TurboMass Gold Quantitation

The MS Method - Full Scan

Scan time = duration of each scan
Inter Scan Delay = time to reset
Try to achieve 10 to 20 scans per peak
Max scan rate = 6200/s

Before looking at the TurboMass Gold integration and quantitation parameters it is necessary to ensure that the MS method has been set up correctly and that an adequate number of scans have been collected across each peak. For a 0.25mm id capillary column peaks can be expected to be as narrow as 3 seconds at base. To obtain 20 scans across the sharpest peaks (the optimum number) a scan needs to be collected every 0.15 seconds. The fast scan speeds available with TurboMass Gold will now allow an adequate number of scans to be taken to ensure good quantitation.

Note that the fastest scan speed is 6200 Daltons/s and so the scan mass range needs to be taken into account.

At high scan speeds TurboMass Gold may need the mass calibration to be re-calibrated at a faster Scan Speed Compensation.

The MS Method - SIR

Dwell Time on each Mass

Delay between each mass
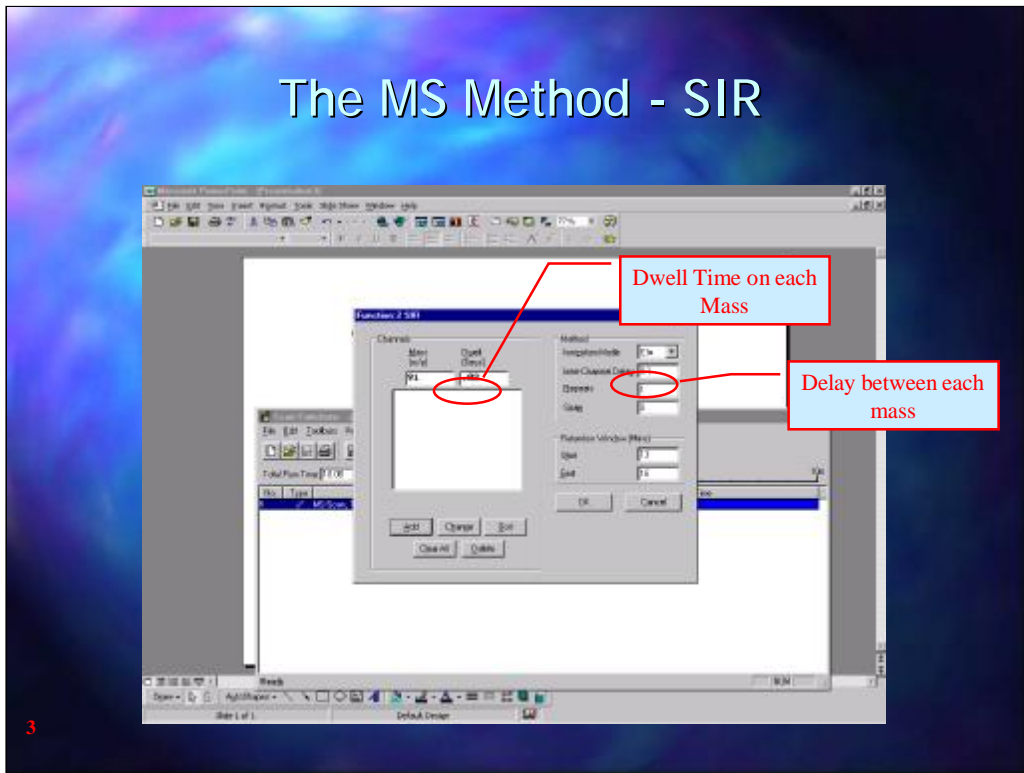
The same rules regarding the number of scans across the peak apply for SIR data as for Total Ion Chromatogram data. In this case the number of scans will be determined by the sum of the dwell times for each ion plus the Inter-Channel delay.

