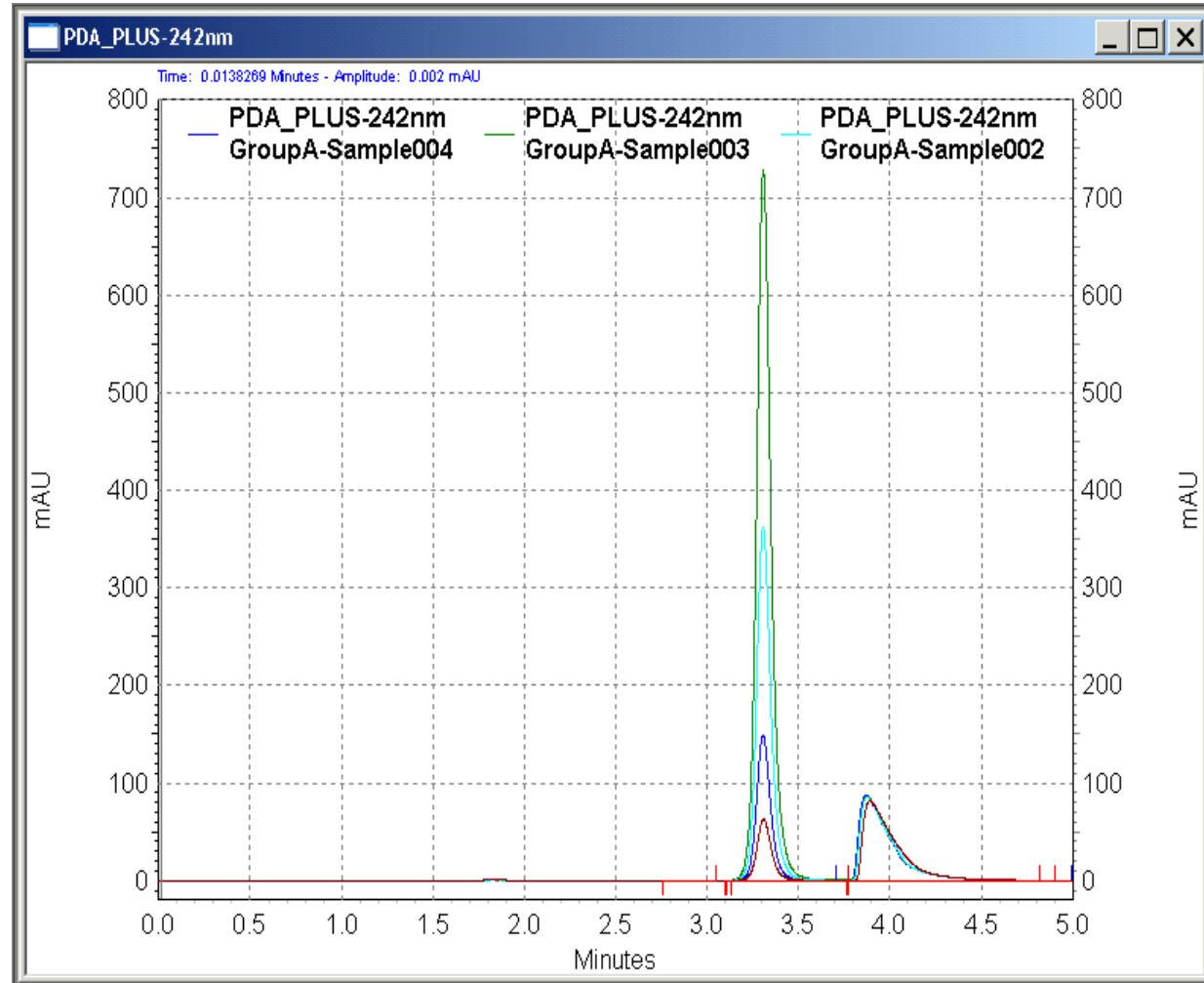


How to Process HPLC Data

After you run the HPLC to collect chromatograms for your samples you need to get information from the data.

The information you need is the peak area for ascorbic acid.

You will use a copy of the HPLC software that *is not attached to the instrument* to get peak areas for all your chromatograms.



Load the CASPiE Instrument Site



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

A screenshot of a Windows Internet Explorer browser window displaying the CASPiE Web Interface login page. The browser's address bar shows the URL "https://instruments.caspie.org/Citrix/MetaFrame/auth/login.aspx". The page header includes the CASPiE logo and the text "Web Interface for MetaFrame® Presentation Server". The main content area is divided into two sections. On the left is a "Log in" form with fields for "User name:" (containing "dmsteffe") and "Password:" (masked with dots), a "Log In" button, and a link for "Advanced Options >>>". On the right is a "Welcome Authorized Users of CASPiE Instrumentation" section with a message about account credentials and a link to "View Instruments". Below that is a "Message Center" section with a message about displaying information or error messages.

