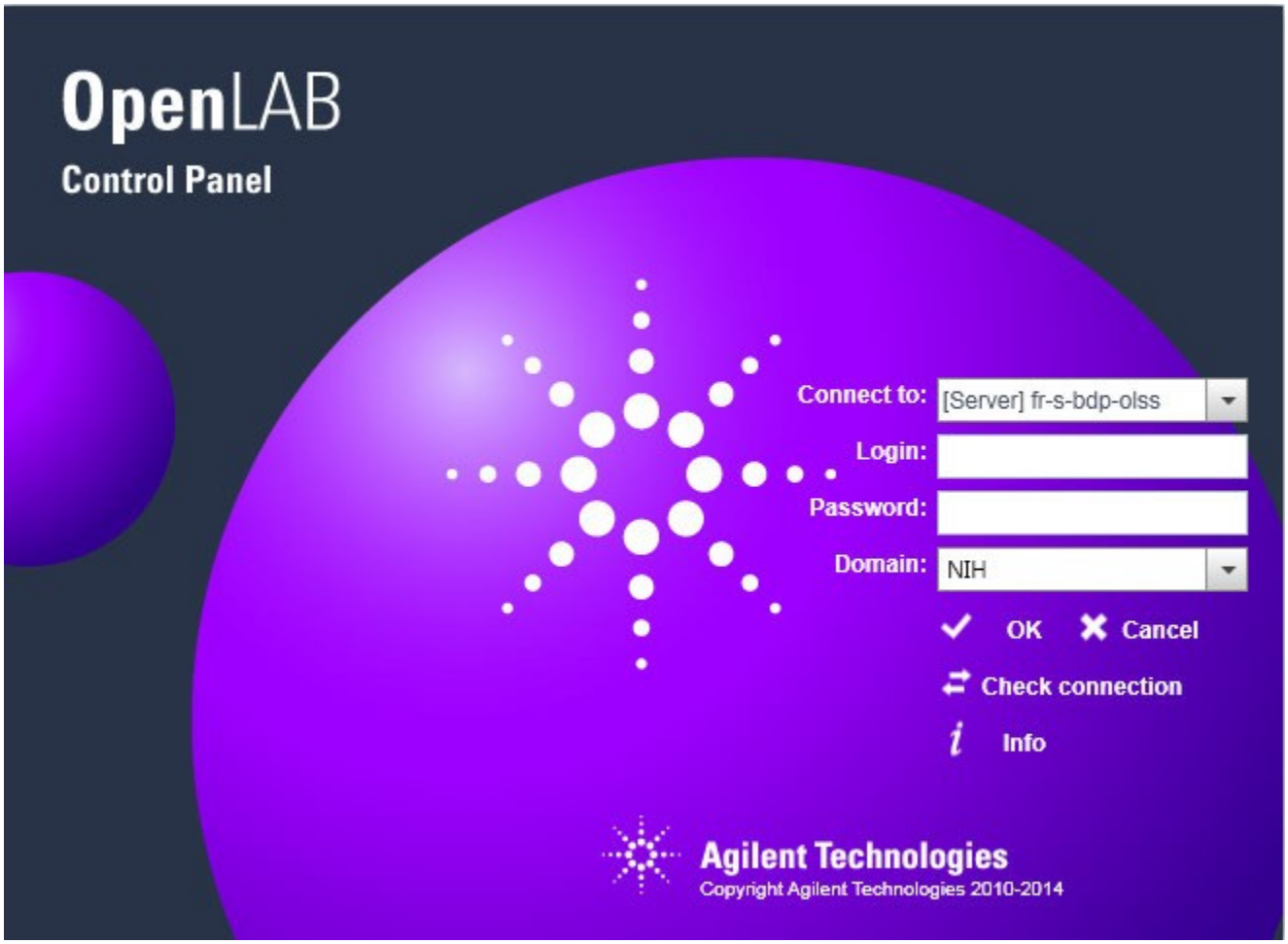


Figure 2 Logging into OpenLAB CDS ChemStation

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

7.4 Successful instrument launch will allow access to ChemStation User Interface (UI)(Figure 3)

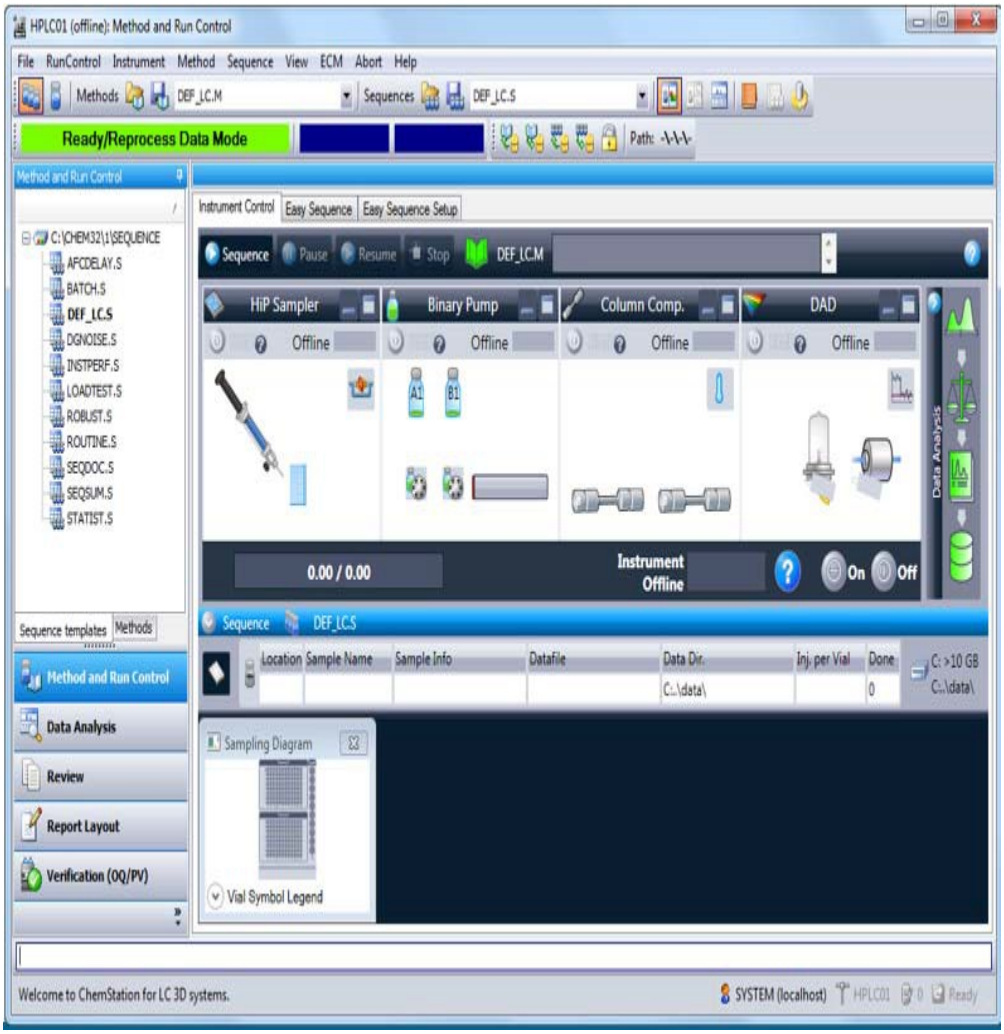


Figure 3: ChemStation User Interface (UI)

Clicking on Method and Run Control Tab will allow access to Method View in ChemStation Explorer. Clicking on Sequence Tab will allow access to Sequence Template View in ChemStation Explorer. The Methods tree in the ChemStation Explorer is divided into two parts. The upper part shows the methods contained in the currently loaded result set.

The lower part shows the methods in the master method directories, which the analyst configures in the Preferences dialog.

The currently loaded method is always shown in bold. By using drag & drop the analyst can easily copy master methods to sequence methods. The entire method including Data Analysis parameters and Acquiring parameters will be copied to the result set.

7.5 Clicking on Data Tab will allow access to Data Analysis View in ChemStation Explorer. From the Data Analysis view click on Methods at the bottom to view available methods. The upper half of the ChemStation Explorer shows the current sequence, shown in bold. The lower half of ChemStation Explorer shows the listing of the available Master Methods. (See Figure 4 and Figure 5 below.)

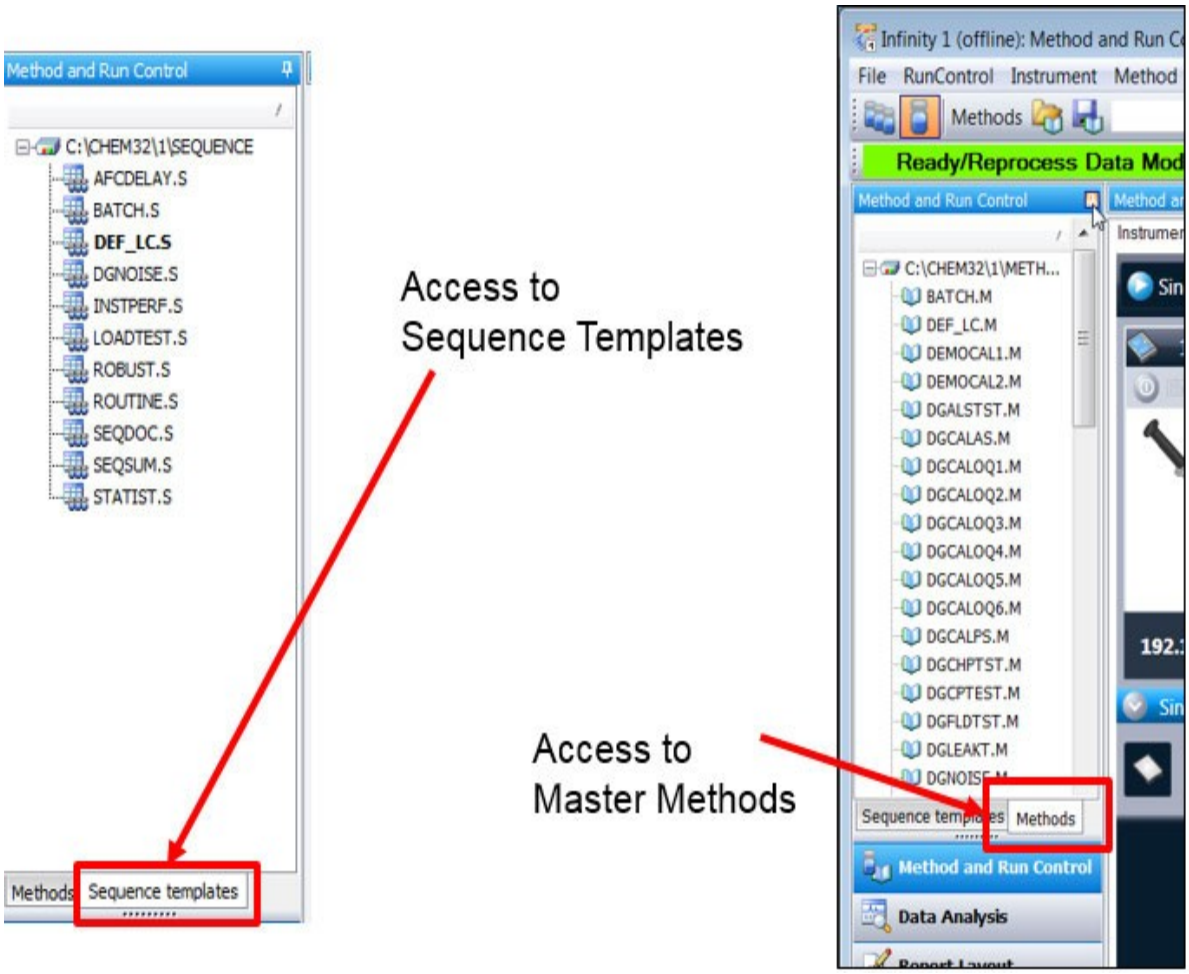


Figure 4: Methods in ChemStation Explorer (Method View)
Sequences in ChemStation Explorer (Sequences View)