Building a Quantitation Method

Click to edit quantitation method
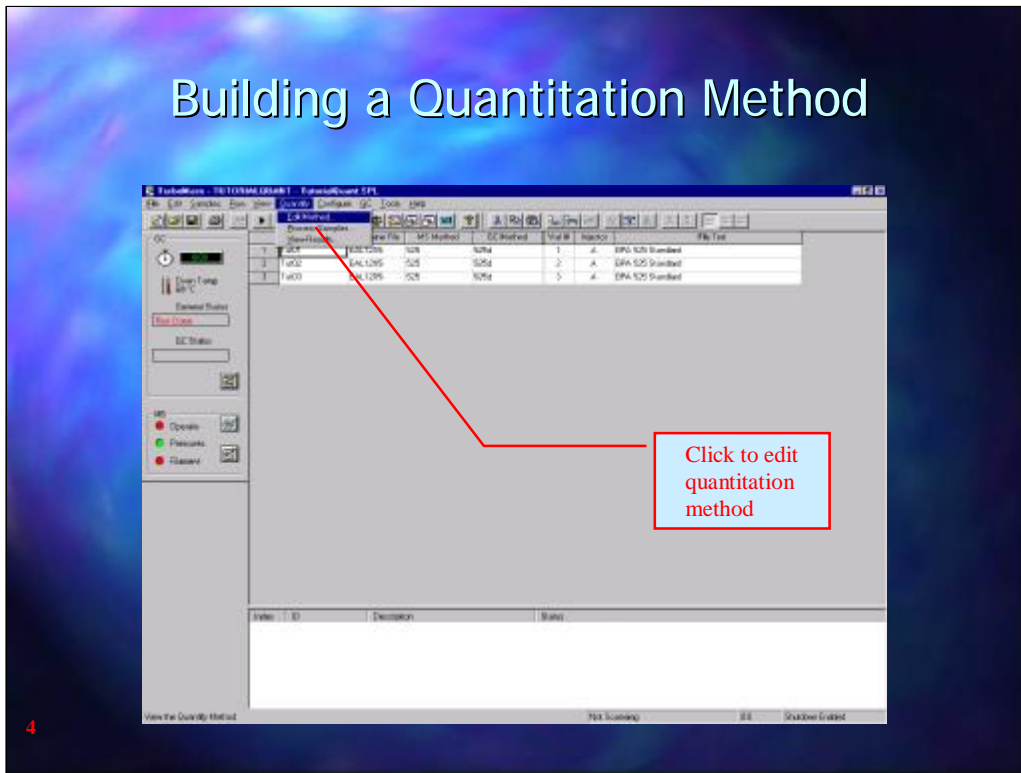
Once the MS method has been set up to collect an adequate number of scans a Quantitation method needs to be built.

This is done from the Quantify drop down menu and Edit Method

The Quantitative Method Editor

Arrange windows so that method editor, chromatogram & mass spectrum can be seen.

Entries to the Quantify Method may be done by opening the chromatogram window and calling up a chromatogram of a calibration standard and the mass spectrum window. When the Quantify Method window is open the right mouse button is used to make entries to the Quantify Method.

Entering Peak Data

Type in component name

Acquisition number entered automatically

Right click on peak crest and t_R is automatically entered.

By selecting a single ion chromatogram the Quantify ion is selected automatically

With a chromatogram open using right mouse click at a peak crest will automatically make an entry into the retention time box. Also, the function number will be entered and if the chromatogram displayed is from a single ion, that ion will also be used for the integration.

The component name needs to be typed in manually.

Entering Peak Data

Alternatively, by clicking and dragging with the right mouse button across the peak, the retention time window for peak identification can be entered. If this is done the mid point of the retention time window will be entered as the retention time.
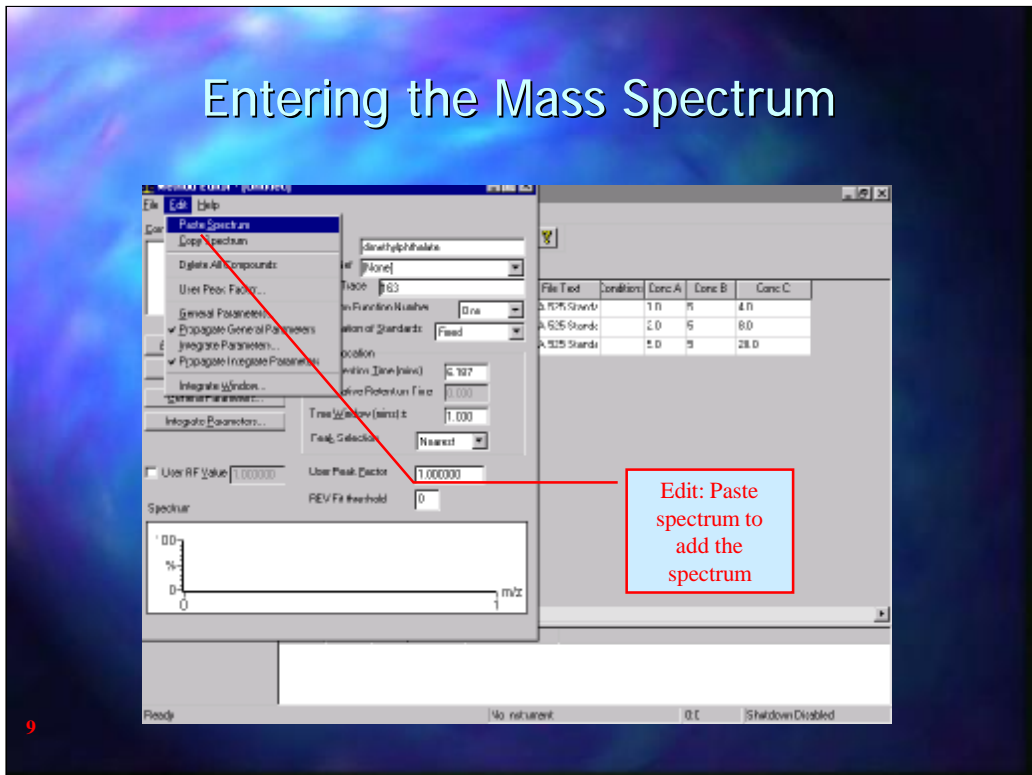
Entering the Mass Spectrum

The mass spectrum for the component may be used to confirm that the peak in the sample is from the correct component. To paste the mass spectrum in the quanitation method it is necessary first to obtain the mass spectrum for the component in the calibration standard.

