Processing Chromatograms

Three processes are available for use on chromatograms; polynomial background subtraction, smoothing, and integration. Background subtraction and smoothing help you improve the presentation of the data. Integration locates peaks, positions baselines, and calculates peak statistics for quantitative work.

Processing multiple chromatograms

The background subtract, smooth and integrate processes can be performed automatically on all the chromatograms within the current window. To enable this operation press the Toolbar button or select **Process All Traces** from the **Chromatogram Process** menu, the menu item will have a tick next to it. To turn off multiple processing reselect the Toolbar button or menu item.

You can choose if you wish to add the processed trace to the current window or if you want to replace the current trace with the processed trace. Pressing this button once causes each subsequent chromatogram or chromatogram process to replace the currently selected trace. Pressing the button a second time causes each subsequent chromatogram or chromatogram process to be added to the traces on display. Note the button is grayed out when the button is depressed.

Subtract

The purpose of Background Subtract

Background Subtract fits a smooth curve through the noise in the chromatogram, then subtracts this curve from the chromatogram, leaving the peaks on a flat baseline.

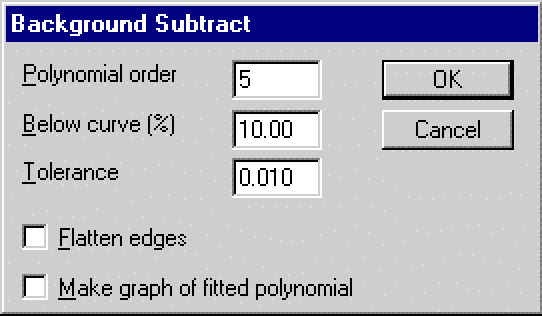

Figure 3.12 *Background Subtract dialog*

The **Polynomial order** control allows you to specify the *degrees of freedom* allowed to the fitted curve. With polynomial order set to 0, a horizontal straight line is fitted. With polynomial order set to 1, a sloping straight line is fitted. The further the background is from a straight line, the higher you must set the **polynomial order** control. But too high a value will cause the fitted curve to begin to follow the peak shapes. Normal operating range for this parameter is 3rd to 20th order.

The **Below curve** parameter allows you to move the background curve up and down in the noise. The curve fit is constrained to place the specified percentage of data points beneath the fitted background curve. Normal operating range for this parameter is 5% - 30%, depending on the abundance and width of peaks in the chromatogram. For fewer, or narrower peaks, increase the value.

The **Tolerance** parameter affects the precision to which the internal arithmetic is performed. It should not normally be altered from its default value of 0.01.

When the **Flatten Edges** parameter is selected MassLynx checks that the polynomial applied is flat or horizontal at the beginning and end of the trace.

The parameters shown in Figure 3.12 produced the background subtracted chromatogram illustrated in Figure 3.14 .