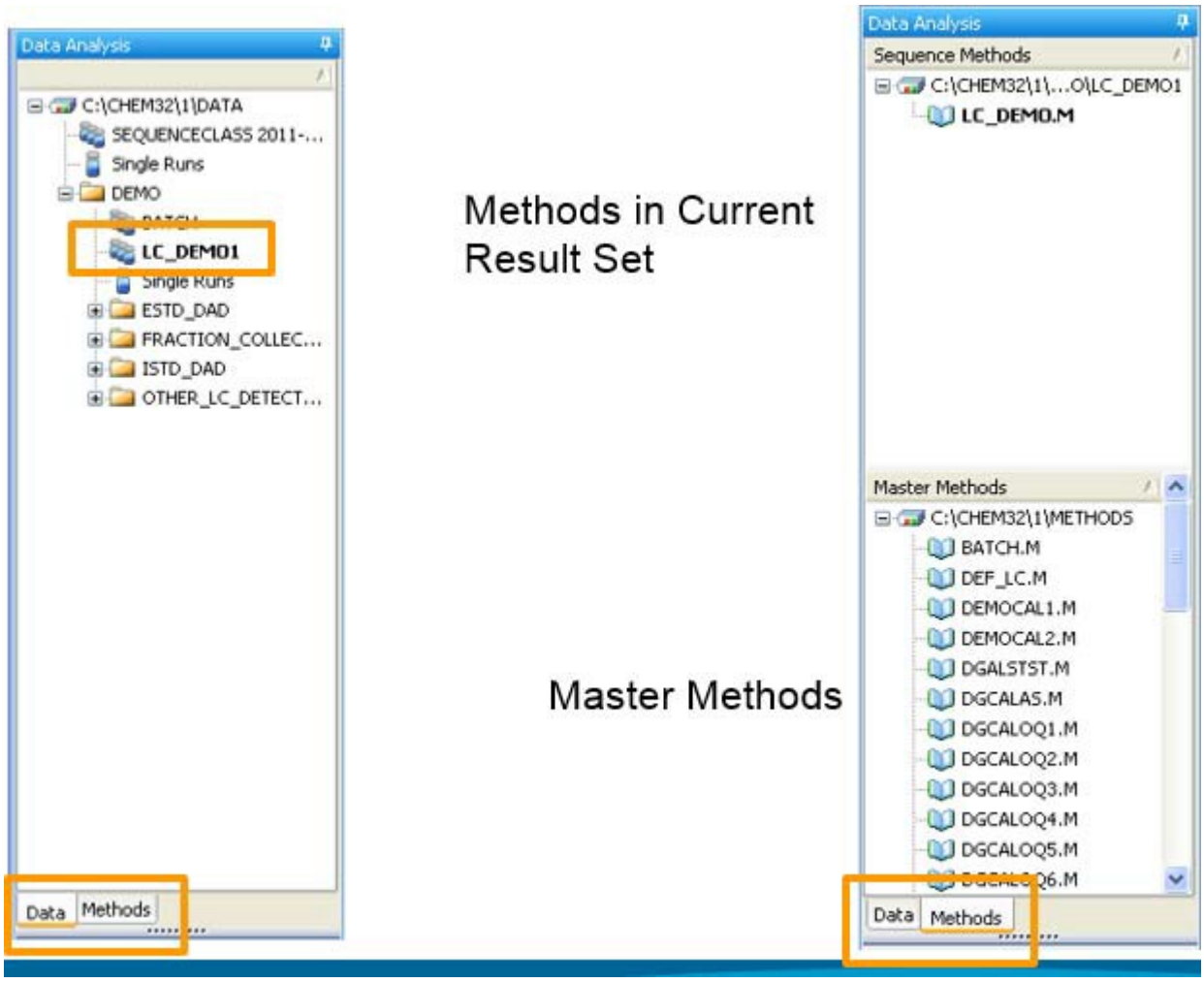
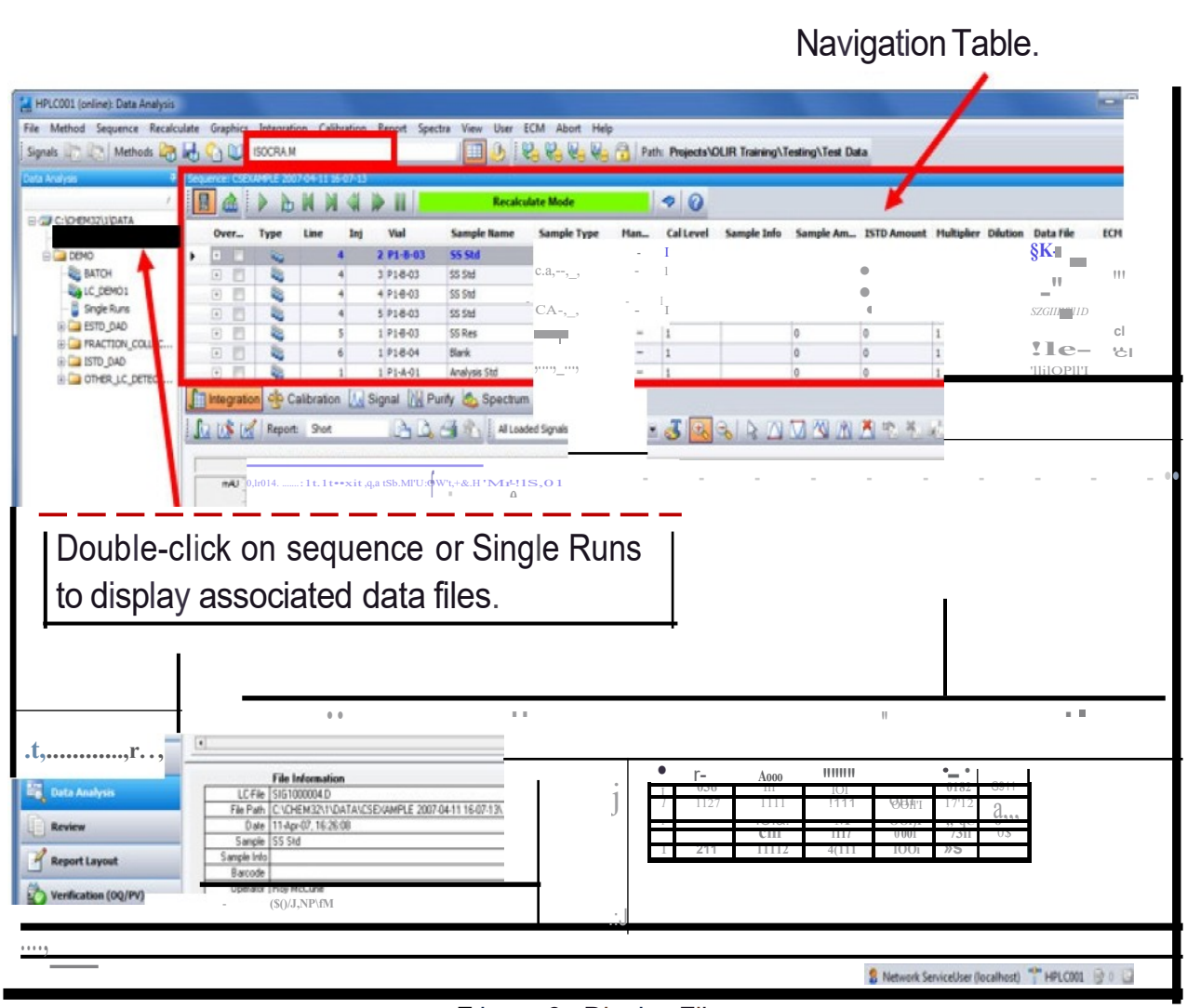


Figure 5 Methods in ChemStation Explorer (Data Analysis View)

The Data Analysis view includes a Navigation Table designed to facilitate data file review. The Navigation Table shows the runs contained in a selected sequence container or single runs within a data folder. You can use the Navigation Table to access individual runs or to automatically scroll through a grouping of data files (Figure 5).

Double-click on the Single Runs or the subdirectory in the Data Explorer to load the files into the Navigation Table. Double-click on the file to load the data file using the specified preferences. Note that the method being used displays above the Navigation Table. Double-Click on sequence or single runs to display associated data files. (Figure 6)



F igure 6:: Display Files

7.6 Creating a Method

7.6.1 Under **" Meth od and Run Control Control** select "Method"

7.6.2 Click on **"Method"** and select **"Edit Entire Method."**

7.6.3 Make sure that all four options in the **"Check Method Sections to Edit"** window are selected. Click on **"OK"**

7.6.4 Add a comment to the **"Method Comm ents"** window. An example may be the name of the product that the new method is specific for. Click on **" OK."**

7.6.5 Select InjectionLocation/Source

7.6.6 In the **" Setup Pump"** window, enter the desired flow rate (ex. = 1 mUminute), the stop time (i.e., total run time) and the post time (the amount of time after each injection to re-equilibrate the column to the original starting conditions In the **" Solvents"** section, enter the percent value for the starting conditions for the corresponding buffer solvent line (A, B, C and/or Das in a Quarternary pump), and enter the name of the buffer (ex. = 85% A /0.1% Trifluoroaceticacid in Water) and 15% B /0.1% Trifluoroaceitc acid in Acetonitrile).

7.6.7 Specify the pressure limits for the new method (the maximum should be slightly lower than the column pressure limit, and the minimum should be set at (0) bar to force shutdown in the event that a buffer reservoir runs dry).

7.6.8 Under the "Timetable" section, enter time "0.00" and the corresponding percent of solvents at time zero. Insert a second line and enter the same post time as previously specified. The operator can also alter flow rates and pressure settings at specific time points within the run, if necessary. Click on "OK." (Figure 7)

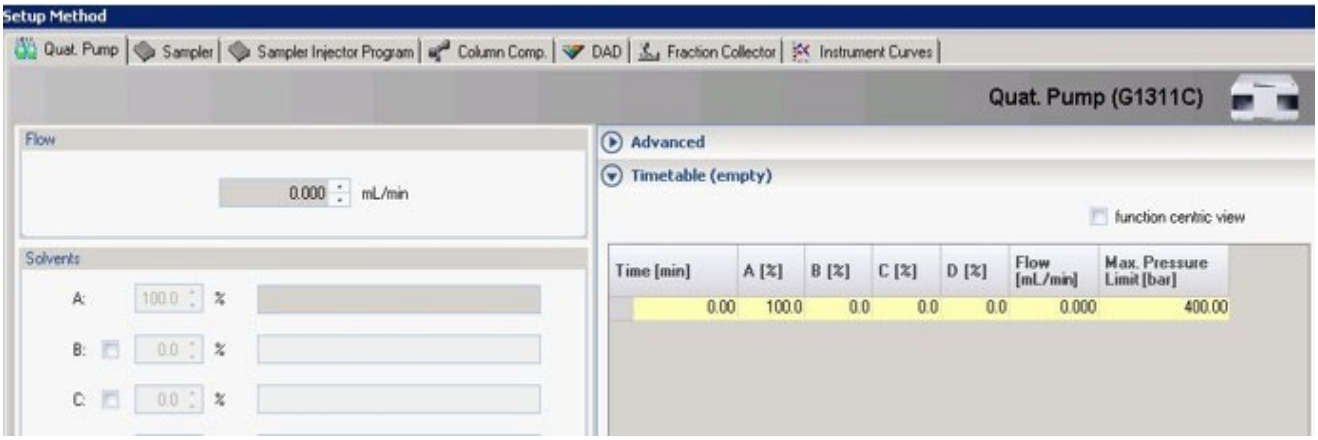


Figure 7: Method Editing Window

7.6.9 Specify the default injection volume for the new method and select "**Injection with Needle Wash**" under "**Injection**" in the "**Setup Injector**" window. Under "**Time**" select "**Same As Pump**" for stop time or enter a specific run time. Enter the chosen value for the post time. Leave the remaining default settings as they are. Click on "**OK**."

