

How to Run the CASPiE HPLC



HPLC stands for High Performance Liquid Chromatography. It is used to separate and quantify complex mixtures. You will use the CASPiE HPLC to quantify ascorbic acid in food samples.

For detailed information about the CASPiE HPLC instrument see http://www.caspie.org/instrument_tutorials.html.

You must have already created and saved a sequence file before you run the HPLC. (Instructions for this are provided in a different document.)

Please Note:

DO NOT make any changes to the instrument that are not instructed in this document. Others will use the instrument after you. Be courteous to your fellow students and leave them a usable instrument!



Load the CASPiE Instrument Site



- To open the HPLC control software launch your web browser and navigate to the CASPiE instrument website at <https://instruments.caspie.org>.
- Enter your Purdue Career account information to login.

A screenshot of a Windows Internet Explorer browser window. The title bar reads "MetaFrame Presentation Server Log In - Windows Internet Explorer". The address bar shows the URL "https://instruments.caspie.org/Citrix/MetaFrame/auth/login.aspx". The browser's search bar contains "Google". The page content includes the CASPiE logo on the left and the heading "Web Interface for MetaFrame® Presentation Server". Below this is a "Log in" form with fields for "User name:" (containing "dmsteffe") and "Password:" (masked with dots). A "Log In" button is at the bottom of the form. To the right of the form is a "Welcome Authorized Users of CASPiE Instrumentation" message, followed by a "View Instruments" link. Below that is a "Message Center" section with a brief description.

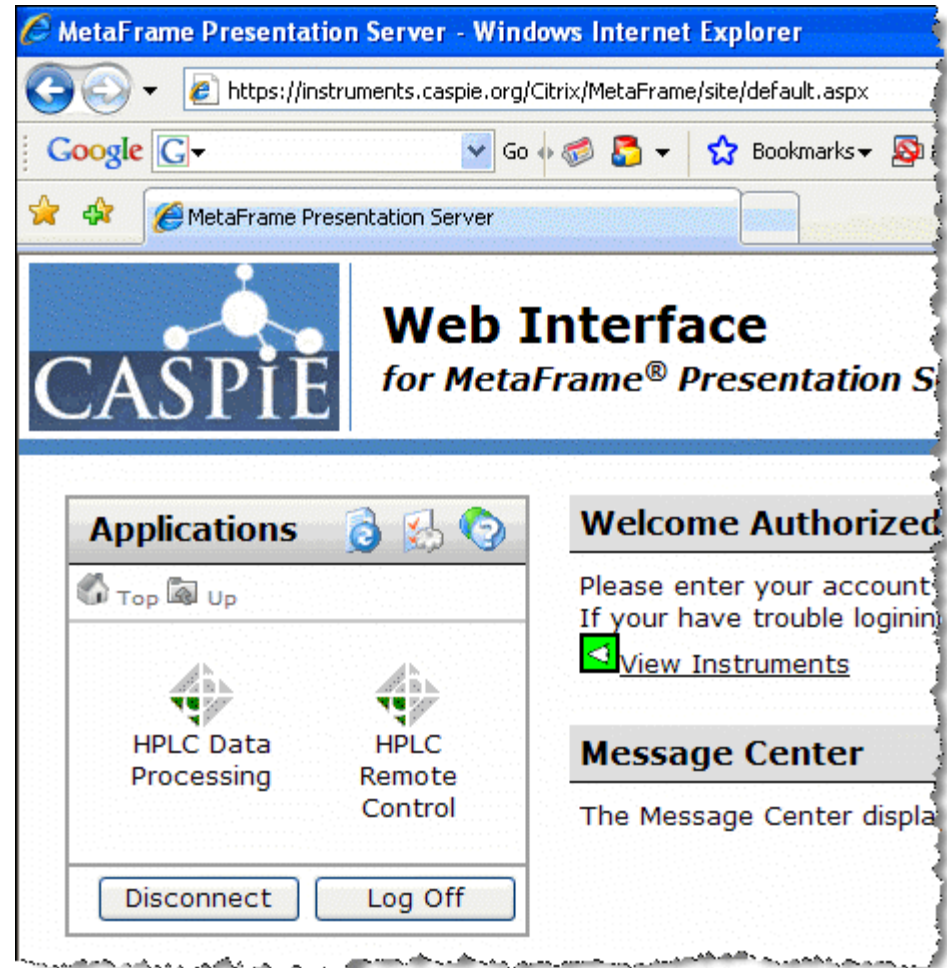
The HPLC Control Software



The software used to control the HPLC instrument is called 'HPLC Remote Control'.

You will only have access to the HPLC instrument for remote control during your scheduled hours.

You will also see the icon for HPLC data processing. You will always have access to the HPLC data processing software during a CASPIE module.

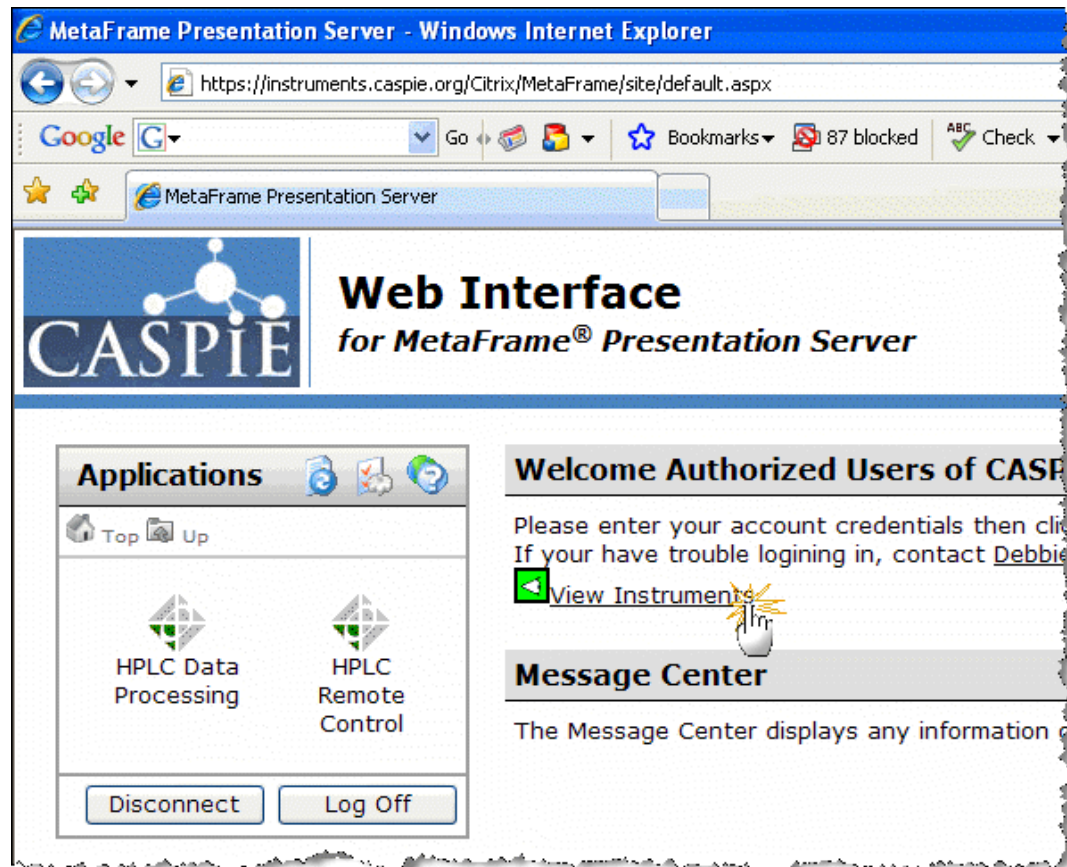


The HPLC Camera

While you are using the HPLC you can view the actual instrument live via an internet camera. (Note that Java is required for loading the camera image.)

