

How to Run a Demonstration Sample on the CASPiE HPLC



HPLC stands for High Performance Liquid Chromatography. It is used to separate and quantify complex mixtures. HPLC is used to quantify ascorbic acid in your food samples.

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

This document gives instruction for running a demonstration sample, not for samples you generate in your laboratory.

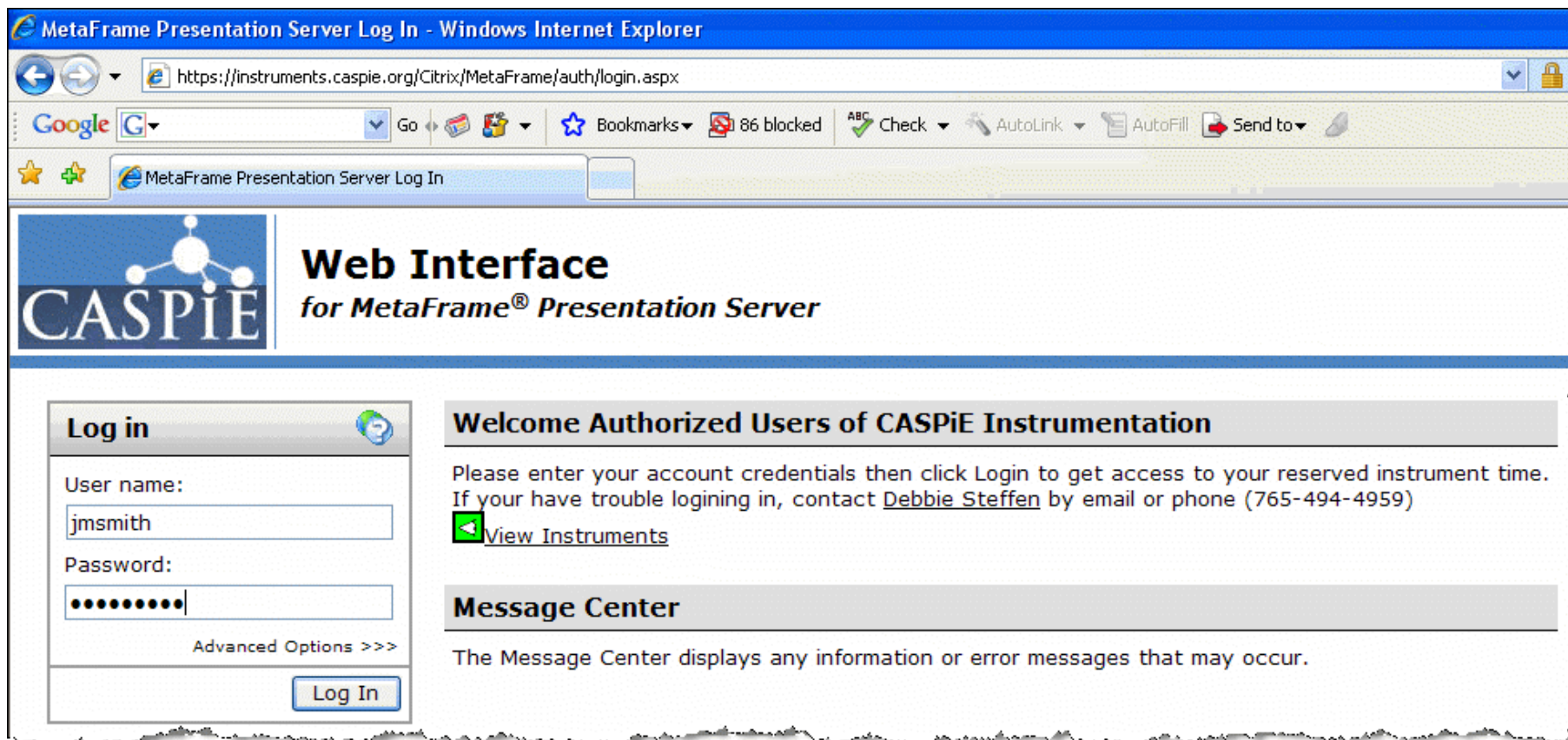
Please Note:

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



The screenshot shows a Windows Internet Explorer browser window with the address bar displaying <https://instruments.caspie.org/Citrix/MetaFrame/auth/login.aspx>. The browser's search bar contains "Google" and the address bar has "MetaFrame Presentation Server Log In" entered. The website header features the CASPiE logo and the text "Web Interface for MetaFrame® Presentation Server".

Log in

User name:

Password:

Advanced Options >>>

Welcome Authorized Users of CASPiE Instrumentation

Please enter your account credentials then click Login to get access to your reserved instrument time. If you have trouble logging in, contact [Debbie Steffen](#) by email or phone (765-494-4959)

Message Center

The Message Center displays any information or error messages that may occur.