7.6.10 In the "**DAD Signals**" window, select a signal (or signals) and enter the desired wavelength(s) for sample detection and the desired bandwidth (Bw). For example, if the operator specifies 215 nm with a Bw of 8, it will monitor from 211 – 219 nm for sample detection. A reference signal and bandwidth must also be specified. This signal will be subtracted from the sample detection signal (i.e., to give real-time baseline correction) so it should be high on the spectral scale, where no sample signal is detected. An example is a reference signal of 400 nm with a Bw of 100 nm (350 – 450 nm reference).

7.6.11 Under "**Spectrum**" select "**Store: All in Peak**", "**Range: 190 to 400nm**" "**Step: 2.0nm**" and "**Threshold: 1.000mAU**". This will store all spectral data relevant to an eluted peak for any spectral analysis that may need to be performed. Under "**Required Lamps**" select "**UV**". Under "**Peak Width**" select "**>0.1 min. (2s)**". Under "**Autobalance**", select "**Prerun**". Under "**Slit**", select "**4nm**". Leave the remaining portions as they are as per the default settings. Click on "**OK**" to continue.

NOTE: Storing spectral data is only necessary for spectral analysis, not for routine purity analysis; one may wish to not collect spectral data in order to save hard drive space.

7.6.12 In the "**Column Thermostat Method**" window, specify a temperature setpoint for the column compartment. Typically, depending on the application and the type of column used, the temperature setting would be 25°C – 30°C (for some reverse-phase HPLC

applications, increasing the column temperature can improve resolution and shorten retention times). Leave the remaining default values as they are. Click on "**OK**" to continue.

7.6.13 In the "**Signal Details**" window, make sure that the detection signal (specified in Step 7.5.10) is listed under the "**Signal Description**" table. If not, then click on the "**Add to Method**" button. It will insert the signal into the table below. Click on the left side of the table at the signal that is not desired to highlight that row, then click on "**Delete Row**". The only remaining signal description in the table should be the one that the operator specified. Click on "**OK**" to continue.

7.6.14 In the "**Edit Integration Events**" window, specify the desired parameters for peak detection. A sensitive, reliable set of starting parameters for peak detection is as follows: Slope Sensitivity: 10, Peak Width: 0.1, Area Reject: 5, Height Reject: 5, and Shoulders: OFF. For most applications, it is a good idea to inhibit the integration events until ~5 minutes after the start of the run. Click on the "**Add a new line to the events table**" icon twice. In the first line, enter: Time: 0, Integration Events: Integration, and Value: OFF. In the second line, enter: Time: 5, Integration Events: Integration, and Value: ON. Click on "**OK**" to continue.

7.6.15 In the "**Specify Report**" window, the default settings should be as follows: under "**Destination**", select "**Printer**" and "**File**"; for file type, select "**.txt**." Under "**Quantitative Results**," select Calculate: Percent, Based On: Area, and Sorted By: RT. Click on the "**Signal Options**" button. In the "**Include**" section, select Axes, Baselines, Tick Marks and Retention Times. Under "**Ranges**," select "**Full**." Click on "**OK**." Leave the remaining default values as they are. The user can specify different report styles under the "**Report Style**" drop-down menu. The default should be "**Short Report**." Click on "**OK**" to continue.

7.6.16 In the "**Instrument Curves**" window, the user can select an instrument data curve to overlay on the chromatograph. However, this feature is optional. Click on "**OK**" to continue.

7.6.17 In the "**Runtime Checklist**" window, select "**Data Acquisition**," "**Standard Data Analysis**," and "**Save Method with Data**." Click on "**OK**" to complete the method setup.

7.6.18 Click on the "**Method**" tab in the main menu and select "**Save Method As…**" Save the method using a file name that is relative to the application. For example, a method created for product XXX maybe designated as XXX.M.or use the QC request form number.

7.6.19 If the user is prompted to insert a comment for the new method, give a brief description of the purpose for creating a method. An example may be "**new method created for product XXX**". Any time a change is made to an existing method, the user will be prompted to and must enter a comment. This is for tracking the method change history required for auditing purposes.

7.7 Creating A Run Sequence

7.7.1 In the Method and Run Control screen, click on "**Sequence Templates**" and select "**Sequence Parameters.**" A path is created automatically for data storage under the "**Data File**" section. Select "Name Pattern" Field to see the available choices to give each chromatogram a unique identity. (Figure 8)

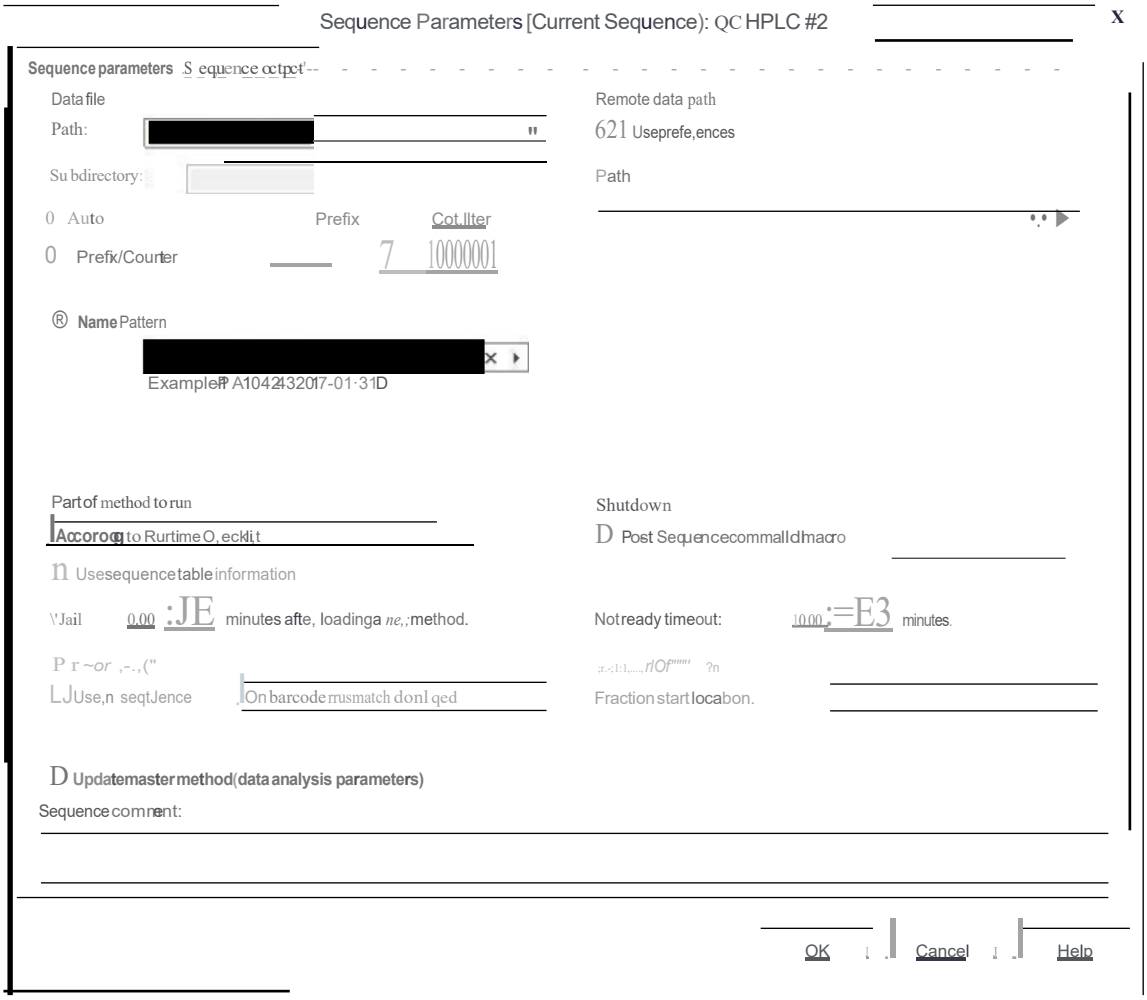


Figure 8: Sequence Parameters

Again, click on the **" Sequence"** tab and select **" Sequence Table".** This will br ing up the sequence table window where the user will enter all of the sample/injection information for the study.

Figures 9-11 Illustrate the basic navigational tools for creating a sequence table.

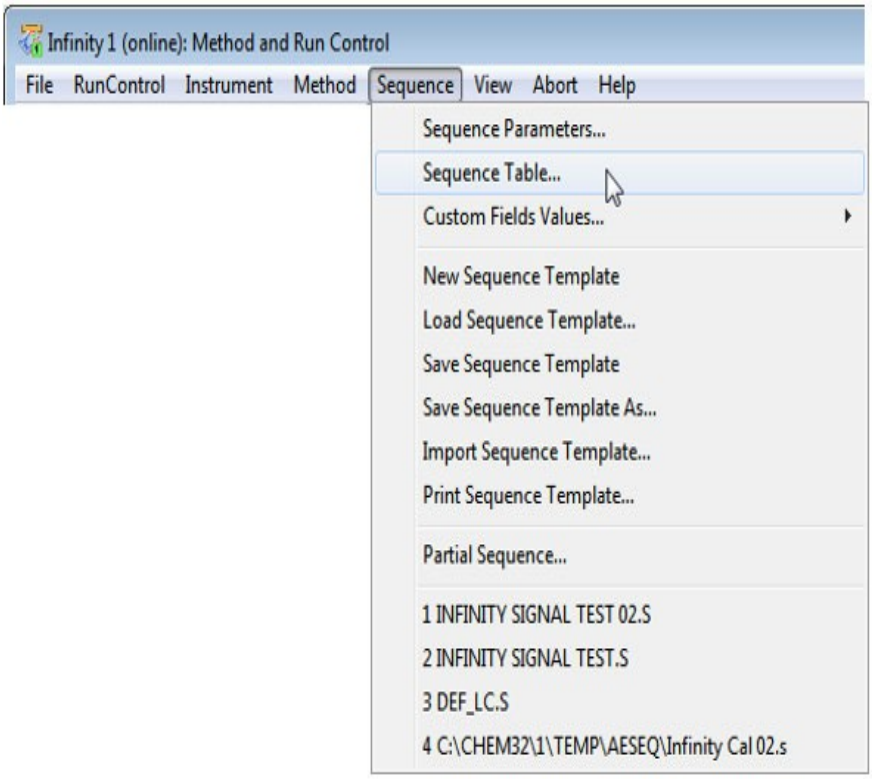


Figure 9: Editing a Sequence Table

The Sequence Table dialog box is used to display the status of the currently running sequence and/or to define exactly how data from each sample in a sequence will be acquired/analyzed. (Figure 9)

