

TurboMass Gold Quantitation

1

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

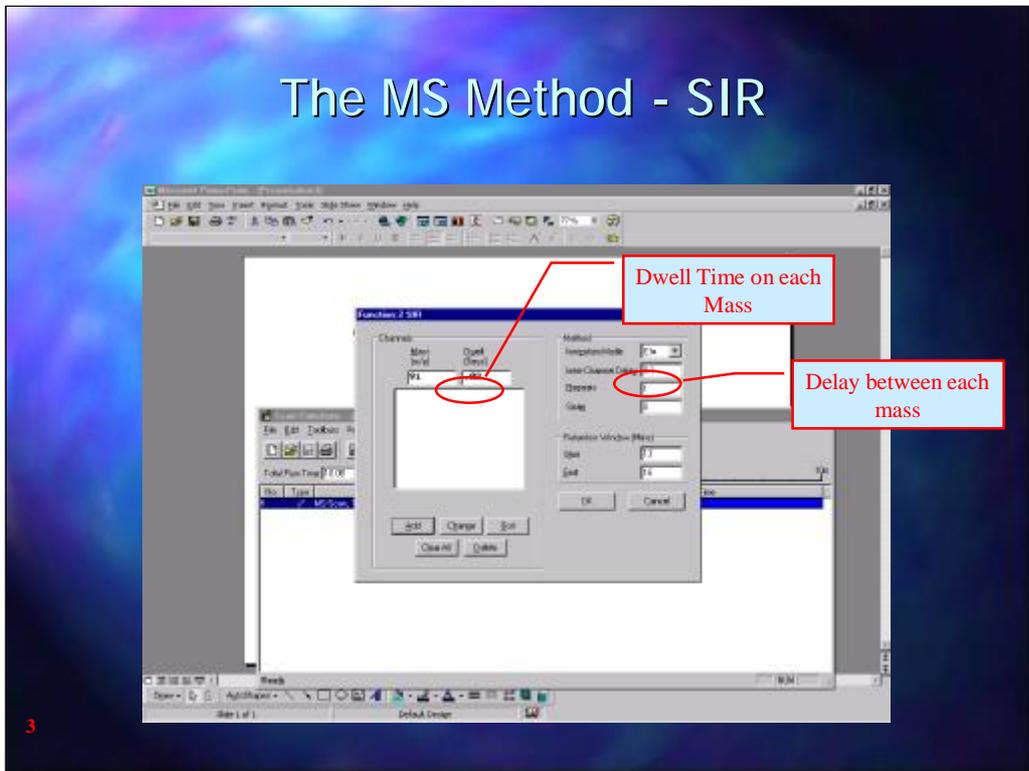
2

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



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.

The Quantitative Method Editor

Method	Std #	Injection	File Path	Sample Path	Concn	Concn Unit	Area
1274	1	A	DNA STD Standard	Standard	1.5	%	4.8
1274	2	A	DNA STD Standard	Standard	2.0	%	8.0
1274	5	A	DNA STD Standard	Standard	5.0	%	20.0

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

6

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

Acquisition Function number refers to acquisition number in MS method

By dragging across the peak with the right mouse button the t_R window will be entered using the centre of the window as the t_R

7

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 screenshot displays two windows from a software application. The top window, titled 'Spectrum - [TUT02]', shows a mass spectrum plot with relative intensity on the y-axis and mass-to-charge ratio (m/z) on the x-axis. A peak is labeled at m/z 153. A menu is open over the plot, with 'Edit; Copy spectrum list' highlighted. The bottom window, titled 'Chromatogram - [TUT02]', shows a chromatogram plot with intensity on the y-axis and time on the x-axis. Two peaks are visible, labeled with retention times 6.29 and 6.42. A red box highlights the 'Edit; Copy spectrum list' option in the chromatogram window's toolbar, with a red arrow pointing from this option to the 'Copy' button in the mass spectrum window's toolbar.

8

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 quantitation 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

The screenshot shows a software window with a menu bar (File, Edit, Help) and a toolbar. The 'Edit' menu is open, displaying options: Paste Spectrum, Copy Spectrum, Delete All Compounds, User Peak Factor, General Parameters, Propagate General Parameters, Integrate Parameters..., Propagate Integrate Parameters, Integrate Window..., Integrate Parameters..., and Integrate Parameters... The 'Paste Spectrum' option is highlighted. A red arrow points from the 'Edit: Paste spectrum to add the spectrum' text box to the 'Paste Spectrum' menu item. The main window area contains a table with columns: File Test, Conditions, Conc. A, Conc. B, Conc. C. The table has three rows of data. Below the table are various input fields and buttons, including 'Use RF Value', 'Spectrum', 'User Peak Factor', and 'REV Fit threshold'. A small plot area is visible at the bottom left of the main window.

File Test	Conditions	Conc. A	Conc. B	Conc. C
A. 10.5 Standards	1 n	5	4 n	
A. 6.25 Standards	2.0	5	6.0	
A. 3.125 Standards	5.0	5	28.0	

Then in the Quantify method window select Edit, Paste Spectrum

Entering the Mass Spectrum

The screenshot shows the 'Quantify Method' window with the following details:

- Compound:** Name: dimethylphthalate, Internal Ref: [None], Quantity Type: 163, Acquisition Function Number: [One], Concentration of Standards: Conc. A
- General Parameters:** Peak Location: Retention Time (min): 6.307, Relative Retention Time: 1.000, Time Window (min): 1.000, Peak Selection: Best Match, Use RF Value: 1.000000, Use Peak Factor: 1.000000, REF Fit threshold: 80
- Spectrum:** A plot showing relative intensity (%) vs m/z. The x-axis ranges from 50 to 250 m/z. A prominent peak is labeled at m/z 163.
- Table:** A table with columns: File List, Conditions, Conc. A, Conc. B, Conc. C. The rows are: A. STD Standards (1.0, 5, 4.0), A. Q25 Standards (2.0, 5, 8.0), A. Sub Standards (2.0, 5, 20.0).

A red box highlights the 'Spectrum' window, and a red arrow points from it to the table in the 'General Parameters' section. A text box next to the arrow says: "Spectrum pasted into method".

10

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

General Method Parameters

The screenshot shows a software window titled "General Method Parameters" with the following fields and values:

- Name: [Search/Modify]
- Internal Ref: [None]
- Quantity Table: [163]
- Acquisition Function Name: [None]
- Concentration of Standards: [Conc. A]
- Peak Location: [Retention Time (min) 16.7]
- Relative Retention Time: [1.000]
- Relative Potention Time: [1.000]
- True Window: [min: 1.000]
- Peak Selection: [Best Match]
- User Peak Factor: [1.000000]
- REV Fit threshold: [80]

A mass spectrum plot is shown at the bottom left, with a peak labeled "163" at approximately 16.7 minutes. A red box highlights the "General Parameters" section with the text "General Parameters entered here".

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

General Method Parameters

Internal or external standard

Internal standard identified in this box

Linear, quadratic or cubic fit selected here

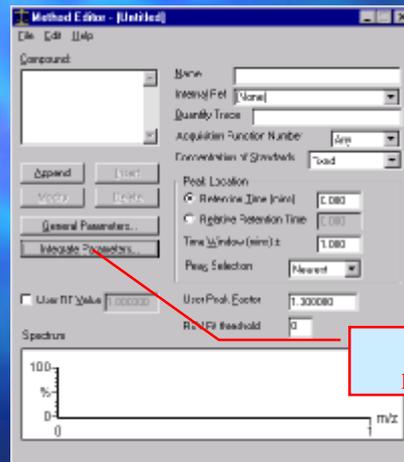
12

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.

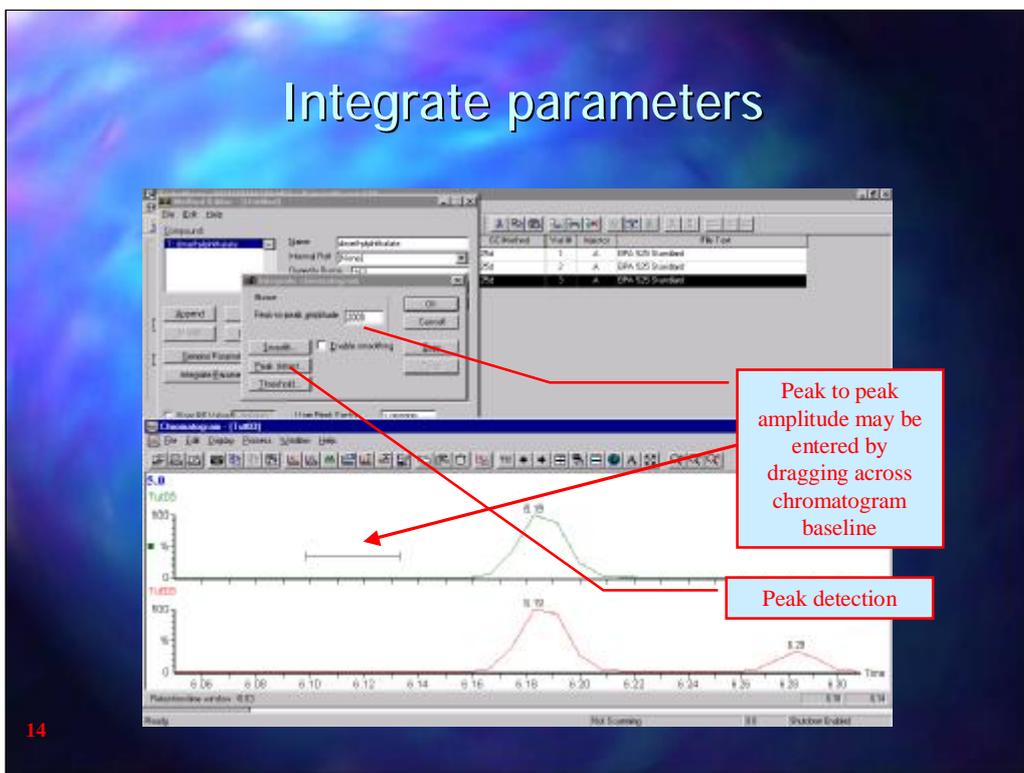
Integrate Parameters



13

The Integrate parameters govern how the peak areas will be calculated

Integrate parameters



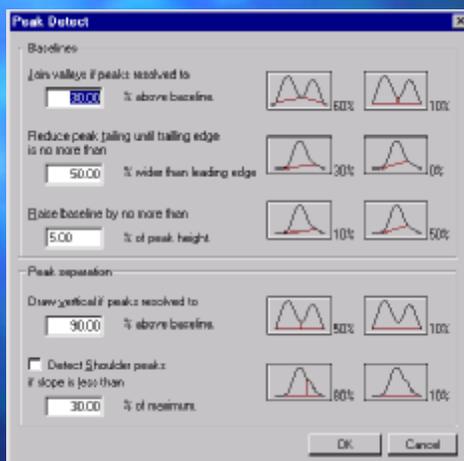
14

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 portion of the baseline that does not include any peaks. The software will automatically calculate the noise level and enter a suitable value 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



15

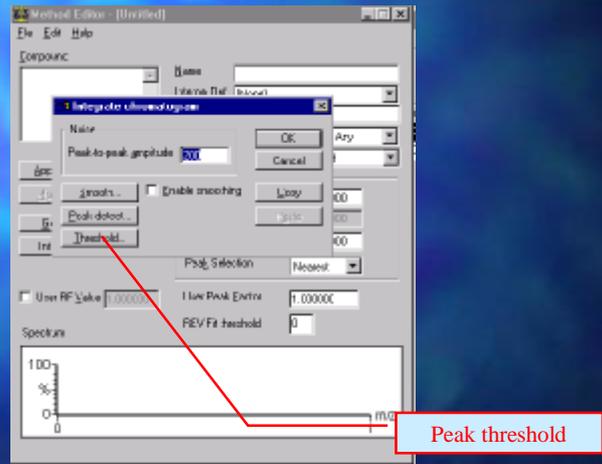
Join valleys determines how partially resolved peaks are integrated. A high value will cause the baseline to be drawn to the valley and a low value will cause a vertical drop line to be drawn between each peak. % above baseline refers to the height of the valley relative to the average peak height.

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.

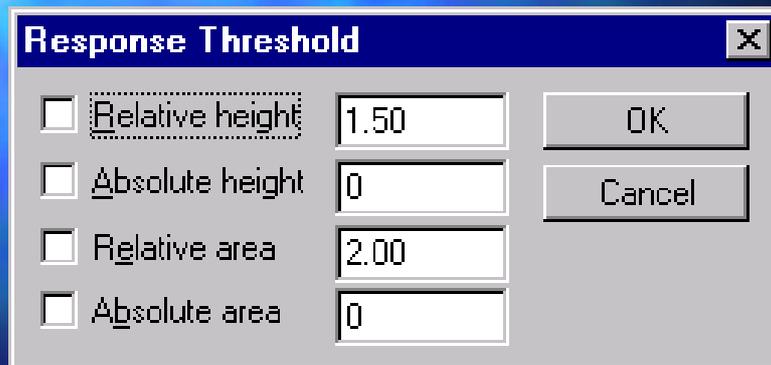
Integrate parameters



16

Threshold governs which peaks are integrated

Peak threshold



Threshold Type	Value
<input checked="" type="checkbox"/> Relative height	1.50
<input type="checkbox"/> Absolute height	0
<input type="checkbox"/> Relative area	2.00
<input type="checkbox"/> Absolute area	0

17

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

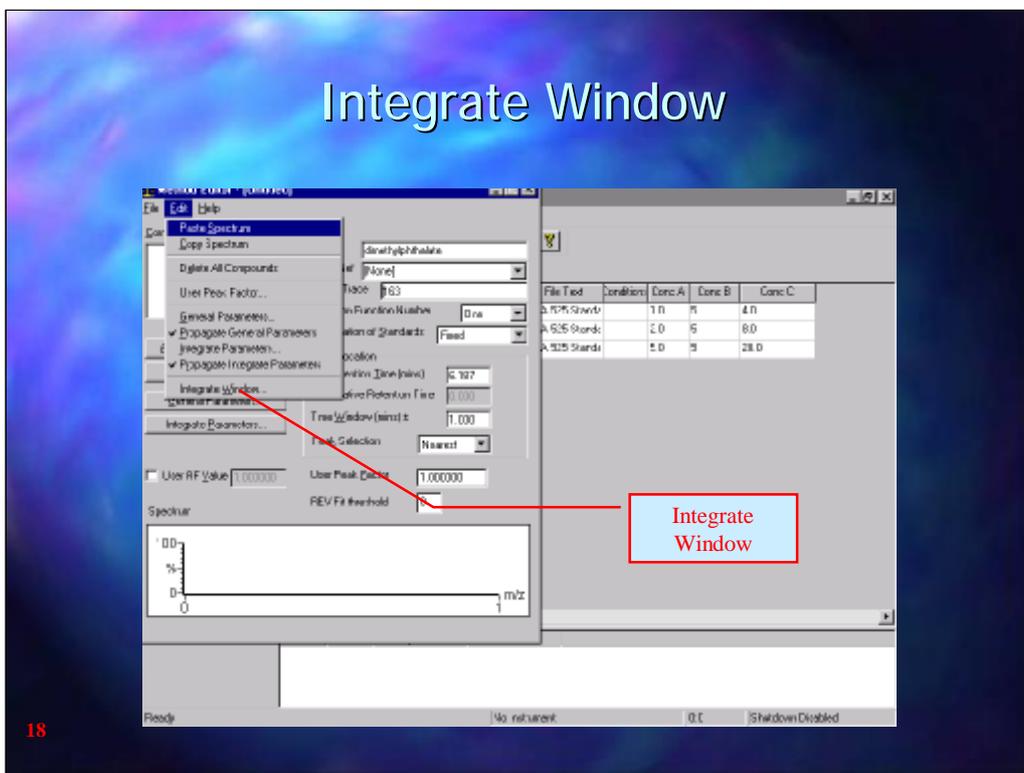
Relative height refers to largest peak in the chromatogram. Any peak with a height less than the relative height compared with the largest peak will be ignored.

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

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

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

Integrate Window



18

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 window 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

The screenshot shows a software window with a sample list and a 'Customize Field Display' dialog box. The sample list has the following columns: File Name, MS Type File, MS Method, GC Method, and Inj ID. The dialog box shows a list of fields to be displayed, including Sample Type, Conc A, Conc B, Conc C, Conc D, Conc E, Conc F, and Conc G. Red arrows point from text boxes to the sample list and the dialog box.

Double Click in grey box to edit columns

Select Sample Type & Conc Columns to match components

19

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

The screenshot shows the 'Method Editor' window with a 'Sample List' table. The table has columns for 'Sample ID', 'Instructor', 'File List', 'Sample Type', 'Conc A', 'Conc B', and 'Conc T'. The first three rows are 'Standard' and the last row is 'Analyte'. A callout box points to the 'Conc A' column, and another callout box points to the 'Sample Type' column.

Sample ID	Instructor	File List	Sample Type	Conc A	Conc B	Conc T
Std 1	A	DPA 525 Standard	Standard	1.0	5	0.0
Std 2	A	DPA 525 Standard	Standard	2.0	5	0.0
Std 3	A	DPA 525 Standard	Standard	5.0	5	0.0
Std 4	A	DPA 525 Standard	Analyte			

Standard or Sample (analyte) specified here

Concentration for Standards specified in Sample list

20

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

The screenshot shows a software window titled "E:\Software - 2011\MSL\MSL01 - E:\msl01\msl01.F1". The main area contains a table with the following data:

File Name	MS Method	Sample	File Size	Sample Type	Conc'd	Conc'd	Conc'd	Conc'd	
1\001	EAL1205	1205	1205	1	A	EPA 821 Standard	Standard	1.0	1.0
1\002	EAL1205	1205	1205	2	A	EPA 821 Standard	Standard	2.0	2.0
1\003	EAL1205	1205	1205	3	A	EPA 821 Standard	Standard	3.0	3.0
1\004	EAL1205	1205	1205	4	A	EPA 821 Standard	Standard	4.0	4.0

A red circle highlights the first row of the table. A red arrow points from this circle to a text box containing the instruction: "Click & drag down rows to be processed".

21

To quantify a number of analyses first click and drag over the samples to be quantified.

Processing Data

Quantitation Method

Calibration data File

Integrate - Measures Peak Areas
Calibrate - Calculates response factors from calibration standards
Quantify - Calculates amounts for analytes

23

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

The screenshot shows the 'View Results' window in a software application. The window title is 'E:\Data\Mass - 20100410\DATA7 - Testdata\Quant 50%'. The main area displays a table with the following data:

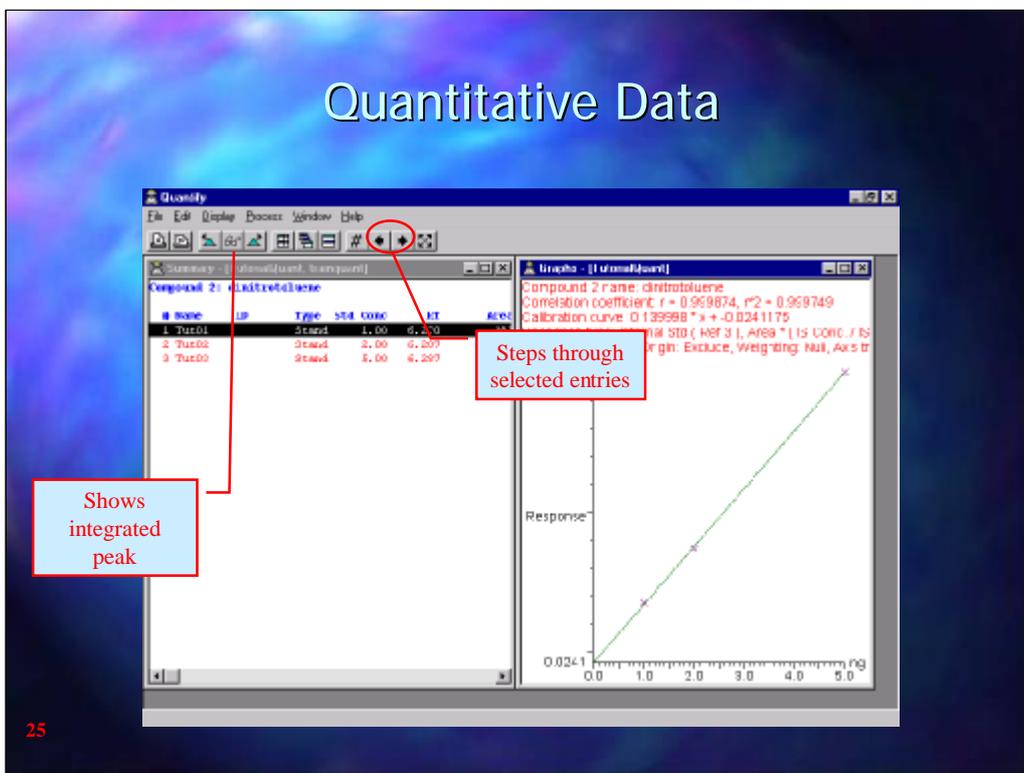
Peak	File	MS	Method	Unit	Vector	File Path	Search Path	Check	Comp	Comp C
1	101	1275	50%	50%	1	A	E:\Data\Mass\Standards	Standard	10	4.0
2	101	1275	50%	50%	2	A	E:\Data\Mass\Standards	Standard	20	8.0
3	101	1275	50%	50%	3	A	E:\Data\Mass\Standards	Standard	30	200
4	101	1275	50%	50%	4	A	E:\Data\Mass\Standards	Standard	40	200

A red box highlights the table, and a red arrow points to the 'View Results' button in the toolbar. A text box with a red border contains the text: 'View Results allows Calibration curves and quantitative data to be read'.

24

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

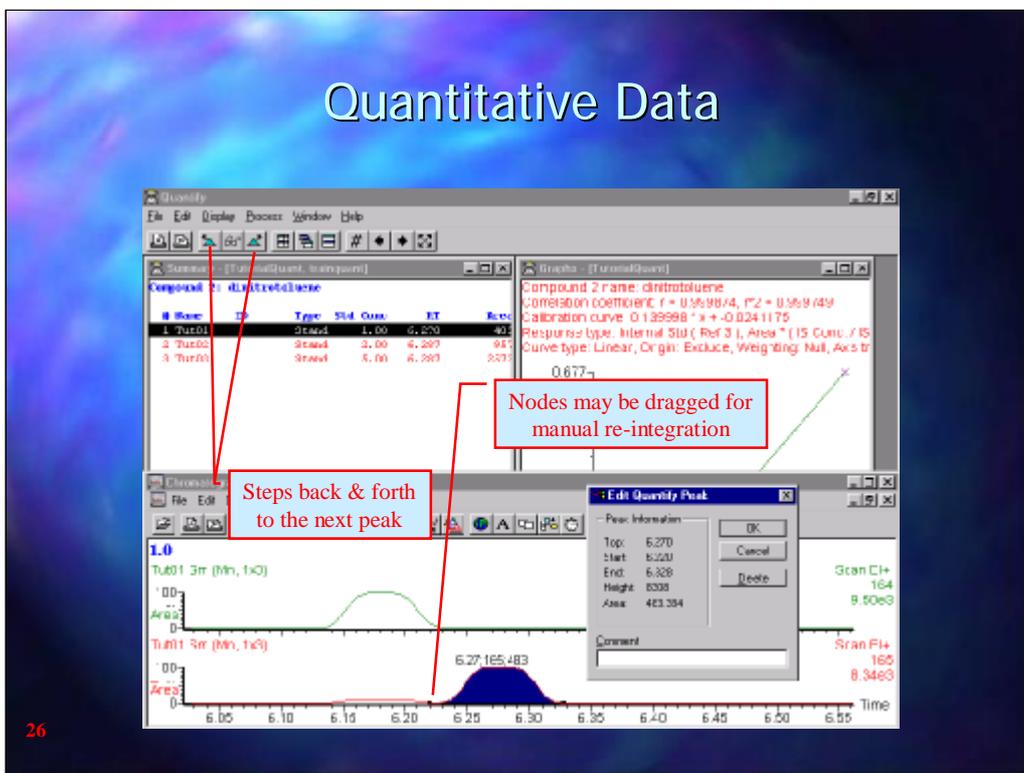
Quantitative Data



25

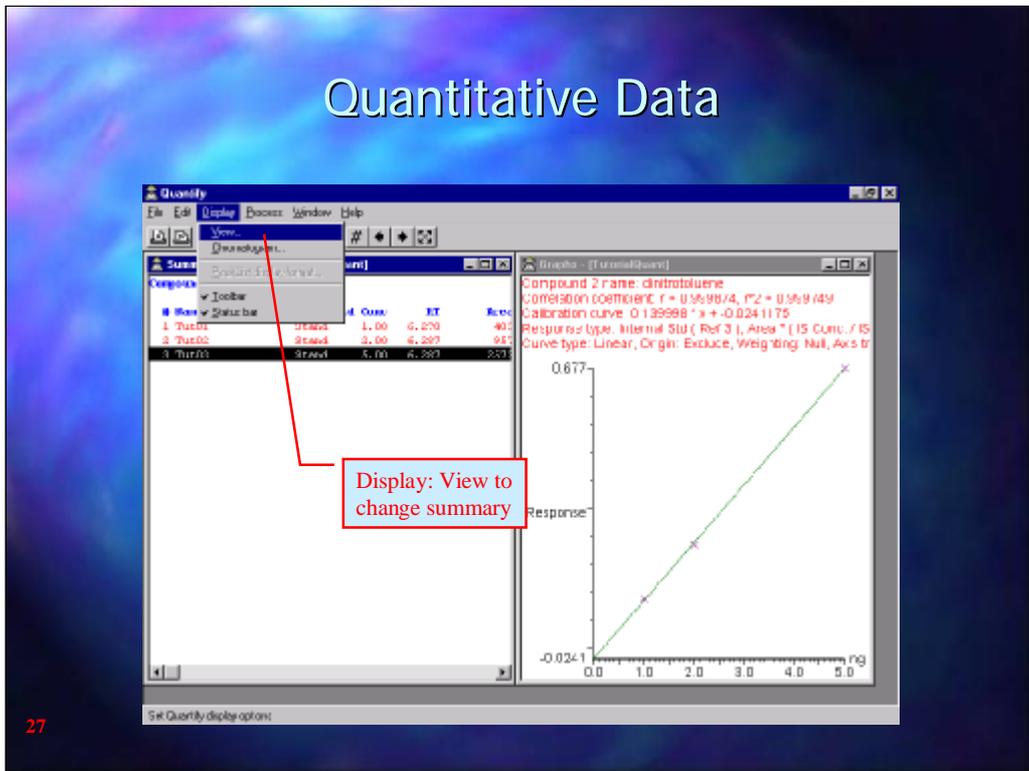
The View results shows the calibration curve and a Summary which may be either by sample or, as in this case, by compound. The  button shows the integrated peak and the  buttons step to the previous or next peak. The   buttons step backwards and forwards through the compounds (summary by compound) or 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.

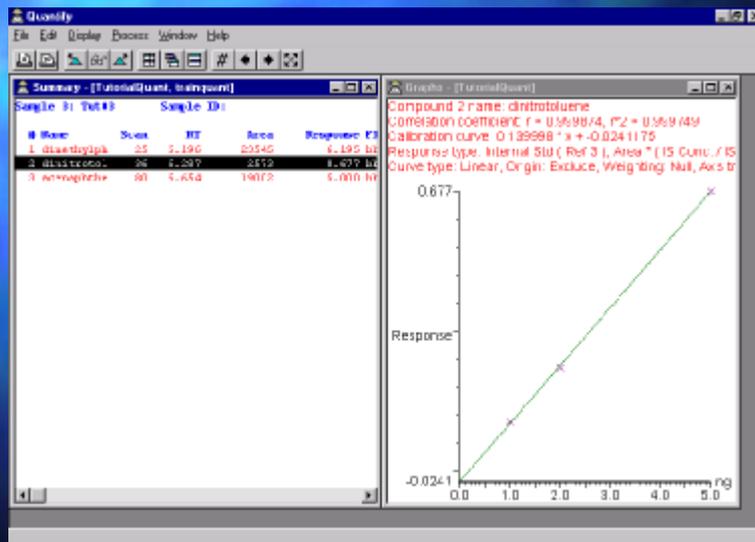
Quantitative Data



27

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

Summary by Sample



29

This shows the Summary by Sample