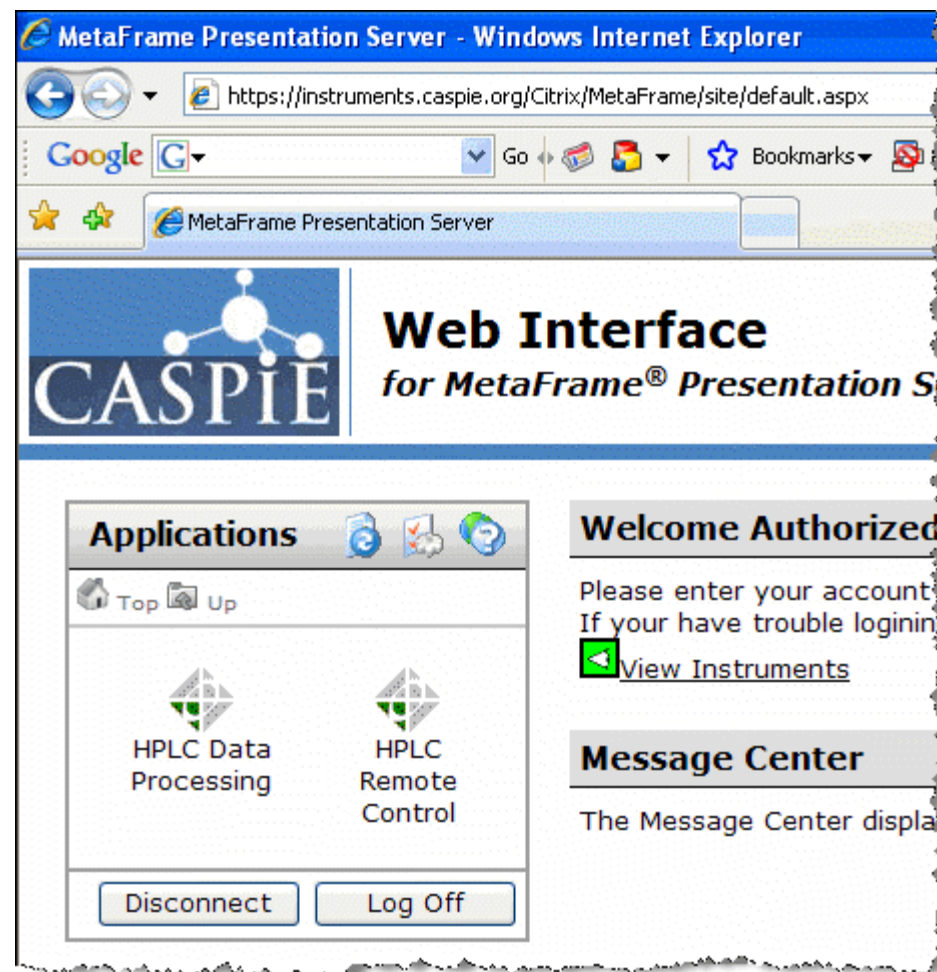
The HPLC Control Software



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

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

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.



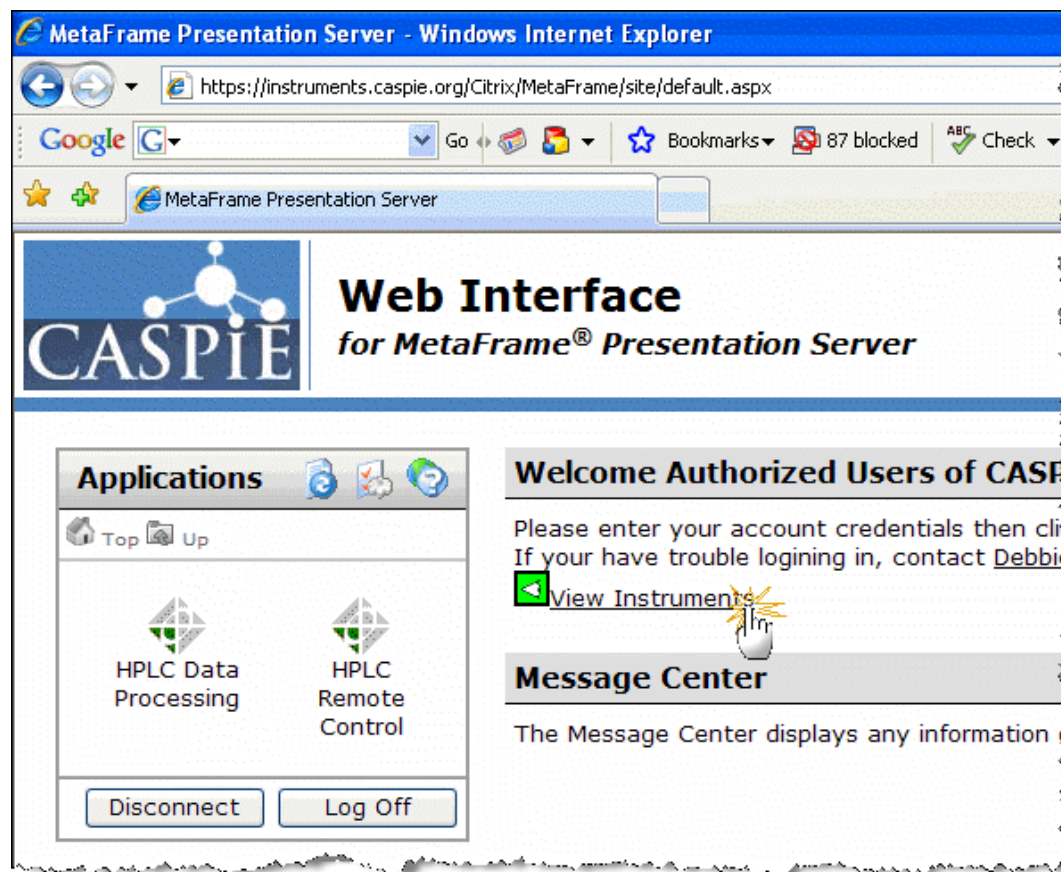
The HPLC Camera



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.

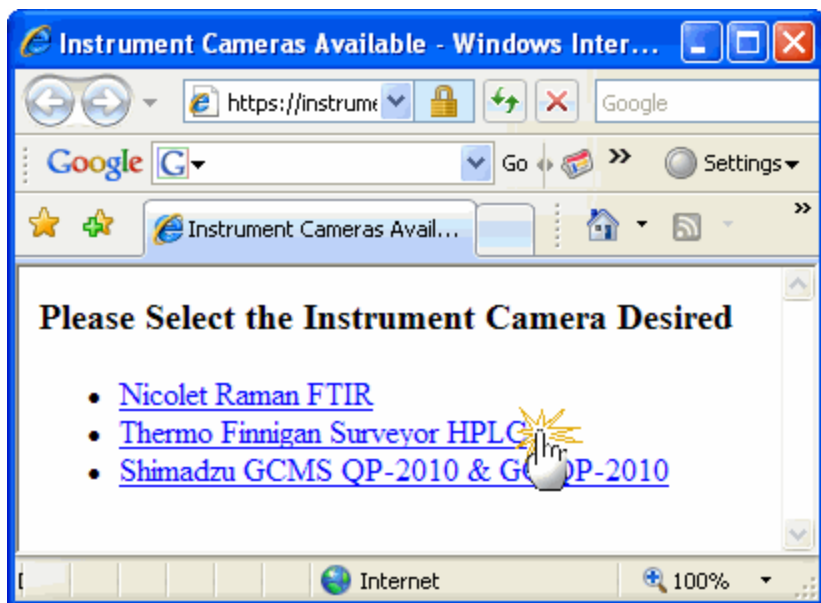
When you click the link another browser window will open.