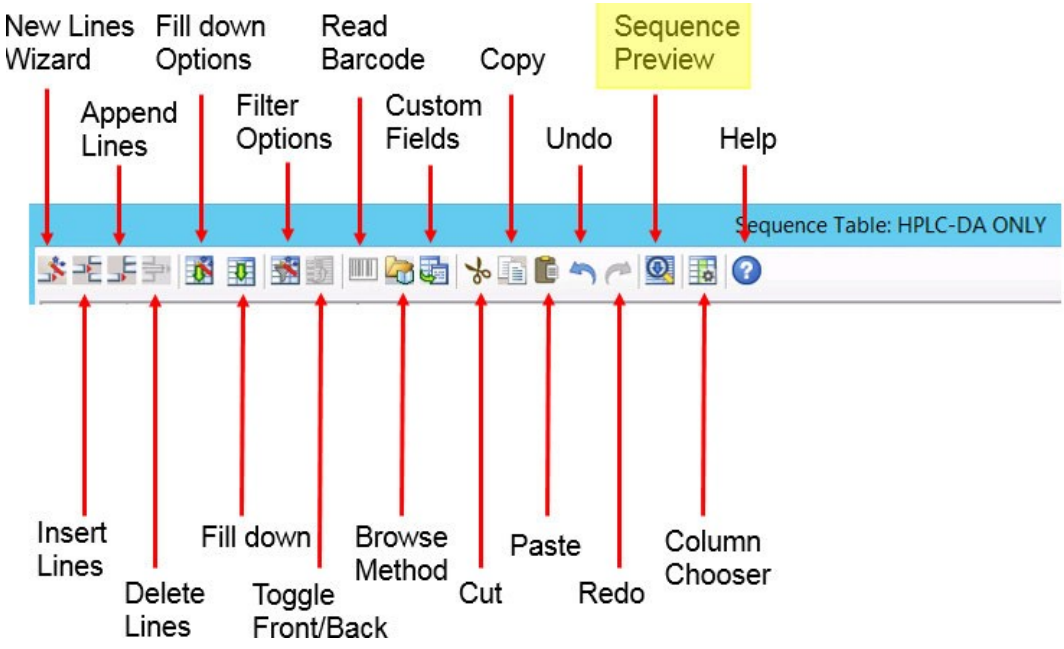


Figure 10: Sequence Table Toolbar

Figure 10 identifies the available functions in a Sequence Table Toolbar.

New Lines Wizard: Displays the **Insert/Filldown wizard**, which allows you to insert one or more lines into the Sequence Table according to a template.

Insert Lines: Inserts an empty sequence line above the selected line.

Append lines: Displays the **Append Lines** dialog box, which allows you to specify the number of empty lines to append to the Sequence Table.

Delete Lines: Deletes the selected sequence line(s).

Fill down Options: Displays the **Filldown Options** dialog box, which allows you to set up the rules for automatically filling multiple lines of the sequence table.

Fill down: Fills down the selected lines according to the rules specified in the **Filldown Options** dialog box.

Filter Option: Displays the **Filter Options** dialog box, which allows you to apply a set of conditions to display a subset of the sequence table

Toggle Front/Back: Toggles the display of a dual-simultaneous injection sequence table between execution order (alternate locations) and separated by injection location. This button is available only when multiple injectors are configured.

Read Barcode: Reads a barcode from the sampler and puts it into the **Sample Name** field of the selected sequence line. When multiple sequence lines are selected, barcodes can be read from a series of samples. If the barcode cannot be read, a? is shown in the **Sample Name** field. The barcode entry applies only to the sequence container, and not to the sequence template.

The barcode tool is available only when a barcode reader is configured.

Browse Method: Displays the **Browse for Master Methods** dialog box, which allows you to select a method to use.

Custom Fields: Displays the **Set up Custom Field Values** dialog box, which allows you to enter the values for the custom fields defined for the sequence.

Cut: Removes the selected line(s) from the sequence table and places them on the clipboard.

Copy: Copies the selected line(s) in the sequence table and places them on the clipboard.

Paste: Inserts the most recently cut or copied lines into the sequence table directly above the currently selected line.

Undo: Undoes the last change to the sequence table.

Redo: Redoes the last **Undo** action.

Sequence Preview: Displays the **Sequence Preview**, which shows the sequence exactly as it will be executed, with all repeat calibrations, control samples and blanks included in the correct sequence.

Column Chooser: Displays the **Column Chooser** dialog box, which allows you to select the columns to display in the Sequence Table.

Help: Displays the online help topic for the Sequence Table.

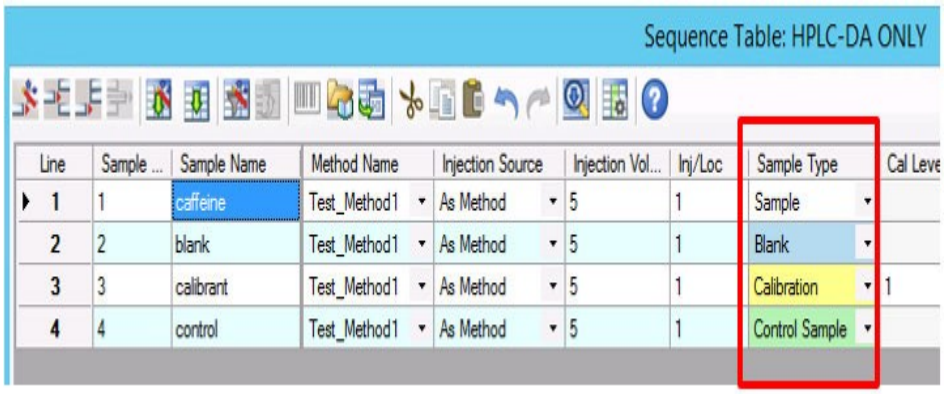


Figure 11: Sequence Table

7.7.2 Ideally, the operator wants to analyze the sample of interest, a commercially-available chromatography standard and/or product standard (positive control) and a blank (negative control), which should be the formulation buffer of the unknown sample. The operator must always set up the sequence to inject the positive control at the beginning and the end of the study, so that the data can be used as a system suitability check for that particular run. After entering all of the information into the sequence table, click on "OK."

7.7.3 For creating short and simple sequences, it is sufficient to simply append lines to the table and enter the parameters needed for each line to define each injection in the sequence. The Location, Method Name, Inj/Location and Sample Type are required for each line and the analyst will be prompted if one of these fields is not set before the sequence table is saved. All of the other columns are optional. (Figure 11)

7.7.4 Click on the "**Sequence**" tab again, and select "**Save Sequence Template As…**" Give the sequence a name that is relative to the study (such as sample ID#, date, etc.). For example, a sequence created for product XXX may be designated as XXX.S. Click on "**OK**." (Figure 12)

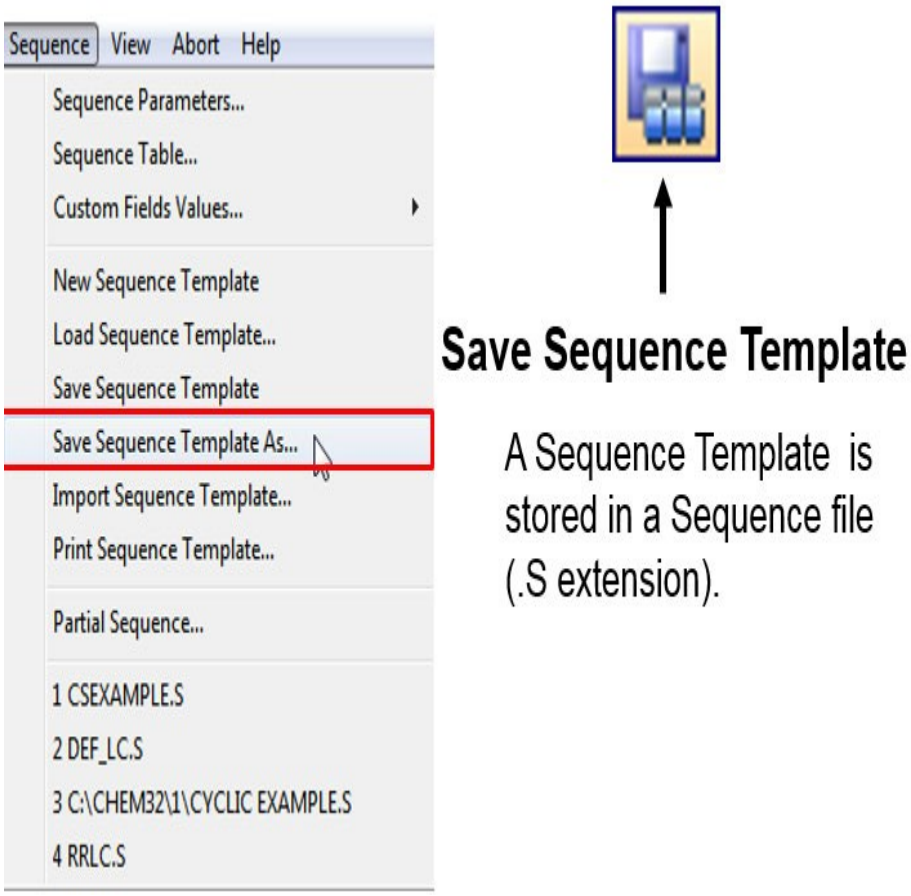


Figure 12: Saving the Sequence File

7.8 Starting a Run

7.8.1 Click on the "**Method**" tab in the main menu and select "**Load Method**." Select the method that has already been created and click on "**OK**." If the method to be used for analysis is already loaded, this part can be skipped.

7.8.2 Click on the "**Sequence**" tab in the main menu and select "**Load Sequence**."

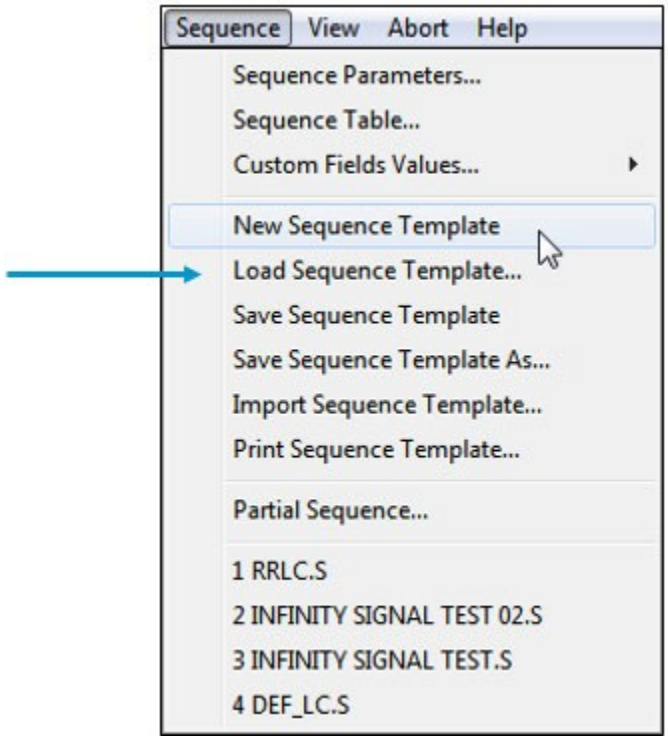


Figure 13: Loading Sequence Template

Select the sequence that has already been created and click on "**OK**." (Figure 13) Again, if the sequence to be used for analysis is already loaded, this part can be skipped.