The camera is accessed via the link labeled 'View Instruments' next to the green eye.

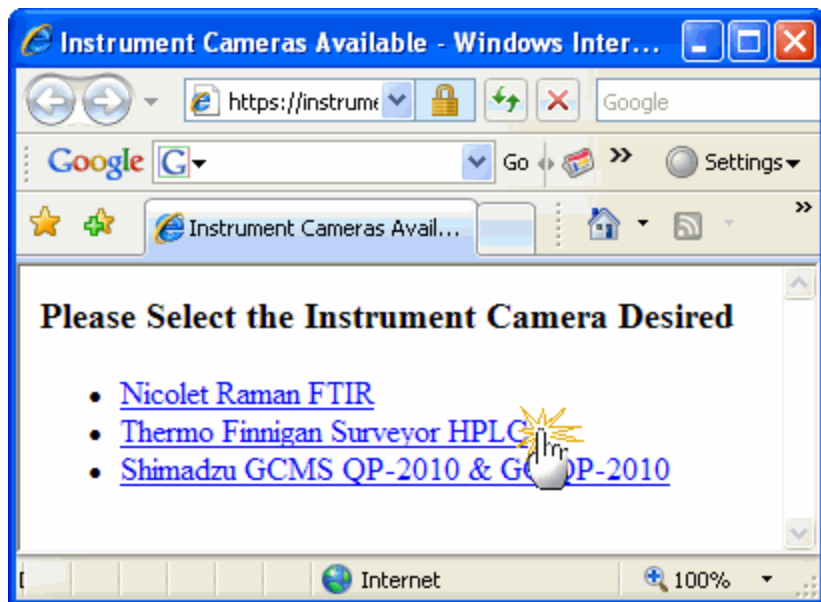
When you click the link another browser window will open.



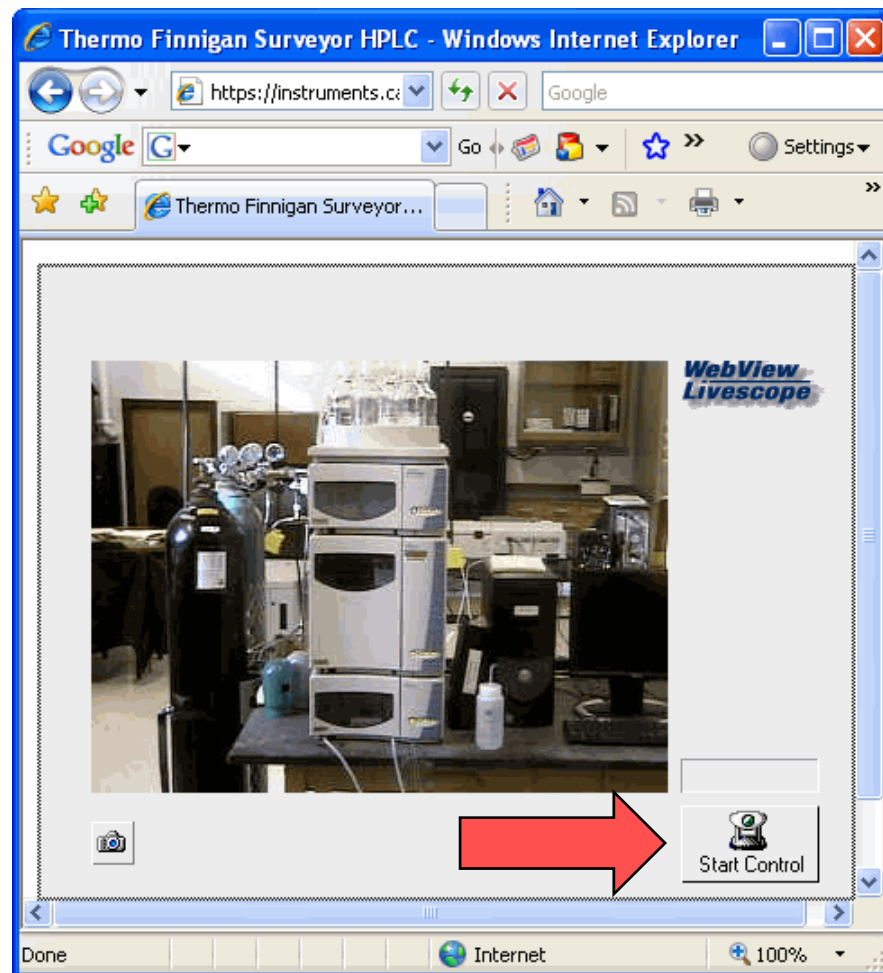
The HPLC Camera

- In the 'Instrument Cameras Available' browser window (a) click on the 'Thermo Finnigan Surveyor HPLC' link to load the camera image.
- To control the camera position click on the 'Start Control' button (b). You may have to click it twice.

(a)