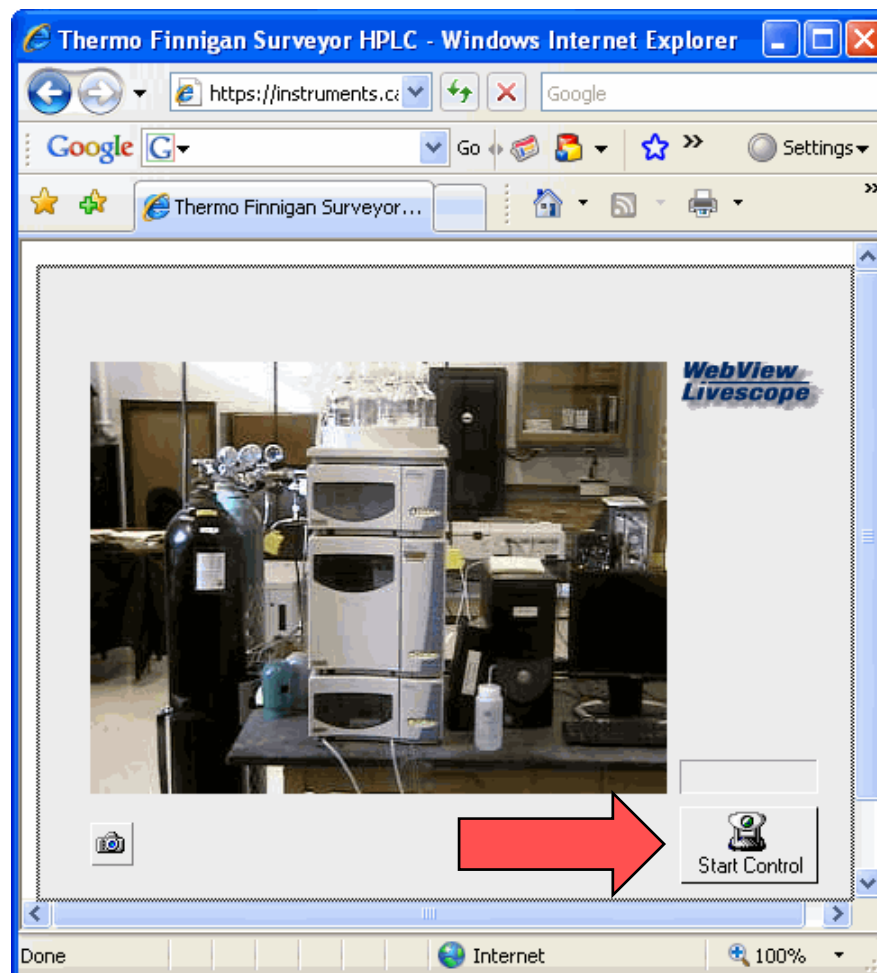
The HPLC Camera

- 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)



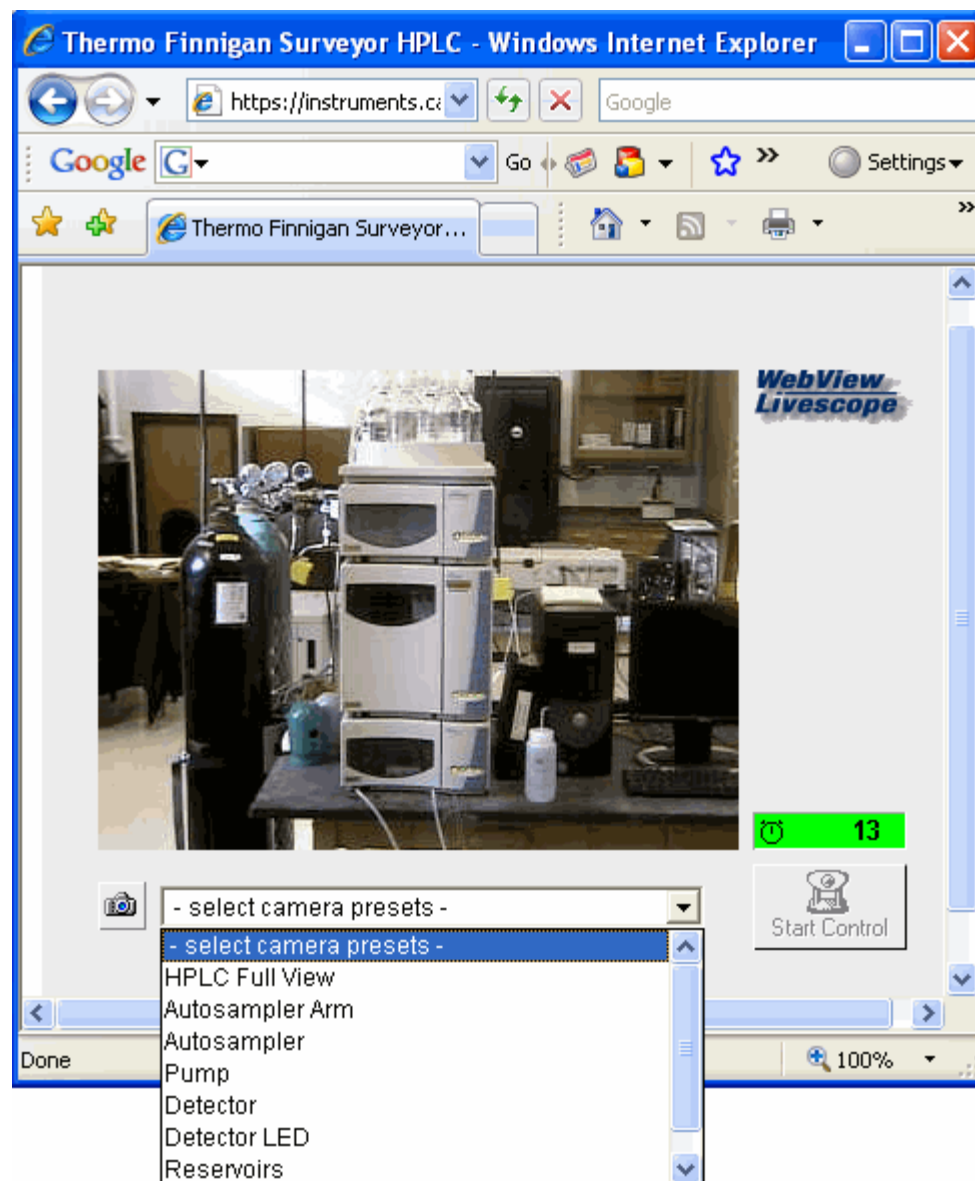
(b)



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.