To do this obtain a mass spectrum by using the combine button from the chromatogram window.

From the Spectrum window select Edit, copy spectrum list.

Entering the Mass Spectrum

Edit: Paste spectrum to add the spectrum

Then in the Quantify method window select Edit, Paste Spectrum

Entering the Mass Spectrum

A copy of the spectrum will be pasted into the Spectrum Window at the bottom of the Quantify Method window.

General Method Parameters

Once all the components have been entered in the Quantify Method the General parameters need to be set up.

General Method Parameters

In the General Parameters the Quantitation type is entered, whether internal standard (relative) or external standard (absolute) and whether the calculations are based on the peak area or the peak height. The concentration units may be entered and the type of calibration curve that is to be used, Average RF, linear, quadratic or cubic fit.

Fit weighting may be used to give more emphasis to lower values rather than the higher values when drawing the line of best fit. 1/x and $1/x^2$ give more emphasis to lower x values whereas 1/y and $1/y^2$ give more emphasis to lower values of y

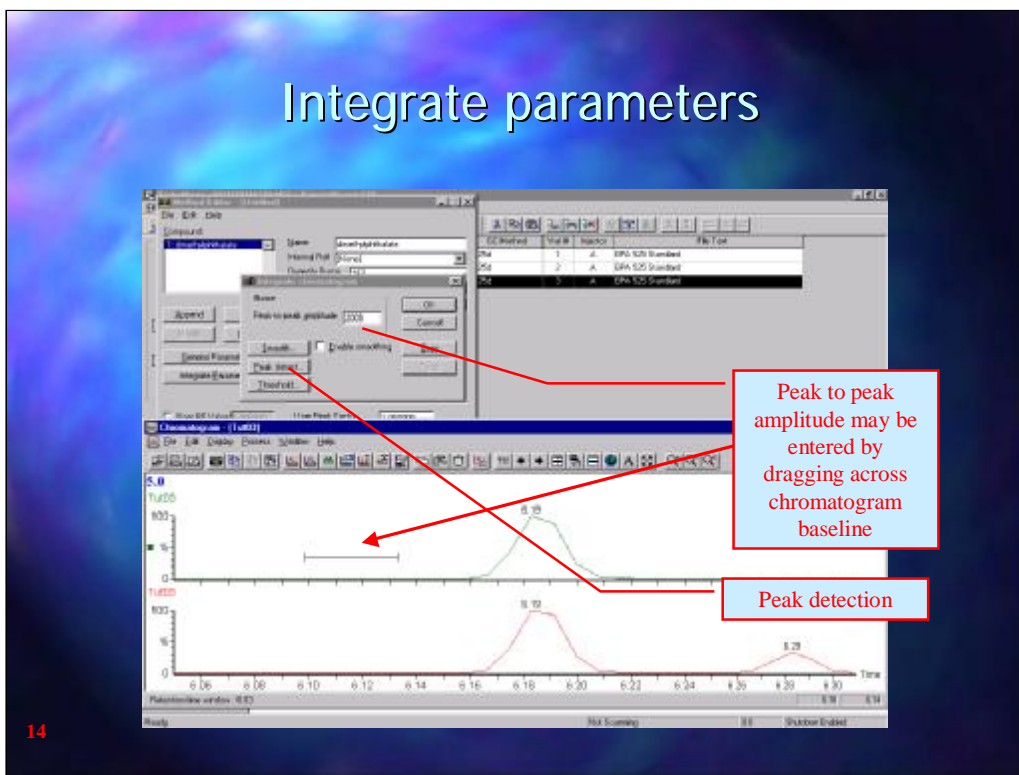Axis transformation allows axis to be plotted in log or 1/x format.
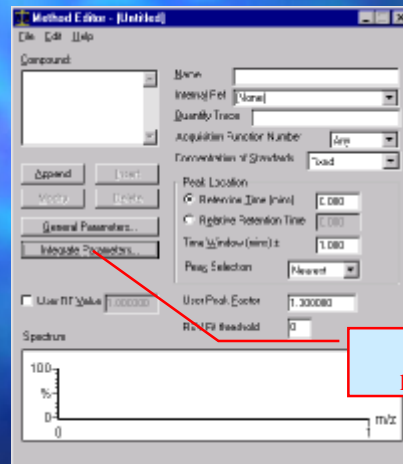
The Integrate parameters govern how the peak areas will be calculated

Integrate parameters

Peak to peak amplitude may be entered by dragging across chromatogram baseline

Peak detection

Peak-to-peak amplitude is used to eliminate the background noise. The easiest way to set this up is to open up a typical chromatogram and then click and drag with the right mouse button over a porti on of the baseline that does not include any peaks. The software will automatically calculate the noise level and enter a suitable val ue for peak-to-peak amplitude.