7.8.3 In the "**Method and Run Control**" screen, there are large icons that represent each component of the 1100 HPLC/1200 RRHPLC systems. Click on the "**ON**" button to the right of these to turn on each system component. Each icon will be displayed in a certain color. The color yellow indicates that the component is busy "warming up." Once each component icon has changed to green, the system is ready to start the run. If any component icon is displayed in a red color, then there is an error with that component. Click on the logbook icon to display the error message. If the problem cannot be resolved, contact the manufacturer for service.

7.8.4 Once the system is ready, click on the "**Start**" icon on the top left of the screen. The component icons will the turn a purple color to indicate prerun activities, then a blue color, indicating that a run is in progress.

7.9 Data Analysis

A report for each injection will be at the default designated printer for that computer or saved as a PDF file. Although the processing/integration parameters have already been written as part of the method, occasionally the chromatograph may have to be manually reprocessed. Some examples of this may be that a minor impurity in the sample was not detected within the parameters that are specified in the method, or perhaps a fluctuation in the baseline was mistakenly integrated as a peak.

To manually reprocess data, click on the drop-down menu on the top left of the screen in the main menu and select “Data Analysis.” Click on “File” and select “Load Signal” (or use the load signal icon on the screen). In the “Folders” section, select the subdirectory that was created for that run to display the data files. Choose the data file for reprocessing and click on “OK.” The chromatograph for that data file will be displayed with all of the file information and the results table.

Just above the chromatograph, several icons will be displayed that are used for manual integration. Integrate the peaks that you wish to include, and remove what has been integrated that is not a peak. Click on “File” and select “Print” and then “Report” (or click on the print report icon at the top right of the screen) to print out a new report with the changes. Keep in mind that ChemStation will not allow any raw data files to be altered or saved. The operator can only change and save the method (i.e., the integration parameters for the method) and/or the sequence information.

Once all of the reports are finished, click on “File” and select “Exit” to close ChemStation. If there is a prompt to shut down the pump and turn off the lamp, select “Yes.”

Archive the data with the Quality Control Test Request Form, project notebook, or other appropriate documentation storage area.

Figures 14-18 illustrate the basic navigational tools available for data table.

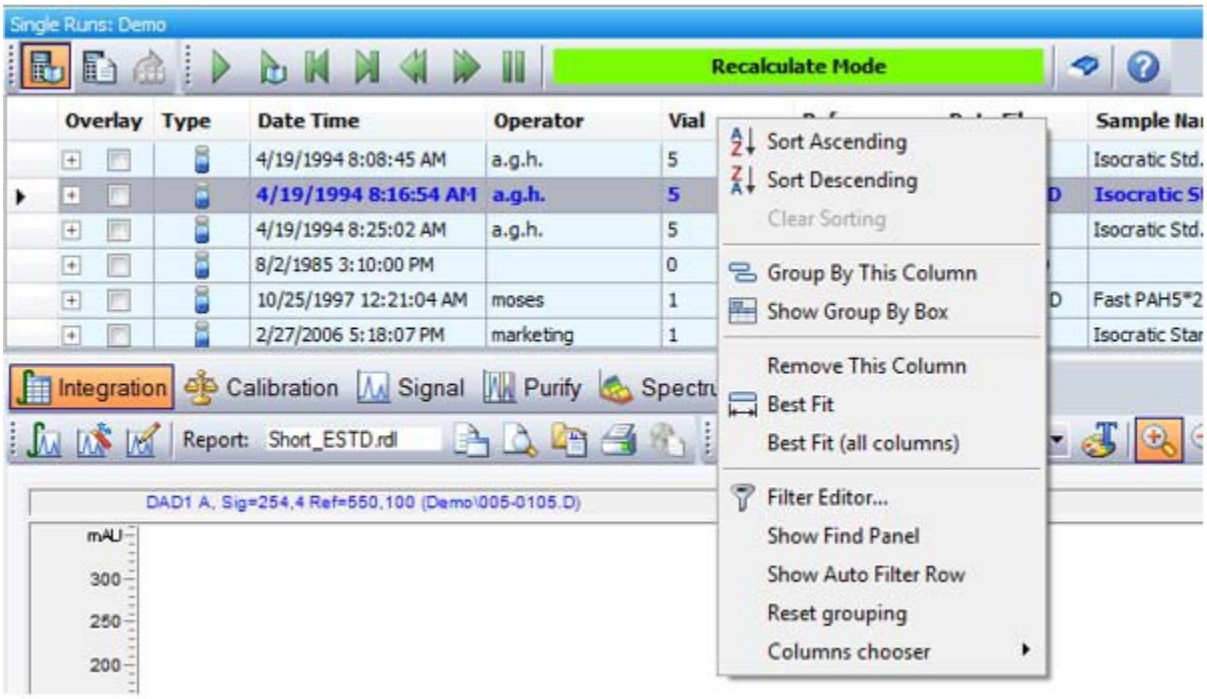


Figure 14 Navigation Table (Options for the Columns)

To access configuration options for the columns, right-click a column header. (Figure 14)

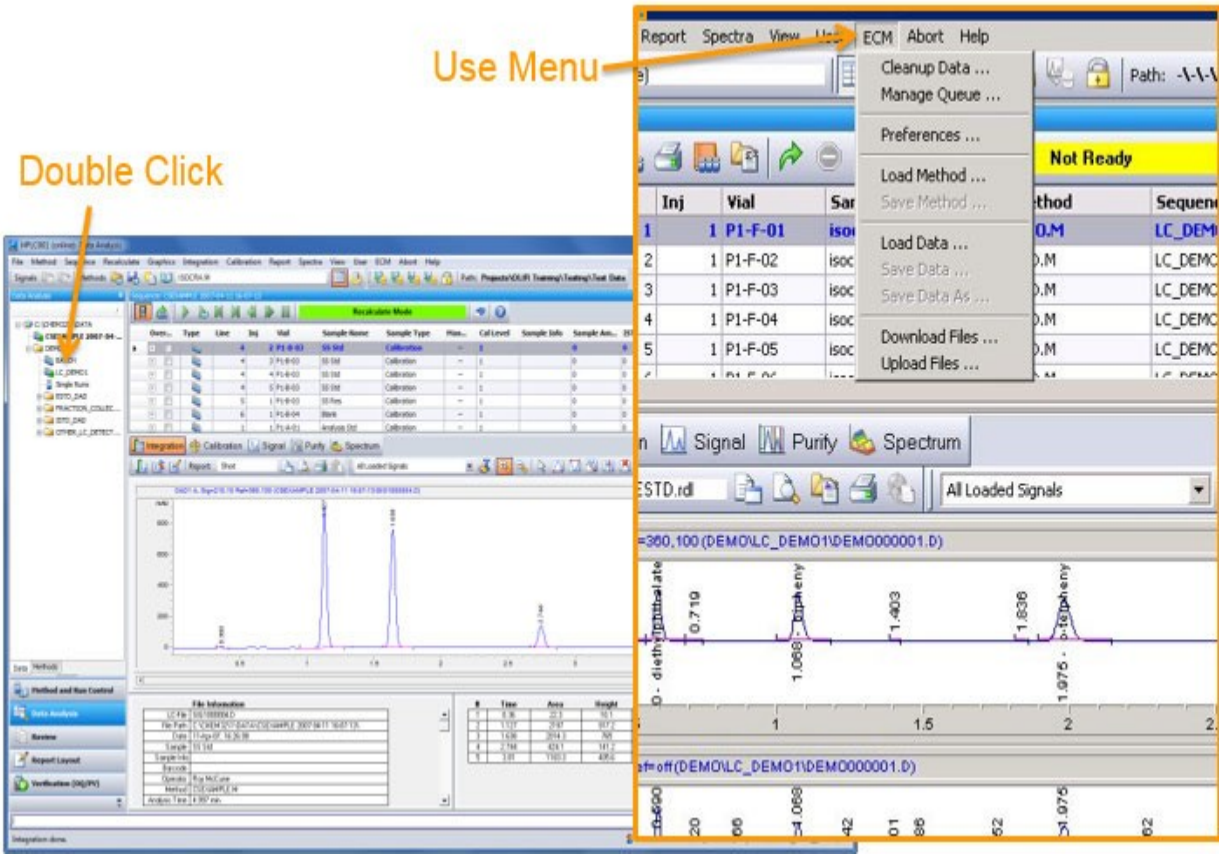


Figure 15 Loading a Data File

Loading a data file can be done in two ways: by using the pull-down menu under heading "ECM (Enterprise Content Manager) or by double clicking on the actual data file located under the data file path (for example: [redacted]). (Figure 15)

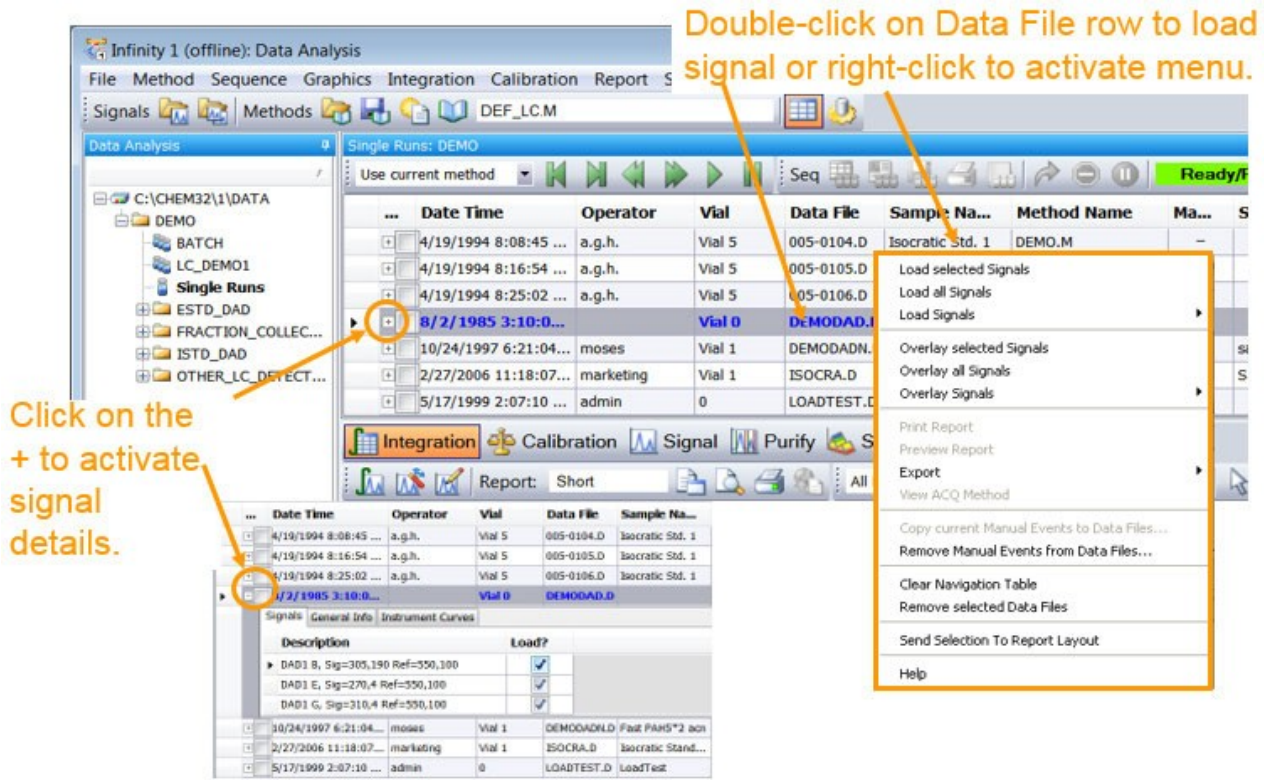


Figure 16 Loading a Specific Signal(s)

Figure 16 illustrates the way a specific signal is loaded for analysis as follows:

Load the signal by double-clicking a row that represents a run. You can load multiple runs by selecting them using **[ctrl]** (for non-contiguous runs) or **[shift]** (for contiguous runs), and selecting **Load Signals** or **Load Selected Signals** from the table's context menu (right mouse click).

