Waters

# MassLynx
# Quantitation (New Version)

Waters

# Quantitation

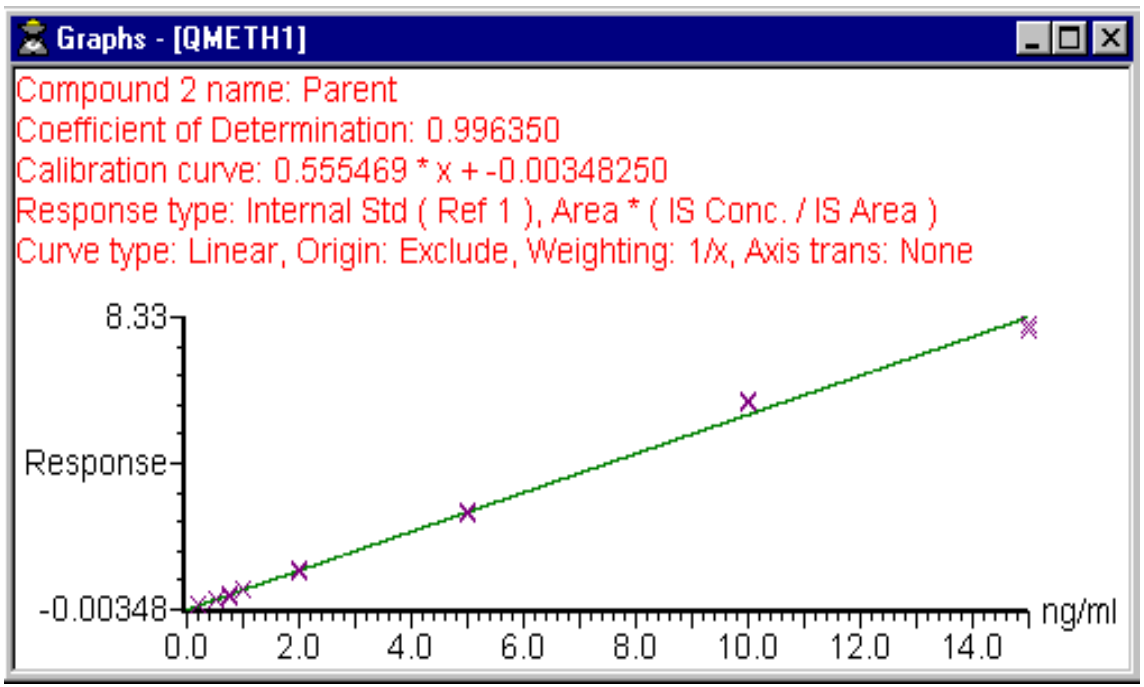


- Determines the concentration of specific analytes within a sample
- Can be done on data acquired through a variety of Acquisition Modes:
  - Multiple Reaction Monitoring (MRM)
  - Single Ion Recording (SIR)
  - Full Scan Acquisition
- QuanLynx and TargetLynx with an EPCAS System is Designed to Be a Part of a 21 CFR Part 11 Compliant Environment.

# How do we quantitate?



- In addition to unknown samples, a set of standards is also run to form a calibration curve.
- MassLynx analyzes the response of unknown samples and compares their response to that indicated by the calibration curve, then calculates the concentrations of the unknowns.



# Steps in Creation of a Calibration Curve for Quantitation

- Integrate peaks in chromatograms
- In each chromatogram, determine the location of the peak relating to a specific compound
- Calculate response factor for the located peak
- Create a Calibration Curve for that compound

# Example Project in MassLynx to Practice On



Project = Quantify.pro

Set of analyses on samples using a MS method that had:

- MRM of 3 channels
  - Internal Standard (294.1 > 64.0)
  - Analyte 1 - Parent Drug (288.1 > 58.0)
  - Analyte 2 - Metabolite (274.1 > 182.1)

# Internal Standards



- Used to account for Experimental Drift
- Can be Added at Various Points in the Analysis
  - In the Original Sample
  - Before Injection by the LC
- Response of Analyte in a Sample is:

$$\frac{\textbf{(Peak Area of Analyte)}}{\textbf{(Peak Area of I.S.) / (Conc of I.S.)}}$$

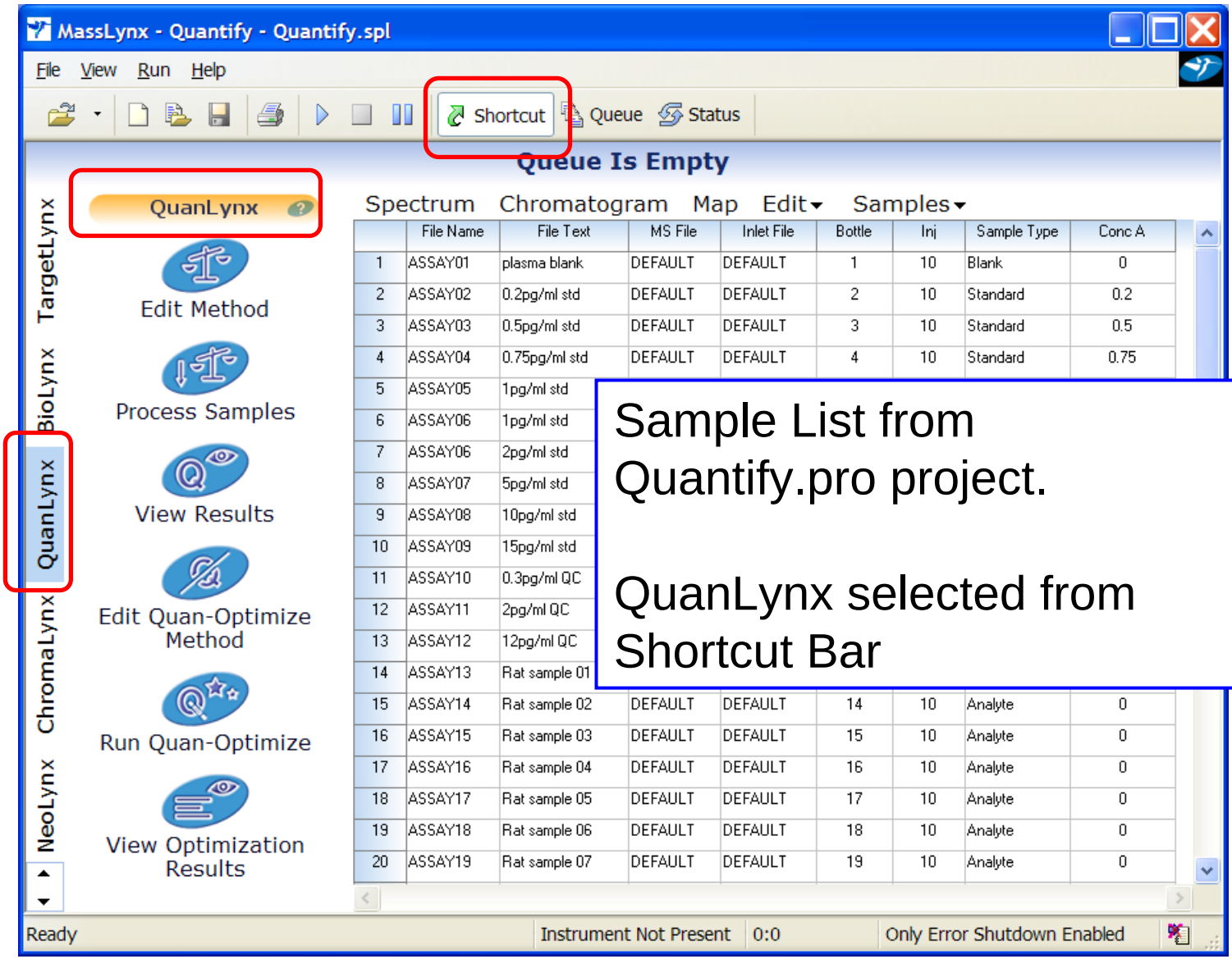
# For our Example: Quantification Steps



1. Enter Sample Types & Concentrations into Sample List
2. Determine Correct Integration Parameters for Chromatogram Peaks.
3. Create Quantification Method.
4. Process Samples.
5. Check Results – Adjust if Needed.
6. Print Out Results – Save Results on in Report File.

# 1. Set up Sample List






Sample List from Quantify.pro project.