The HPLC Data Processing Software

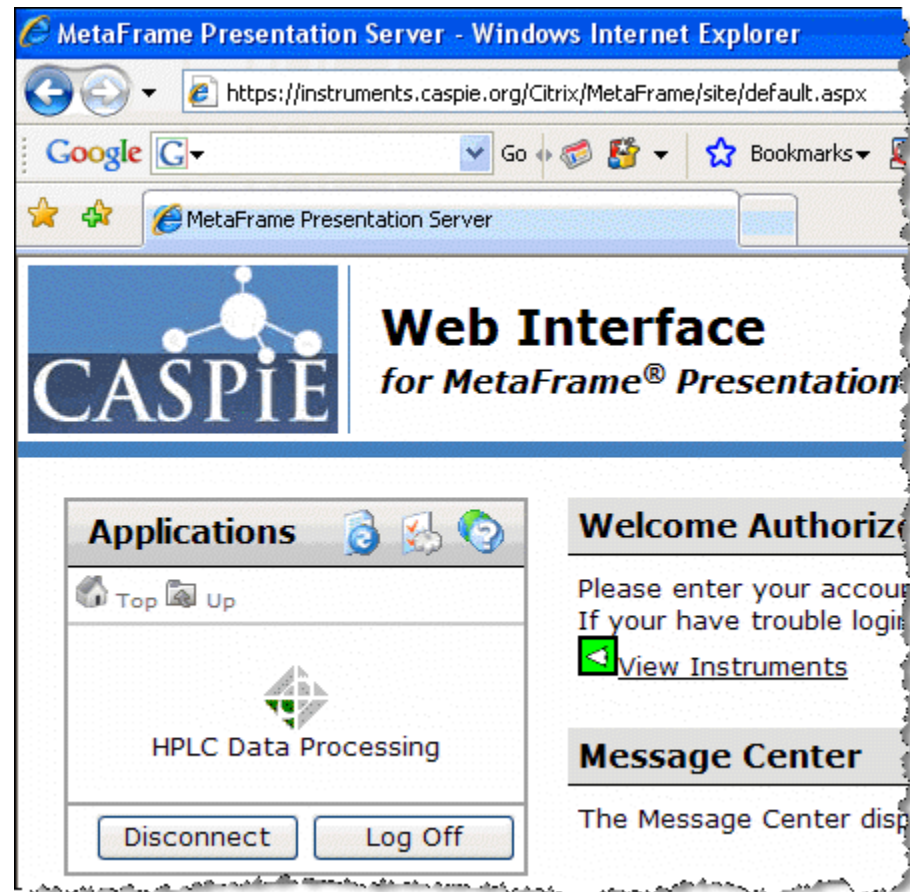


You will use a copy of the HPLC software that *is not attached to the instrument* to process your data files. It is the same version used to create the HPLC sequence file.

The version of the software not attached to an instrument is called 'HPLC Data Processing'.

You will always have access to the HPLC data processing software during a CASPIE module. You will only have access to the HPLC instrument for remote control during your scheduled hours.

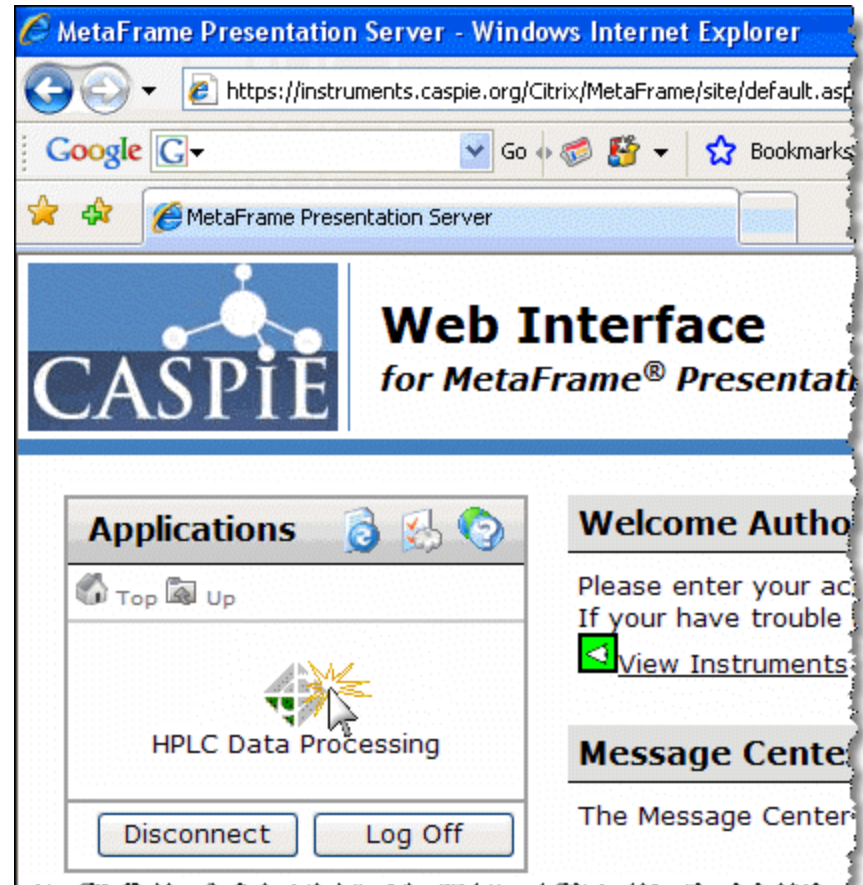
Important Note:
You must have the Citrix client installed on your computer to proceed!