The right mouse click functions that are available include:

- **Load selected Signals** – Allows you to load only the signals marked in the **Signals** tab of the **Sample Details.**
- **Load all Signals** – Loads all signals from the selected runs.
- **Load Signals** – Allows you to select signals to load from the submenu.
- **Overlay selected Signals** – Allows you to overlay only signals marked in the **Signals** tab of the **Sample Details**.
- **Overlay all Signals** – Overlays all signals from the selected runs.
- **Overlay selected Signals** – Allows you to select signals to overlay from the submenu.
- **Print Report** – Prints a report to the currently selected printer.
- **Print Preview** – Displays a print preview of the report.

Each **Navigation Table** line can be expanded by clicking the + (plus) sign at the left of the line to configure signal-specific options:

- **Signal** – Lists the acquired signals and allows you to specify the signals to be loaded. The signal display selection applies to each run individually. You must then right-click on **Load** and select **Load Selected Signals**.
- **General Info** – Lists the header details about the run.
- **Instrument Curves** – Allows you to select the instrument data curves to be displayed along with the chromatogram/electropherogram and in the printout.
- **Export** – Allows you to export file in AIA, CSV or DIF format. Select the format for file export from the submenu.
- **View ACQ Method** – Displays the acquisition method parameters in a separate window. Note that this item is enabled only for data acquired with revision B.03.01 with **Unique Folder Creation ON**.
- **Copy current Manual Events to data files** – Allows you to copy the current manual integration events to one or more selected data files.
- **Remove Manual Events from data files** – Allows you to remove manual integration events from one or more selected data files.

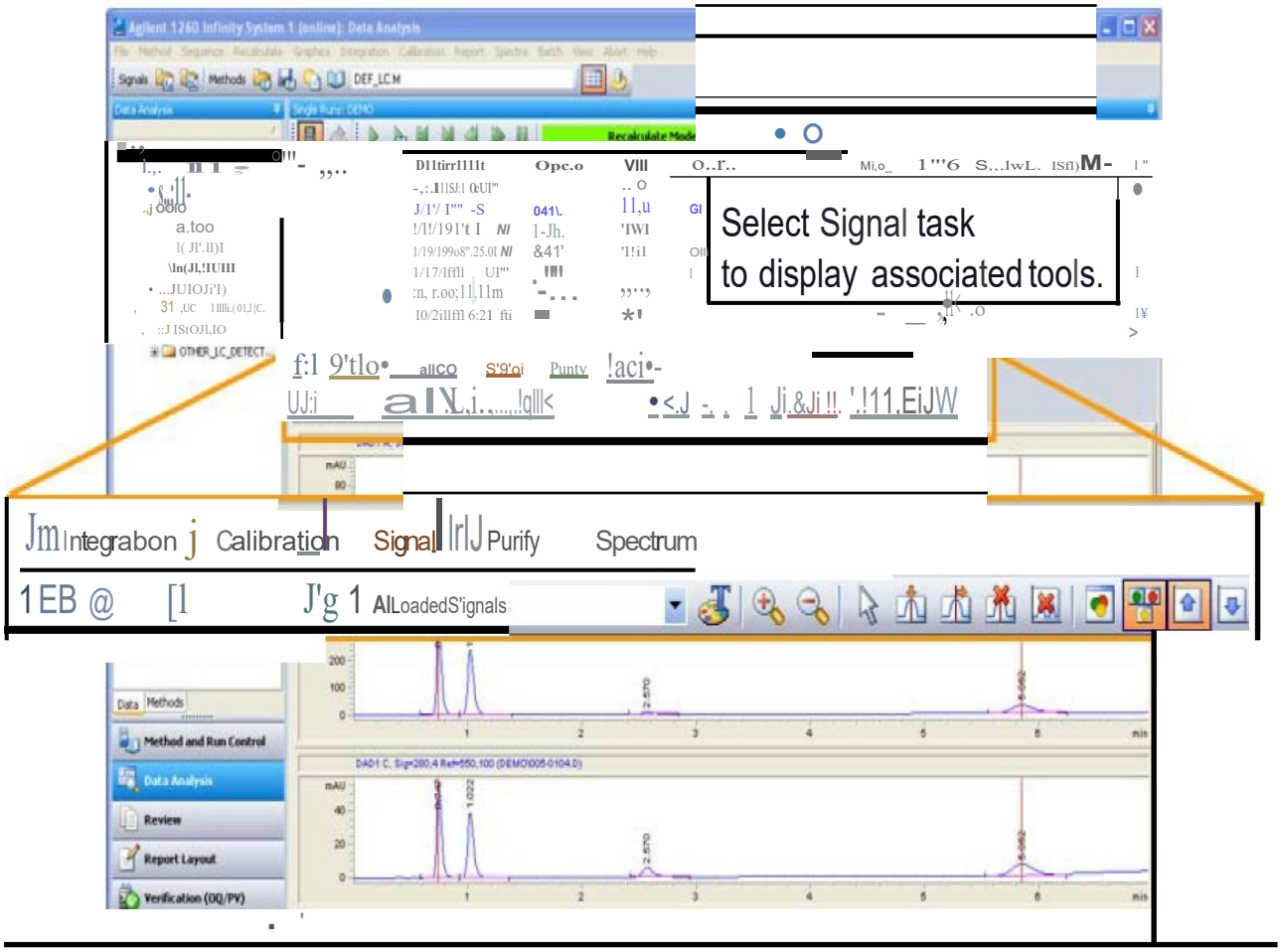


Figure 17 Data Analysis Options

Figure 17 shows the available tools for data analysis. Clicking on each tool's icon describes what that tool is used for. For example, the icon looking like a magnifying glass with a plus sign in the middle will allow expansion of the base line of a particular section of the chromatogram.

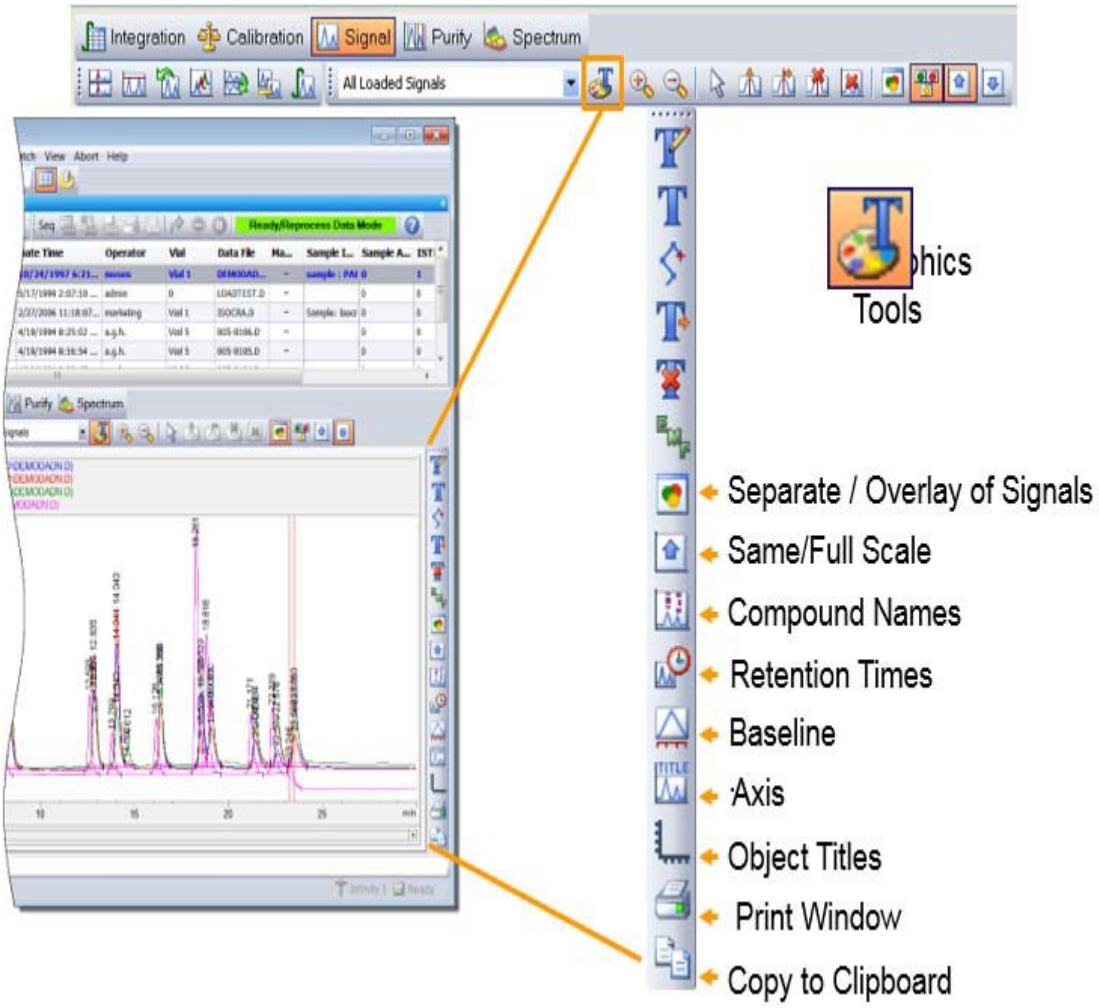


Figure 18: Signal Options Tools Use the Graphics tool set to graphically work with your chromatogram. The options found in the **Signal Options** dialog box are displayed in the right margin when you select the Graphics Task tool. This tool bar is dockable, meaning it can be moved and docked in other places on the screen. (Figure 18)

8.0 Report Layout

8.1 OpenLAB CDS allows for two types of report templates: Intelligent and legacy (also known as classic) reports. Please see figures 19-24 and Agilent manual (pages 179-202) for detailed information on creating each type of report.