QuanLynx selected from Shortcut Bar

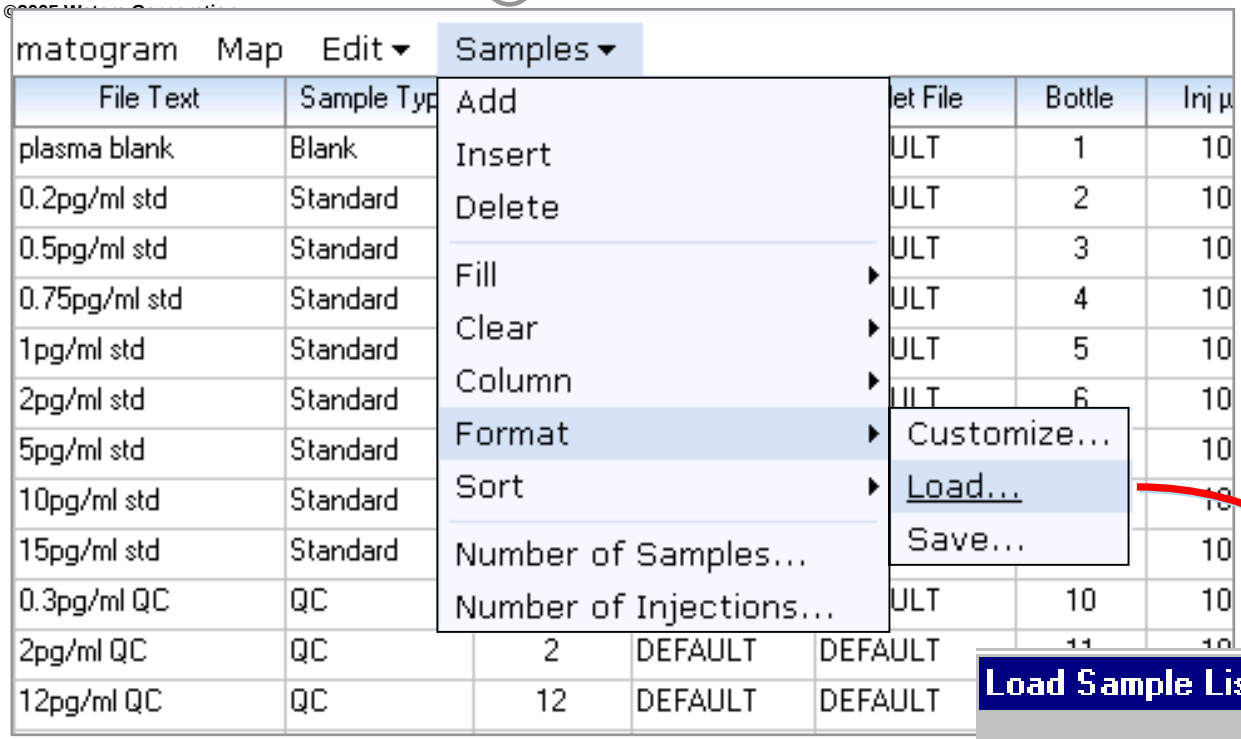
# 1. Set up Sample List



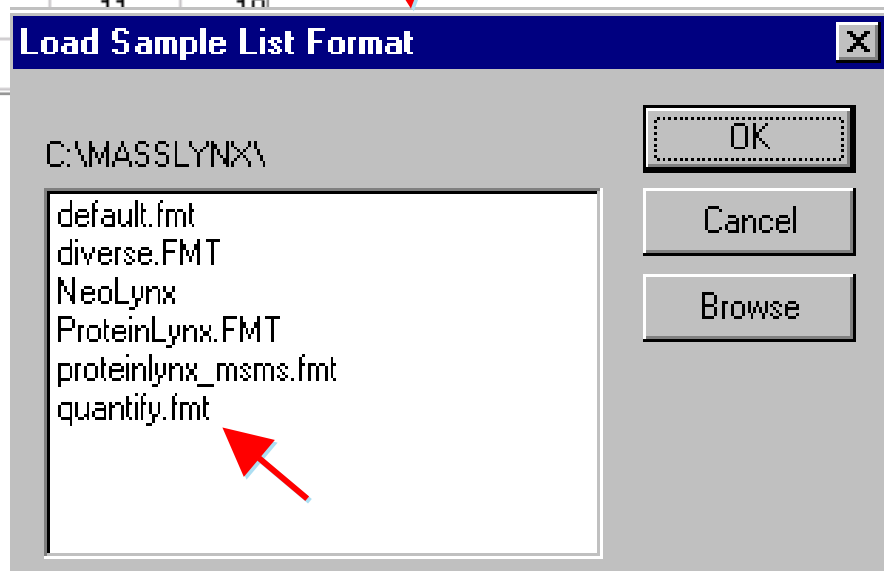
- Standard Sample list plus two additional categories:
  - Sample Type
  - Concentration A (B, C, D….)

| | File Name | File Text | MS File | Inlet File | Bottle | Inject Volume | Sample Type | Conc A |
|---|---|---|---|---|---|---|---|---|
| 1 | ASSAY01 | plasma blank | DEFAULT | DEFAULT | 1 | 10.000 | Blank | 0 |
| 2 | ASSAY02 | 0.2pg/ml std | DEFAULT | DEFAULT | 2 | 10.000 | Standard | 0.2 |
| 3 | ASSAY03 | 0.5pg/ml std | DEFAULT | DEFAULT | 3 | 10.000 | Standard | 0.5 |
| 4 | ASSAY04 | 0.75pg/ml std | DEFAULT | DEFAULT | 4 | 10.000 | Standard | 0.75 |
| 5 | ASSAY05 | 1pg/ml std | DEFAULT | DEFAULT | 5 | 10.000 | Standard | 1 |
| 6 | ASSAY06 | 2pg/ml std | DEFAULT | DEFAULT | 6 | 10.000 | Standard | 2 |
| 7 | ASSAY07 | 5pg/ml std | DEFAULT | DEFAULT | 7 | 10.000 | Standard | 5 |
| 8 | ASSAY08 | 10pg/ml std | DEFAULT | DEFAULT | 8 | 10.000 | Standard | 10 |

# 1. Set up Sample List – Adding Extra Columns




Use the "***Samples / Format / Load*** " menu item and load in a format that already has these fields.




Alternatively, you can 'right click' on the sample list and use the "***Customize Display"*** item on the 'pop-up' menu and add these columns.

# 1. Review of Sample Types

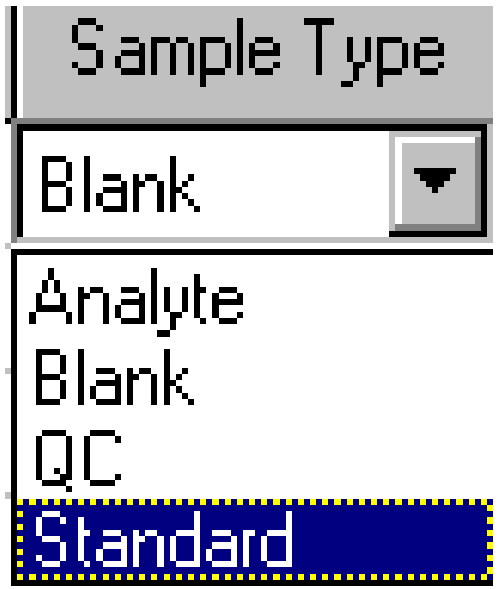


| | |
|---|---|
| Blank - | Solvent or matrix, insures that system is clean and/or shows endogenous material in sample. |
| Standard - | Sample of a known concentration, used to form calibration curve. |
| Analyte - | Sample of unknown concentration. |
| QC - | Quality Control - Known concentrations, used to test the validity and accuracy of the calibration curve. |

# 1. Specify Sample Types and Concentrations



- Pull Down menu within the sample list. Specify whether the sample is a Blank, Standard, Analyte or QC.




Alternatively, just type the first letter of the sample type (example for ‘Analyte’ type ‘A’) and hit enter.

Concentration A or (B, C…)

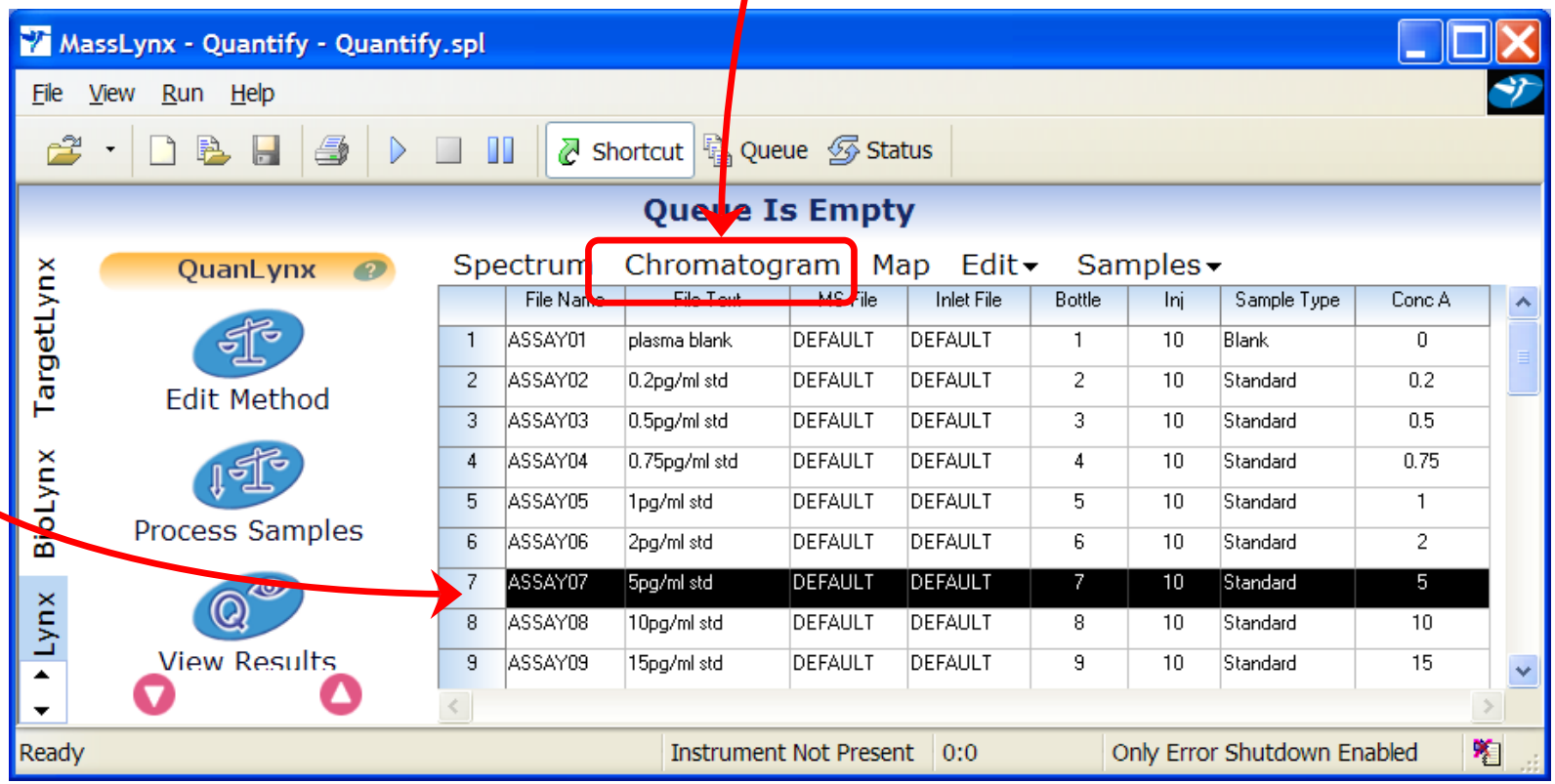
- The known concentrations of Standards or QC’s must be entered into this column.

| Conc A |
|---|
| |
| 0.2 |
| 0.5 |
| 0.75 |
| 1 |
| 2 |
| 5 |
| 10 |
| 15 |
| 0.3 |
| 2 |
| 12 |

# 2. Determine Correct Integration Parameters for Chromatogram Peaks



- Go to the Sample List and highlight a Standard in the middle of the concentration range.
- Click on the Chromatogram button.



# 2. Peak Integration-Display All Traces

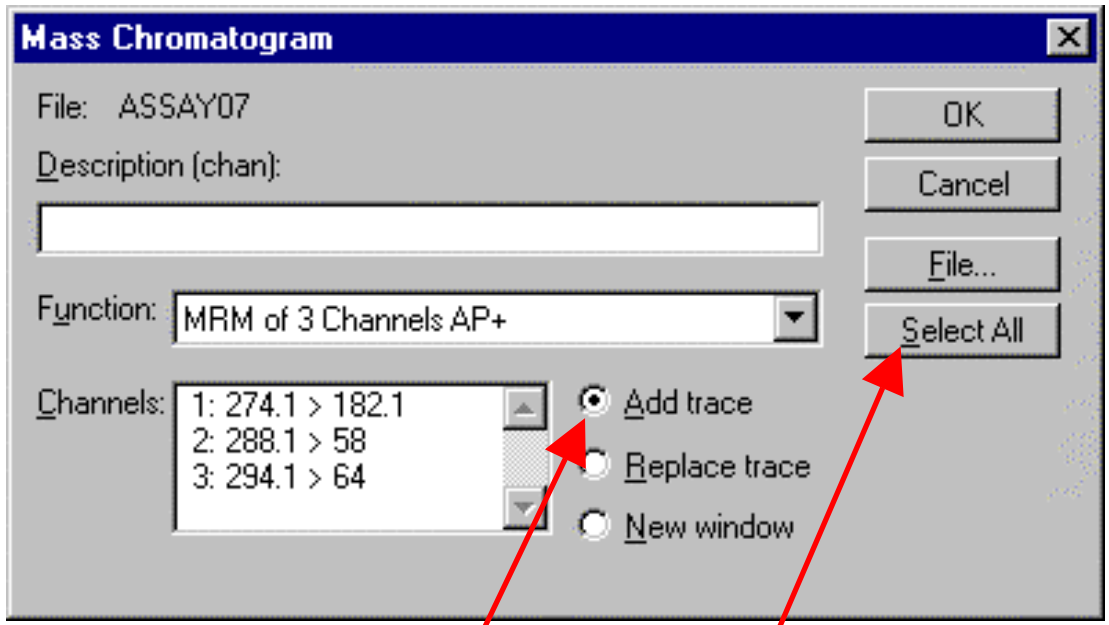


- The TIC for the highlighted sample will be brought up.
- Click ***Display, Mass*** from the top of the chromatogram window.

click ***Add Trace*** and ***Select All***

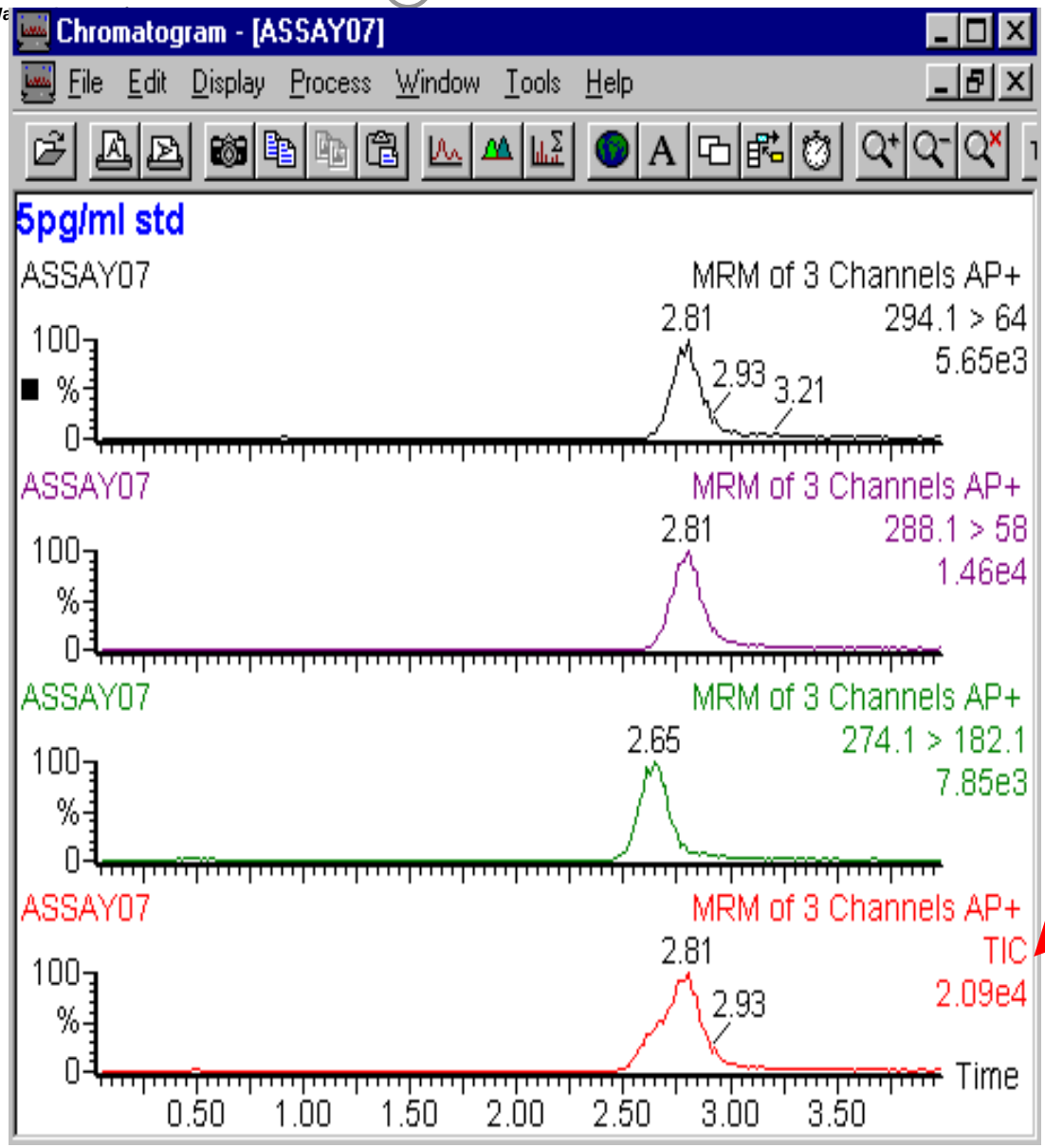
to bring up all

of the transitions.



# 2. Three Ion Chromatograms Should Now Be Shown






- Each individual transition will now be displayed.
- Delete the TIC to simplify the screen. (If the TIC is still displayed)

# 2. Setup Peak Integration-Noise



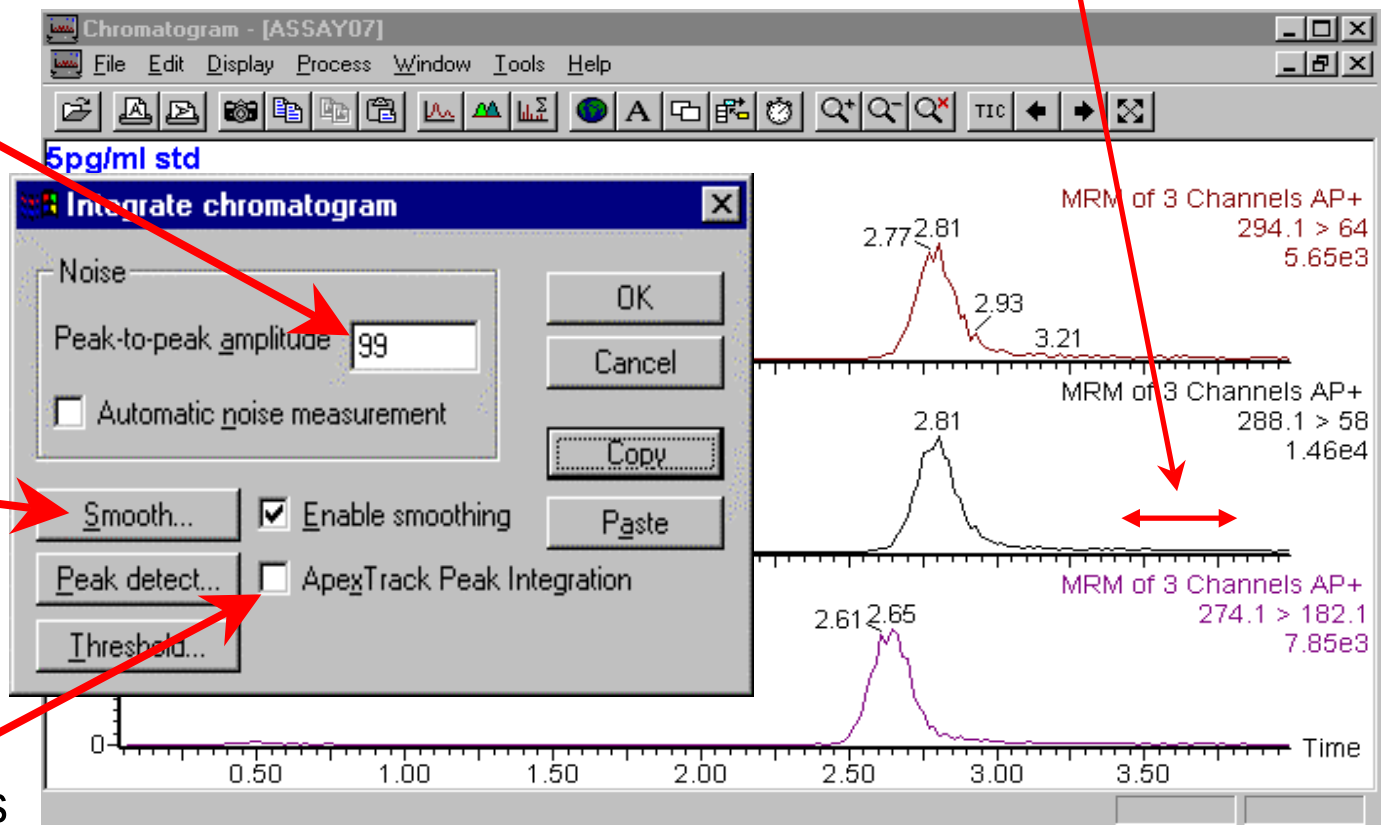
To setup the Integration use the (***Process, Integrate***) menu item to get the 'Integrate Chromatograms' dialog box. First determine the baseline noise by grabbing some noise (right click and drag) over a quieter area of the chromatogram .