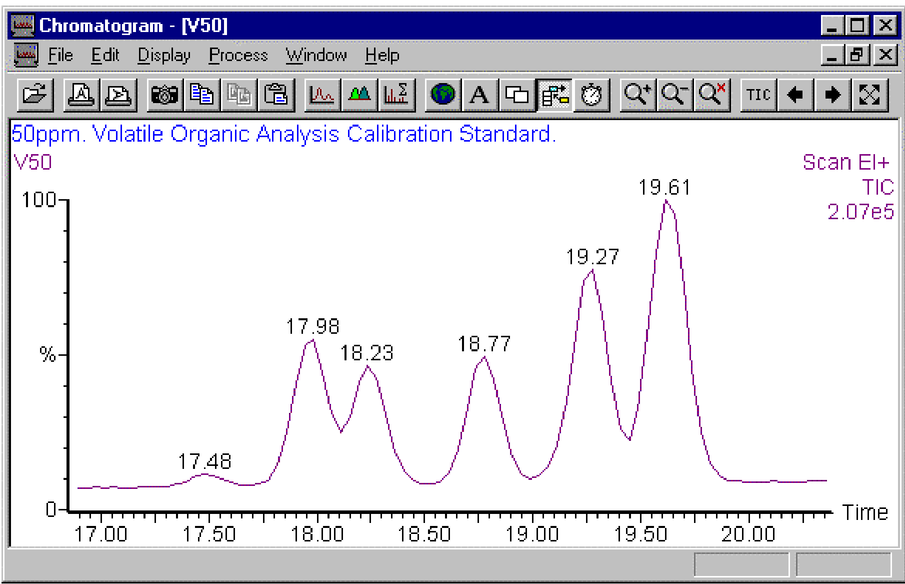

Figure 3.13 *Unprocessed Total Ion Chromatogram*

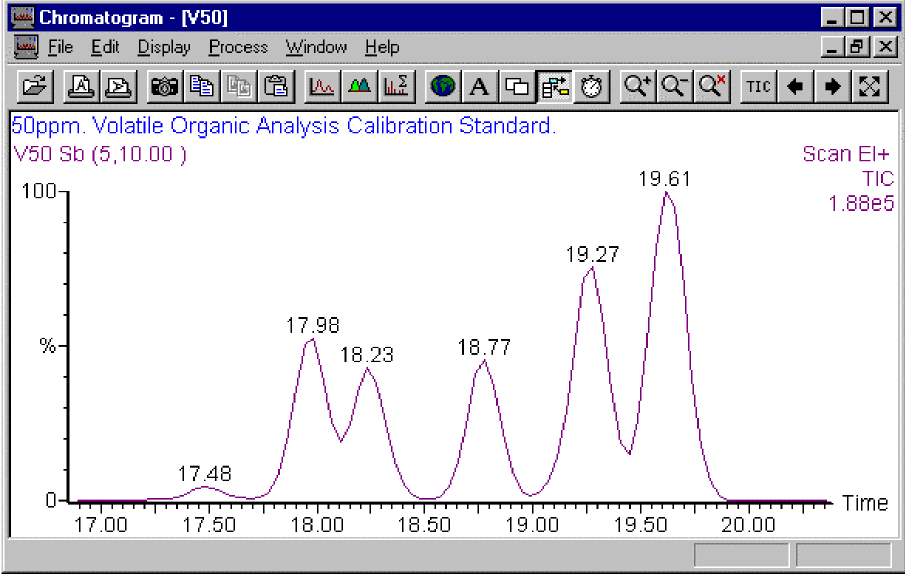

Figure 3.14 *Background Subtracted Chromatogram. Parameters as shown in* ***Figure 3.12***

Checking the results of Background Subtract

You can check the operation of the background subtraction process with a given set of parameters by selecting the **Make graph of fitted polynomial** check box. This causes the same calculation to take place, but rather than displaying a chromatogram with the background curve subtracted, the curve itself is displayed. By choosing **Overlay graphs** and **Link vertical axes** from the Chromatogram Display View dialog, a display like Figure 3.15 can be produced, enabling the fit of the baseline to the noise to be examined. The parameters shown in Figure 3.12 were used.

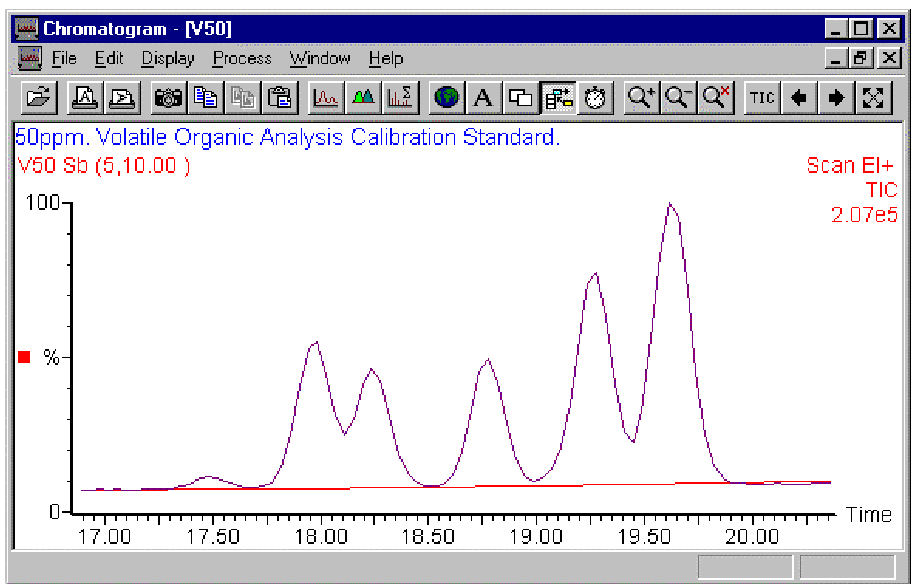

***Figure 3.15** Checking the operation of Background Subtract*

To subtract the background from a chromatogram

1. Choose **Subtract** from the Chromatogram **Process** menu.
2. Set the **polynomial order** parameter as described above.
3. Set the **below curve** parameter as described above.
4. Press the **OK** button.

The Subtract status dialog box indicates the progress of the subtract algorithm. After every iteration, the **convergence** value in the dialog box is updated. The algorithm terminates when **convergence** is less than **tolerance**.

With higher order polynomials, background subtract will sometimes have difficulty converging on a solution. There is a pre-set upper limit of 300 iterations. If background subtract does not seem to be making progress, you can press the **Cancel** button in the status box, and try again with a lower-order polynomial.

Smoothing Chromatograms

Smoothing improves presentation and aids interpretation of a chromatogram by increasing the apparent signal-to-noise ratio.

Two types of smoothing are available for chromatograms: **Moving Mean** and **Savitzky Golay**. Both methods slide a window along the chromatogram, averaging the data points in the window to produce a point in the smoothed chromatogram. Moving Mean takes the arithmetical mean of the intensities of the data points in the window. Savitzky Golay takes an average of the intensities weighted by a quadratic curve. This tends to enhance peak and valley shapes, as well as preserving the height of the peaks better than the Moving Mean. However, Savitzky Golay does tend to produce small artifacts on either side of the real peaks.

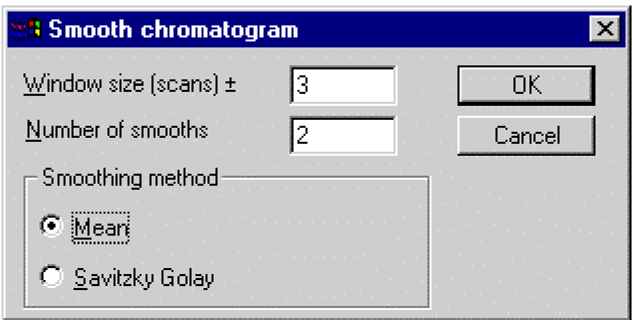

Figure 3.16 Chromatogram Smooth Parameters dialog

■ To smooth a chromatogram

1. Choose **Smooth** from the **Chromatogram Process** menu.

2. Set the **Window size** parameter. The number you specify is the half-width of the smoothing window in scans. This parameter can be set automatically by clicking the right mouse button, and dragging across a chromatogram peak at half height (Figure 3.18).

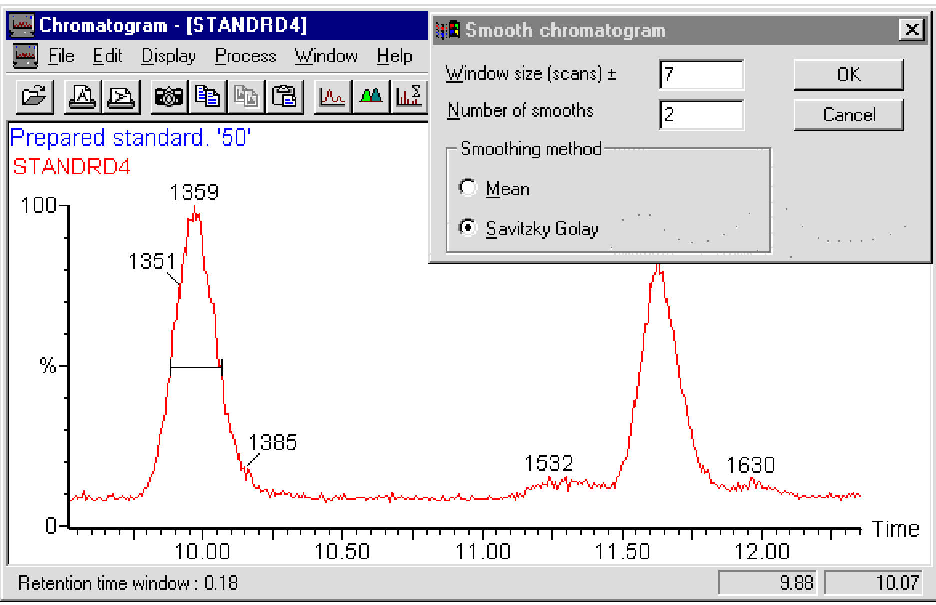

Figure 3.17 Setting the Window size parameter

3. Select a smoothing method.

4. You may wish to alter the number of times the smooth is repeated, by changing the **Number of smooths** parameter from its default value of two. Increasing this parameter gives a heavier smooth.

5. Press the **OK** button.

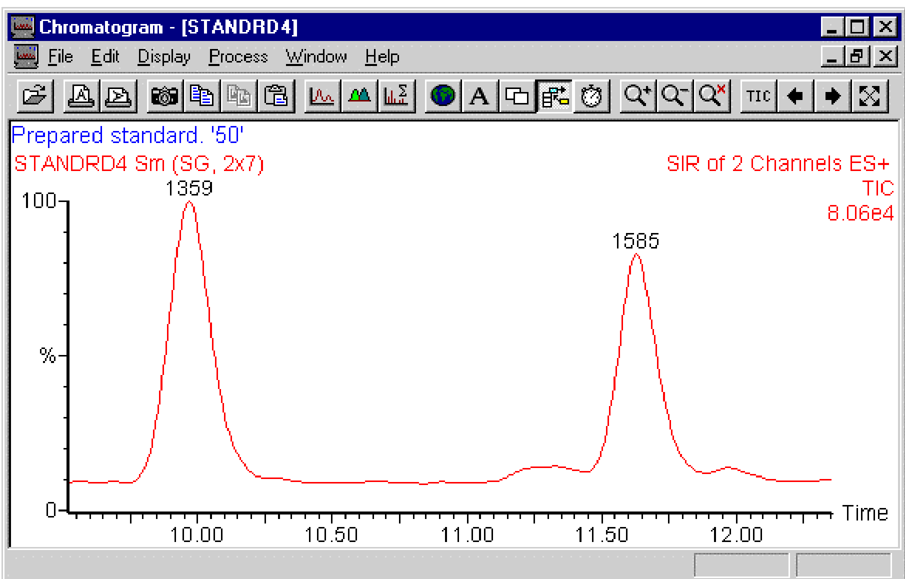

Figure 3.18 *Results of chromatogram smoothing*

Integrating Chromatograms

The integration process locates the peaks in a chromatogram, draws baselines and calculates peak heights and areas for quantification.

You can integrate a chromatogram with the current parameters by clicking on the button in the Toolbar. You can use the Integrate dialog to change the parameters. **Note:** The integration process operates only on the currently displayed range and not on the whole chromatogram.

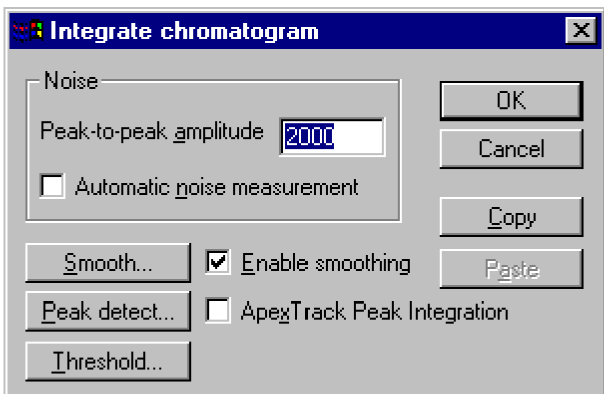

Figure 3.19 *Integrate chromatogram dialog*

The **Copy** button allows you to copy the current integration parameters to the Clipboard. These parameters can then be pasted into another application such as the Quantify Method Editor.

The **Paste** button allows you to paste a set of integration parameters from the Clipboard.

The **Integrate chromatogram** dialog requires the user to enter the **Peak-to-peak noise amplitude**. This value is used by the integration software to prefilter the chromatogram. A suitable value can be measured directly from the chromatogram by clicking the right mouse button, and dragging the mouse across a section of noise in the chromatogram. The sensitivity of the integration algorithm can be fine-tuned by manually adjusting this value.

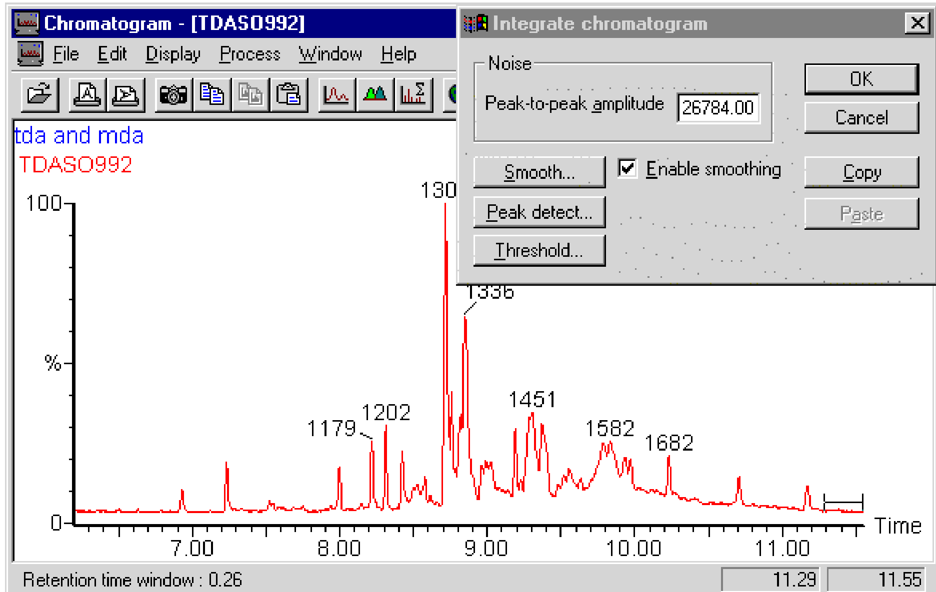

Figure 3.20 Setting the peak-to-peak noise amplitude

You may choose to smooth the chromatogram before integrating by selecting the **Enable smoothing** check box. The parameters for the smooth may be examined and altered by pressing the **Smooth..** button. For more information, see "Smoothing Chromatograms" on page 133.

Check the **ApexTrack Peak Integration** box to use an alternative peak detection algorithm.

Small peaks may optionally be removed by setting one of the four available threshold parameters. Press the **Threshold..** button to examine or modify these parameters.

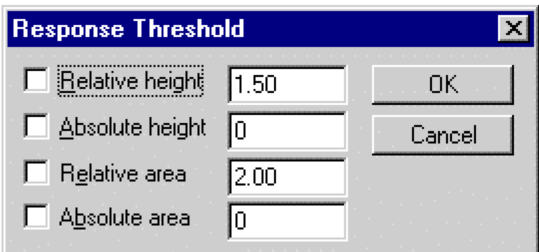

Figure 3.21 The Response Threshold dialog

Relative height Check this box to remove peaks whose height is less than the specified percentage of the highest peak.

Absolute height Check this box to remove peaks whose height is less than the specified value.

Relative area Check this box to remove peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Check this box to remove peaks whose area is less than the specified value.

You may examine and modify the parameters controlling the positioning of baselines and separation of partially resolved peaks by verticals (droplines) by pressing the **Peak detect...** button.

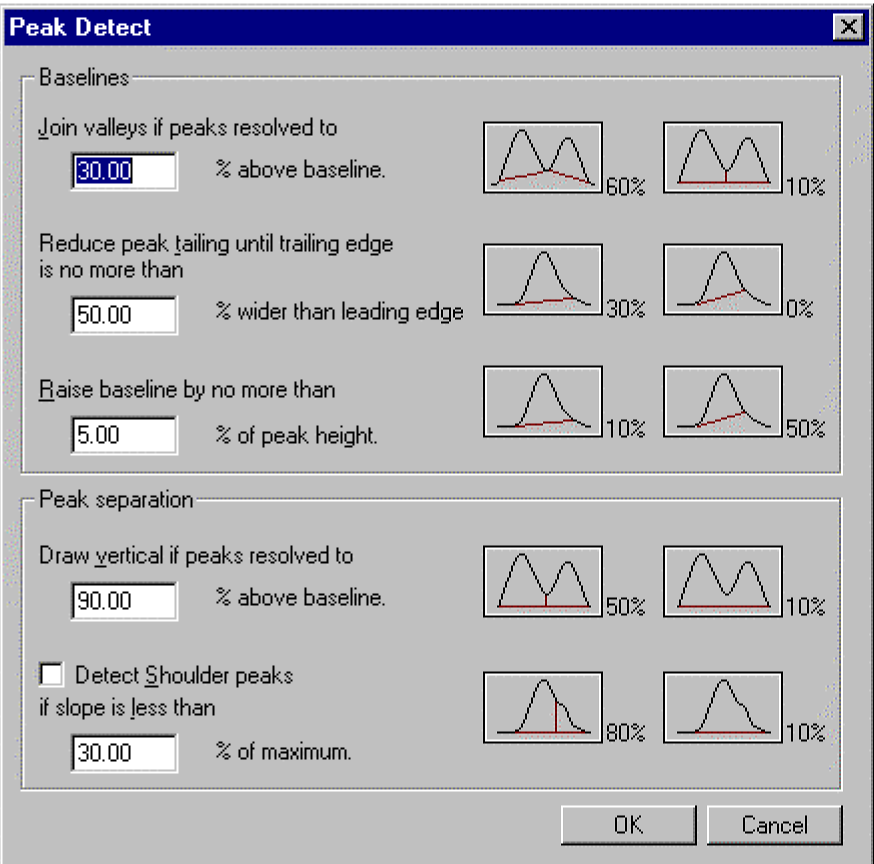

Figure 3.22 *The Chromatogram Peak Detect dialog*

The **join valleys** parameter affects how baselines for partially resolved peaks are drawn. The larger the value of this parameter, the more peak baselines will be drawn up to the valleys between unresolved peaks. The default value for this parameter is 30%, and normal operating range is 5%–75%.

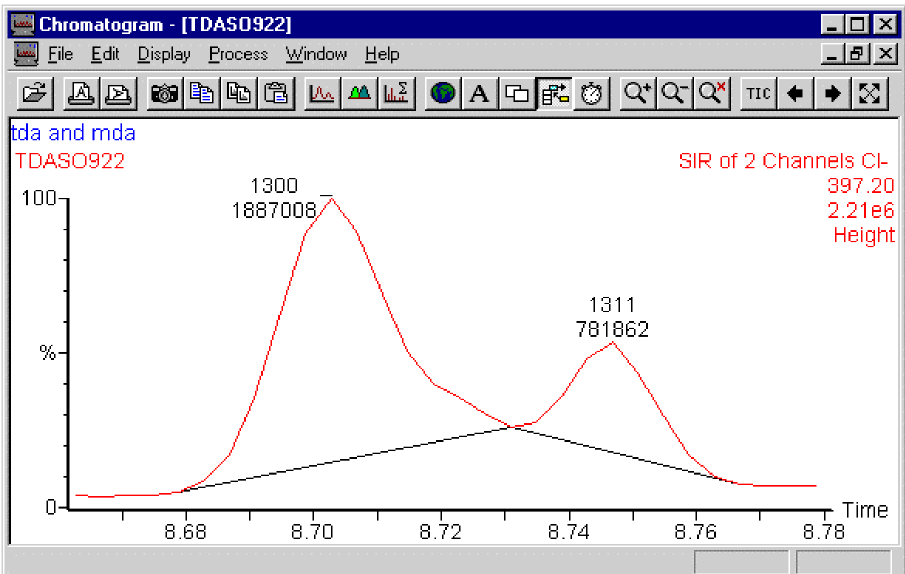

Figure 3.23 *Join valleys parameter set to 60%*

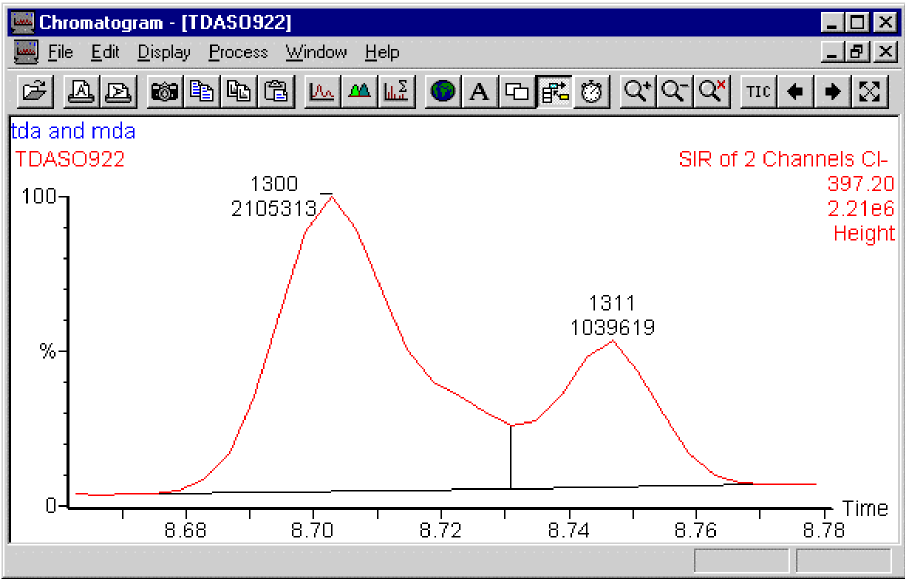

Figure 3.24 *Join valleys parameter set to 15%*

The **reduce peak tailing** and **raise baseline** parameters allow control over the positioning of baseline end points. In the example below, the pronounced tail on the peak at 5.42 mins is reduced by decreasing the value of the **reduce peak tailing** parameter from 150% to 50%. The default value for this parameter is 50%, and normal operating range is between 25% and 300%.