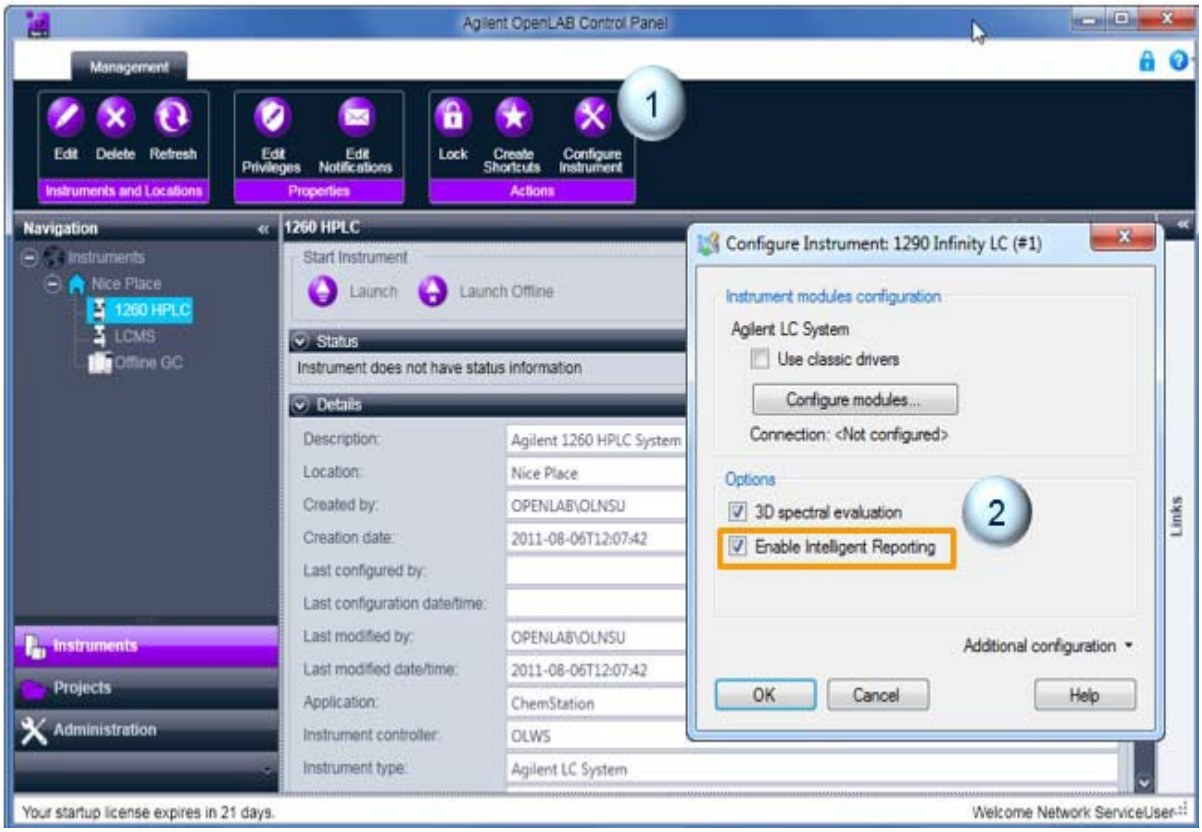
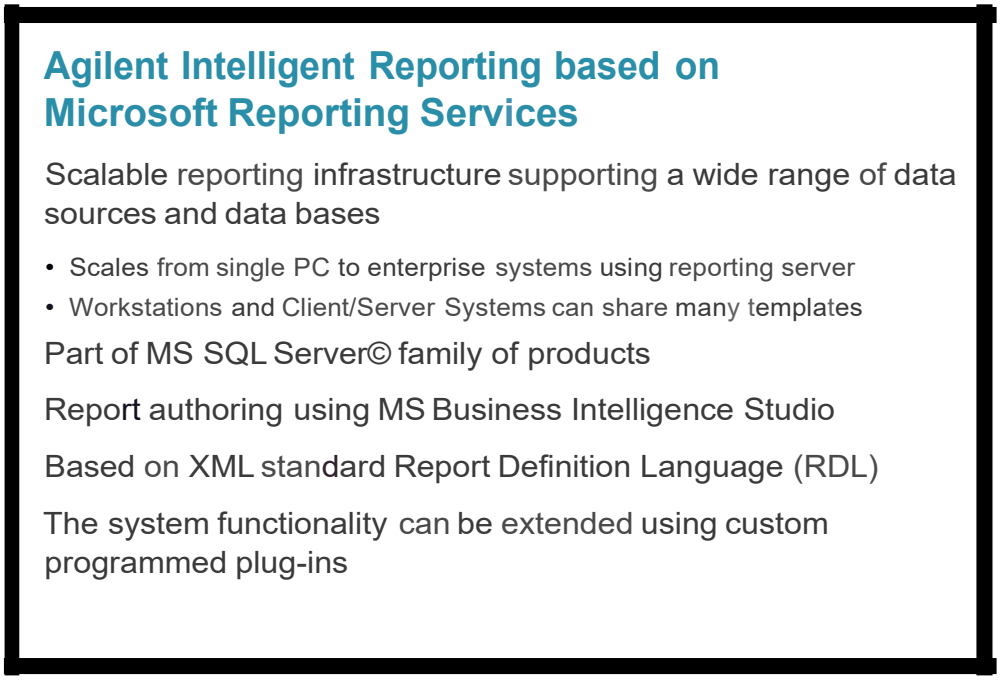


Figure 19 Intelligent or Legacy Report Templates

The OpenLAB ChemStation (C series) is capable of generating two report templates. One is the new report template or Intelligent reporting. The second type of reporting is the legacy report template for those instances (or instruments) that report templates created in earlier revisions must be used. These templates can be used alongside the new templates, but if you wish to edit legacy templates in the C series ChemStation you must go to the configuration and disable **Intelligent Reporting**.

To disable Intelligent Reporting open the **Agilent OpenLAB Control Panel** and select **Configure Instrument** (1). Remove the check mark next to **Enable Intelligent Reporting** (2) (Figure 19).

Figure 20



Agilent Intelligent Reporting is based on Microsoft Reporting Services. (Figure 20). These services provide a scalable reporting infrastructure that can be used to generate reports from a single PC all the way to enterprise-scale reporting.

This provides a major advantage for Agilent instrument users in that templates created on the larger client/server systems are generally interchangeable with those created at the workstation level. This not only makes it easier to share templates, but also means that your reporting can scale as your system scales.

Microsoft Reporting Services are part of the MS SOL Server family of products and use MS Business Intelligence Studio as the authoring tool. The report templates are based on an XML standard report definition language (RDL).

The use of standards-based tools offers substantial advantage when it comes to interchanginginformation between systems, or extending the system with custom programmed plug-ins. Many IT organizations work with Microsoft Reporting Services. Thus, the skills these groups develop for larger corporate projects translate easily to analytical reporting.

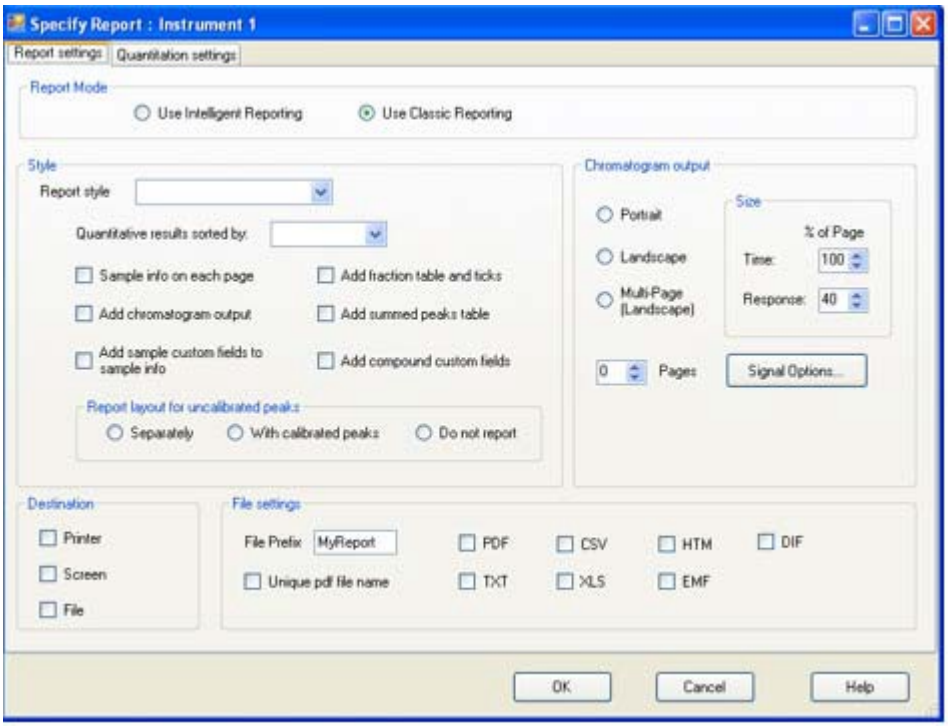


Figure 21: Specify Report (Classic reporting)

Either classic or Intelligent reporting can be used within one method. The parameters used to calculate results move to a new Tab named ***Quantitation Settings***.

These settings are:

- Calculation type (e.g. Percent, ESTD, ISDT, …)
- Calculation base (Area, Height)
- Calculation factors

Depending on the selected report mode (classic or Intelligent) a different set of report parameters is available (Figures 21 &22).

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

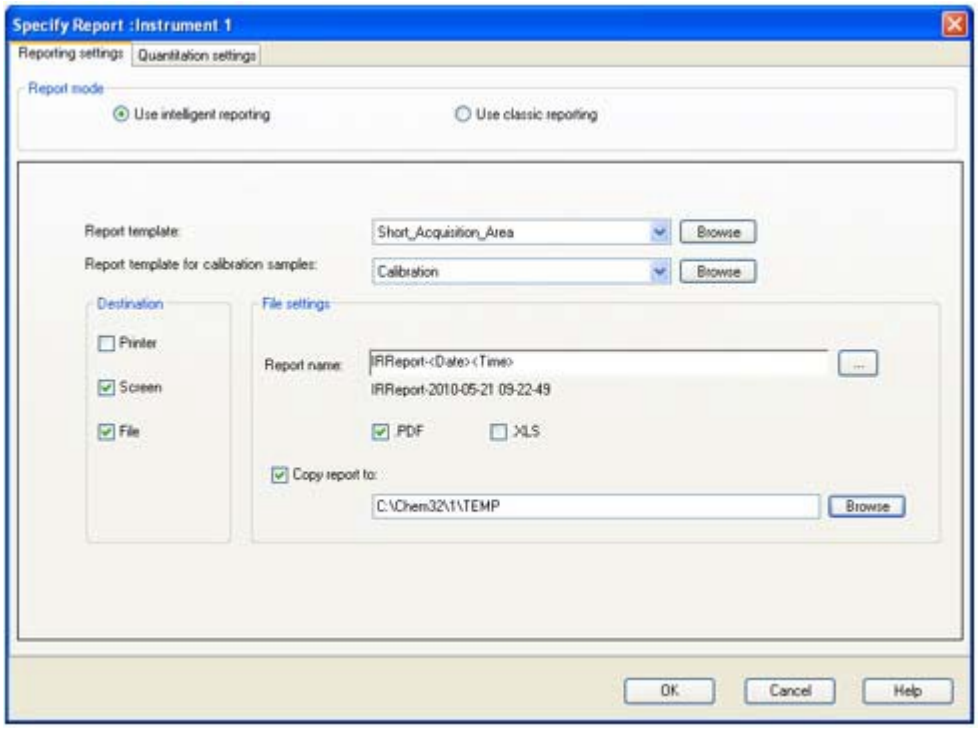


Figure 22: Specify Report (Intelligent Reporting)