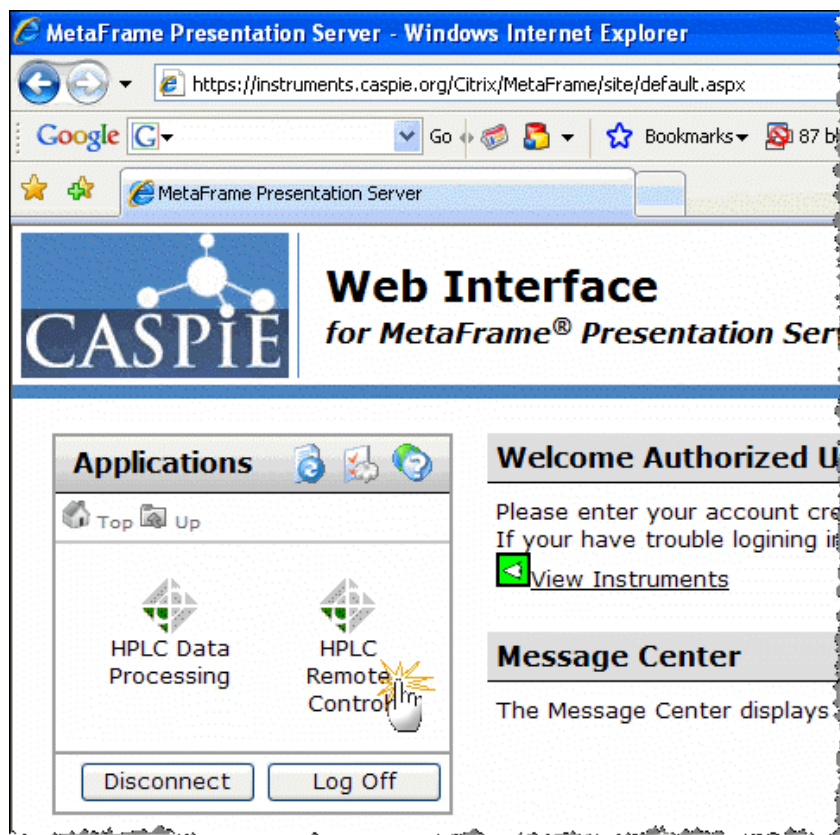


Launch the HPLC Remote Control Software

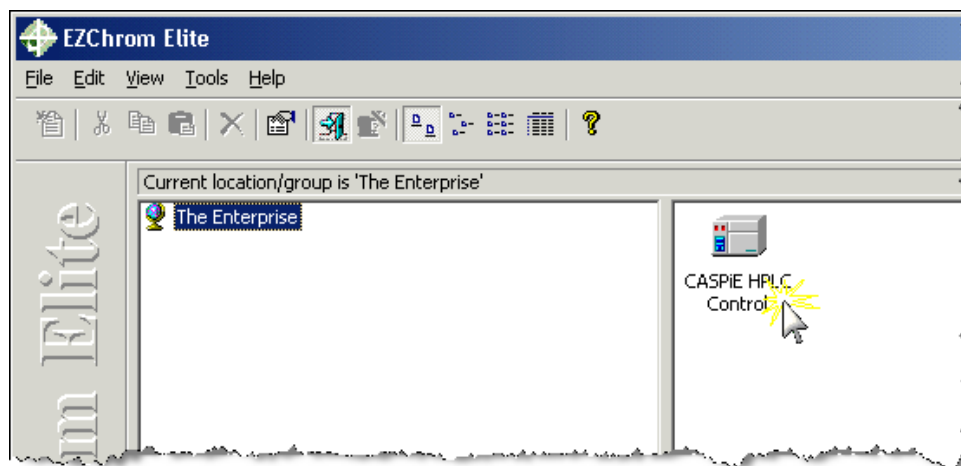


- To launch the HPLC remote control software go back to the CASPIE Web Interface browser window (a) and click once on the 'HPLC Remote Control' icon.
- After the EZChrom Elite software launches double-click on the "CASPIE HPLC Control" icon to connect to the instrument (b).

(a)