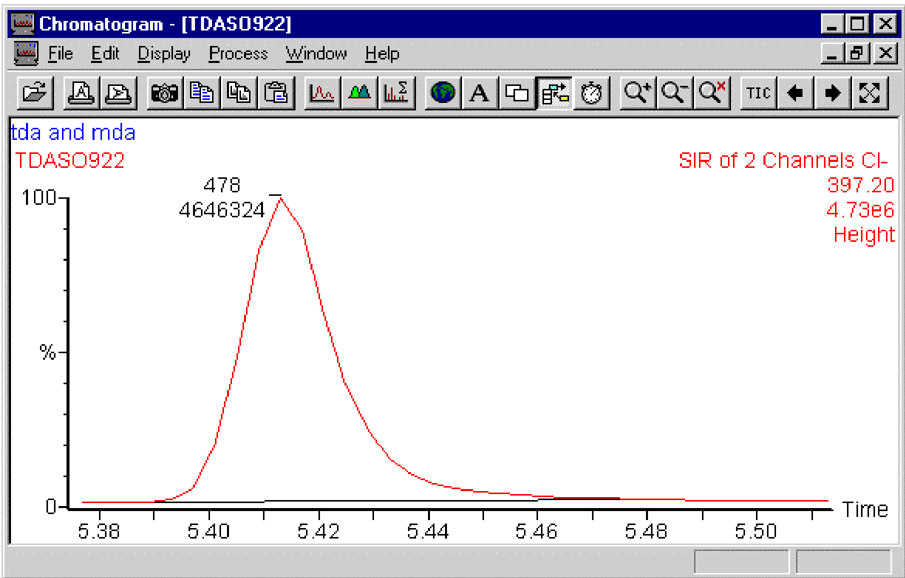

Figure 3.25 *Reduce peak tailing parameter set to 150%*

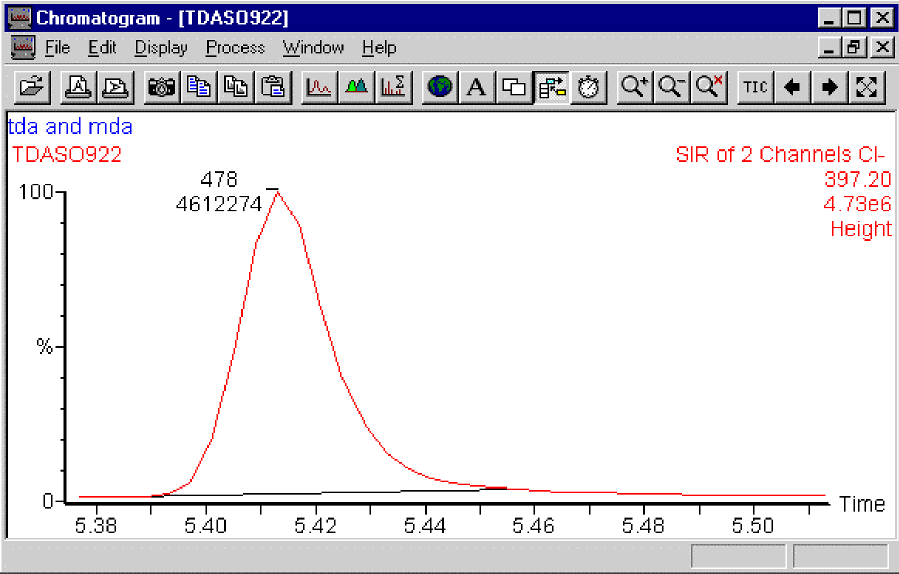

Figure 3.26 *Reduce peak tailing parameter set to 50%*

The **raise baseline** parameter prevents the baseline end point being moved too high up the peak. To prevent the baseline endpoints moving up the peaks, reduce the value of this parameter. The default value is 5%, and normal operating range is 5% - 20%. This parameter is only relevant when the **reduce peak tailing** parameter has a small value (less than 50%). In the example below, the **reduce peak tailing** parameter has been set to 25%.