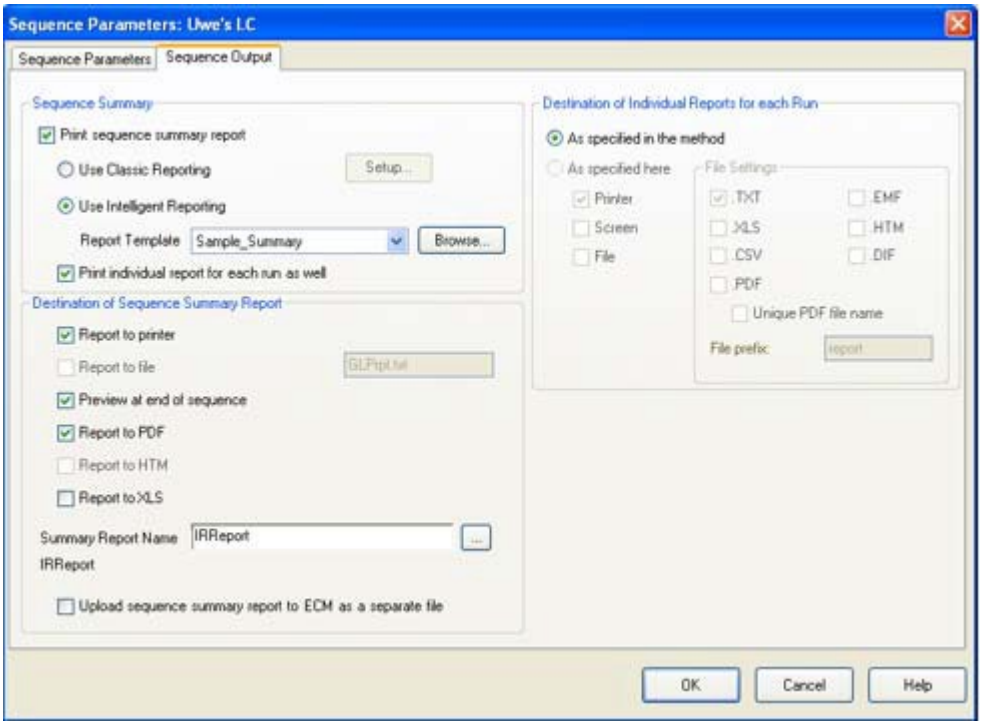


Figure 23: Specify Report (Sequence Summary)

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

The user can use either ***Intelligent Reporting*** or classic reporting for the sequence summary report.

When the *OpenLAB Intelligent Reporter* groupbox is no longer visible, it is still possible to launch the OpenLAB Intelligent Reporter client via the toolbar / menu,

In case of ***Intelligent Reporting*** the user needs to define

- The report template to be used
- The report destination (printer, PDF, XLS, or preview to screen)
- Whether individual method reports should be generated (according to the individual method)

By using the option <*Print individual reports for each run as well*> it is possible to mix ***Intelligent Reporting*** and classic reporting. For example, you can define an ***Intelligent Reporting*** sequence summary template and use classic report styles for the individual single injection reports.

The settings in the Sequence Summary Parameters dialog will **NOT** be used when ***Intelligent Reporting*** is selected. (Figure 23)

9.0 Important Note

How to properly logoff or disconnect from OpenLAB ChemStation.

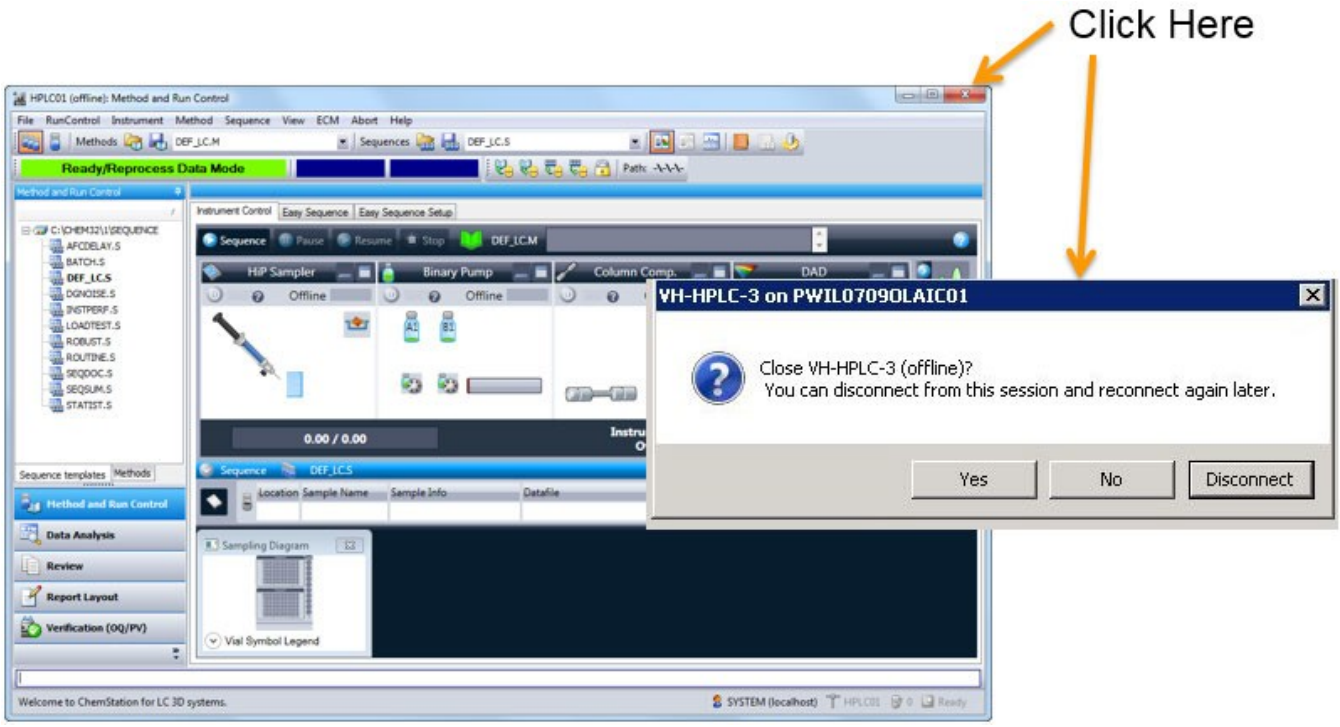


Figure 24: Proper Logging off or disconnect from ChemStation

There are two options for termination a ChemStation session (Figure 24). In Option 1, choose “Disconnect” if instrument is actively acquiring data.

In Option 2, choose “‘yes” ONLY to shut down the ChemStation.

10.0 Troubleshooting

NOTE: Consult the current system operating manual to address any of these cases.

10.1 Poor Peak Shape, Loss of Resolution, or Peak Splitting.

10.1.1 The most likely causes of poor peak shape, loss of resolution, or peak splitting are column void, sample overload, sample-mobile phase incompatibility, and dead volume from improperly assembled fittings and connectors.

10.1.2 Ensure that:

- The mobile phase is the proper pH for the analytes and the column.
- Analytes are soluble in the mobile phase.
- Analytes are not overloaded onto the column.
- All connecting fittings are well-sealed.

10.2 High Pressure

10.2.1 A restricted flow path is the probable cause of high pressure. Make sure that all components in the mobile phase and the sample are compatible with one another, filtered, and that precipitation cannot occur.

10.2.2 To isolate the likely component causing the high pressure, replace the column with a union and measure the total system pressure.

10.2.2.1 If pressure without the column is high, disconnect all connections starting at the end of the flow path until the section causing the high pressure is isolated.

10.2.2.2 If the pressure without the column is acceptable, try removing particulate matter from the inlet frit by running the column in reverse by running the column in the reverse flow direction at a maximum of half the normal flow rate, with the outlet disconnected from the detector. If the pressure is still too high, clean the column (refer to the Manufacturer’s Manual).

10.3 Retention Time Drift

10.3.1 Retention time drifts mainly as a result of inadequate system equilibration.

10.3.2 Make sure that the column has been adequately equilibrated in freshly made mobile phase.

10.4 Unstable Baseline

10.4.1 A wide variety of factors cause unstable baseline. The most likely ones are as follows.

- Column not adequately equilibrated in mobile phase (Make sure that the column is equilibrated in freshly made and degassed mobile phase. Use HPLC-grade components to prepare mobile phase.)

- Pump delivering unstable flow (consult the operator's manual or contact Agilent).
- Leaks in the system.
- Low detector lamp energy.
- Air bubbles in the system.
- Contamination in the detector cell.
- Mobile phase is not being mixed properly.
- Mobile phase is contaminated.

10.4.2 Make sure that: the column is equilibrated in freshly made and degassed mobile phase. Use HPLC-grade components to prepare mobile phase.

10.5 Poor Sensitivity

10.5.1 Probable causes contributing to poor sensitivity are low detector energy, contamination of detector cell, incorrect detector or integration settings, degraded analyte, inconsistent injection volume, or too much extra-column volume.

10.5.2 Ensure that:

- The HPLC system (detector, injector, etc.) is clean, calibrated, and operating properly.
- All liquid connections have zero dead volume.

10.6 Ghost Peaks

10.6.1 Ghost peaks are indicators of a contaminated column or system, carryover from prior injections, late-eluding peak from the sample, or pump oil is leaking into the system.

10.6.2 Make several 0 µL injections (turn injector valve) to determine the source(s) of the ghost peak(s).

10.6.2.1 If peak decreases with each injection, the contamination is from a previous sample injection.

- Clean the entire system (excluding the column) with isopropanol.
- Clean the column. (Refer to the Manufacturer's instructions)
- Add a strong solvent flush at the end of the run to remove any possible late eluting compounds.

10.6.2.2 If the peak stays the same size with each injection, the contamination is from the mobile phase (especially from water and ion-pair reagents).

- Clean the entire system (excluding the column) with isopropanol.
- Clean the column. (Refer to the Manufacturer's instructions)
- Replace every component of the mobile phase, including the glassware and solvent reservoirs.
- Check the pump to make sure that it is not leaking oil into the lines.

10.6.2.3 If no peak is seen with a 0 µL injection, the ghost peak is in the sample.

- Add a strong solvent flush at the end of the run to remove any possible late eluting compounds.
- Filter sample, if feasible.
- Make sure that all components of the sample are soluble in the mobile phase and sample solvent.

10.7 Negative Peaks (peaks that dip below the baseline)

10.7.1 The following factors contribute to the appearance of negative peaks:

- Incorrect integrator or detector settings.
- Mobile phase has a higher UV absorbance or greater refractive index than the analyte.
- Refractive index of sample solvent differs too much from the mobile phase.

10.7.2 Make a 0 µL injection (turn injector valve).

10.7.2.1 If the negative peak is seen, then it is due to the refractive index change caused by the momentary stop in flow or increase on pressure when the valve switches.

10.7.3 Make injections of sample solvent only.

10.7.3.1 If negative peak is seen, the sample solvent has less absorbance or a drastically different refractive index than the mobile phase. Change all components of the mobile phase, making sure that they are fresh.

10.7.3.2 If no negative peak is seen, then there is a component in the sample that has less absorbance or a drastically different refractive index than the mobile phase. Adjust the detector wavelength. Change all the components of the mobile phase.

11.0 References and Related Documents

11.1 Agilent OpenLAB CDS ChemStation Edition: Concepts and Workflows.

11.2 Agilent OpenLAB CS ChemStation Edition: Reference to Operational Principles

11.3 Agilent OpenLAB Data Analysis: Reference

11.4 Agilent G4635AA OpenLAB ECM Intelligent Reporter: Manual for Advanced Report Template Designers

11.5 OpenLAB Enterprise Content Manager: Administrator’s Guide

11.6 OpenLAB Enterprise Content Manager: User’s Guide

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted]