Star Chromatography Workstation
Version 6

ProStar 325 UV-Vis Detector

Control Software

Using the ProStar 325 UV-Vis Detector

This section describes the configuration and use of the ProStar 325 UV-Vis Detector with the Star Chromatography Workstation. For information on the operation of the Star Workstation, please refer to the Star Workstation Data Acquisition with LC Control manual.

Configuring the 325 Detector

1. **Connect the Detector to Your Star Workstation**

    The detector communicates with the Star Workstation using Ethernet communications. Up to 4 ProStar 325 detectors can be attached to one Star workstation PC. If the detector is attached directly to the PC, the crossover cable that is shipped with the ProStar 325 detector can be used. If the detector is attached to an ethernet hub or switch, a patch cable can be used. Both raw data and synchronization signals for starting and stopping the detector are sent through the Ethernet connection.

2. **Connecting the ProStar 325 Detector to your Varian ProStar 210/218 CIM or ProStar 701 Fraction Collector**

    In order to get the detector signal into the Dynamax modules for display and to properly assign fraction log entries, you will need to attach one of the analog output channels to the input of the ProStar 701 Fraction Collector and the other to the pump CIM. The analog signal cable, part number 110744200, plugs into the detector analog out and provides outputs for the CIM and the fraction collector.

3. **Setting up Ethernet Communications**

    For general information about setting up the Ethernet communications, please see the *Ethernet Communications Setup* section of the Star Workstation Data Acquisition with LC Control manual. In addition, the following steps should be used to establish communications between the ProStar 325 and the Star Workstation.

    a. Start with the ProStar 325 detector powered off. Check the back of the instrument so you know the MAC address of the detector. Make sure that detector is attached to the workstation either directly or through a network.

    b. On the System control screen, pull down the Instrument menu and select **Setup Ethernet Communications**. You will see the following screen.

Setup Ethernet Ports for 79ZWS11 : 10.190.70.235

Address	Module Type	IP Address or Domain Name	Status	Actual IP
44	325	10.190.70.234	Online	10.190.70.234
45	None / 325 / 800	10.190.70.236	Not Responding	10.190.70.236
Config...	800	10.190.70.237	Online	10.190.70.237
47	None		No Address	

IP Address Management (BOOTP Server)
4 IP Addresses are being assigned from this Workstation.   Setup...

Select the Workstation's IP Address...

☑ Prompt for Setup if needed   < Back   OK   Cancel

c. Select 325 from the **Module Type** menu and enter the IP address that you want to assign to this module.

d. Press the **Setup** button on the right side of this menu and you will see the following screen.

Setup BOOTP Server at 10.190.70.235

☑ Manage IP addresses from this Workstation
☐ Require password entry for this dialog box

	Ethernet Address	IP Address	Host Name
1	00.60.93.f0.01.0d	10.190.70.236	test2
2	00.60.93.f0.01.07	10.190.70.234	test1
3	00.50.c2.00.13.03	10.190.70.237	800
4	00.60.93.f0.01.13	10.190.70.233	bench
5	00.60.93.00.8c.90	0.0.0.0	<unnamed>
6			
7			

Add
Insert
Delete

Manually enter an IP Address and Host Name corresponding to each Ethernet Address in the table. Use this feature when individual IP Addresses and Host Names have been reserved for use by each Module, but IP Address and Host Name management is not performed by a Network Administrator.

◉ Assign IP addresses manually
○ Assign: 0 IP addresses starting from: 0.0.0.0

This Workstation will assign these settings to each Ethernet Module
Subnet Mask: 255.255.255.0
Gateway: 10.190.70.1
Domain: <unnamed>

Ok   Advanced...   Cancel

e. Make sure that **Manage IP Addresses from this Workstation** is checked.

f. Watch this screen until the Ethernet address that corresponds with the MAC address on the rear of the instrument appears.

g. On the line with the appropriate Ethernet address, type in the IP address that you entered on the previous page.

h. Type in a host name for the detector and then click on any other entry on the screen.

i.   Click the **OK** button on the bottom of this screen and the **OK** button at the bottom of the previous screen

j.   Close system control and turn off the ProStar 325.

k.   Re-start system control and turn on the ProStar 325. You should see the ProStar 325 icon with the assigned address appear on the configuration screen.

l.   Drag the ProStar 325 icon from the *Available Module area* to an instrument. Double-click in the Instrument area to view the System Control Status Window.