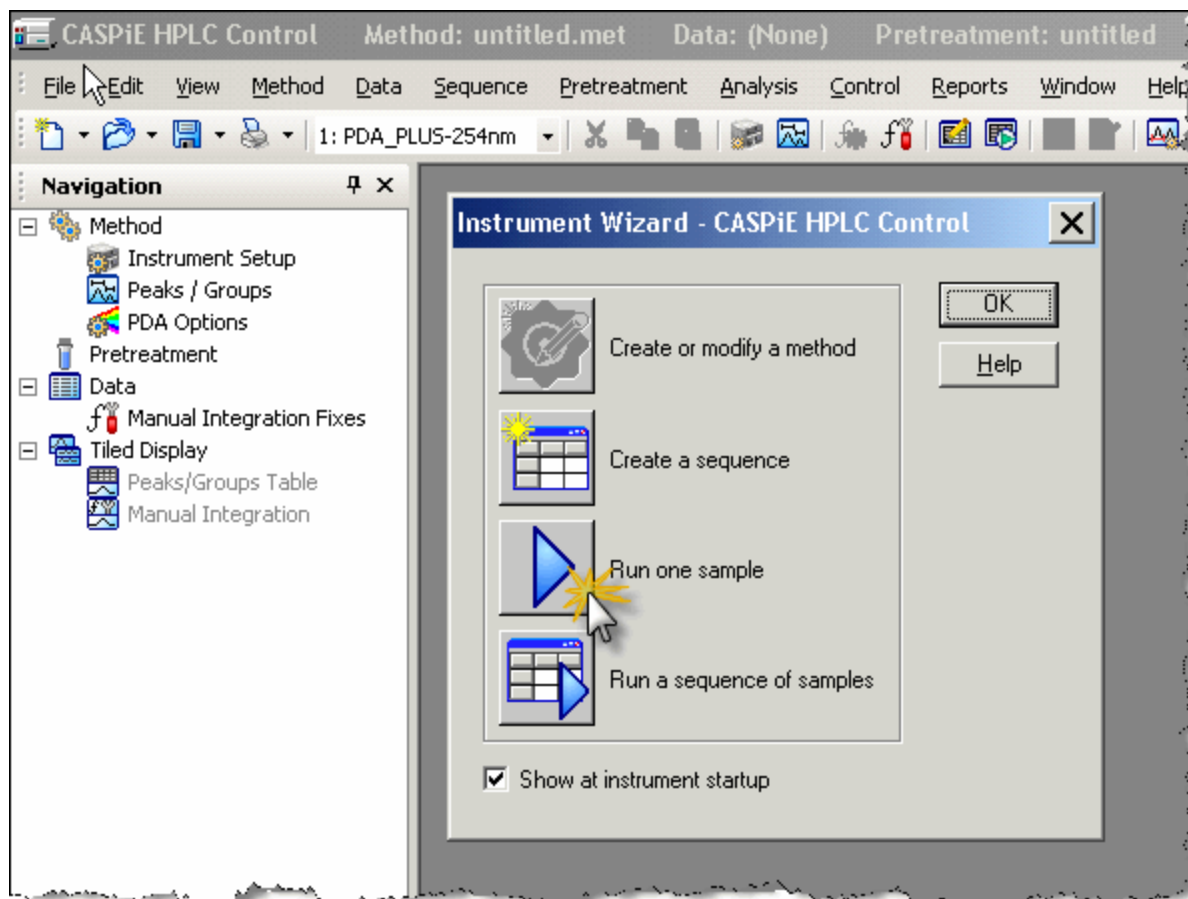


(b)



Instrument Wizard

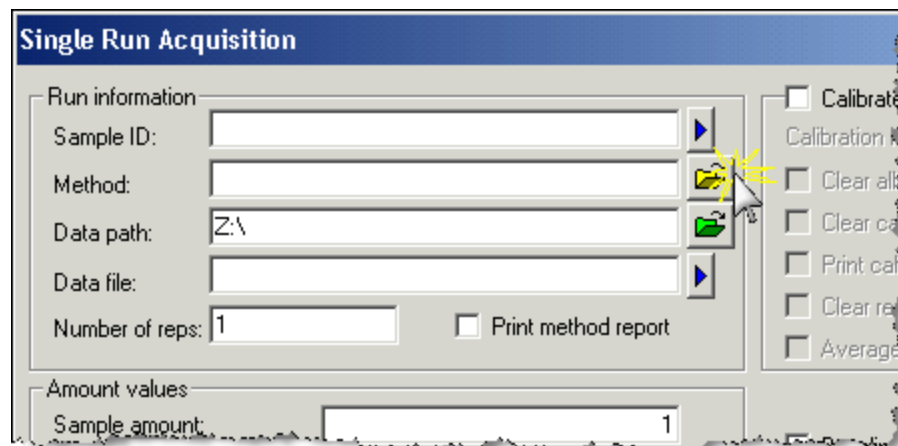
- The next screen will have an 'Instrument Wizard' window.
- Click on the 'Run one sample' icon.
- A new window will open called 'Single Run Acquisition'.



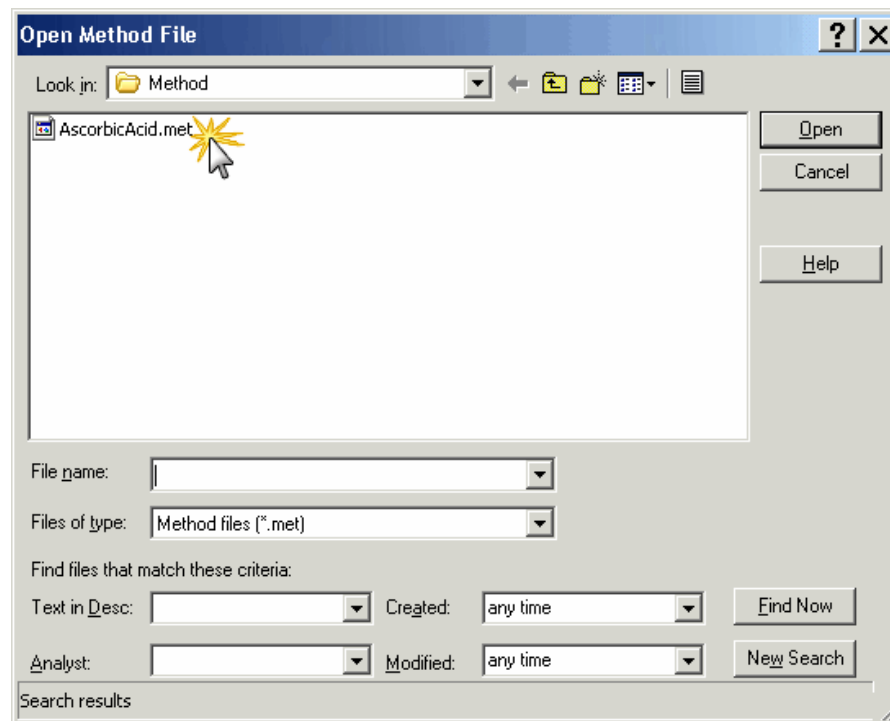
Single Run Acquisition Window

- In the 'Single Run Acquisition' window click on the file open icon to the right of the method box (a).
- The Open Method File window will open. Select the AscorbicAcid.met file and click 'Open' (b).

(a)



(b)



Enter Sample Information



- Enter a sample ID (1) and data file name (2) of your choosing.
- In the 'Vial' box (3) enter 'A;A1'. *Correct entry is very important because this is the location of the demonstration sample.*
- Keep the default entries in the remaining information boxes. The injection volume should be 10 μL .