Launch HPLC Data Processing Software

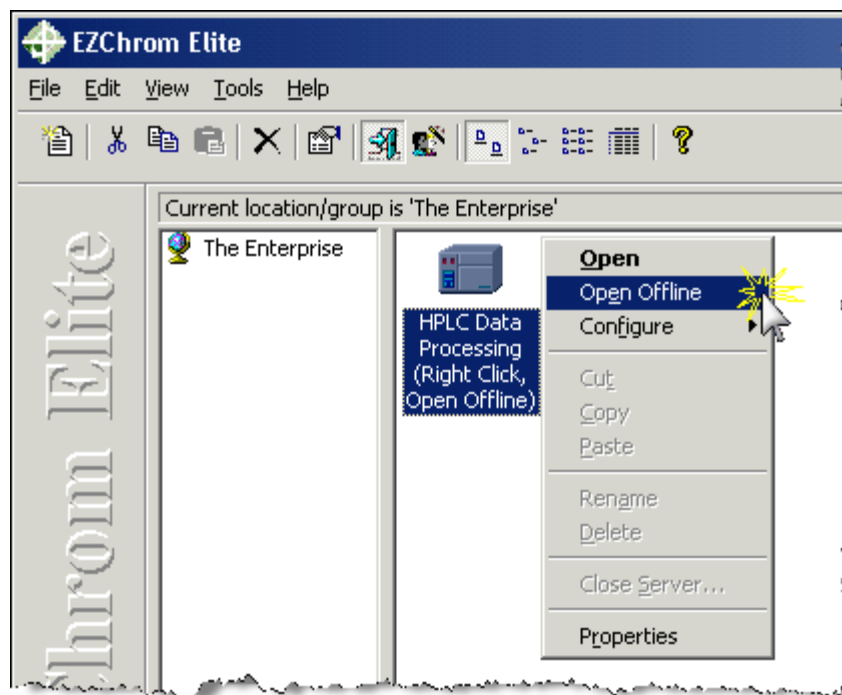
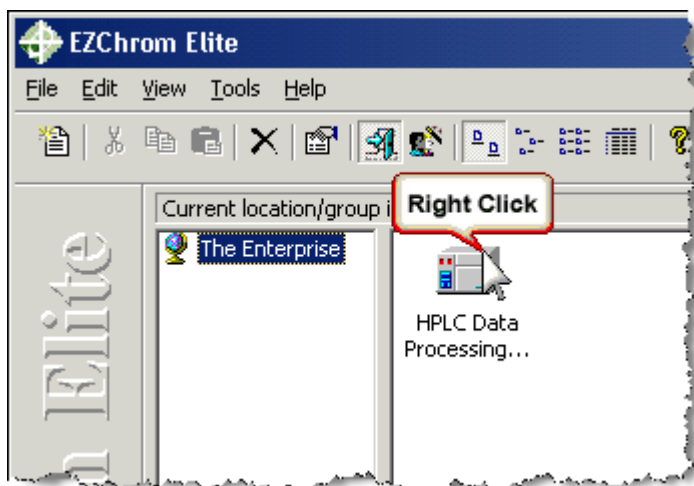


- You should see at least one icon in the 'Applications' box.
- Launch the 'HPLC Data Processing' software by clicking once on the icon.
- If this icon is not present ask your TA for help.



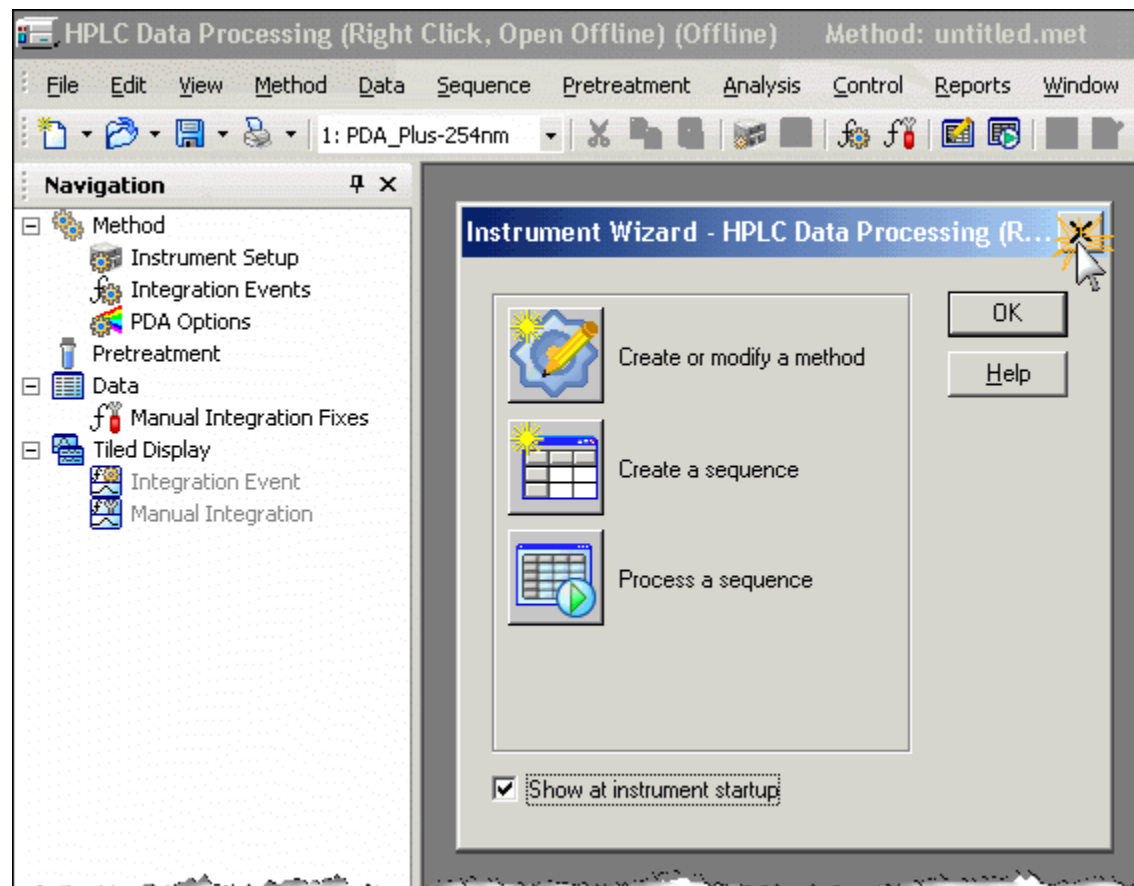
Open Software 'Offline'

- Because this software is not attached to an instrument it must be opened 'offline'.
- To do this **right click on the icon and select 'Open Offline'**. If you have a Macintosh select File| Open Offline...



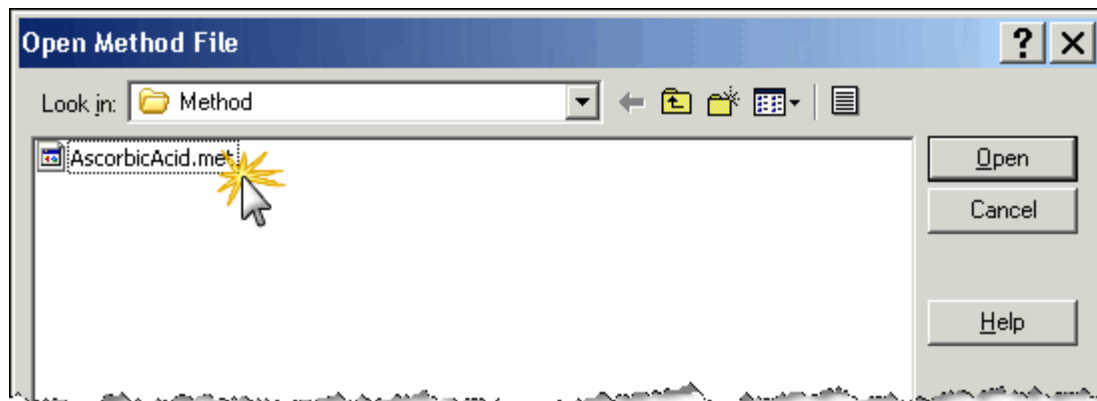
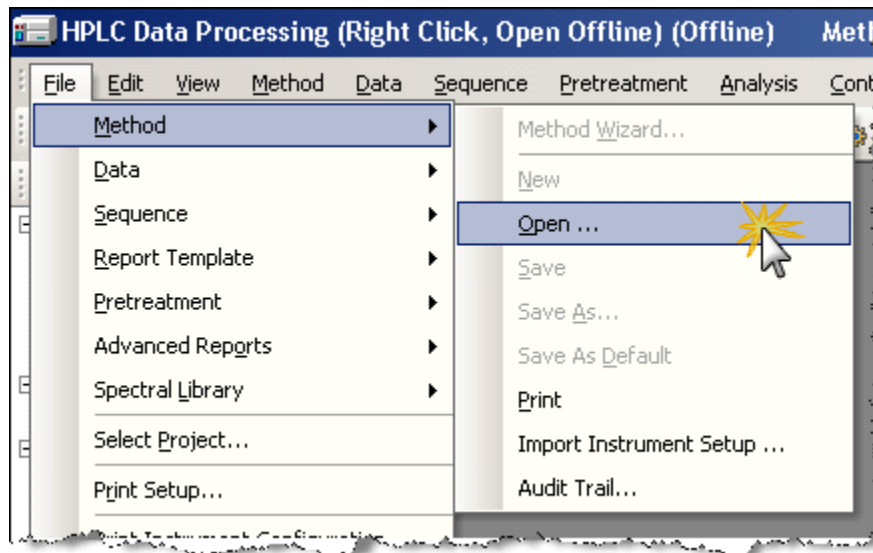
Instrument Wizard – Close

- The next screen will have an ‘Instrument Wizard’ window.
- Close the Instrument Wizard window.