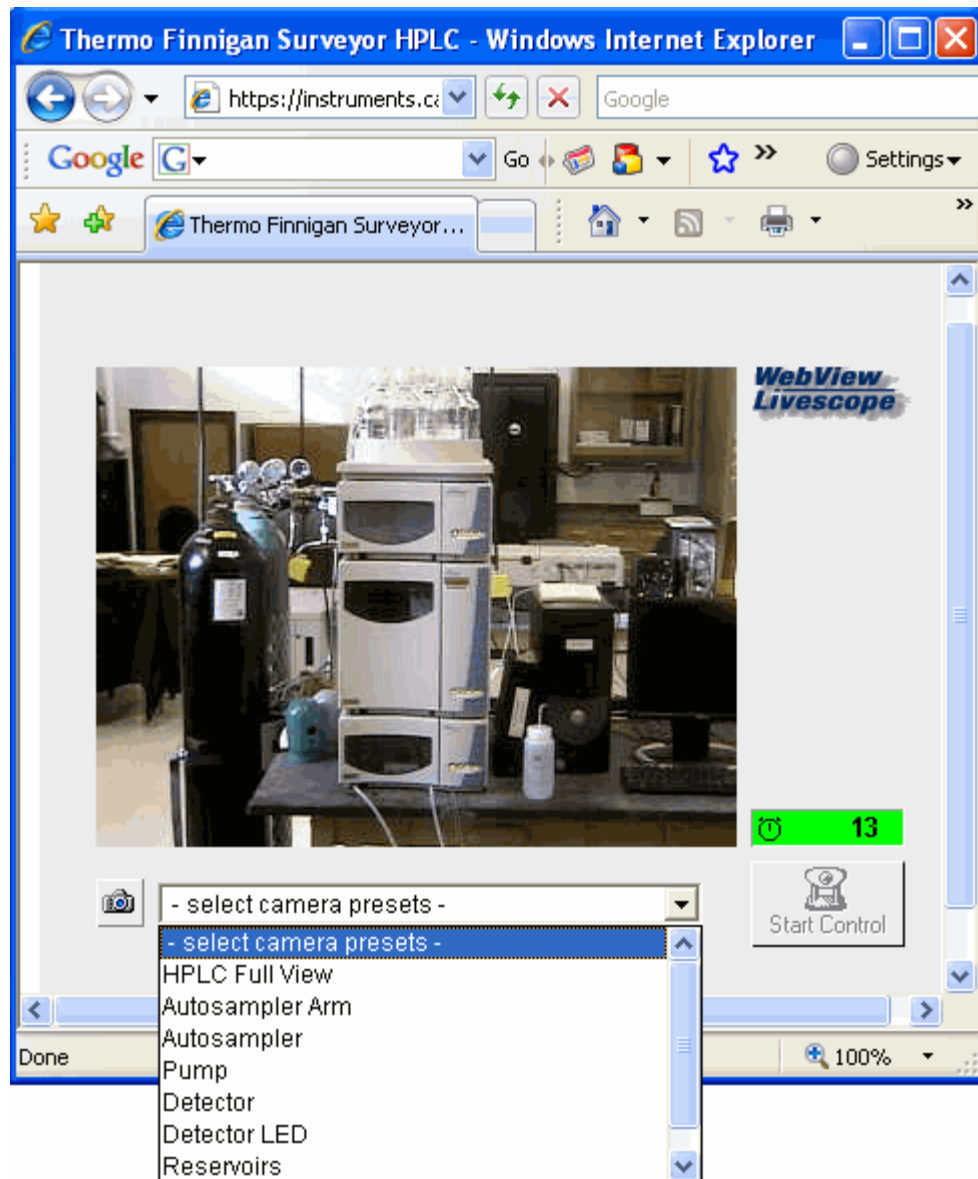


(b)



The HPLC Camera Presets

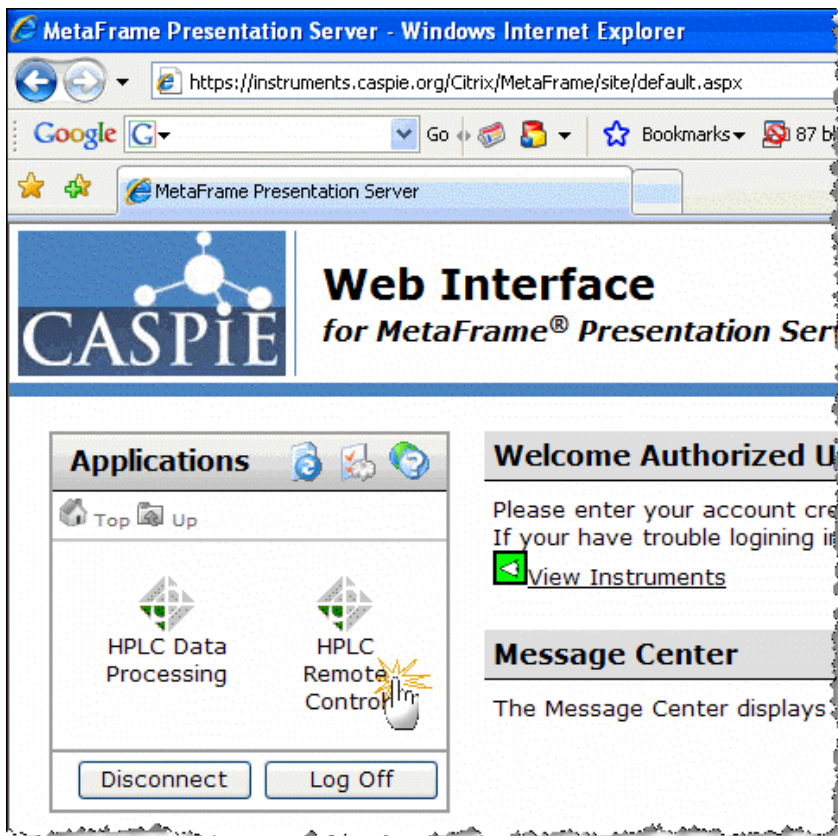
- When you have control of the camera there will be a green countdown clock above the 'Start Control' button.
- Click on the '- select camera presets' drop down and select one of the presets to move the camera to a different position.
- Only preset positions are available for viewing.



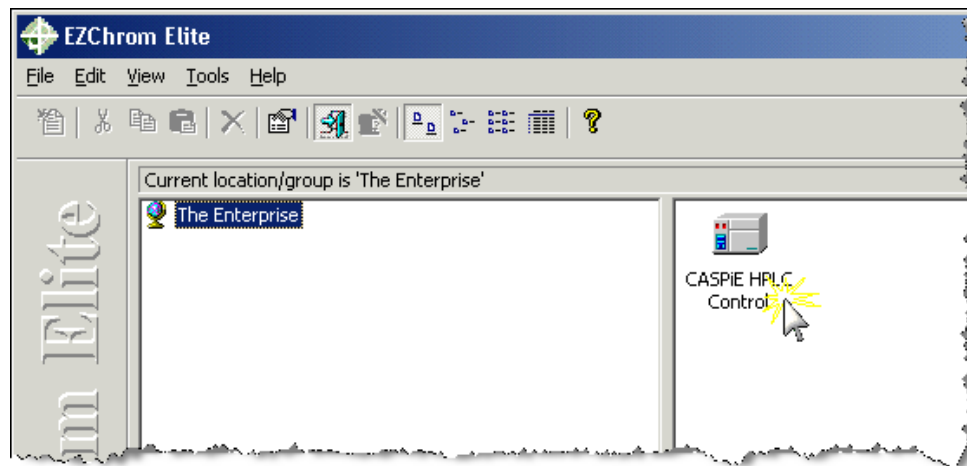
Launch the HPLC Remote Control Software

- To launch the HPLC control software go back to the CASPIE Web Interface browser window (a) and click once on the 'HPLC Remote Control' icon.
- After the EZChrom Elite software launches double-click on the "CASPIE HPLC Control" icon to connect to the instrument (b)

(a)

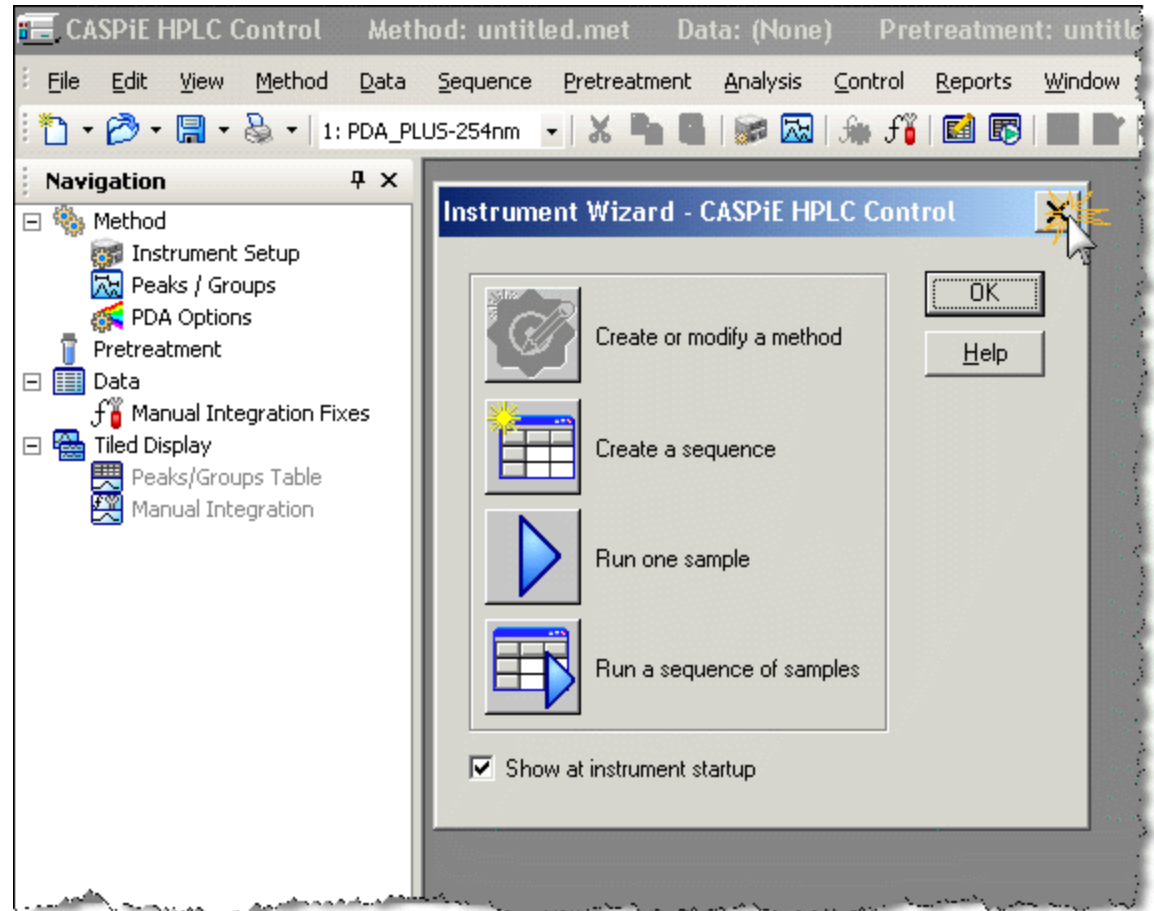


(b)



Instrument Wizard – Close

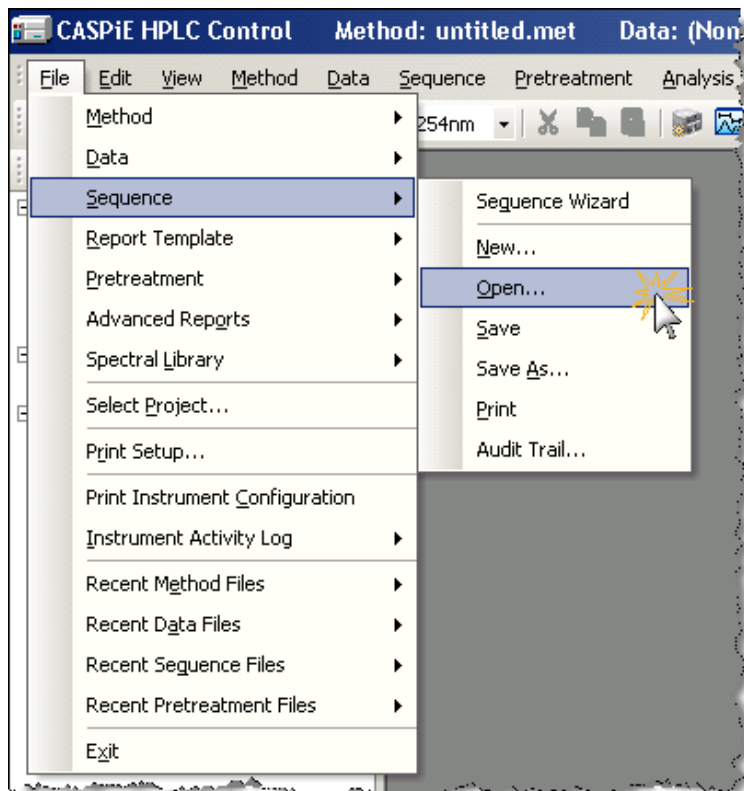
- The next screen will have an ‘Instrument Wizard’ window.
- Don’t do anything with it. Just close the Instrument Wizard window.



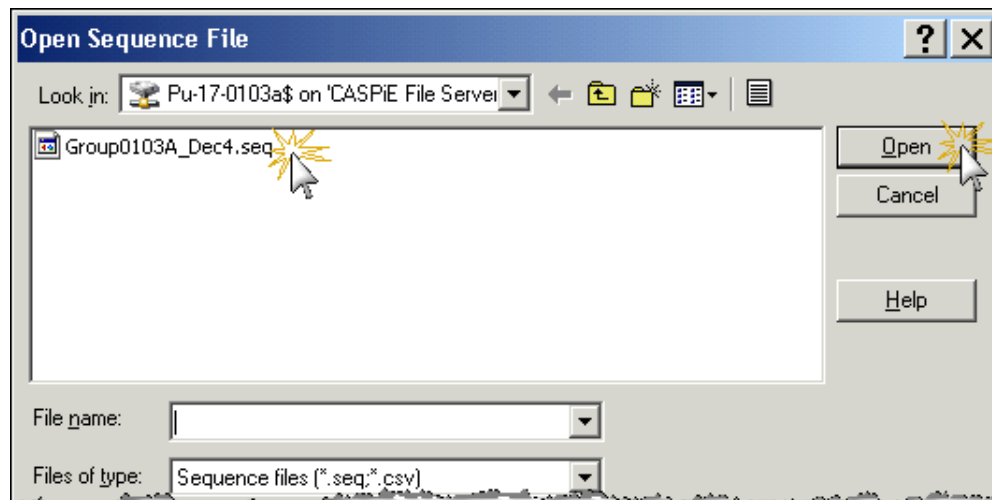
Open a Sequence File

- Now open the sequence file you made previously.
- From the menu select File| Sequence| Open (as shown in image a).
- Select your previously saved sequence file and click 'Open' (b). A window will open displaying your sequence.

(a)

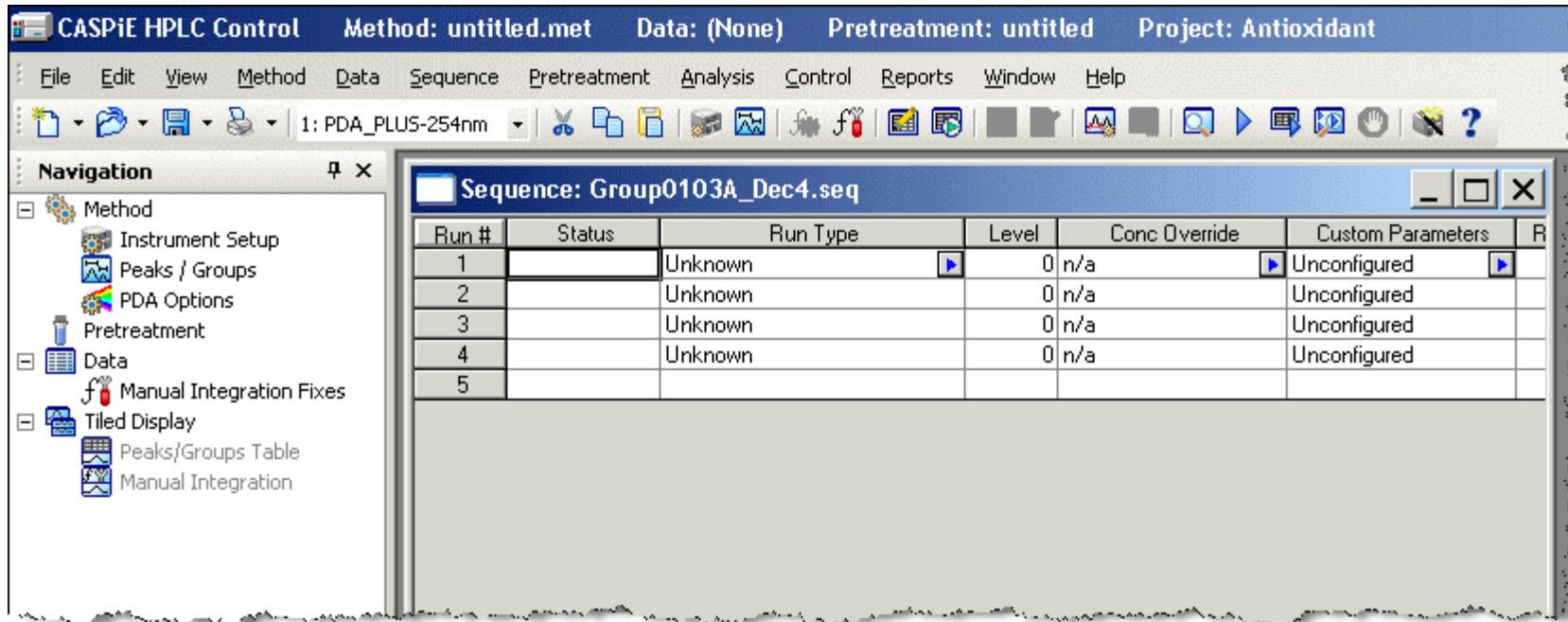


(b)



Sequence File

- You will want to keep this window visible during data collection so you can monitor the progress of the sequence run. This is explained in more detail later.
- You can resize and reposition the Sequence window as desired.



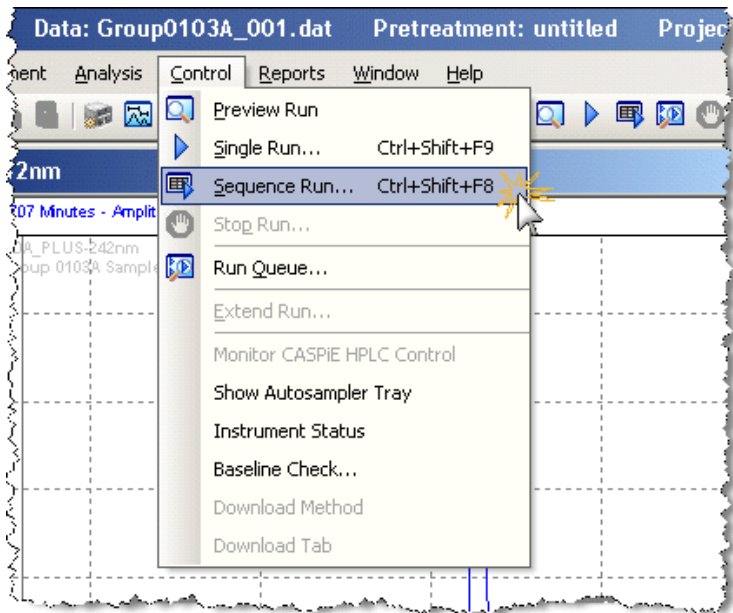
The screenshot displays the CASPIE HPLC Control software interface. The title bar shows 'CASPIE HPLC Control' with sub-headers for 'Method: untitled.met', 'Data: (None)', 'Pretreatment: untitled', and 'Project: Antioxidant'. The menu bar includes File, Edit, View, Method, Data, Sequence, Pretreatment, Analysis, Control, Reports, Window, and Help. The toolbar contains various icons for file operations and control functions. On the left, a 'Navigation' pane lists categories like Method, Data, and Tiled Display. The main window, titled 'Sequence: Group0103A_Dec4.seq', contains a table with the following data:

Run #	Status	Run Type	Level	Conc Override	Custom Parameters	R
1		Unknown	0	n/a	Unconfigured	
2		Unknown	0	n/a	Unconfigured	
3		Unknown	0	n/a	Unconfigured	
4		Unknown	0	n/a	Unconfigured	
5						

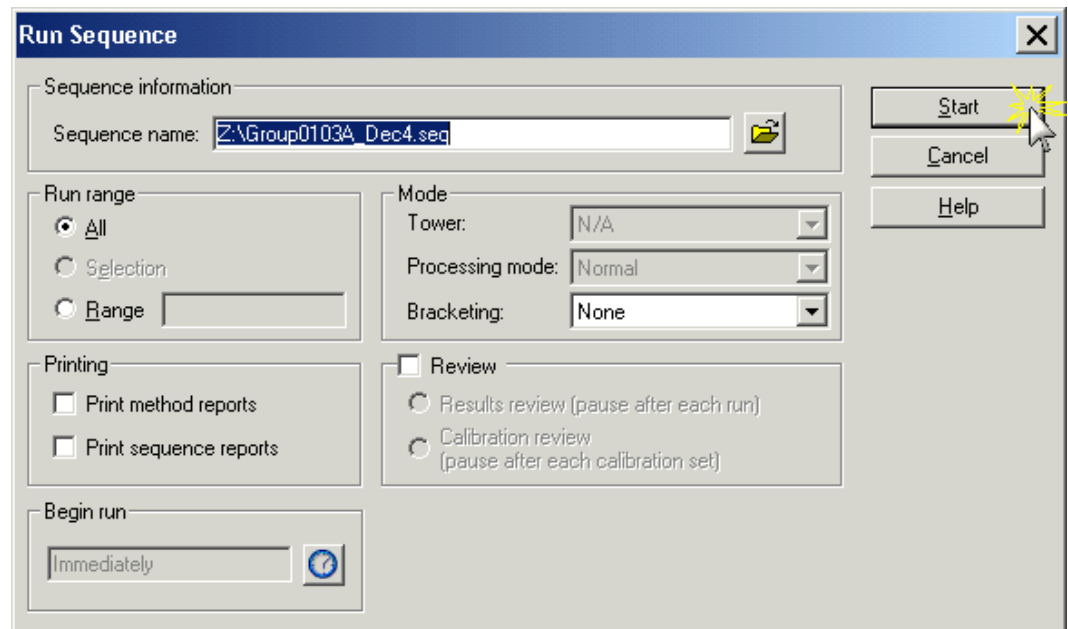
Sequence Start

- To initiate your sequence, from the menu select Control | Sequence Run (a).
- The Run Sequence window (b) will open with your sequence file displayed in the Sequence name box.
- Start the run by clicking the 'Start' button in the Run Sequence window.