The screenshot shows the 'Single Run Acquisition' window with the following fields and values:

- Run information:**
 - Sample ID: **1** Demo Sample
 - Method: K:\Method\AscorbicAcid.met
 - Data path: Z:\
 - Data file: **2** DemoSample04-20-09
 - Number of reps: 1
 - Print method report
- Amount values:**
 - Sample amount: 1
 - Internal standard amount: 1
 - Multiplication factors: 1 1 1
 - Dilution factors: 1 1 1
- Autosampler:**
 - Use program: [empty]
 - Vial: **3** A;A1
 - Injection volume: 10 μL

Start Run



- Double check the vial location; it should be A;A1.
- To initiate your run, click the start button in the 'Single Run Acquisition' window.

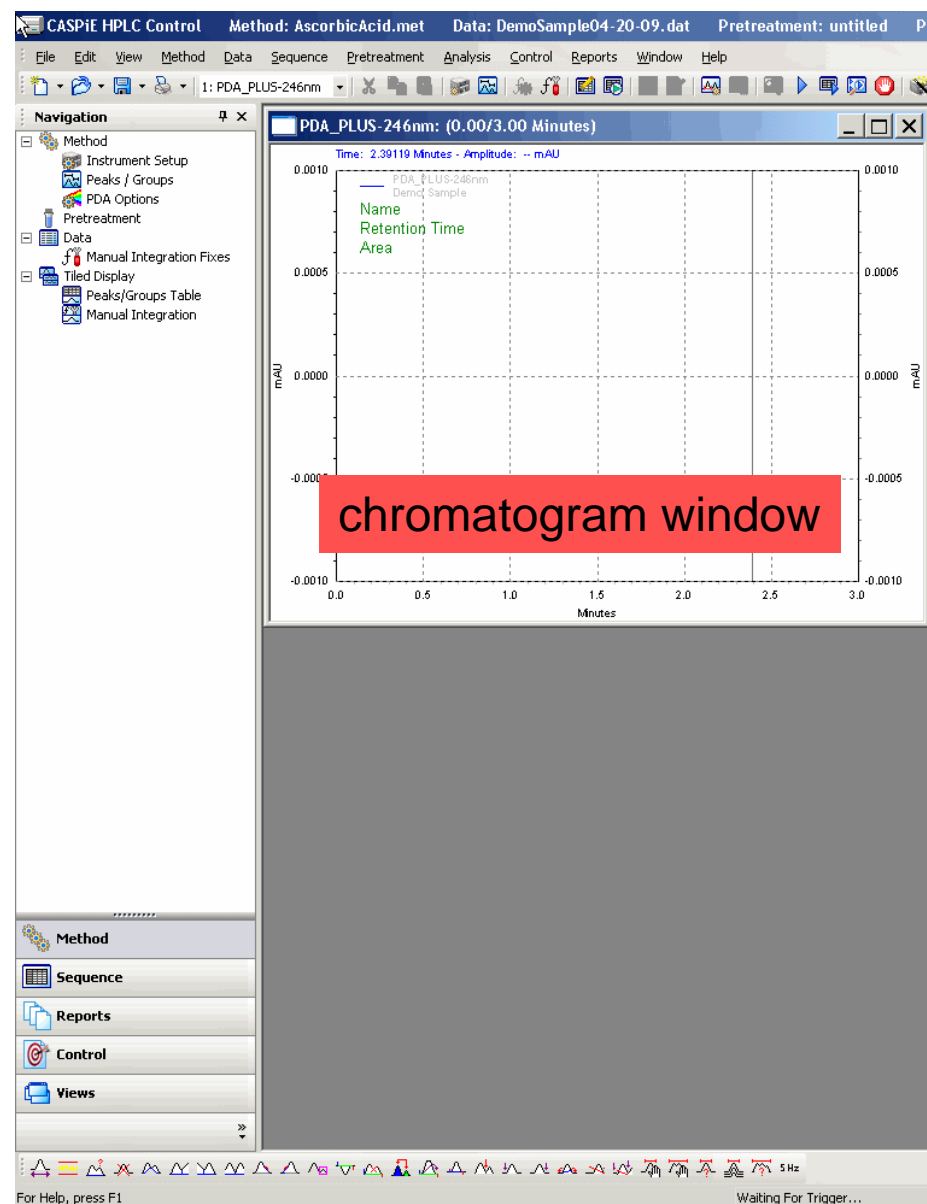
The screenshot shows the 'Single Run Acquisition' dialog box with the following fields and options:

- Run information:**
 - Sample ID: Demo Sample
 - Method: K:\Method\AscorbicAcid.met
 - Data path: Z:\
 - Data file: DemoSample04-20-09
 - Number of reps: 1
 - Print method report
- Amount values:**
 - Sample amount: 1
 - Internal standard amount: 1
 - Multiplication factors: 1, 1, 1
 - Dilution factors: 1, 1, 1
- Autosampler:**
 - Use program: [empty]
 - Vial: A;A1
 - Injection volume: 10 μ L
- Calibration:**
 - Calibrate
 - Calibration level: 1
 - Clear all calibration
 - Clear calibration for level
 - Print calibration report
 - Clear replicates
 - Average replicates
- Baseline Check:**
 - Baseline Check
- Begin run:**
 - Immediately
- Buttons:** Start, Cancel, Help, Description...

Chromatogram Window



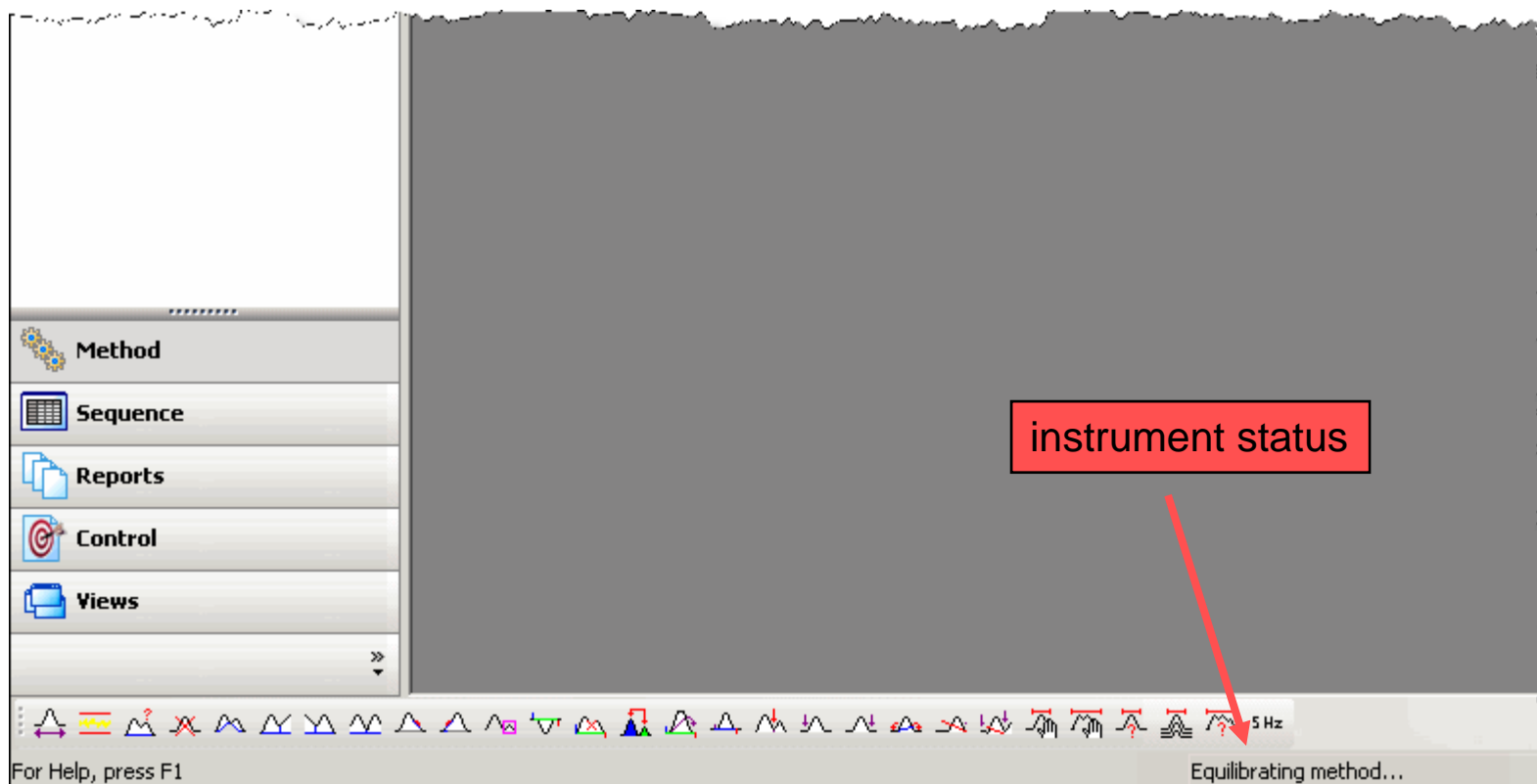
- After the run is started a chromatogram window labeled 'PDA_PLUS-242nm' will open.
- The chromatogram window will update during the run.



Instrument Status

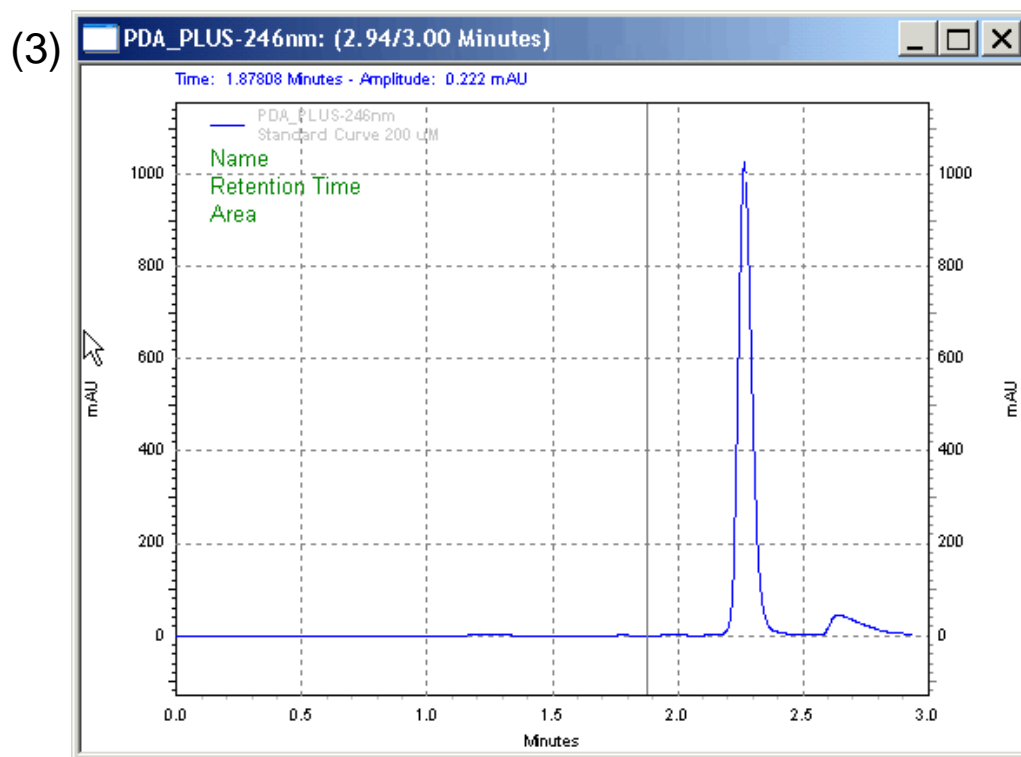
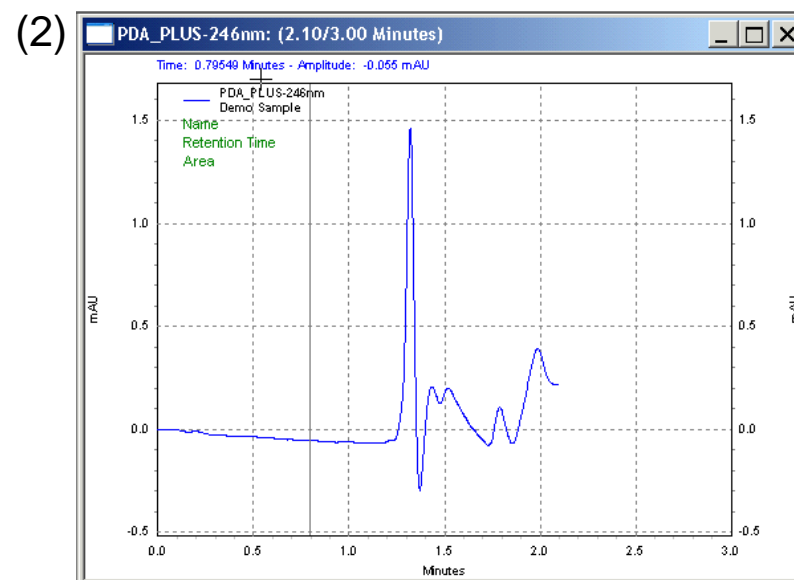
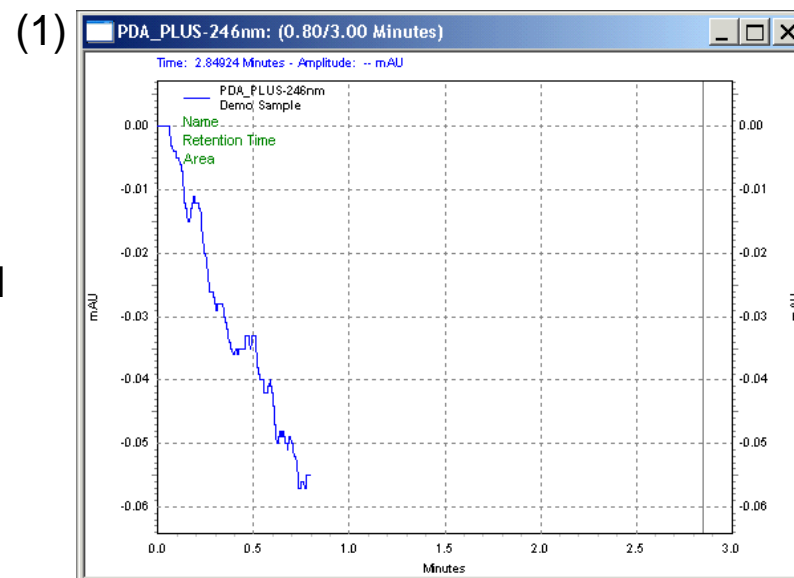