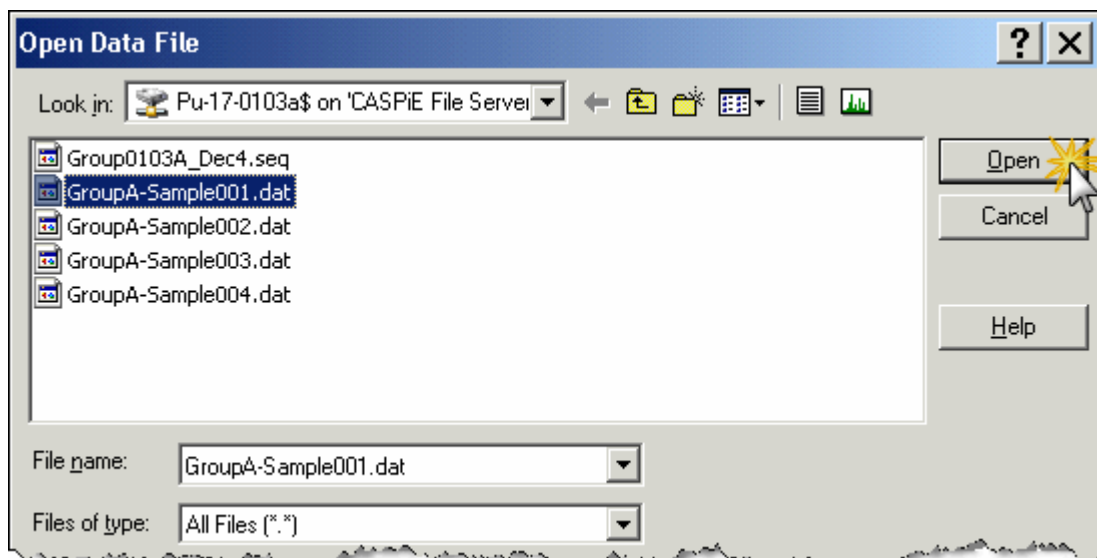
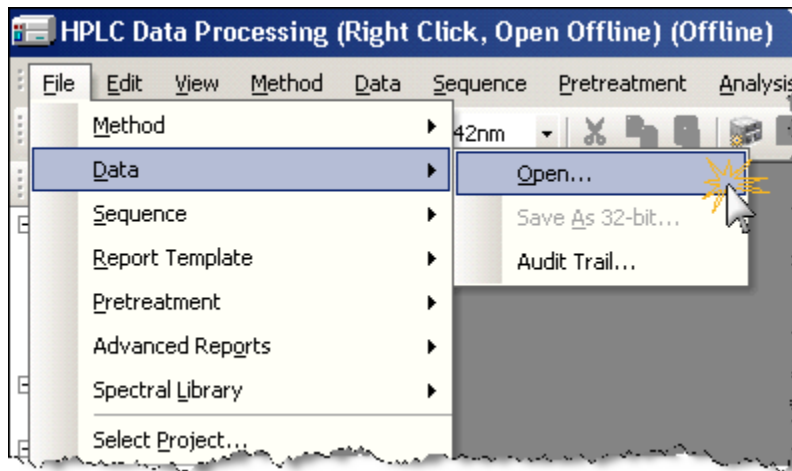
Open Method File

- **Select File| Method| Open.**
- **Select AscorbicAcid.met from the 'Open Method File' window and click Open.**
- **The 'Instrument Setup' window will open. You don't need to do anything with this window and may close it if you wish. (Note that all the information in the method will still be available to the program.)**



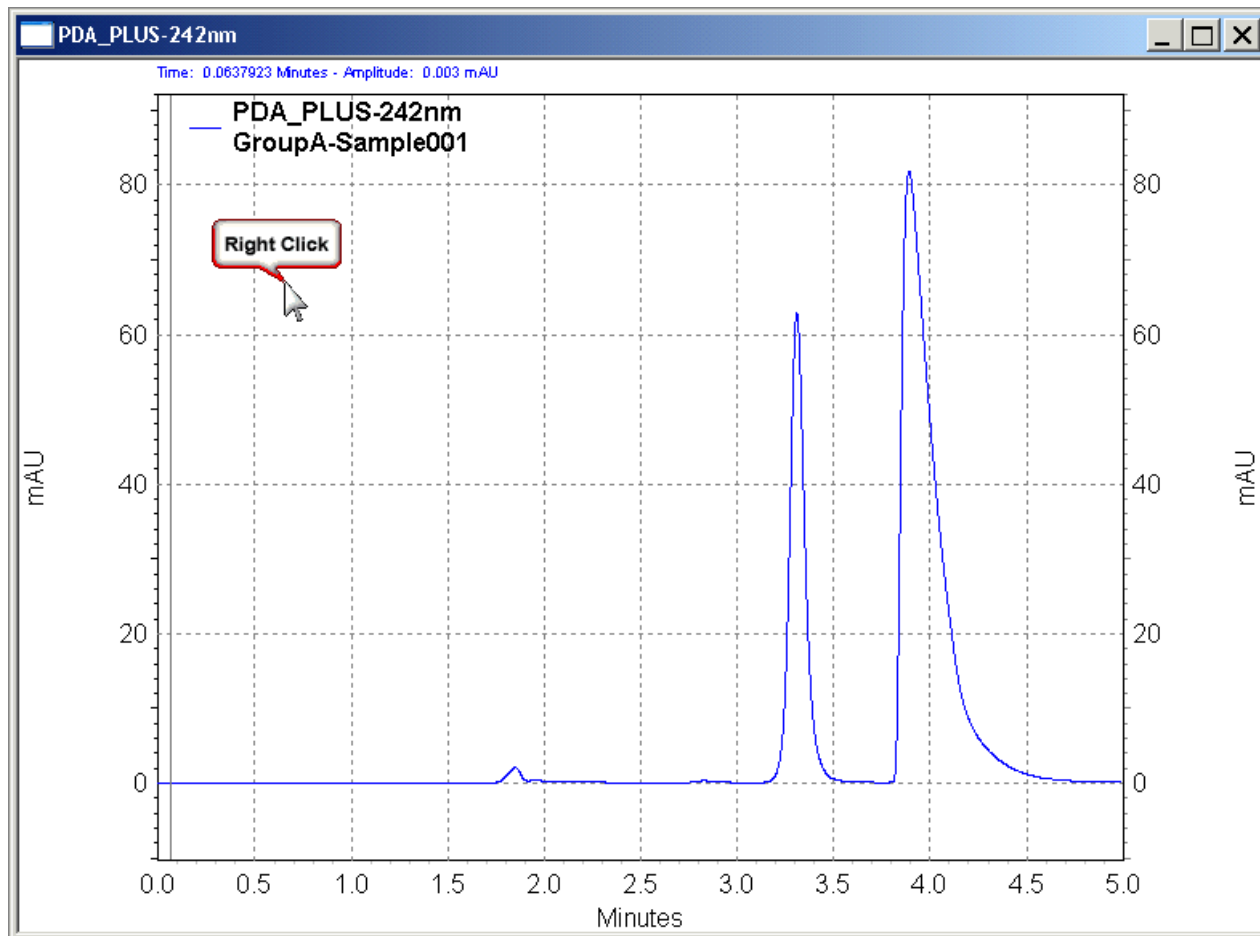
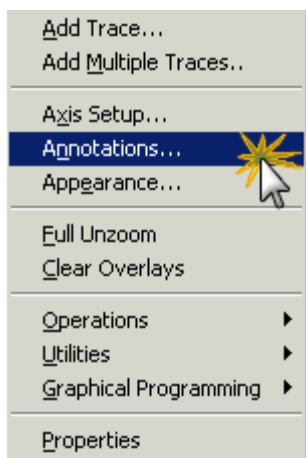
Open a Data File