The ProStar 325 Detector Status Window

Shown below is the Status Window for the ProStar 325 detector.
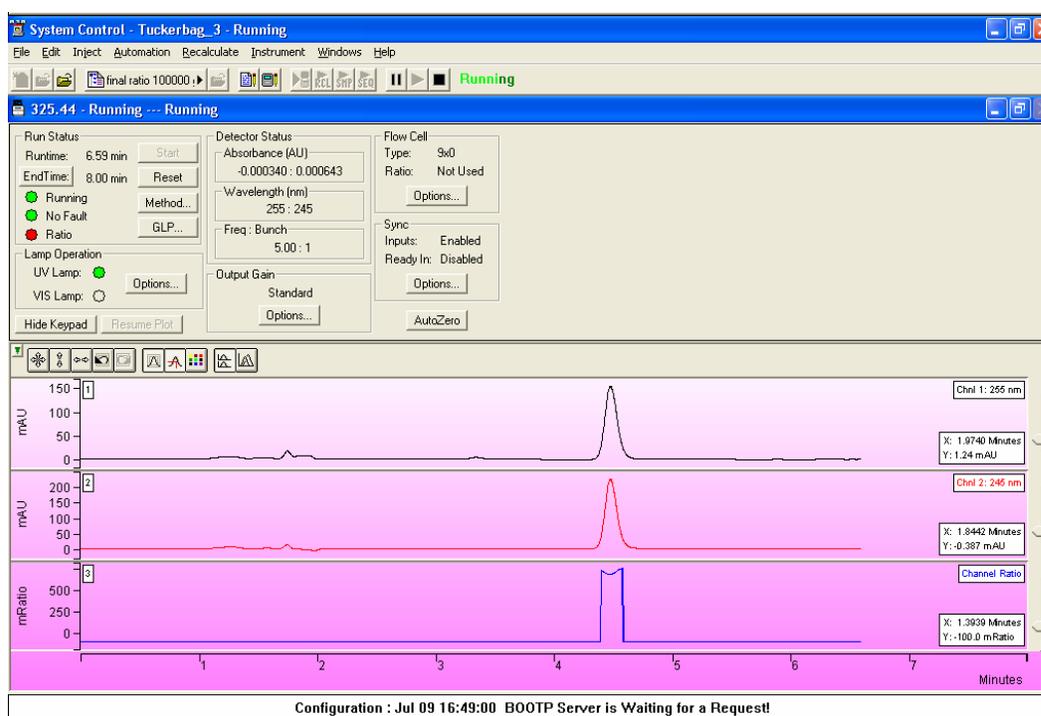
ProStar 325 Status Screen

Description of the ProStar 325 Detector Status Window

*   On the bottom of the ProStar 325 detector is the chromatogram display window. This window will display the real time out of the detector both during a run and between runs. It will not display anything during the initial detector calibration phase when the lamp is first turned on. The detector will display a single chromatogram, two chromatograms or two chromatograms and a wavelength ratio depending on what was selected in the active method. The detector can sequence between single, dual and ratio modes in any series of methods.

*   On the left of the ProStar 325 status window is the **Run Status** that includes the usual Workstation buttons (Start, Reset, Method Edit).

- The **End Time** button can be used to change the end time for the current run after you have activated the Method. The software will not let you set the end time to a time exactly equal to a line in the detector time programmed table.

- The Status lights visually show the state of the detector. If wavelength ratio mode is activated in the method currently running, the Ratio light will indicate whether the detector is presently calculating a wavelength ratio. The software will only calculate a ratio when it detects a peak. (See the method editor section of this document for more information.

- The **GLP** button will display the GLP information as shown below. This information will be appended to the data file in the instrument run log for every run.


GLP Status Screen

- Numerical Detector Status information is displayed in the Detector status box. If the instrument is operating in dual channel mode, there will be two wavelengths and two absorbance readings. In dual channel mode, the absorbances are designated channel 1 and 2 in the ProStar 325 method. Also listed in Detector status section is the frequency of data acquisition per channel in points per second and the bunch rate.

Lamp Operation Options Screen

- The status of the UV and Visible lamp is displayed in the lamp operation section of the status screen. If the circle is a dark gray, the lamp is installed but not on. If the button is green, the lamp is on. When the lamp is first turned on, the button will flash for a while before going completely green.

- Pushing the **Options** button in the Lamp Operation section displays the above screen. The status of the lamps is displayed. To turn a lamp on, click on the lamp on box next to the lamp. When the lamp is first turned on, the detector will go through a calibration at all of the wavelength ranges. These will be reflected in changes in the Absorbance and Wavelength sections of the Detector Status display. This will take 2 to 4 minutes. During this time, the detector will not take any inputs.

- The **Lamp Operation Options** screen also has a check box for New Lamp. When this is checked, the lamp hours will be reset to 0 and the date when the lamp was installed will be set to the current date. This is displayed in the **GLP** screen and put in the run log for every run.

- The user can select the detector action in case of a fault. The fault could be an internal fault within the detector or a fault generated by another instrument and conveyed to the detector through the fault sync signal.
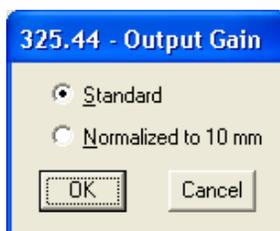
Output Gain Control Screen

- Clicking the **Output Gain** button will display the Output Gain control screen. This screen operates only for the 9x0 analytical flow cell. The user can select either the standard output or an output normalized to a 10 mm flow cell path length. This will multiply the output signal by 1.111. This makes it easy to compare peaks seen on the ProStar 325 detector with peaks generate on a detector with a 10 mm flow cell. Selecting the normalized mode does not improve the signal to noise because the entire signal is multiplied by 1.111. Normally this is set to Standard.
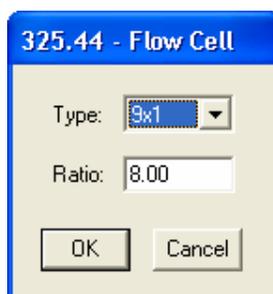
Flow Cell Options Screen

- The user must select the flow cell that they are using from the (**Flow Cell Option** button). All 4 flow cell types, 9x0, 9x1, 4x0 and 4x0.15 as well as 0x0 are selectable. The information selected here is recorded in the instrument run log in the Star data file. If a prep flow cell is selected, the user will be able to enter a ratio. The ratio is used for extended range operation. (Please see the detector Operator's manual for more information on extended range operation.) The ratio for each prep flow cell is determined at the factory and is included with the flow cell. The detector will always operate in extended range mode with a prep flow cell.
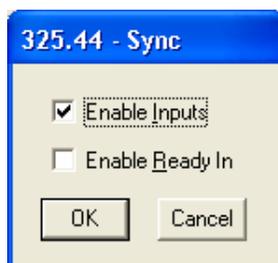
Sync Options Screen

- The hardware sync signals on the rear panel of the detector can be controlled using the **Sync Options** screen. If the general inputs are enabled, the detector could be started from a contact closure and could receive a fault signal directly from another instrument. If the Enable Ready In is active, the detector will not go to the ready state until the ready inputs are shorted together. This is only used if the detector must wait for another instrument, which is not controlled by the Star workstation to get ready before the detector becomes ready. See the ProStar 325 Operator's manual for more information on Sync signals.

- The button to **AutoZero** the detector signal going to the workstation and the analog outputs is located on the right side of the Status screen.

Building a ProStar 325 Detector Method
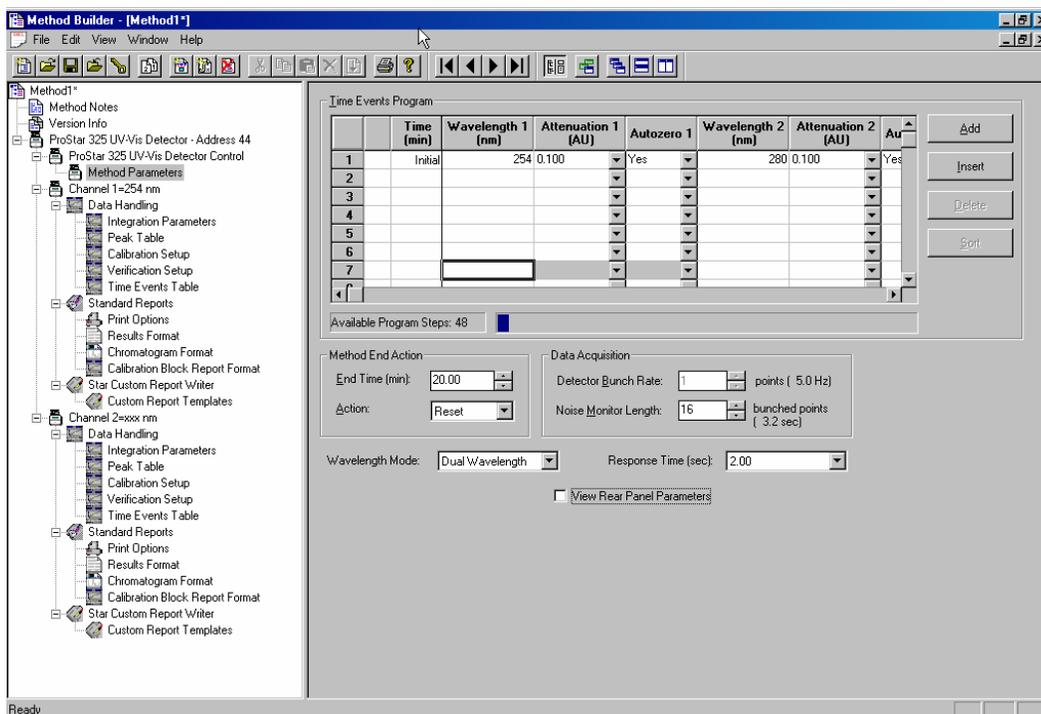
Above is the Workstation Method Builder window for the ProStar 325. Since the ProStar 325 can acquire data from two different wavelengths at the same time, the method can have two data handling sections as shown here.

The window consists of two distinct areas: The **Parameters** and the **Time Events Program**.

The **Timed Events Program** will allow you to enter up to 48 time-programmed steps. The maximum time is 1440 minutes. All programmed steps must be at least 0.01 minutes apart.

Time Events consist of the following parameters:

- Time (min): This is the time when a time programmed event will take place. The units are minutes to a resolution of 0.01 minutes from 0.01 minutes to 1440 minutes. There is always an Initial line.

- Wavelength 1: This is the wavelength for data collection in single channel mode. It is the first wavelength in dual channel mode and it is the wavelength used as the numerator for absorbance ratioing.

- Attenuation 1: This is the number of Absorbance units per volt for the 1 volt full scale output on the rear of the detector.

- AutoZero 1: This tells the detector to AutoZero the signal at the set time. This is usually used in conjunction with a time programmed wavelength change.

- Wavelength 2, Attenuation 2, AutoZero 2: These parameters are used for the second channel when the detector is operated in either dual channel or wavelength ratio mode.

Also shown on this screen is the first level of detector parameters. These parameters are used and are visible all of the time.

- Method End Action – End Time (min): The method end time can be set at any time from 0.01 to 1440 min. It cannot be set to one of the time-programmed lines. It can be earlier than the last time-programmed line. The detector end is not dependent on the end time of any of the other modules associated with the instrument.

- Method End Action - Action: At the end of a method, the detector can be set to either stay on the final wavelength or reset to the initial wavelength of the method. Reset is the preferred entry and the default.

- Data Acquisition – Detector bunch rate: The detector will collect data at 20 points per second (20 Hz) in single channel mode. The detector bunch rate can reduce the amount of data stored for each run, by adding individual points together in the final RUN file. This is only active in single wavelength mode. In dual wavelength mode, the detector bunch rate is fixed at either 5 point per second per channel for a method, which doesn't have wavelength differences greater than 200 nm at any one time, and 2.5 points per second per channel for a method, which has wavelength differences greater than 200 nm at any one time. In general, the bunch rate should yield at least 15 data point per peak for good quantitation.

- Data Acquisition – Noise Monitor Length: The noise monitor length is the number of points being used to monitor the noise in the detector for the purposes of chromatographic data handling. A large number of points will give a slightly lower noise level but a longer monitor time. The default is 16 is usually best.

- Wavelength mode – Single, Dual or Ratio: The wavelength mode determines the number of channels of data that are acquired and whether a real time ratio of the absorbances of the two wavelengths is calculated. In single wavelength mode, only one time programmed channel of data are gathered. In dual wavelength mode, two time programmed channels of data are gathered. In ratio mode, two time programmed channels of data are gathered and a ratio of absorbances is calculated as described later in this document.

- Response time (sec): 2 sec, 1 sec, 0.5 sec, 0.05 sec. The response time controls the digital filtering done on the data. A response time of 2 sec will result in the least noise while 0.05 sec will result in the most noise.
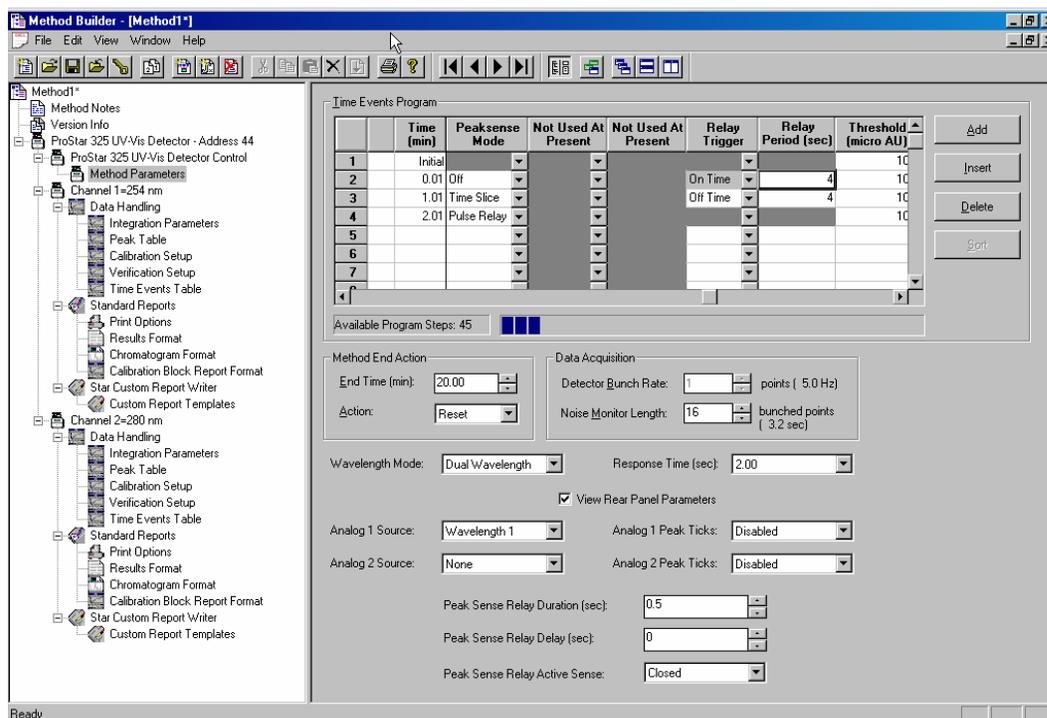
- By clicking on the **View Rear Panel Parameters** check box, entries that control the peak sense relay and the analog outputs are displayed. If you will not be using these parameters, you do not need to display the entry fields.