Smooth is also used to minimise the noise level. Two options are available; mean, where a number of scans are specified and the average abundance of these scans is used to provide a smoothed data point. Savitski- Golay smoothing may also be used. This involves a more complex calculation to smooth the background. It generally gives a better smoothing than using mean but it does require at least 15 scans across each peak to work properly.

Peak detect is used to determine the start and end of each peak.

Peak detection
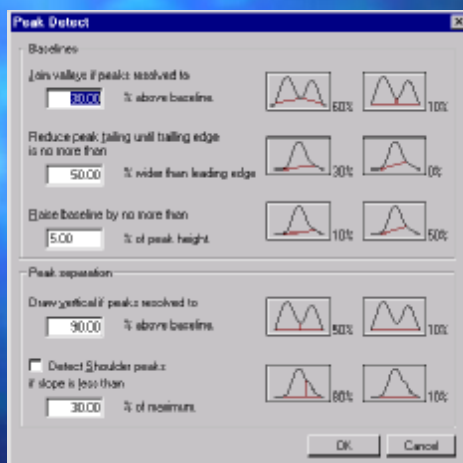
Join valleys determines how partially resolved peaks are integrated. A high value will cause the baseline to be drawn to the valley and a low value will cause a vertical drop line to be drawn between each peak. % above baseline refers to the height of the valley relative to the average peak height.

Reduce tailing refers to to parameters. % wider than leading edge is measured at 10% of peak height and is a comparison of the peak width after a vertical drop line from the peak crest relative to the width before the peak crest.

The second parameter specifies how high the baseline can go relative to the height of the peak.

Peak separation determines whether partially resolved peaks are integrated as separate peaks or combined as one peak. A high value for Draw Vertical will ensure that peaks are integrated separately.
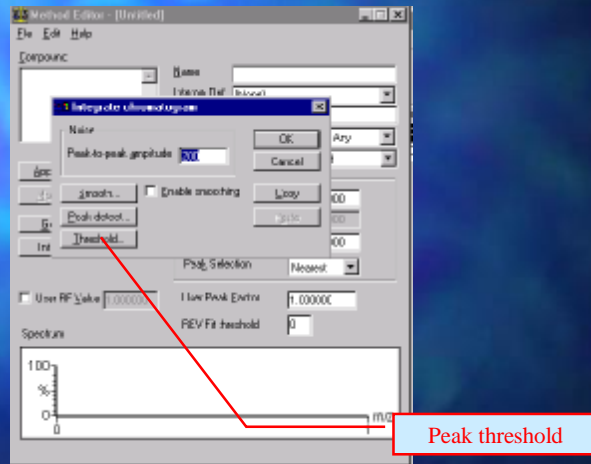
Threshold governs which peaks are integrated

Peak threshold

Response Threshold

☐ Relative height   `1.50`   OK

☐ Absolute height   `0`   Cancel

☐ Relative area   `2.00`

☐ Absolute area   `0`

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To use the threshold values the relevant threshold needs to be selected.

Relative height refers to largest peak in the chromatogram. Any peak with a height less than the relative height compared with the largest peak will be ignored.

Absolute height will ignore peaks that are less than the specified abundance

Relative area refers to the peak area relative to the largest peak un the chromatogram.

Absolute area will ignore anything with an area count less than the value specified.

Integrate Window
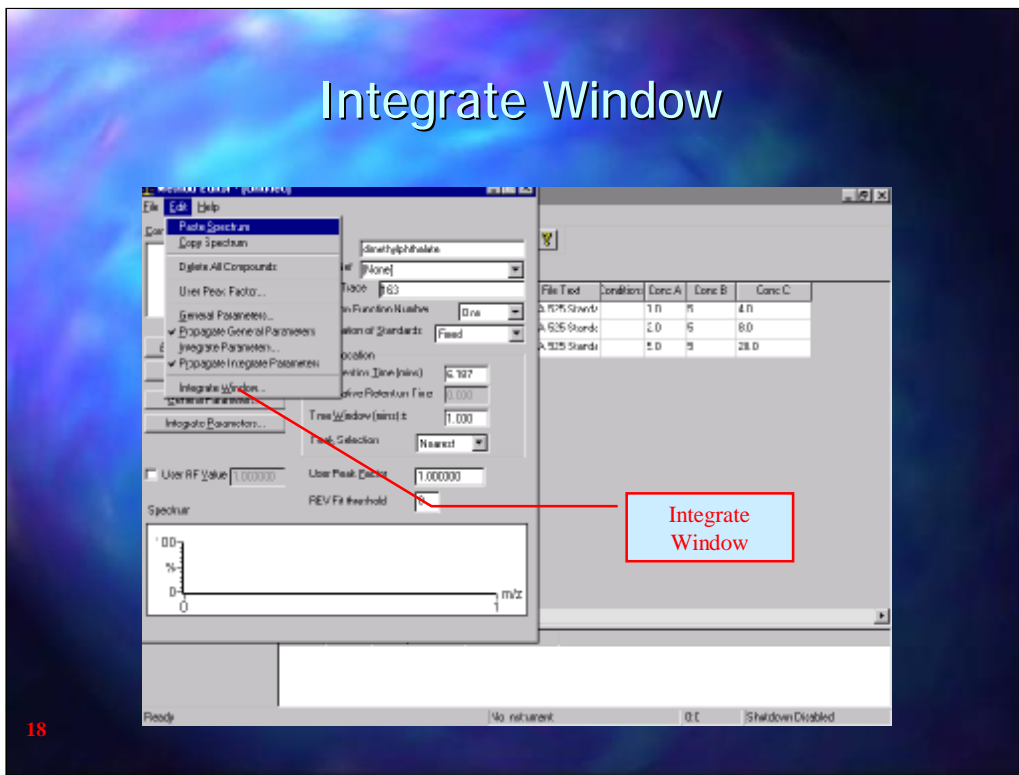
Under the Edit drop down menu is an entry for Integrate Window. This is a window in which the start and the end of the peak must fall. It is entered as a multiple of the Time window and the default window is set to 2 times the time window. This is usually adequate but if the peak is particularly broad or if the time widow is too narrow the baseline under the peak may not include the whole width of the peak. If this is the case then the Integrate window will need to be widened.

Modify sample List Display

Double Click in grey box to edit columns

Select Sample Type
&
Conc Columns to match components

Before calibration and integration can occur the sample list will need to be modified with some new columns added. This is done by pointing to any grey heading box at the top of the sample list and double clicking with the right mouse button. A list of columns will be displayed. Ensure that the SAMPLE TYPE and an adequate number of CONC A to CONC T columns to cover the number of components are ticked.

Entering Calibration Amount

Concentration for Standards specified in Sample list

Standard or Sample (analyte) specified here

The last part of the Quantify method is to enter the weights of the calibration standards. The amounts of each standard are entered in the sample list under Conc A through to Conc T. In the Quantify method the concentration of standards specify which Concentration column to use for each compound.

Also in the sample list under Sample type each run is identified as a Standard or a sample (analyte)

Processing Data

Click & drag down rows to be processed

To quantify a number of analyses first click and drag over the samples to be quantified.
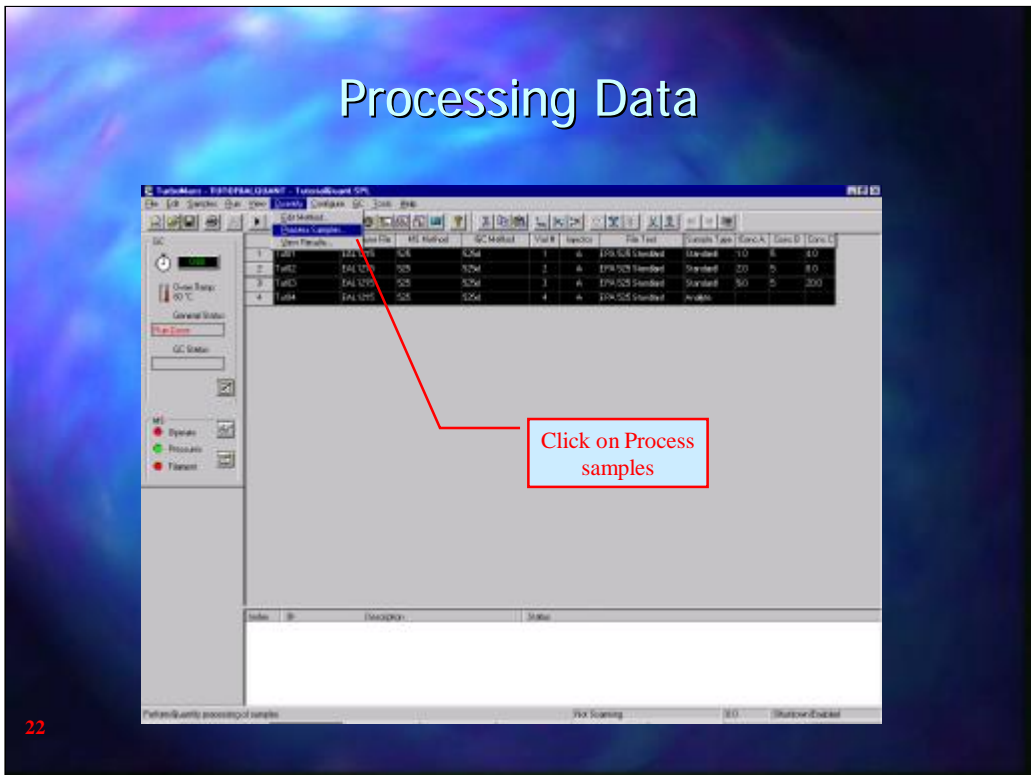
Then Click on Process Samples under the Quantify Drop down menu

Processing Data

Integrate samples will calculate the area under each peak

Calibrate standards will calculate the response values from the rows that are identified as standards.