- **Select File| Data| Open.**
- **Select any of the files with the .dat extension and click Open.**
- **A Data window will open with the selected chromatogram.**



Setup Data Window

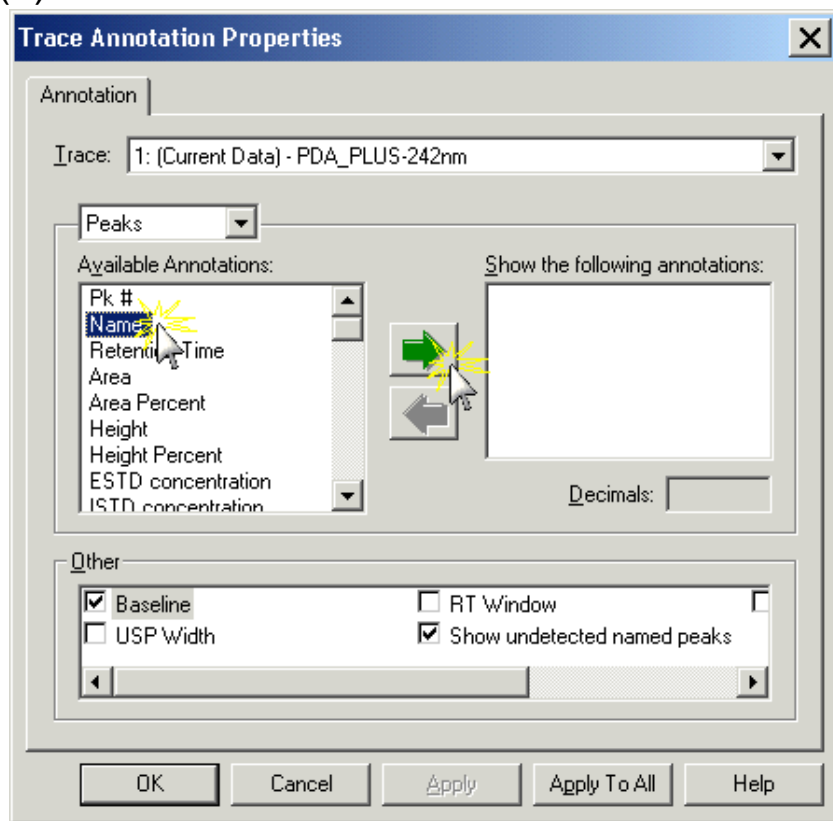
- The data window has the title 'PDA_Plus-242nm.'
- Right click anywhere in the data window and select 'Annotations'.



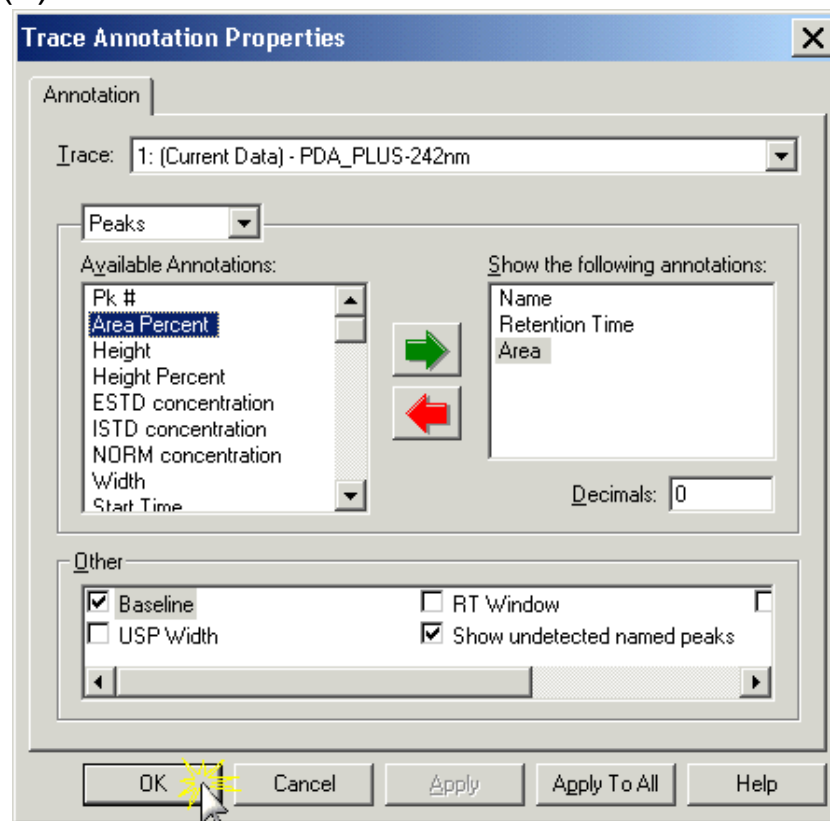
Trace Annotation Window

- In the “Trace Annotation Properties” window (a) select “Name” then click the green arrow. Repeat for “Retention Time” and “Area”.
- Name, Retention Time, and Area will be in the “Show the following annotations” box (b).
- Click OK to finish. You only need to select the annotations once; the software will remember your settings from now on.