(a)

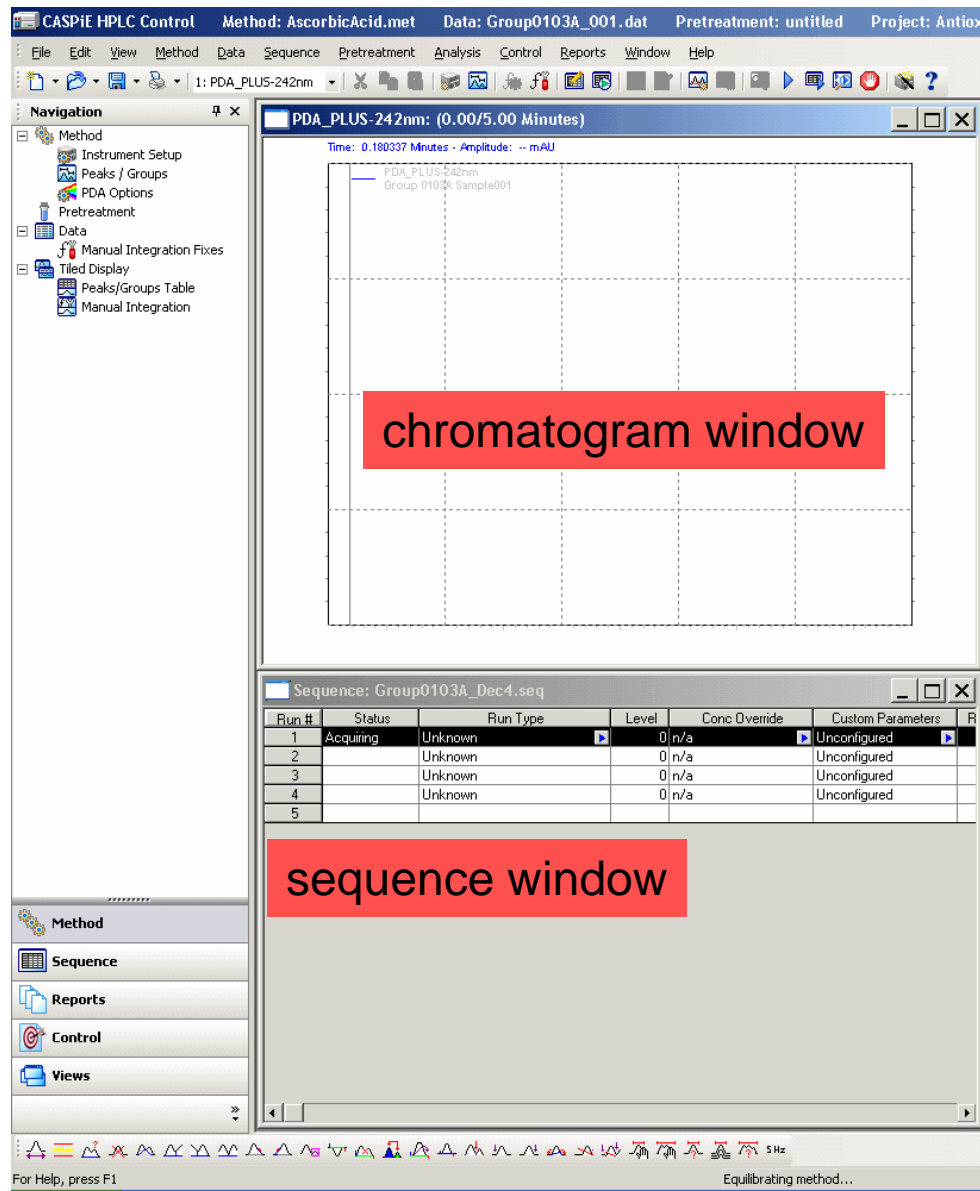


(b)



Chromatogram & Sequence Windows

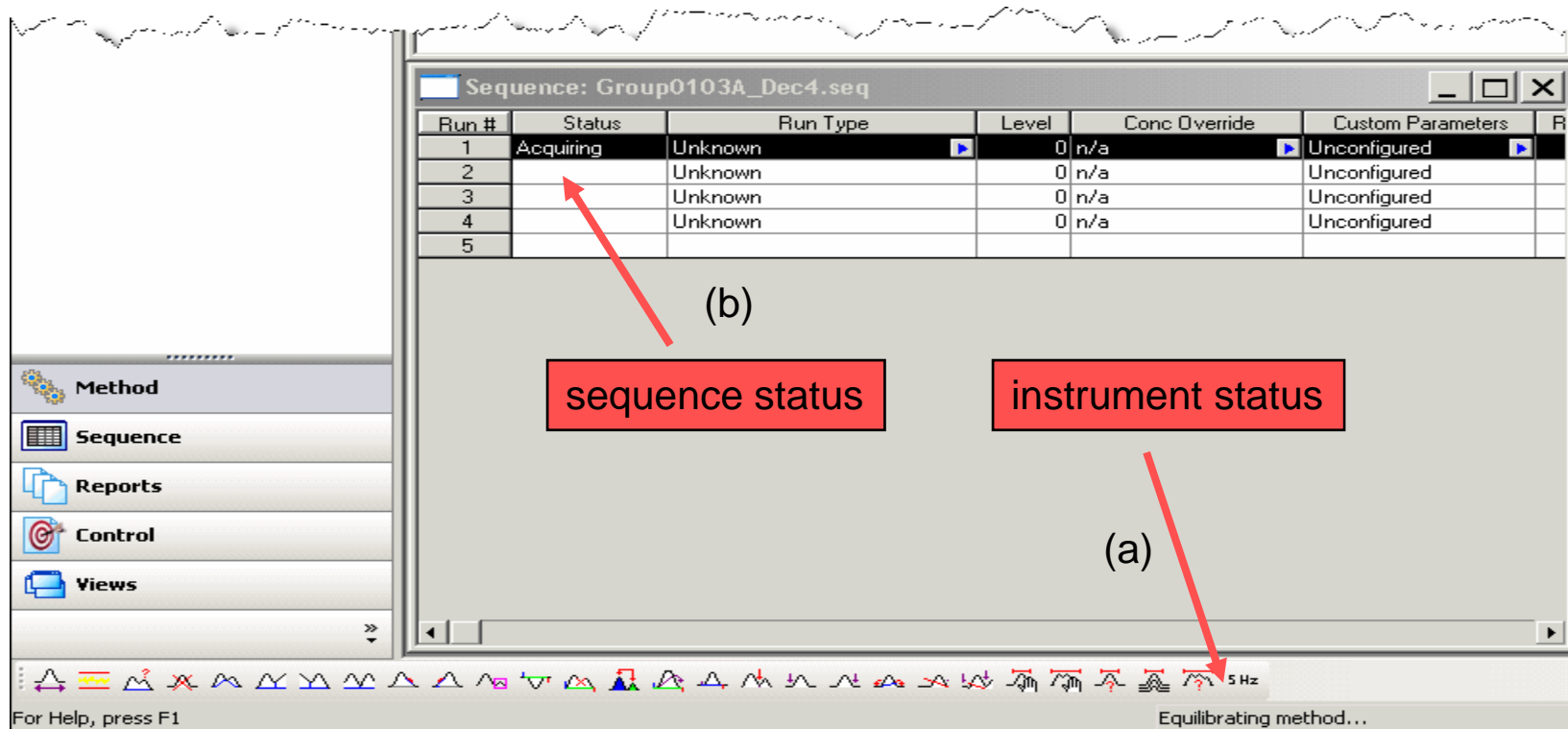
- After the sequence run is started a chromatogram window labeled 'PDA_PLUS-242nm' will open. There should be two windows open, the chromatogram and sequence windows.
- You can resize the windows to make them both visible.
- The chromatogram and sequence windows will update during the run.