Quantify Samples will calculate the amounts for each sample.

Method shows the Quantify method that will be used to process the data and the Browse button allows another method to be selected. Likewise Curve is name given for the calibration data which again can be changed by using the Browse button.

View Results

View Results allows Calibration curves and quantitative data to be read
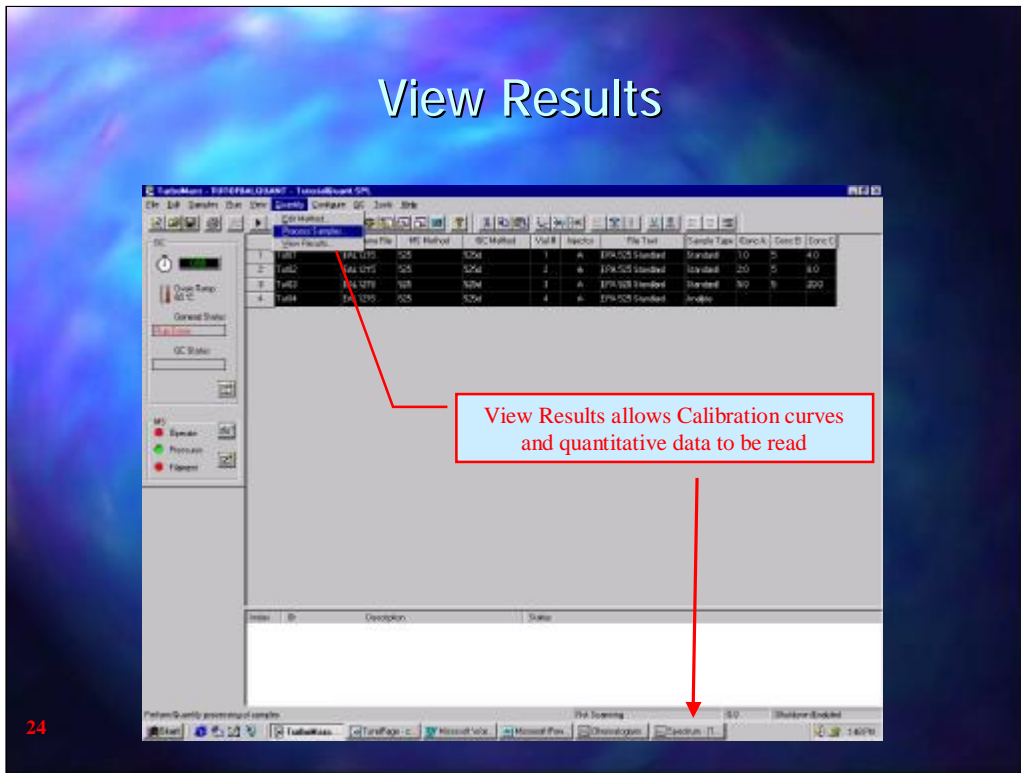
The processed data can be accessed from the View Results from the Quantify drop down menu or by clicking on Quantify on the Windows toolbar