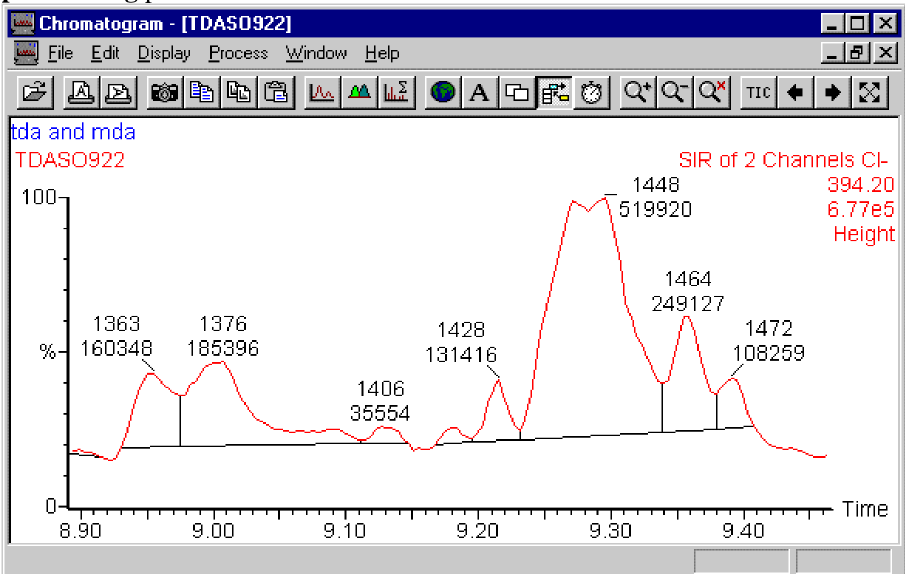

Figure 3.27 *Raise baseline parameter set to 50%*

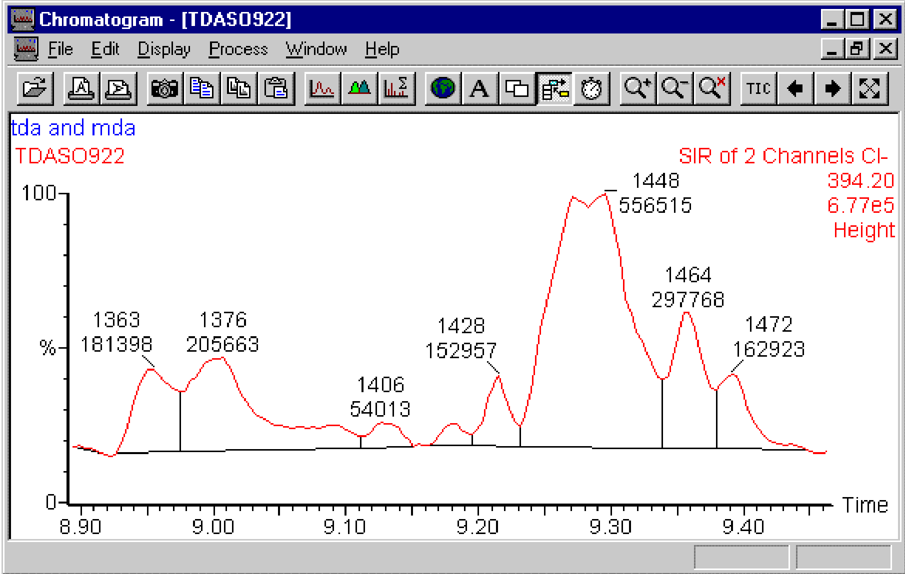

Figure 3.28 *Raise baseline parameter set to 5%*

The **draw vertical** parameter determines how well resolved peaks must be before they are separated by a dropline (or baselines are drawn up into the valleys, depending on the value of the **join valleys** parameter). If you wish poorly resolved peaks to be separated, increase the value of this parameter. The default value is 90%, and normal operating range is 50%–100%.

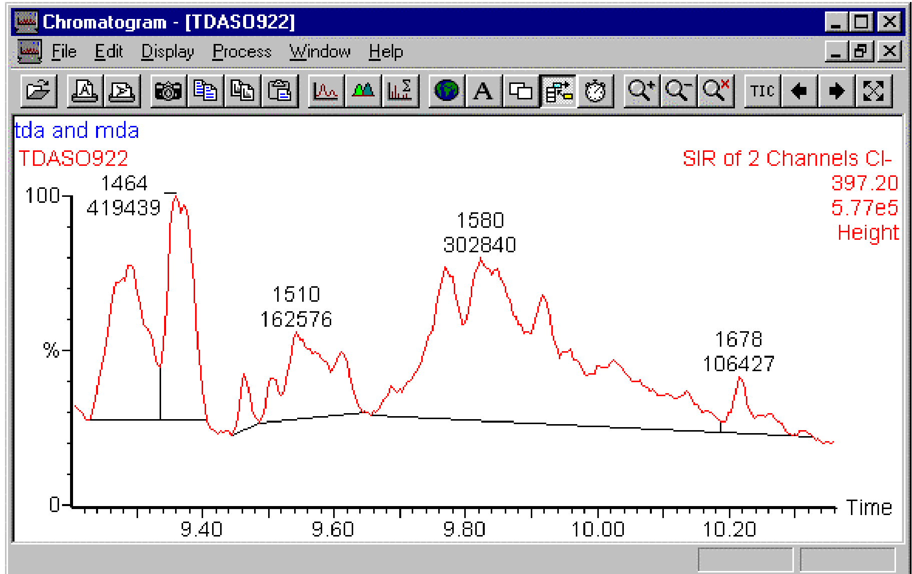

Figure 3.29 Draw verticals parameter set to 50%.