(for example)

Peak to Peak Amplitude of Noise Will Be Filled In

Next Click on Smooth

Note Apex Track Peak Integration is Available

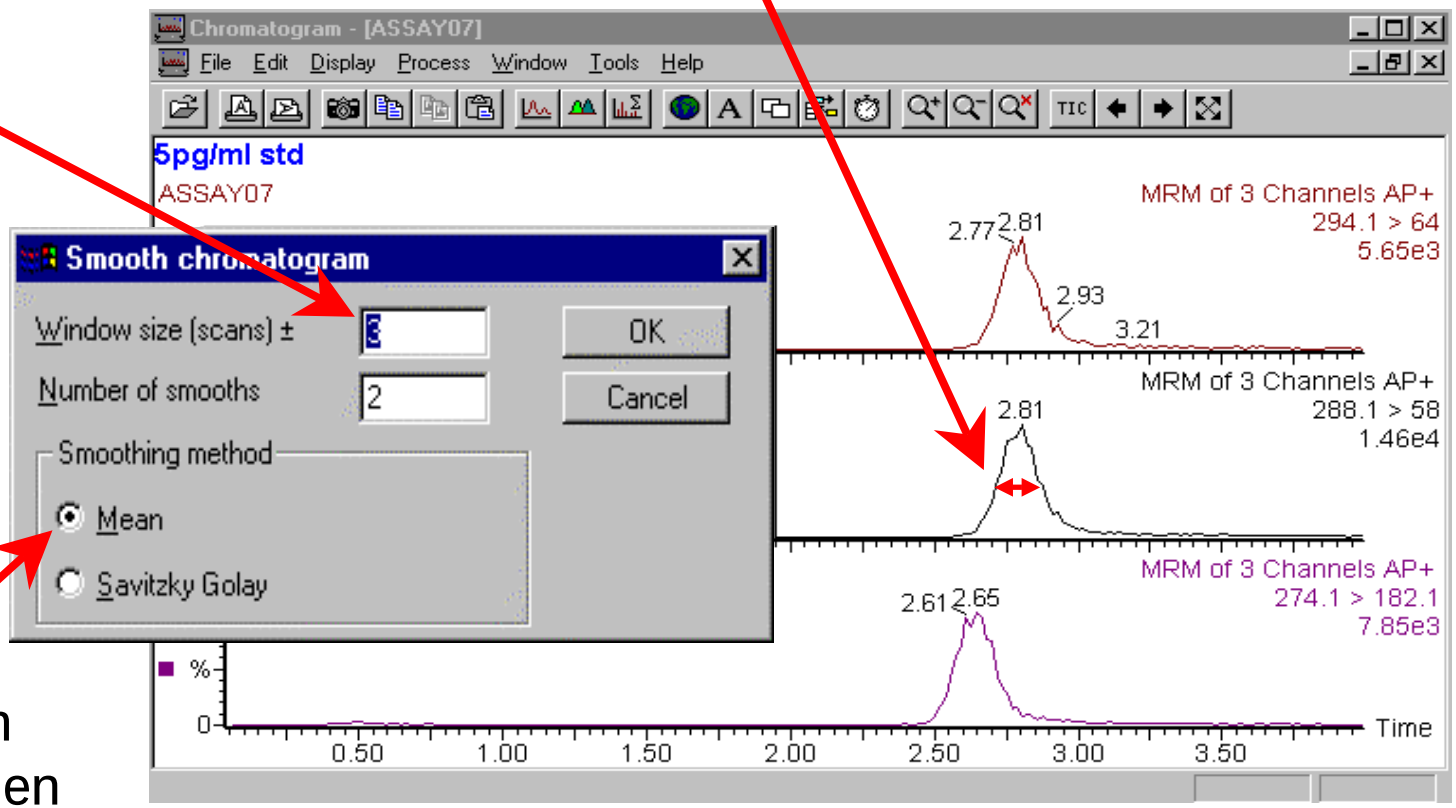


# 2. Setup Peak Integration-Smoothing



Continue setting up Integration Process: After Clicking on ***Smooth***, Right click and drag over the peak at half height.

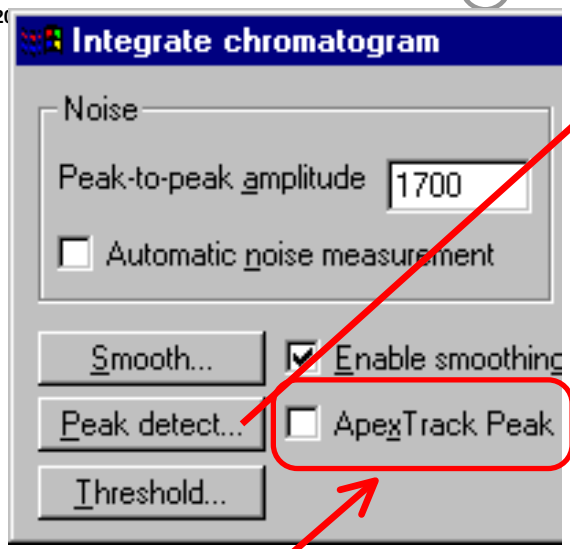
Window Size Will Be Filled In



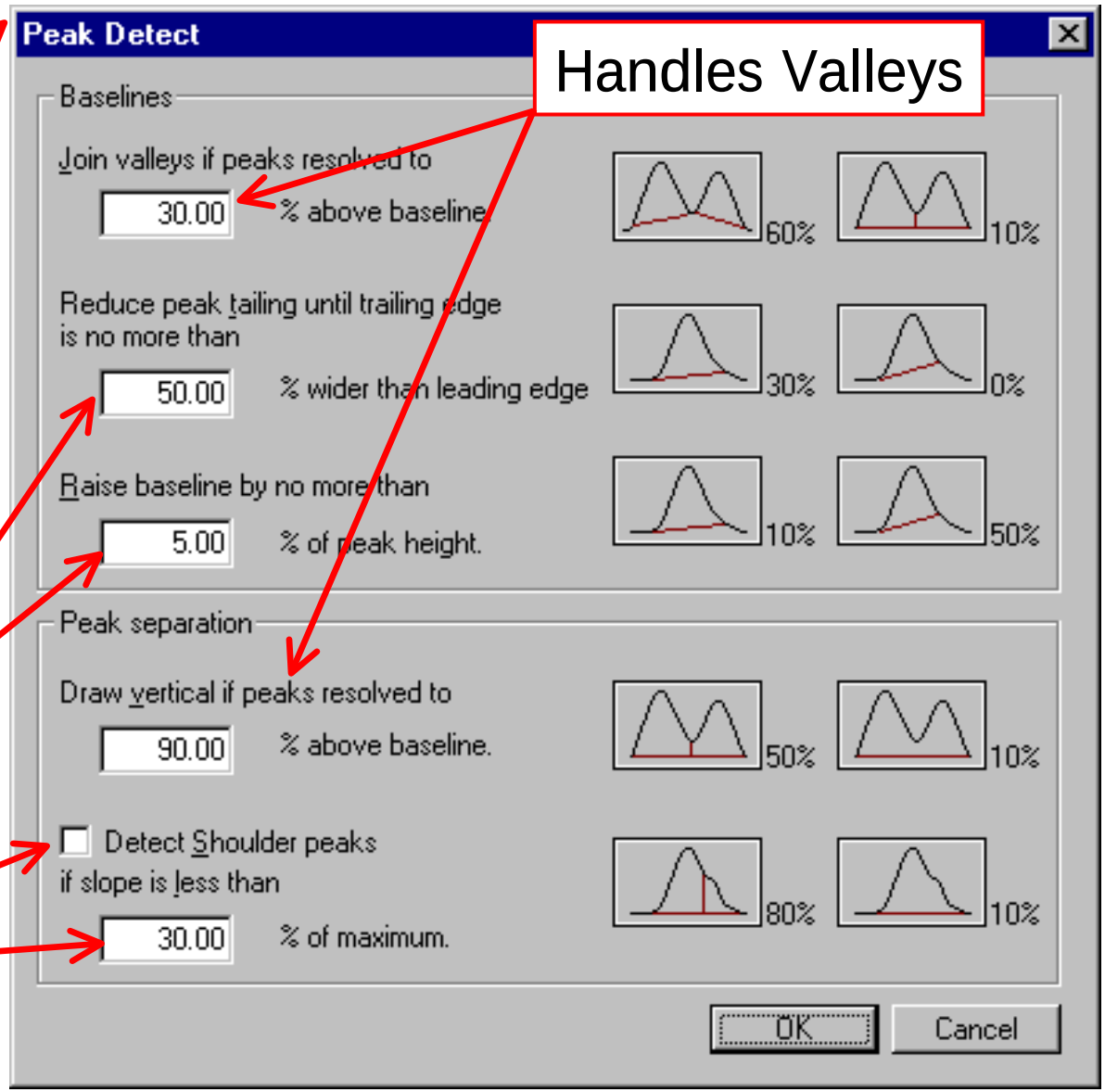

Use Mean Method When Smoothing Chromatograms

Remember the correct window size

# 2. Setup Peak Integration – Peak Detect




Without Apex Peak Integration

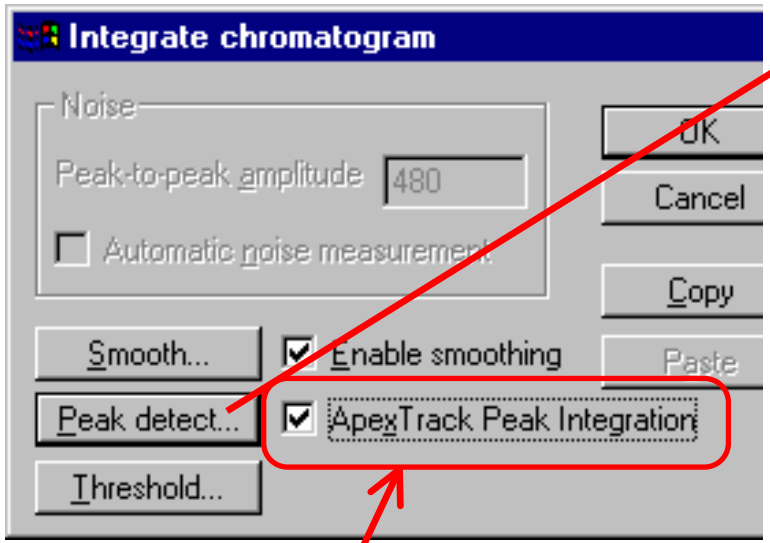



Setups Baseline

Shoulder

# 2. Setup Peak Integration – Peak Detection with Apex Integration

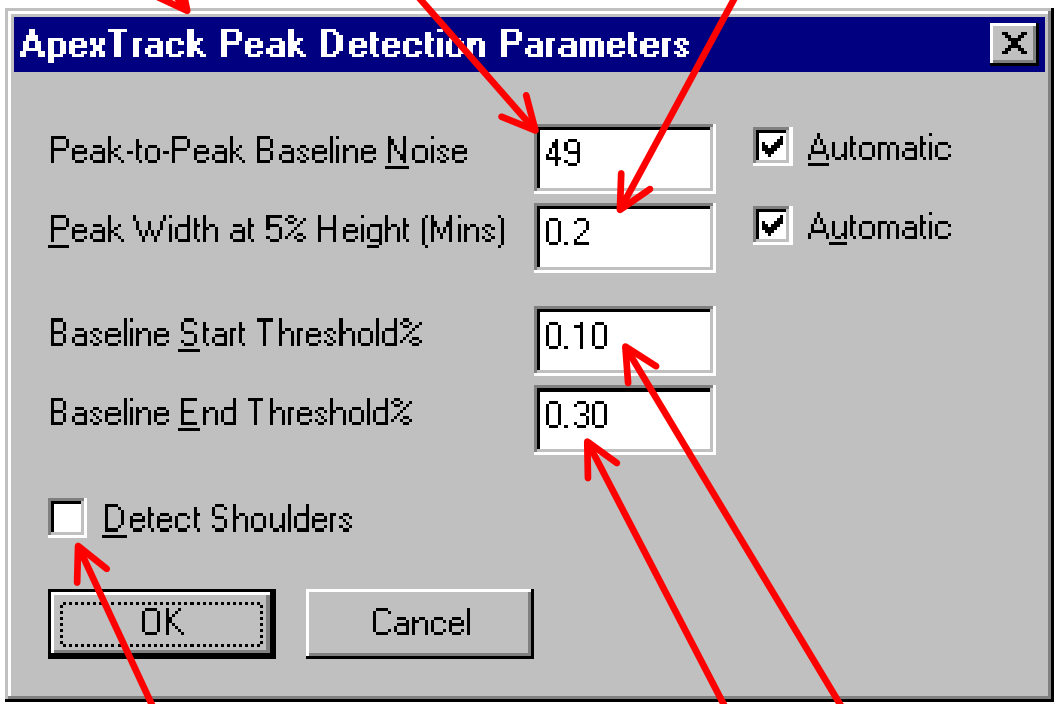





If Apex Peak Integration is selected, 'noise' is handled in the peak detect setup. Noise and Peak width can be entered or you can have them calculated for you.

Noise

Peak Width

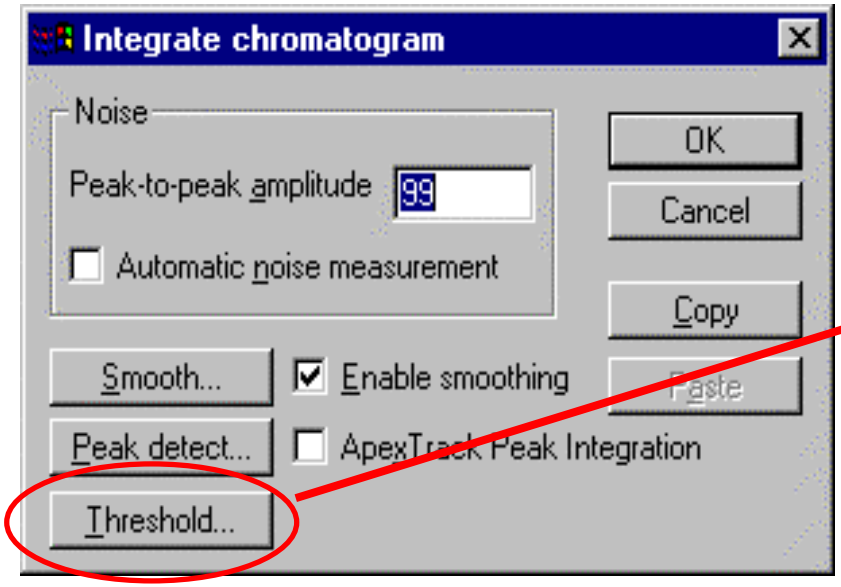


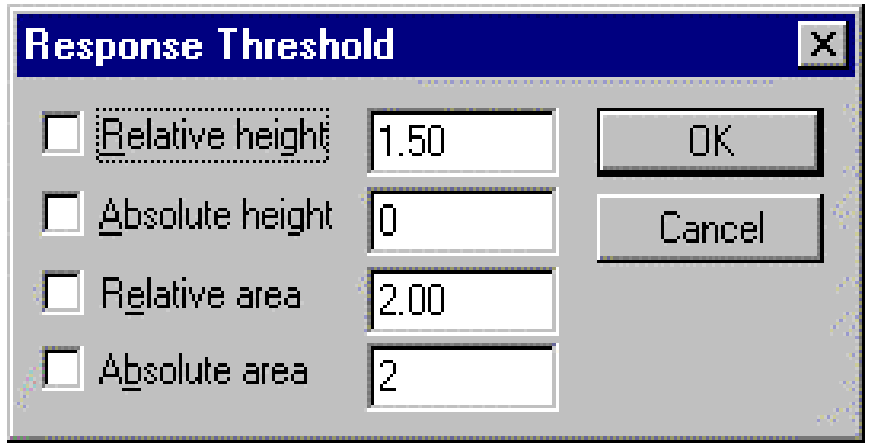

'Check' if you want Shoulders detected

Set starting and ending Baseline levels

# 2. Quant Method Editor – Integration Parameters






Peak Threshold parameters can also be adjusted in the Method Editor.
Specify Criteria to Discriminate Peaks from Noise.

For ‘Relative’ values, enter the percentage of the largest peak (base peak) a peak must exceed to be integrated.
For example, check ‘Abs area’ and enter 20% of the peak area for your lowest standard. Any peak with an area lower than this will be considered noise and not integrated.

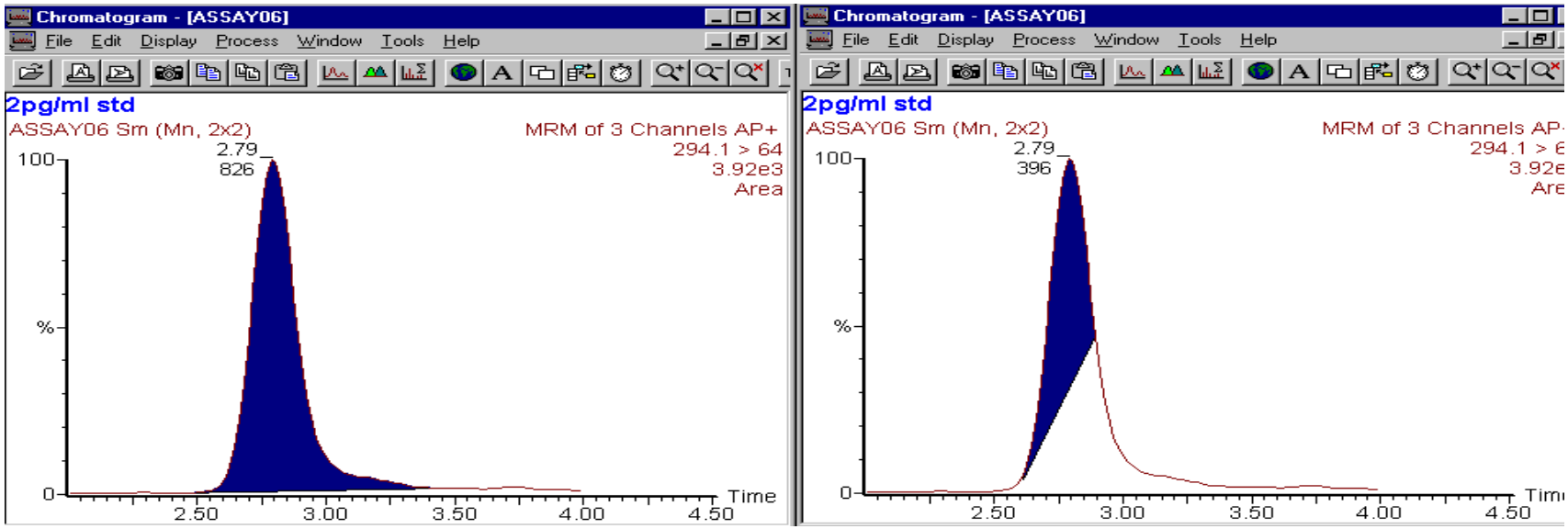
# 2. Setup Peak Integration Parameters



- Click ***OK***, the peak of interest will be integrated.
- Review the integration - is it acceptable? If not, repeat the integration with different parameters (noise, peak detect, thresholding) until satisfactory results are obtained.
- Once an acceptable integration is attained, you may want to test it on a low range standard and a high range standard to insure that parameters are adequate for the full range of response.

# 2. Review Peak Integration





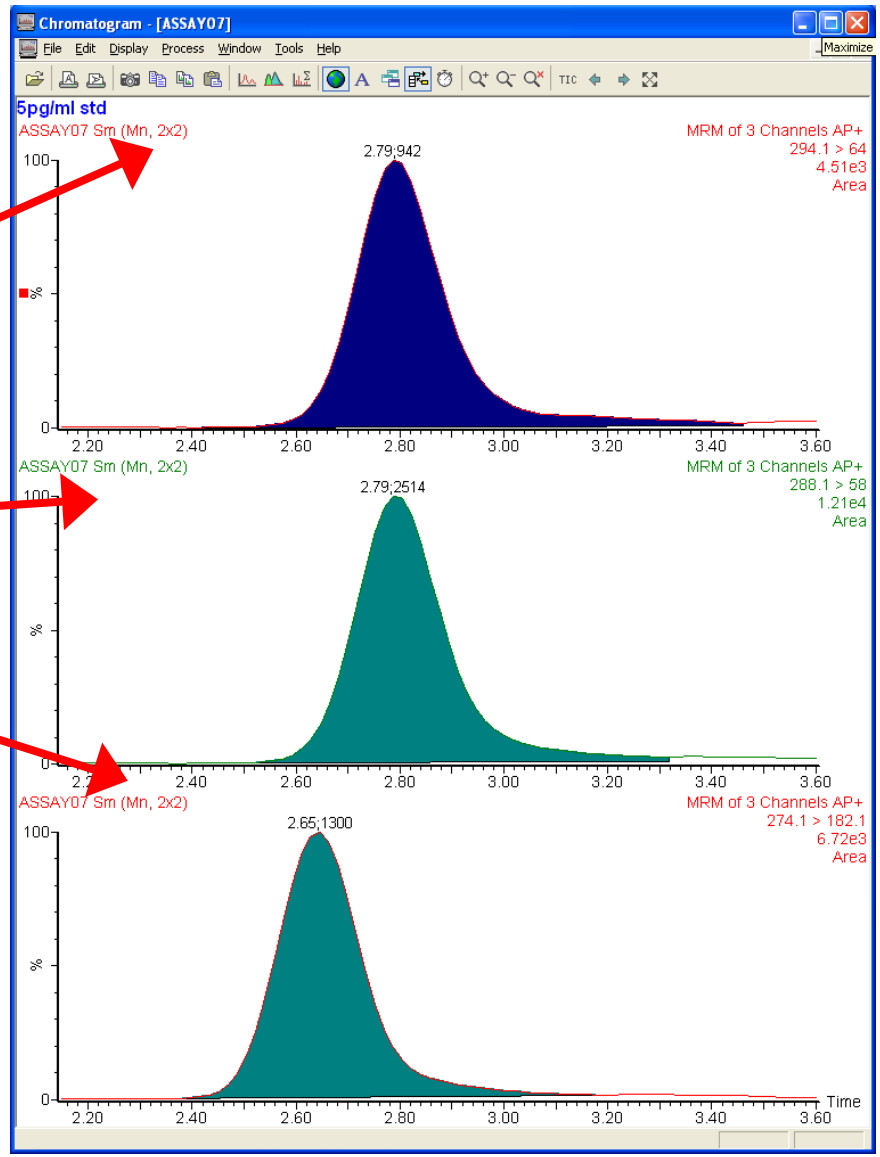

Here's an example of a well integrated peak and a poorly integrated peak.