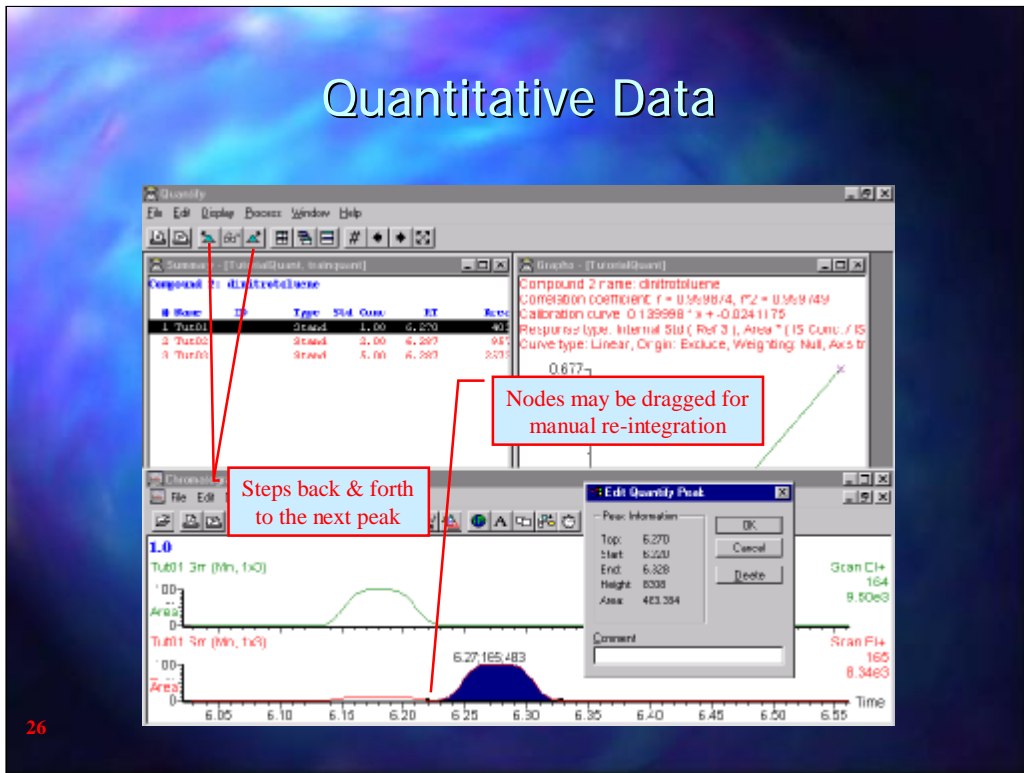
Quantitative Data
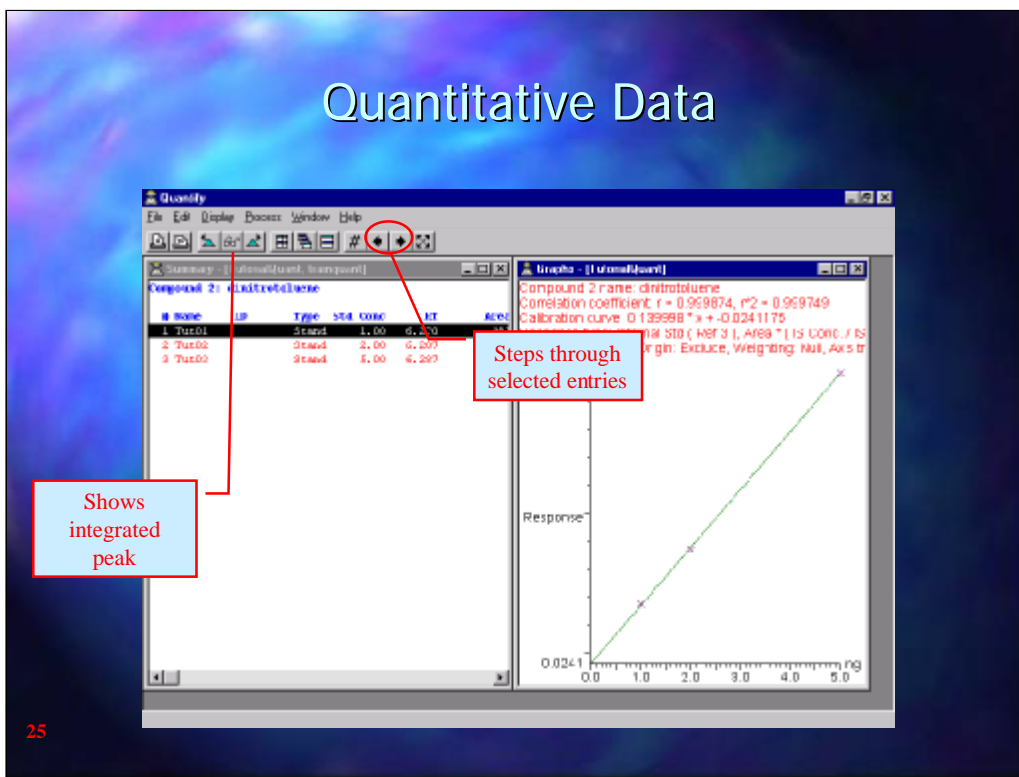
The View results shows the calibration curve and a Summary which may be either by sample or, as in this case, by compound. The 🔭 button shows the integrated peak and the 📈 📈 buttons step to the previous or next peak. The ◀ ▶ buttons step backwards and forwards through the compounds (summary by compound) or through the samples (summary by sample)
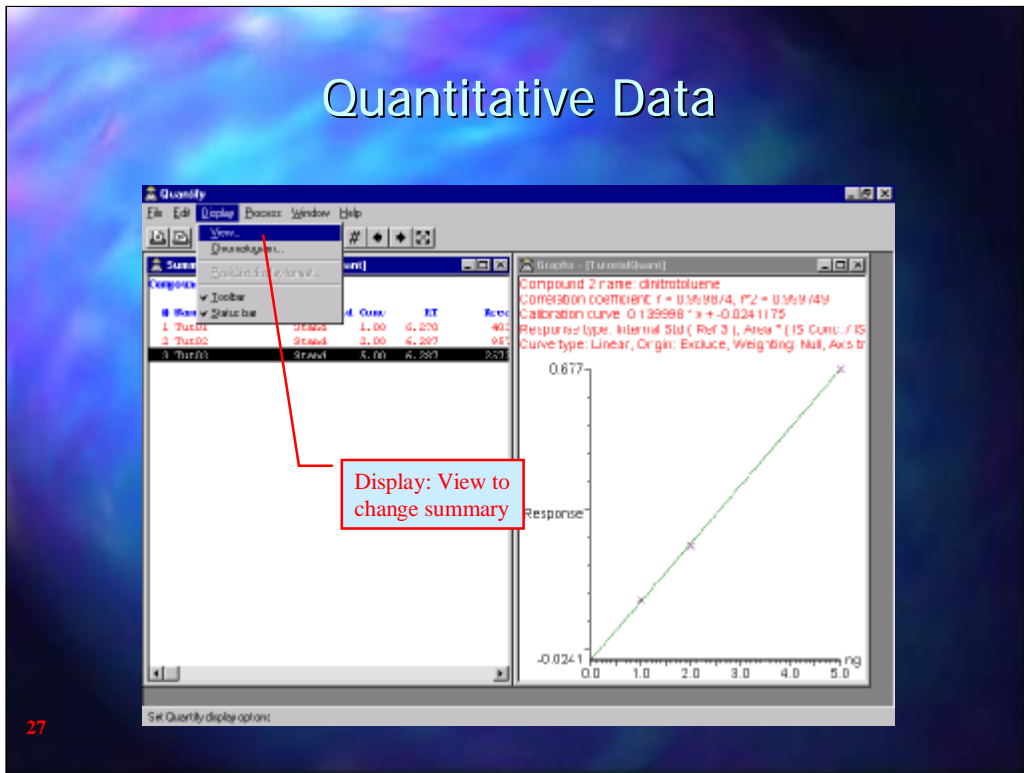
Quantitative Data