The screenshot displays the CASPIE HPLC Control software interface. The main window is titled 'CASPIE HPLC Control' and shows the 'PDA_PLUS-242nm: (0.00/5.00 Minutes)' chromatogram window. The chromatogram window is currently empty, with a red box overlaid on it containing the text 'chromatogram window'. Below the chromatogram window is the 'Sequence: Group0103A_Dec4.seq' window, which contains a table of sequence runs. The table has columns for Run #, Status, Run Type, Level, Conc Override, and Custom Parameters. The table shows five runs, all with a status of 'Acquiring' and 'Unknown' run type. A red box is overlaid on the bottom of the sequence window containing the text 'sequence window'. The interface also includes a navigation pane on the left with options like Method, Peaks / Groups, PDA Options, Pretreatment, Data, Tiled Display, Peaks/Groups Table, and Manual Integration. At the bottom, there is a status bar with a 5 Hz frequency indicator and the text 'Equilibrating method...'. The bottom left corner of the screenshot contains the text 'For Help, press F1'.

Run #	Status	Run Type	Level	Conc Override	Custom Parameters
1	Acquiring	Unknown	0	n/a	Unconfigured
2		Unknown	0	n/a	Unconfigured
3		Unknown	0	n/a	Unconfigured
4		Unknown	0	n/a	Unconfigured
5		Unknown	0	n/a	Unconfigured

- At the start of the run the instrument will take a few seconds to get the sample ready.
- The status of the instrument during a run is shown at the bottom of the screen (a).
- The progress of the sequence run is shown in the Sequence window (b).



The screenshot displays a software interface with a sequence window and a control panel. The sequence window, titled "Sequence: Group0103A_Dec4.seq", contains a table with the following data:

Run #	Status	Run Type	Level	Conc Override	Custom Parameters	R
1	Acquiring	Unknown	0	n/a	Unconfigured	
2		Unknown	0	n/a	Unconfigured	
3		Unknown	0	n/a	Unconfigured	
4		Unknown	0	n/a	Unconfigured	
5						

Red arrows point from the text labels to the corresponding elements in the interface:

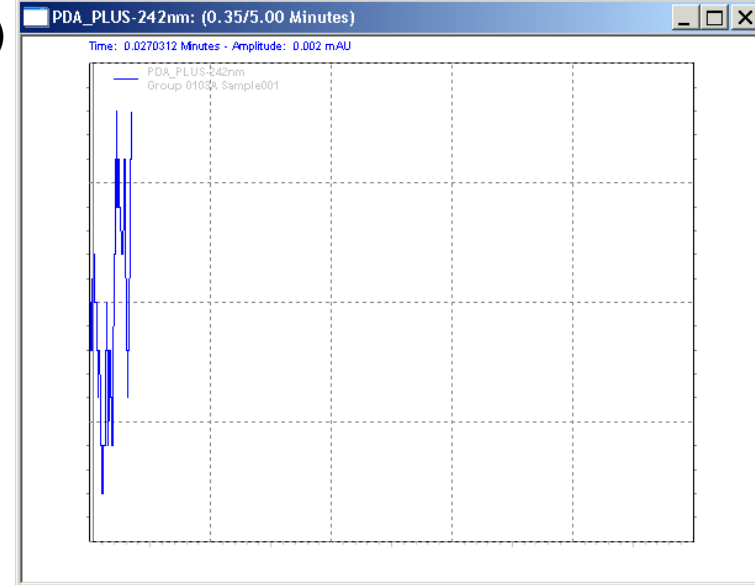
- (b) points to the "Acquiring" status in the first row of the table.
- (a) points to the "Equilibrating method..." text at the bottom right of the interface.

Other interface elements include a sidebar with "Method", "Sequence", "Reports", "Control", and "Views" buttons, and a toolbar at the bottom with various icons and a "5 Hz" label.

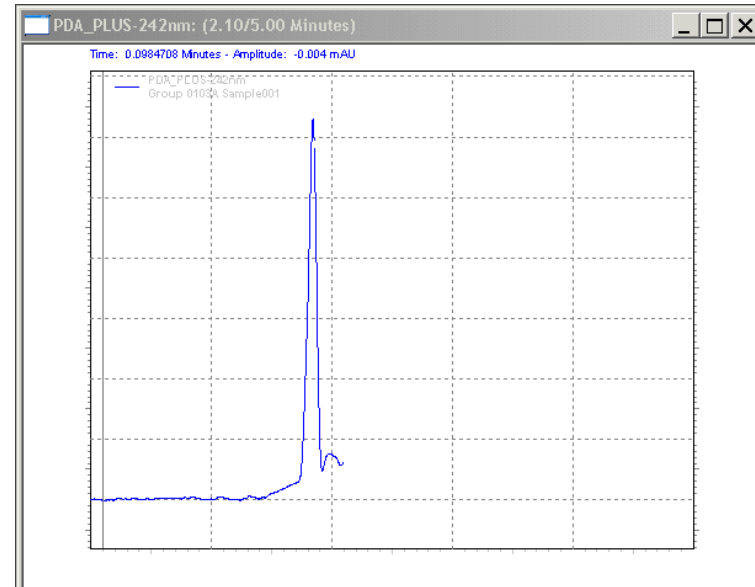
Data Collection

- It will take 5 minutes to collect each chromatogram.
- An example chromatogram collection is shown here.
- The y axis will autoscale as the maximum signal changes.

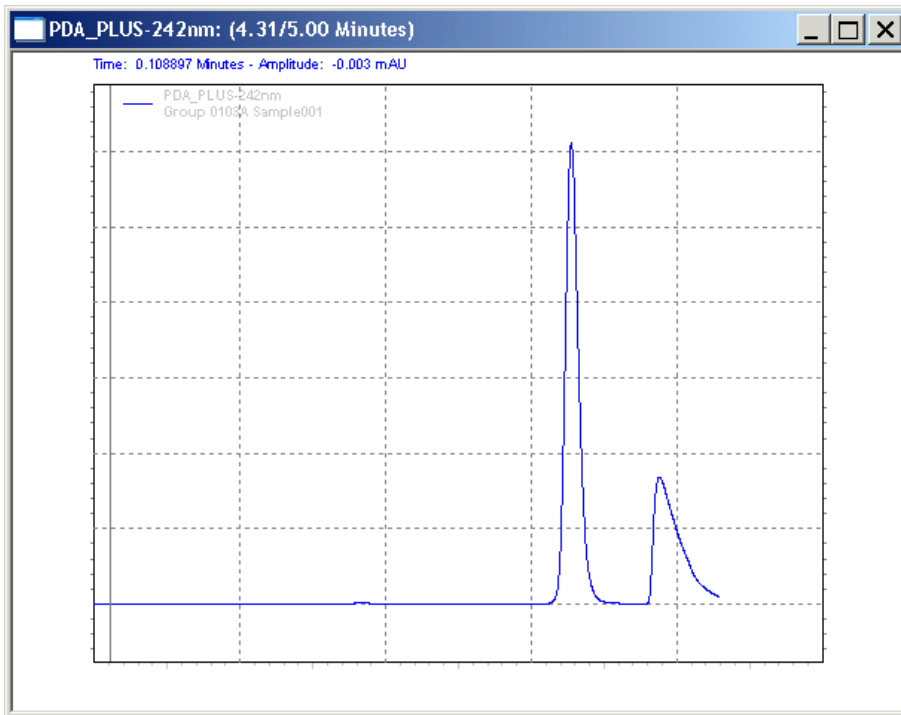
(1)