# 2. Review Peak Integration- Example



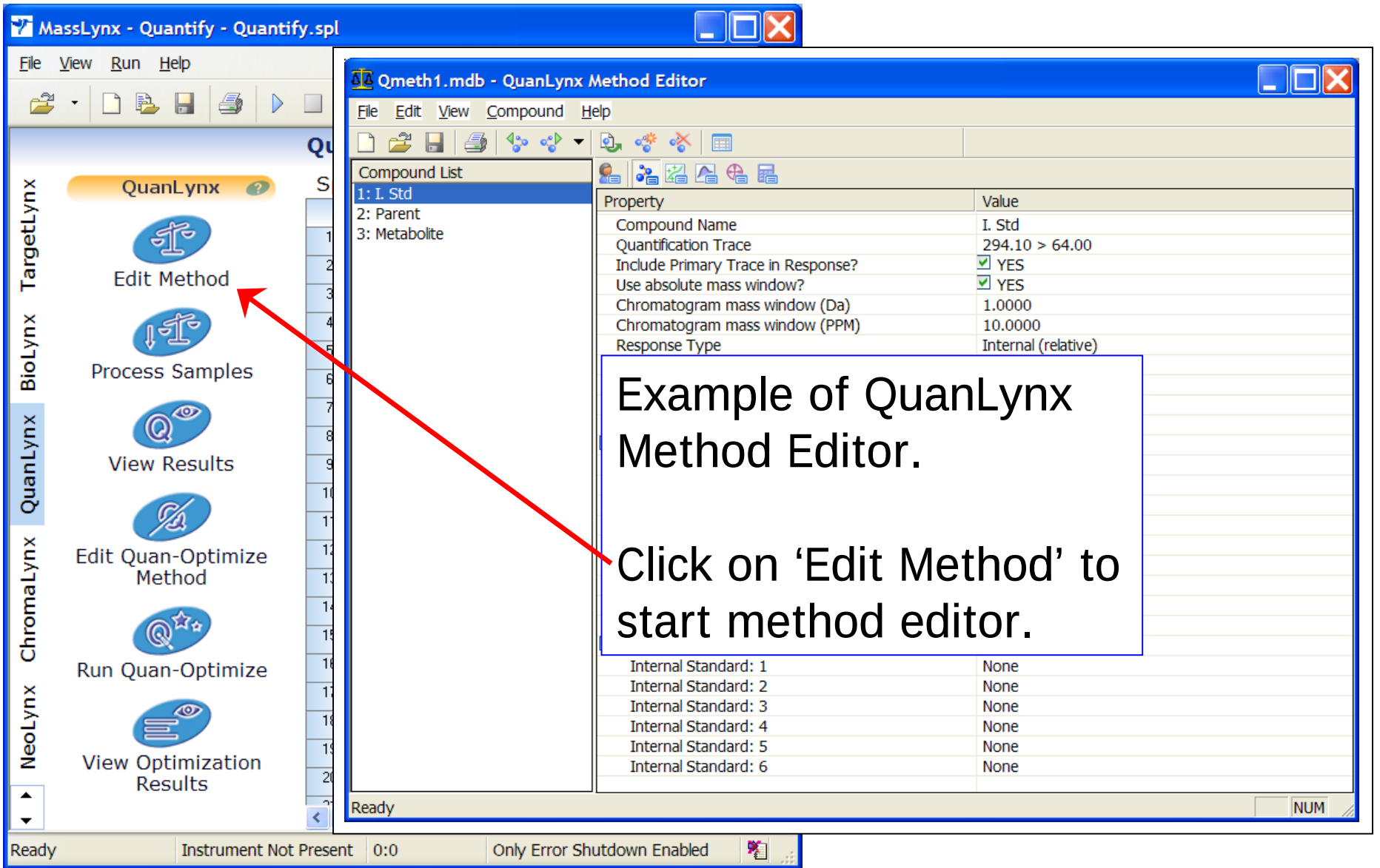
Example of Peak Integration for Quantify Project

Note Smoothing Parameters for each transition



# 3. Build Quantitation Method



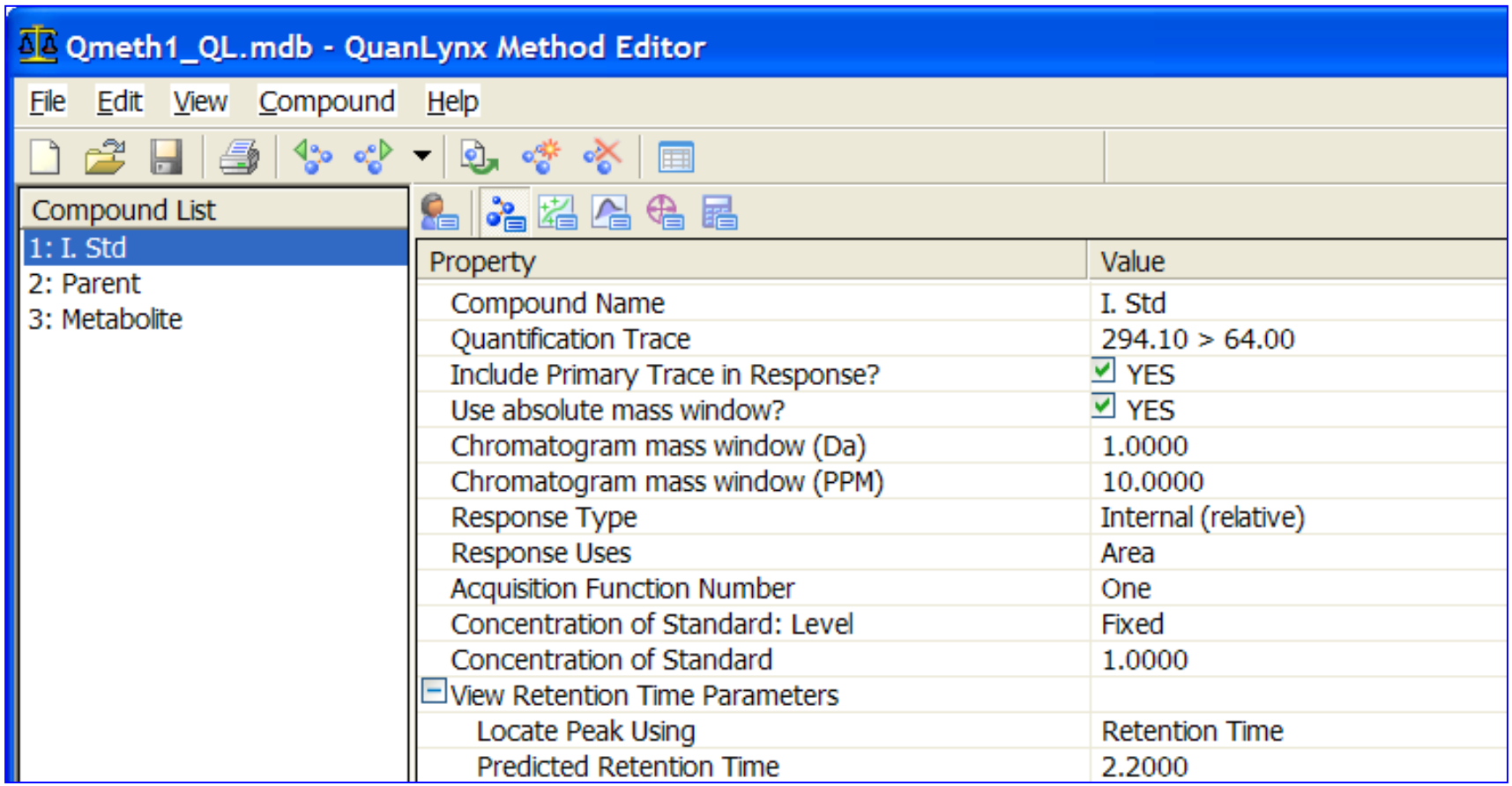


# 3. Build Quantitation Method



The different compounds in the assay are listed on the left.

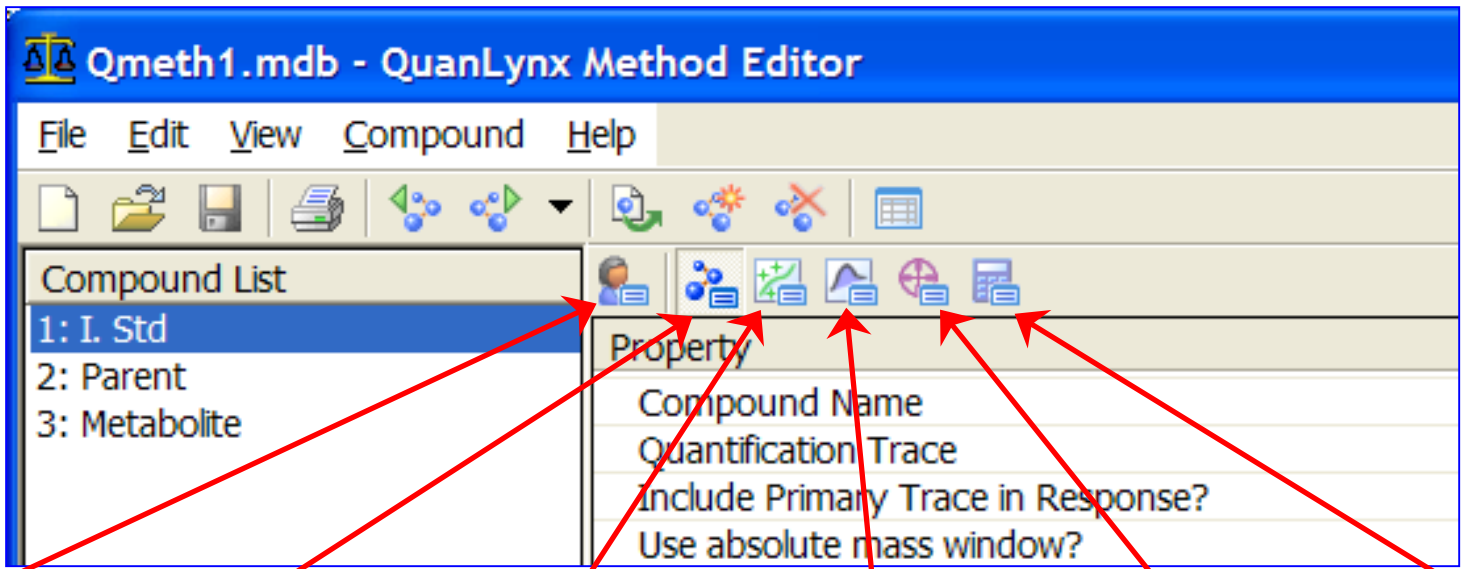
Click on a compound in the list and the parameters that describe how to quantitate that compound are listed on the right.