The peak may be manually edited if the integration is not good by clicking and dragging the "nodes" at the start and end of each peak.
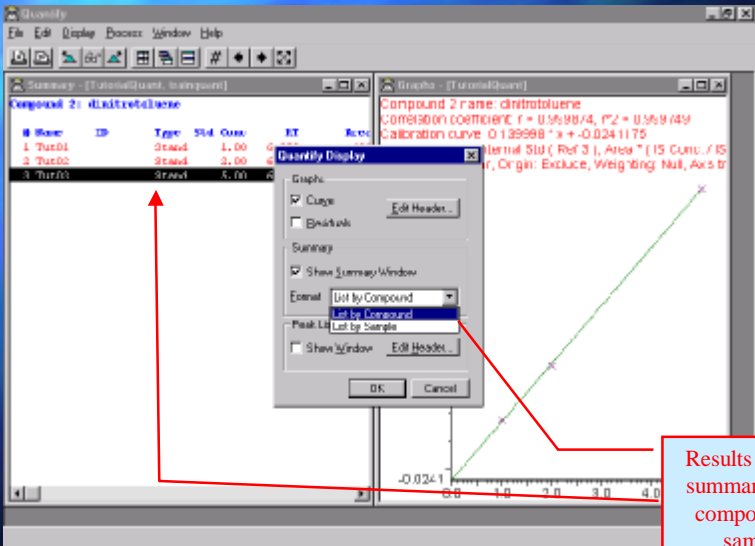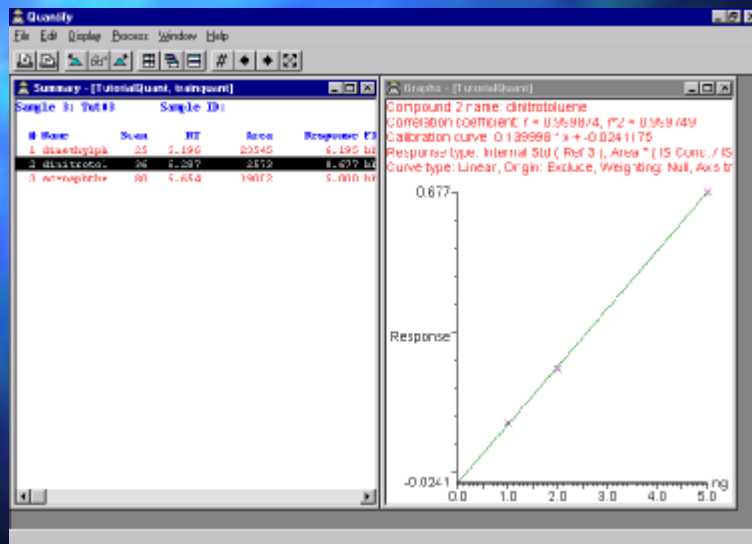
Quantitative Data

Under the Display drop down menu and selecting View the summary window may be changed to View by Sample or View by Compound.

Quantitative Data

Results may be summarised by compound or sample

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Summary by Sample

This shows the Summary by Sample