- Analog Source1 and 2: Wavelength 1, Wavelength 2, none: These entries specify the output signal for the two analog outputs on the rear panel of the detector. Wavelength 2 is only useful if the detector is operating in dual channel mode. The same signal can be output (e. g. Wavelength 1) can be output from the two analog channels even if the detector is operating in single wavelength mode.

- Analog 1 and 2 Peak Tics – Disabled and Enabled: IF enabled is selected, the detector will output a decrease detector signal whenever the peak sense relay is triggered.

- Peak sense relay duration time (sec) – 0.1 to 99.9 sec: This is the length of time that the peak sense relay will be on when it is triggered.

- Peak Sense Relay Delay (sec) – 0 to 99 sec: This is the length of time that the peak sense relay will be delayed between the time that it is triggered and the time that the relay is actually activated.

- Peak Sense Relay Active Sense – Open or Closed: When the peak sense relay is active, it will either be shorted (closed) or it will be open.

The **Time Events Program** table can be scrolled over to reveal more detector options.

- Peak sense mode – Time slice, Pulse Relay, Off: If Time Slice is activated, the peak sense relay will be activated starting at the time on the relay trigger entry. The relay will be pulsed based on the entries in the parameters section and the Relay period. If the relay period is 10 sec and the peak sense duration is 1 sec, the peak sense relay will be activated every 10 seconds and stay activated for 1 sec. This will start when the On Time relay trigger is activated and will stop either when the peak sense mode is turned off or when the Relay Trigger is set to Off Time. If Pulse Relay is activated, the peak sense relay will be activated once at that time.

- Relay Trigger – On Time, Off Time. If the Time Slice Peak Sense entry is activate, the Relay trigger will control when the relay starts pulsing and when it stops pulsing.

- Relay Period (sec) 1 to 999 sec: The Relay Period is the period of time between pulses of the peak sense relays when the Time Slice mode is turned on.

The last entries in the Time Programmed table are shown above.

- Threshold (micro AU) 1 to 100,000,000: The threshold is the value in micro AU above which the driver will calculate the ratio of absorbances of the two wavelength channels if the detector is in Ratio mode. See the section below for more information on absorbance ratioing.

- Relay 1, 2, 3, 4: The 4 relays on the back of the ProStar 325 can be turned on and off independently. In the On setting, the relay is closed, in the Off setting, the relay is open.

# Absorbance Ratioing on the ProStar 325 Detector

The ProStar 325 detector has the ability to ratio the absorbance from two different channels as the peaks are eluted from the column. If you are collecting the peaks, this can be used to estimate the purity of a peak. If the purity of the peak begins to change, you can shift the collection of the peak into another vessel.

In order to successfully use absorbance ratioing to estimate peak purity, you must carefully set several parameters including the wavelengths to monitor and the peak threshold.

In order to get a meaningful absorbance ratio, you must select the two wavelengths carefully. The compound you are looking at and the potential impurities must absorb at the two wavelengths. If they do not, you may not see much of a change of ratio as the impurity is eluted.

You must also choose the threshold level carefully. The threshold level is used to limit the calculation of the ratio to the times when the ProStar 325 driver has detected peaks on channel 1. If the software would calculate and display a ratio continuously, the display would be very confusing. Most of the time, the software would be ratioing the noise. This ratio could vary significantly with minimal signal variation, making it very difficult to interpret the ratio.