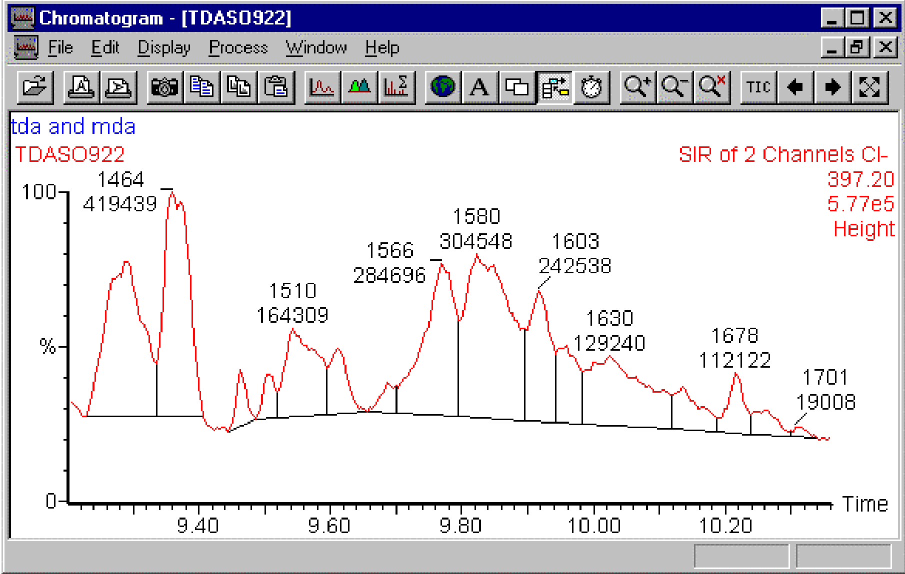

Figure 3.30 Draw verticals parameter set to 95%.

You can optionally attempt to detect completely unresolved peaks, or shoulders, by selecting the **detect shoulder peaks** check box. The algorithm will detect a shoulder if the slope of the shoulder top is less than the specified percentage of the steepest slope on the peak. Therefore, to make shoulder detection more sensitive, increase the value of this parameter. The default value is 30%, and normal operating range is 20%–90%.

■ To integrate a chromatogram

1. Display the chromatogram range you wish to integrate over.

2. Choose **Integrate** from the Chromatogram **Process** menu.

3. Enter a value for **Noise amplitude**. To calculate this value, display a section of the chromatogram that contains only background. Click at one end of this section with the right mouse button, drag the mouse pointer to the other end and release it. The integration software will calculate the **Noise amplitude** and update the value shown in the control.

4. Optionally, select the **Enable smoothing** check box, and examine or modify the smoothing parameters by pressing the **Smooth...** button.

5. Optionally, set up one or more thresholds to remove small peaks by pressing the **Threshold** button to bring up the **Response Threshold** dialog.

6. Press **OK** to exit the dialog and perform the integration. The integration software will smooth the chromatogram trace if requested, locate the peaks, draw baselines and calculate peak statistics.

Editing Detected Peaks

You can use the **Edit Integrated Peaks** dialog box to alter the results of integration by changing the position of an individual baseline, adding a single peak, or deleting one or all peaks.

■ To display information about an integrated peak

A single click with the left mouse button on a peak will display the **peak top position**, **peak height** and **peak area** in the status bar at the bottom of the chromatogram window.

Peak Annotation can be displayed using any combination of peak top time, peak top scan, peak response height and peak response area by choosing **Peak Annotation** from the Chromatogram **Display** menu.

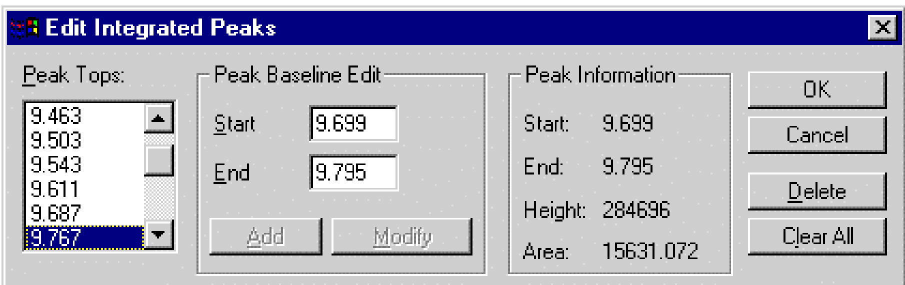

***Figure 3.31** Edit Integrated Peaks dialog*

■ To edit a peak baseline

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

2. Select the peak whose baseline you wish to edit by clicking the right mouse button on a peak in the chromatogram, or by selecting from the Peak Tops list box with the left mouse button.

3. Alter the **Start** or **End** point by typing in new values with the keyboard, or select a range with the right mouse button, and press the **Modify** button.

   You can also alter the range by clicking on one of the end markers (boxes) with the left mouse button and dragging it to the required position.

4. The figures in the **Peak Information** group will update to reflect the edited baseline.

■ To add a new peak

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

2. Type the start and end points of the new peak's baseline into the **Start** and **End** controls or select a range with the right mouse button.

3. Press the **Add** button

4. The figures in the **Peak Information** group will update to reflect the new peak.

To delete a single peak

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu
2. Select the peak you wish to delete by clicking the right mouse button on a peak in the chromatogram, or by selecting from the peak tops list box with the left mouse button.
3. Press the **Delete** button.

To delete all the peaks

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu
2. Press the **Clear All** button.

When you are happy with your changes to the integrated peaks, press the **OK** button in the **Edit Integrated Peaks** dialog. Pressing the **Cancel** button aborts the edit and discards your changes.