# 3. Build Quantitation Method



Use the buttons on the tool bar to decide which parameters for a compound you wish to view. 'Click' on the button on the left to display all of the parameters for a compound. 'Click' on one of the other buttons to display only a subset of the parameters.




All Params (Display all params)

Compound Params (e.g.Name, m/z Trace)

Calculation Factors (Line fitting params)

Integration Params (e.g. smoothing, peak detect)

Target Ion Params (confirmatory ions)

Calculation Factors (e.g. S/N)

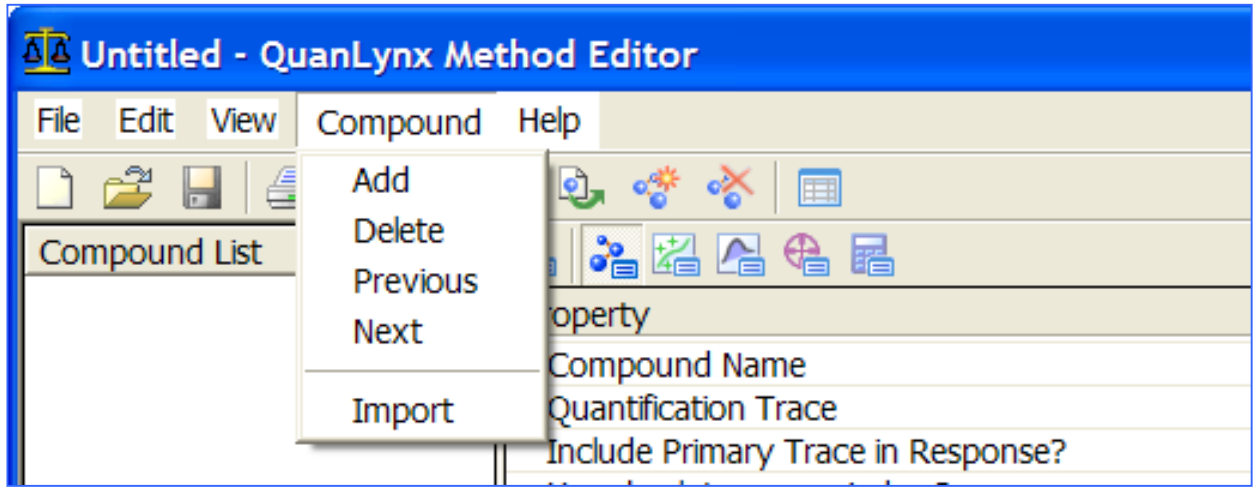
# 3. Build Quantitation Method



For this example, use the ‘File/New’ menu item and create a new method.

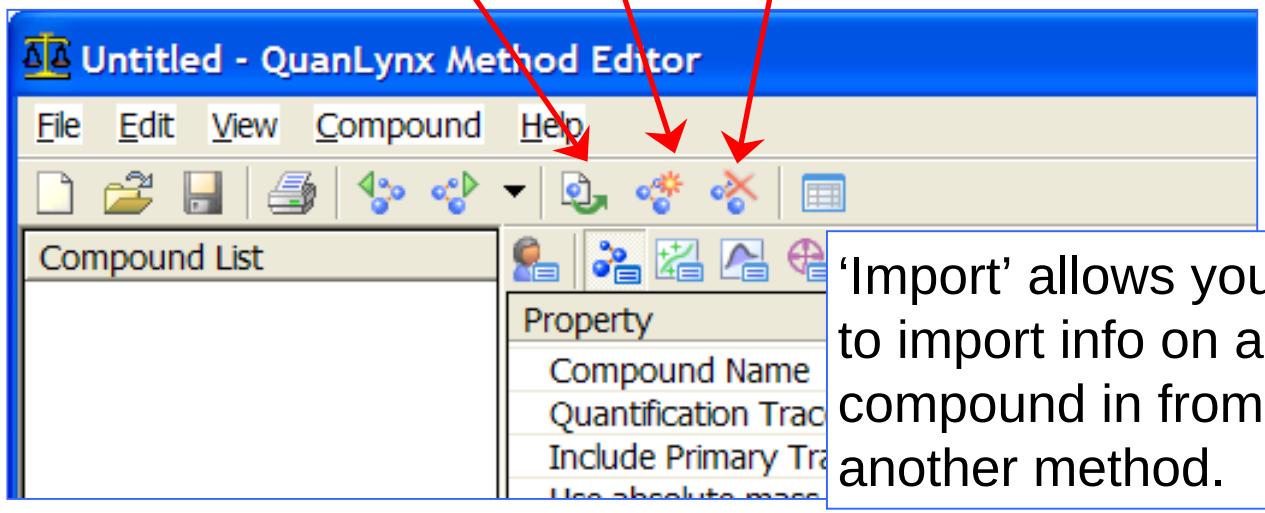
‘Click’ on the ‘Compound Properties’ button to display the name and ‘trace’ fields.

To add a compound use the drop down menu or use the ‘Add’ button on the tool bar.




Compound Buttons

Import Add Delete

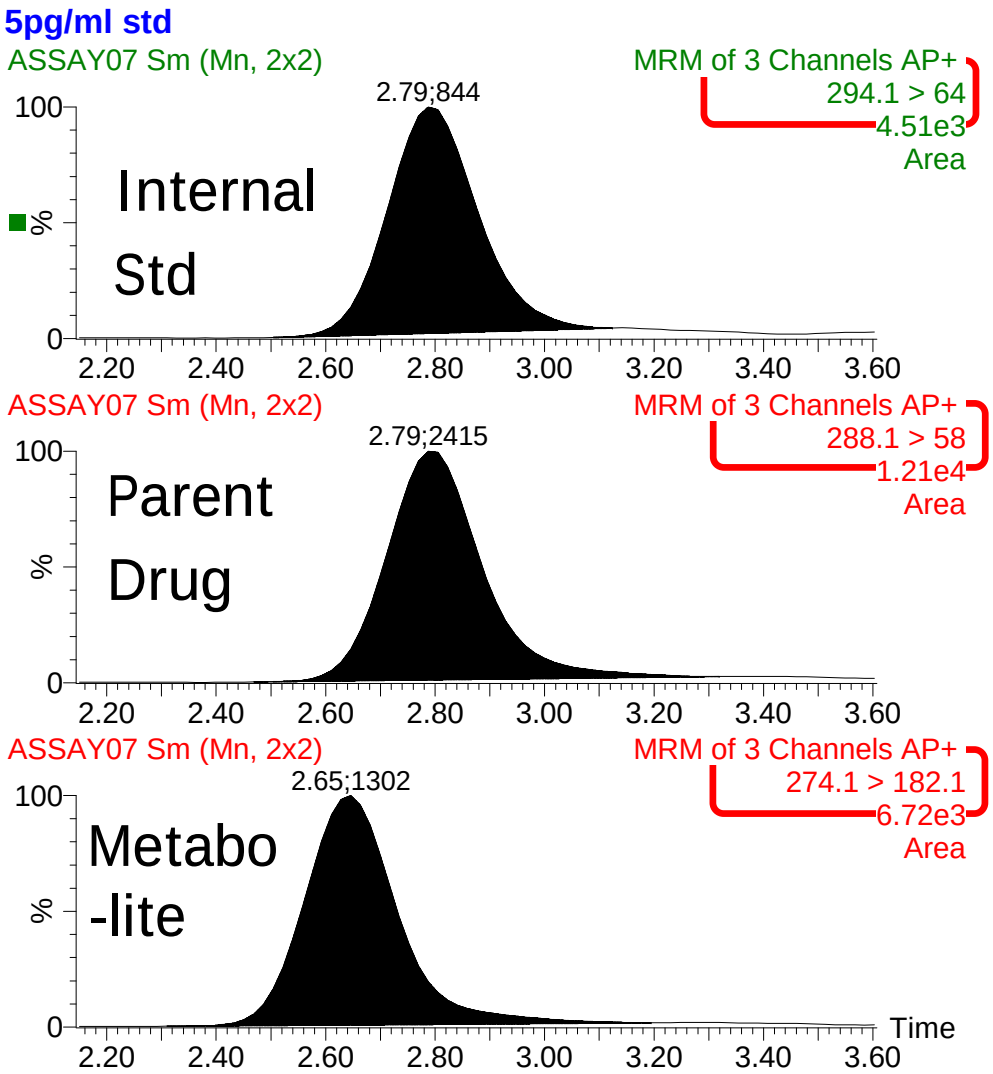



‘Import’ allows you to import info on a compound in from another method.

# 3. Quant Method Editor – Add Info on Compounds



For this example, we are going to first enter quant parameters for the internal standard, next enter quant parameters for the parent drug and take care of the metabolite last.