(a)

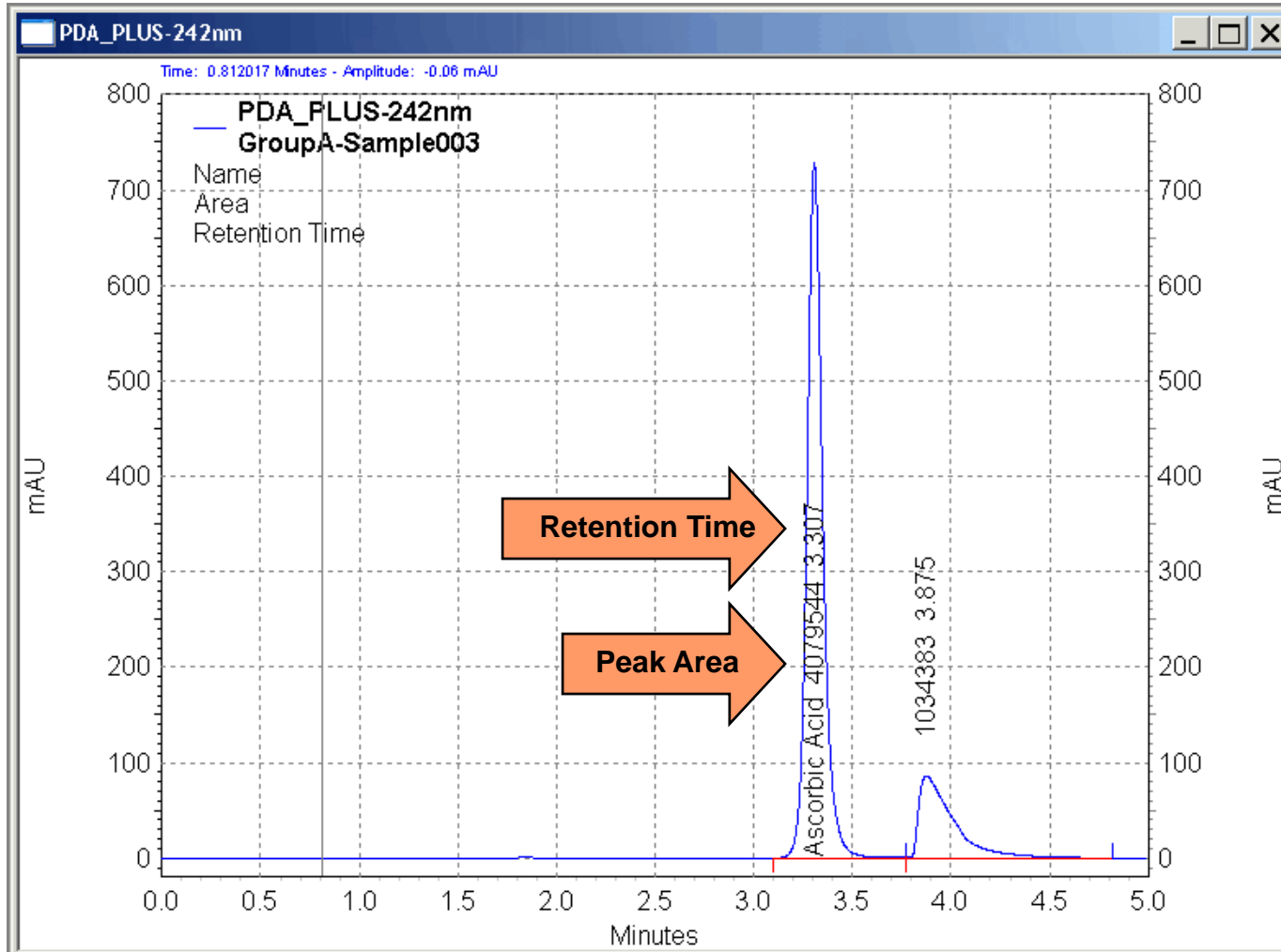


(b)