(2)

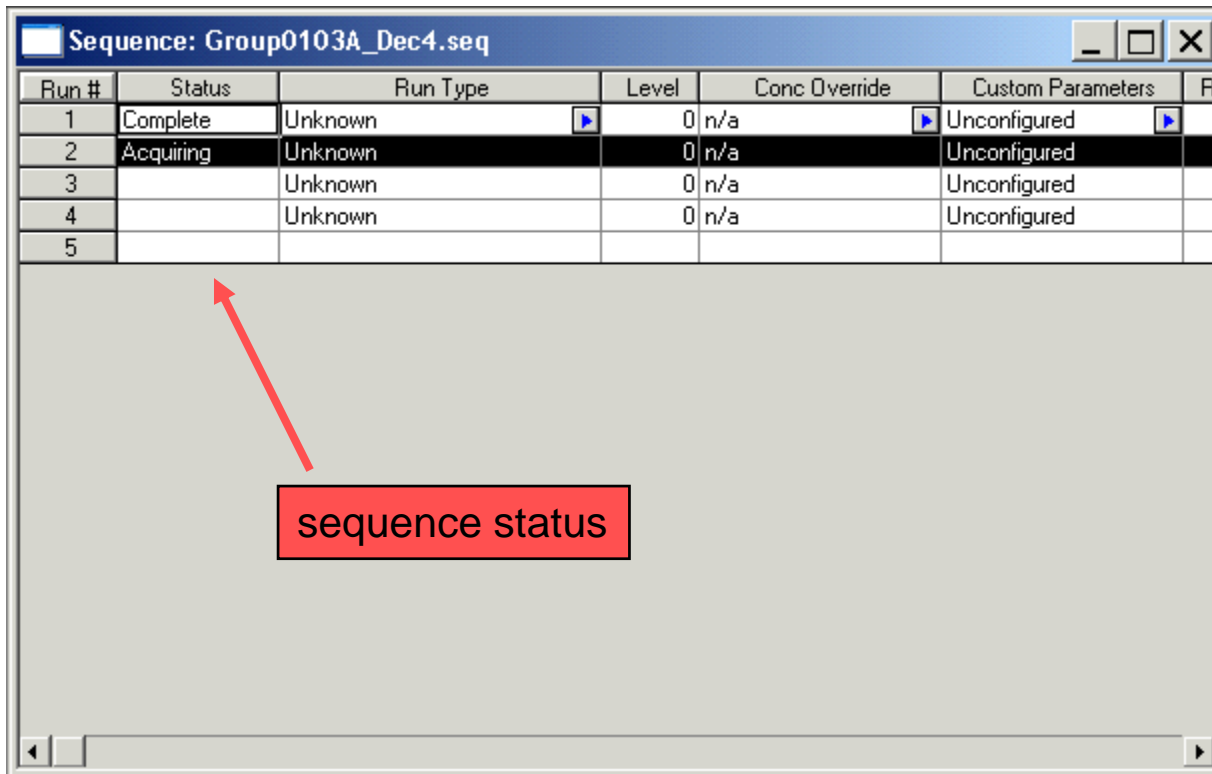


(3)



Sequence Run Progress

- Monitor the progress of the run by noting the 'Status' column in the Sequence window.
- The instrument will automatically proceed down the list of samples in the sequence file. You need only make sure each sample is 'Complete.'

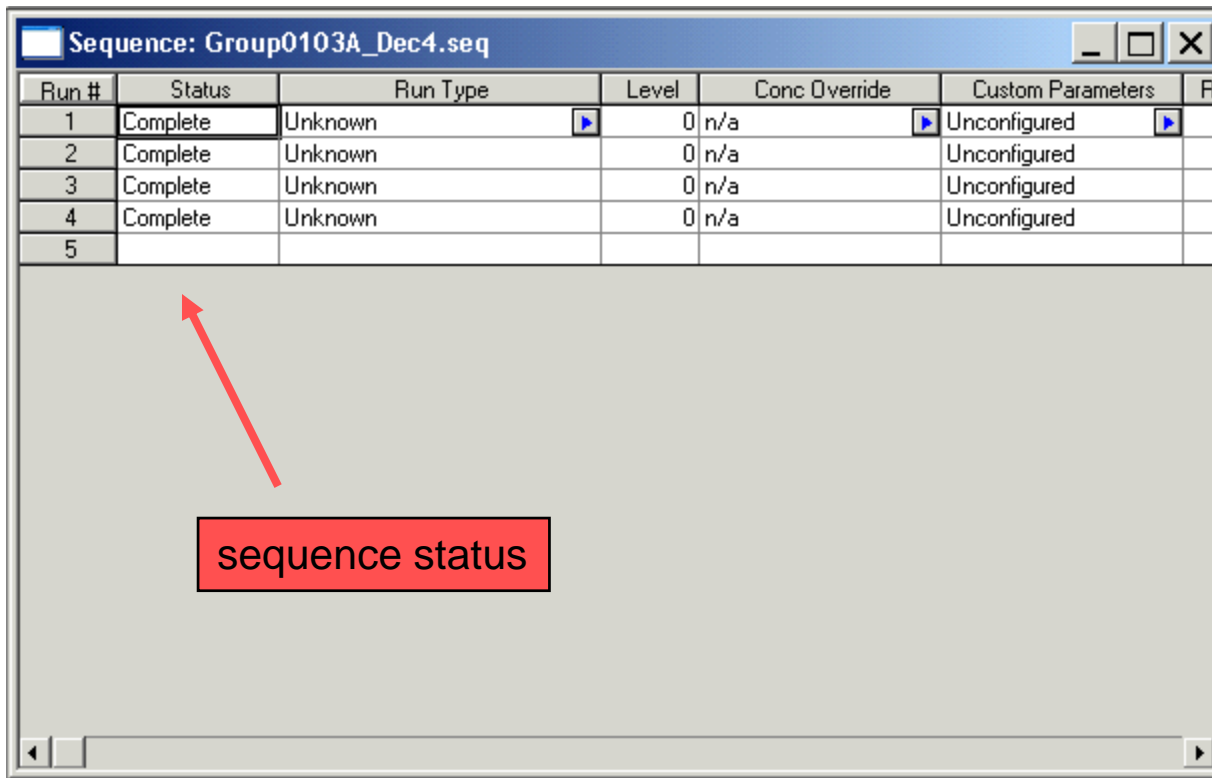


Run #	Status	Run Type	Level	Conc Override	Custom Parameters	R
1	Complete	Unknown	0	n/a	Unconfigured	
2	Acquiring	Unknown	0	n/a	Unconfigured	
3		Unknown	0	n/a	Unconfigured	
4		Unknown	0	n/a	Unconfigured	
5						

sequence status

Sequence Run Finished

- When the sequence is finished and chromatograms are collected for all your samples the sequence status column will show 'complete' for all runs.
- You are now finished collecting data and can exit the program.



Run #	Status	Run Type	Level	Conc Override	Custom Parameters	R
1	Complete	Unknown	0	n/a	Unconfigured	
2	Complete	Unknown	0	n/a	Unconfigured	
3	Complete	Unknown	0	n/a	Unconfigured	
4	Complete	Unknown	0	n/a	Unconfigured	
5						

Close Windows

- Remember, DO NOT make any changes to the instrument.
- Exit all EZChrom Elite HPLC software windows.