- At the start of the run the instrument will take a few seconds to get the sample ready.
- The status of the instrument during a run is shown at the bottom of the screen.
- When the sample is running the status will read 'Running sample...'



Data Collection

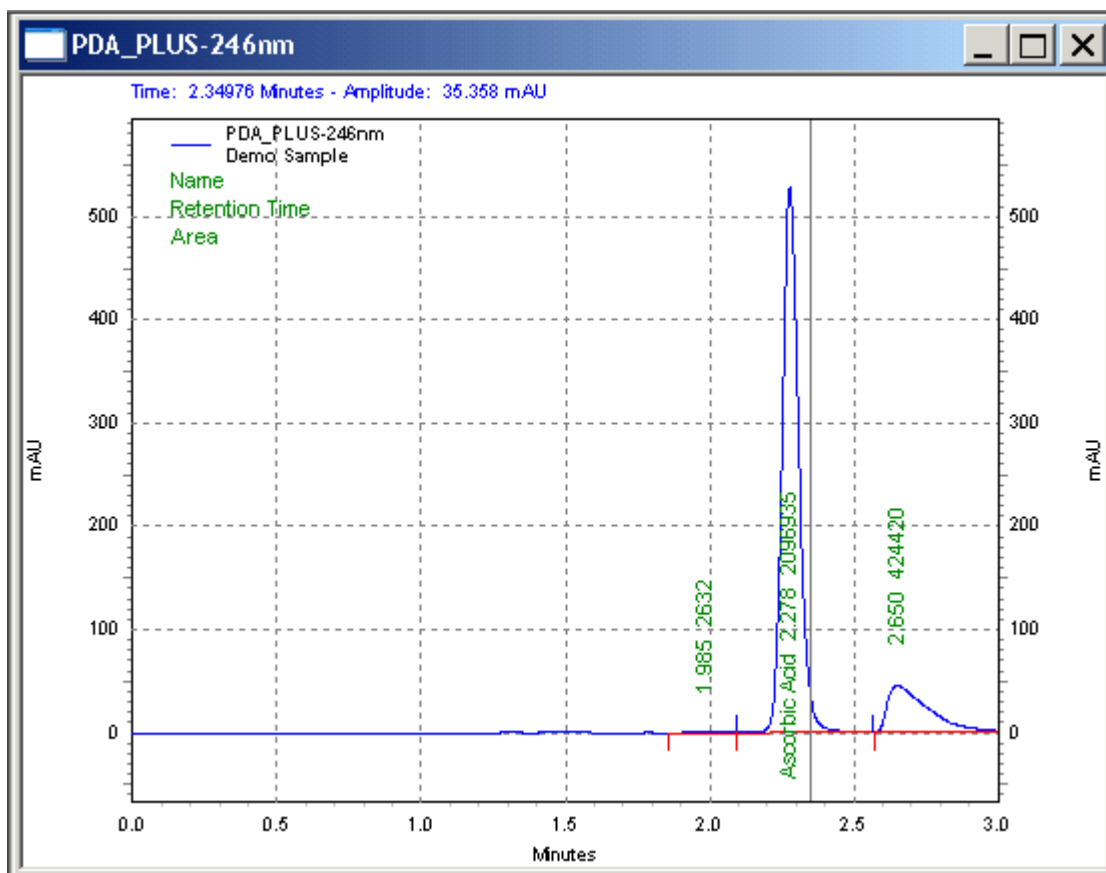
- It will take 3 minutes to collect the chromatogram.
- An example chromatogram collection is shown here.
- The y axis will autoscale as the maximum signal changes.



Run Finished



- When the run is finished the software will integrate the peaks and display the results.
- You are now finished collecting data and can exit the program.



Close Windows

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