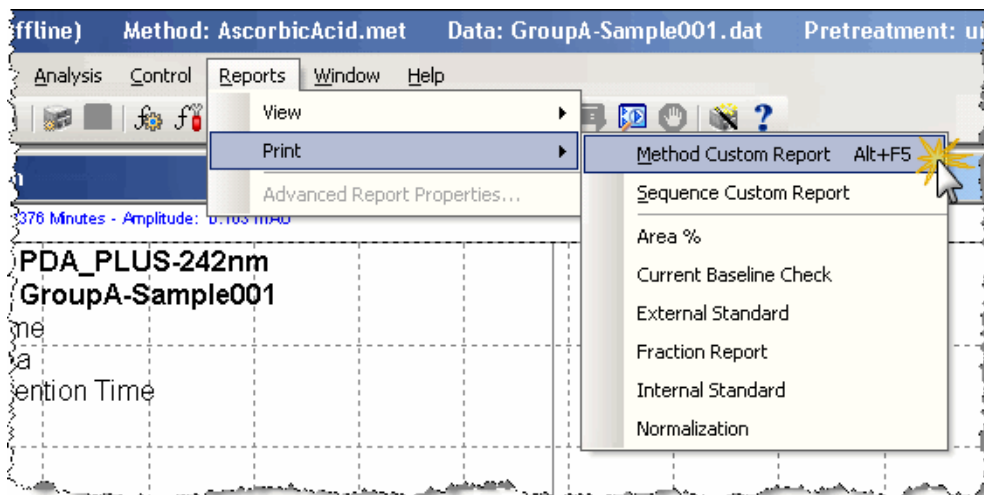
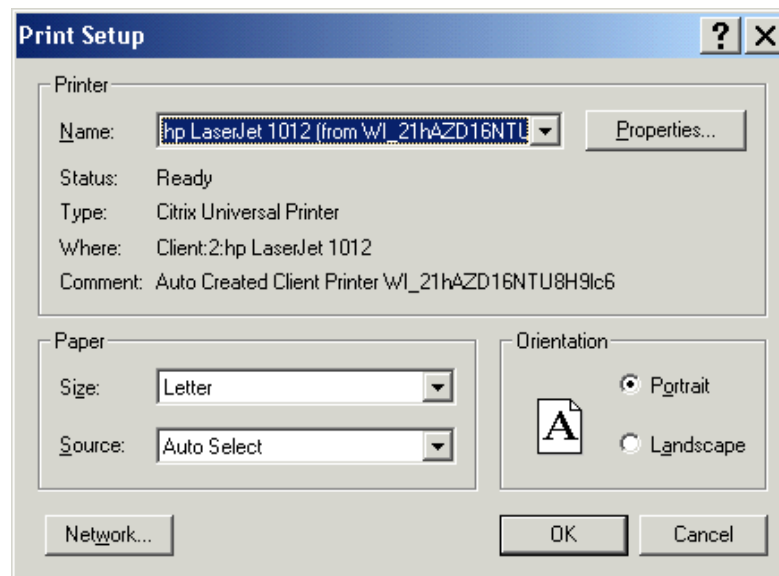
Analyze Chromatogram

- From the top menu select Analysis| Analyze. The chromatogram will now be labeled. The baselines are shown in red.



Print Report

- Before you print the selected chromatogram first check the printer settings. From the menu select File| Print Setup. Make sure the name of your local printer is shown.
- Don't worry about all the letters next to the printer name; this is normal.
- From the menu select Reports| Print| Method Custom Report.

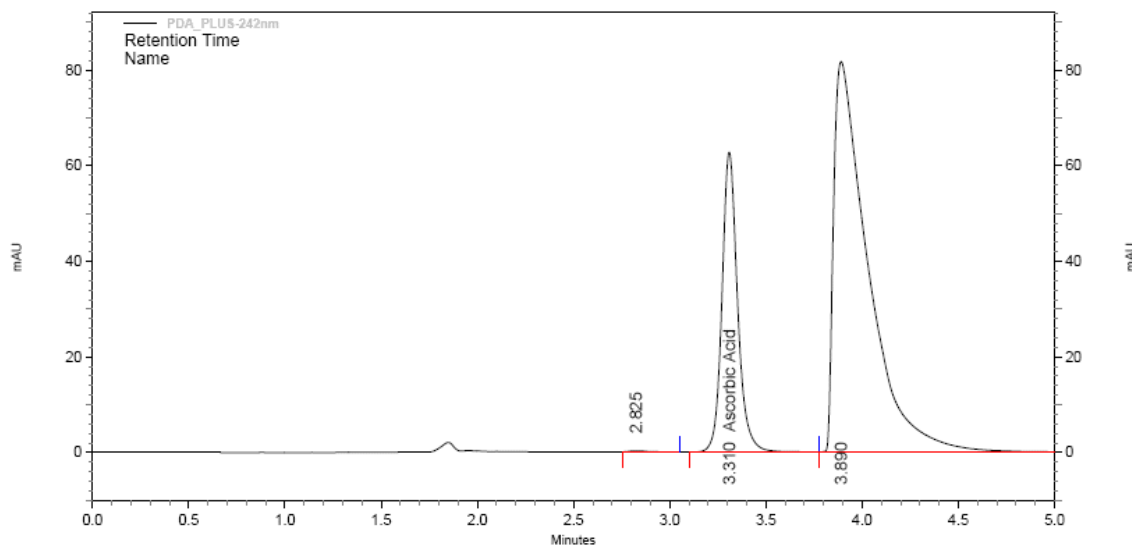


The Report

- The printed report will look like this.
- Print reports for your remaining chromatograms by repeating the steps under “Open a Data File”, “Analyze Chromatogram,” and “Print Report.”
- If you have trouble printing ask your TA for help.

Run Report

Sample ID: GroupA-Sample001
Data File: Z:\GroupA-Sample001.dat
Method: K:\Method\AscorbicAcid.met
Run Time: 12/4/2007 11:34:54 AM
Vial: A;D1
Inj. Vol.: 10 uL



PDA_PLUS-242nm Results

Name	Retention Time	Area
Ascorbic Acid	3.310	353126

Close Windows

When you have finished printing exit all EZChrom Elite HPLC software windows.