The threshold is the detector signal in microAU above which the driver will calculate the absorbance ratio. This is shown below
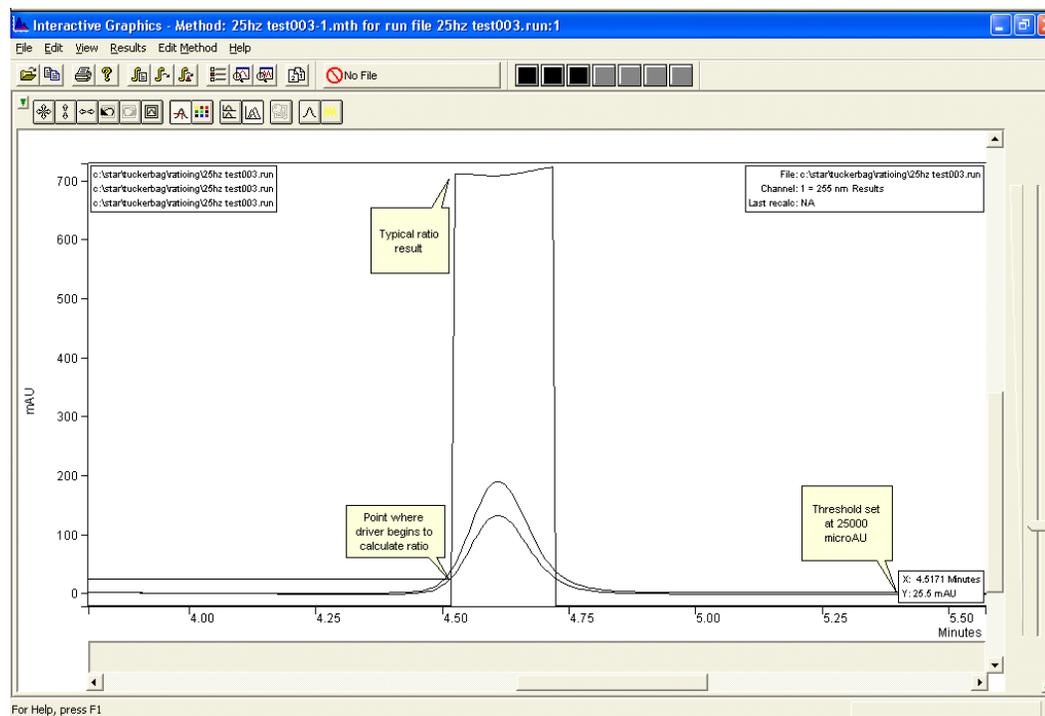
Here a threshold of 25000 microAU is used to detect the presence of a peak and start the ratioing process. Note that the cursor in Interactive Graphics is set at the start of the ratio and the signal reading is 25 mAU (25000 microAU).

In order to calculate the ratio, the driver has to correct for potential baseline offsets. Therefore, after the peak is detected using the threshold, the driver will look back 40 points on channel 1 to find the lowest point. Once it has found the position of the lowest absorbance point on channel 1, it will take that value and subtract it from the channel 1 signal across the peak. It will also take the absorbance value on channel 2 at that time and use it as the baseline, subtracting it from the absorbance values that are used for the ratio.

Although this baseline correction improves the resultant ratio, it is not ideal. It would be better if the entire change of signal across the peak could be used to correct the ratio. However, because ratios are calculated as the peaks are eluting, it is only possible to use the information at the start of the peak to correct for baseline offsets. A typical real time ratio is shown in the figure above. Although there are some changes in the ratio across the peak, this peak should probably be considered pure.

The ratio is calculated as the absorbance on channel 1 divide by the absorbance on channel 2. As long as the absorbance on channel 1 is less than the absorbance on channel 2, the ratio will vary between 0 and 1. If the absorbance on channel 2 becomes less than the absorbance on channel 1, the ratio will become greater than 1. The ratio can become very large. In order to make it easier to display and interpret the ratio, the Star driver will compress the calculated ratio using the following rule.

If the calculated Ratio (R) is less than 1, then the displayed ratio (R_d) is set as follows:

$$\text{Eq.1} \quad R_d = R$$

If the calculated Ratio (R) is greater than 1, then the displayed ratio (R_d) is set as follows:

$$\text{Eq.2} \quad R_d = 2 - 1/R$$