# Hint. It will be easier if you:

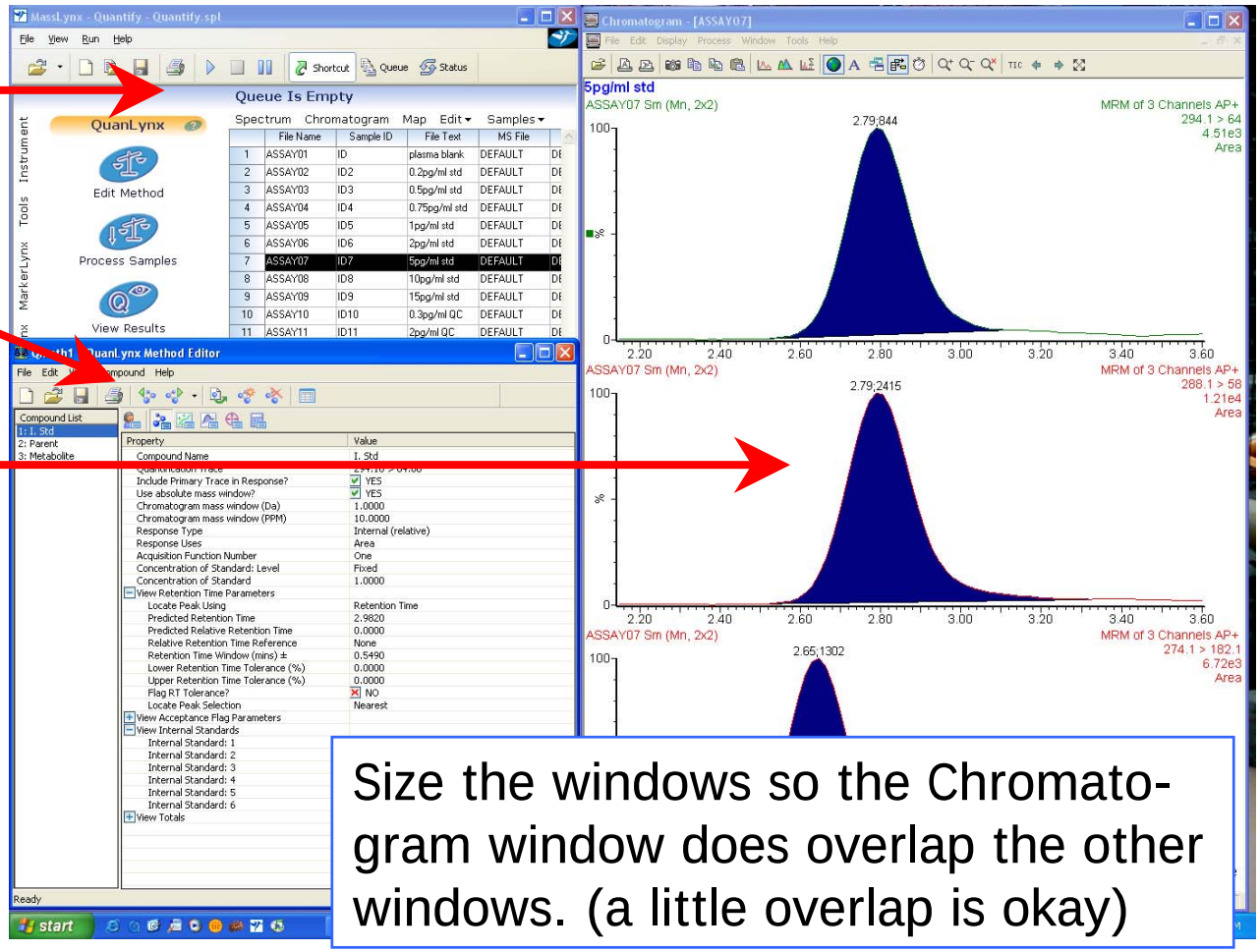


Reduce the size of the Sample List window so this window along with the Method Editor dialog box occupies half of the screen and the chromatogram window occupies the other half of the screen.

Main MassLynx Sample List Window

Method Editor Dialog Box

Chromatogram Window (pick a standard from the middle of the conc. range and display the channels from all of the compounds.)



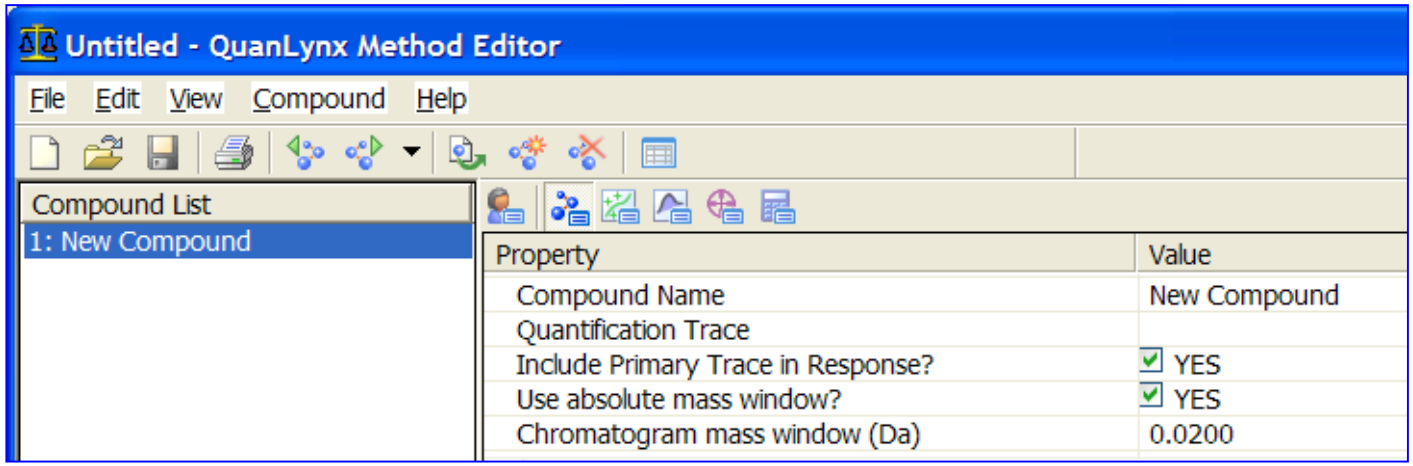

Size the windows so the Chromato-gram window does overlap the other windows. (a little overlap is okay)

# 3. Build Quantitation Method
## Add First Compound to a New method



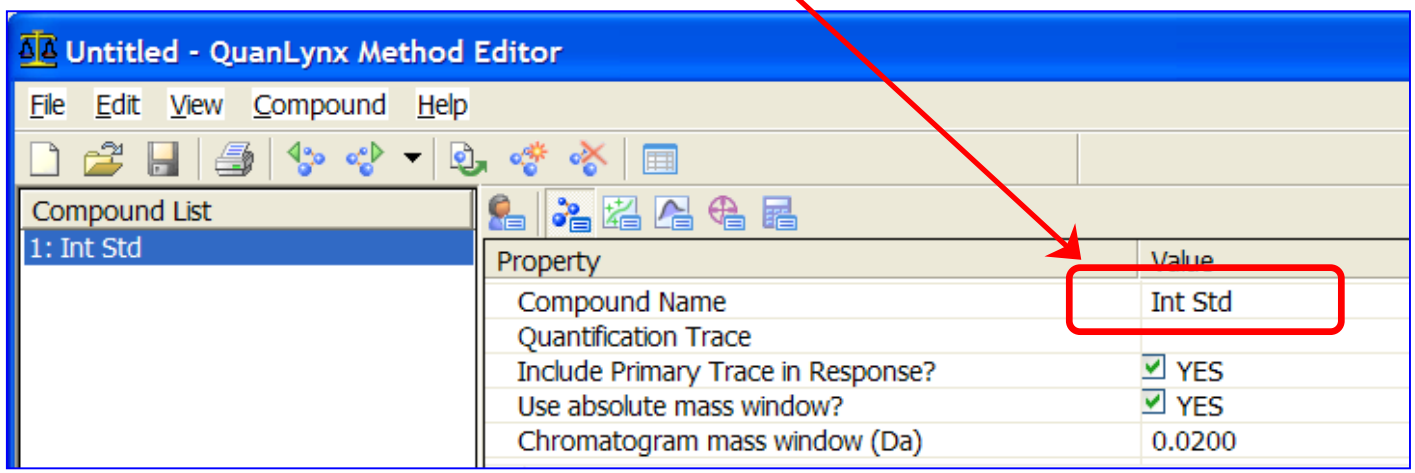
Click on the ‘Compound/ Add’ menu item or the ‘Add’ button to add a compound to this method.

Example of new method after clicking on ‘Add’.



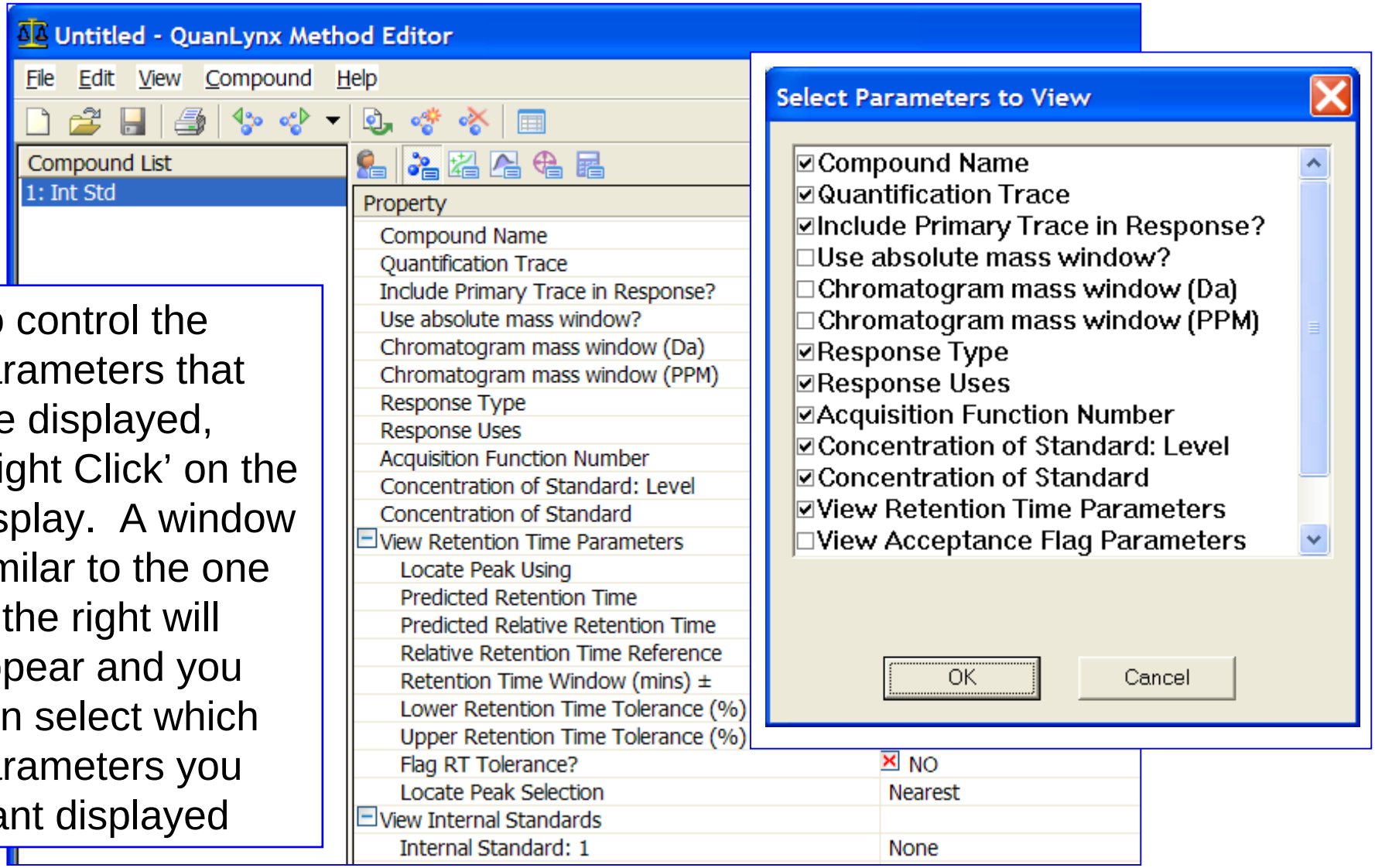

Next type in the name for your compound (we will do the “Int Std” first in this example)

Example after entering name for our compound.



# 3. Build Quantitation Method



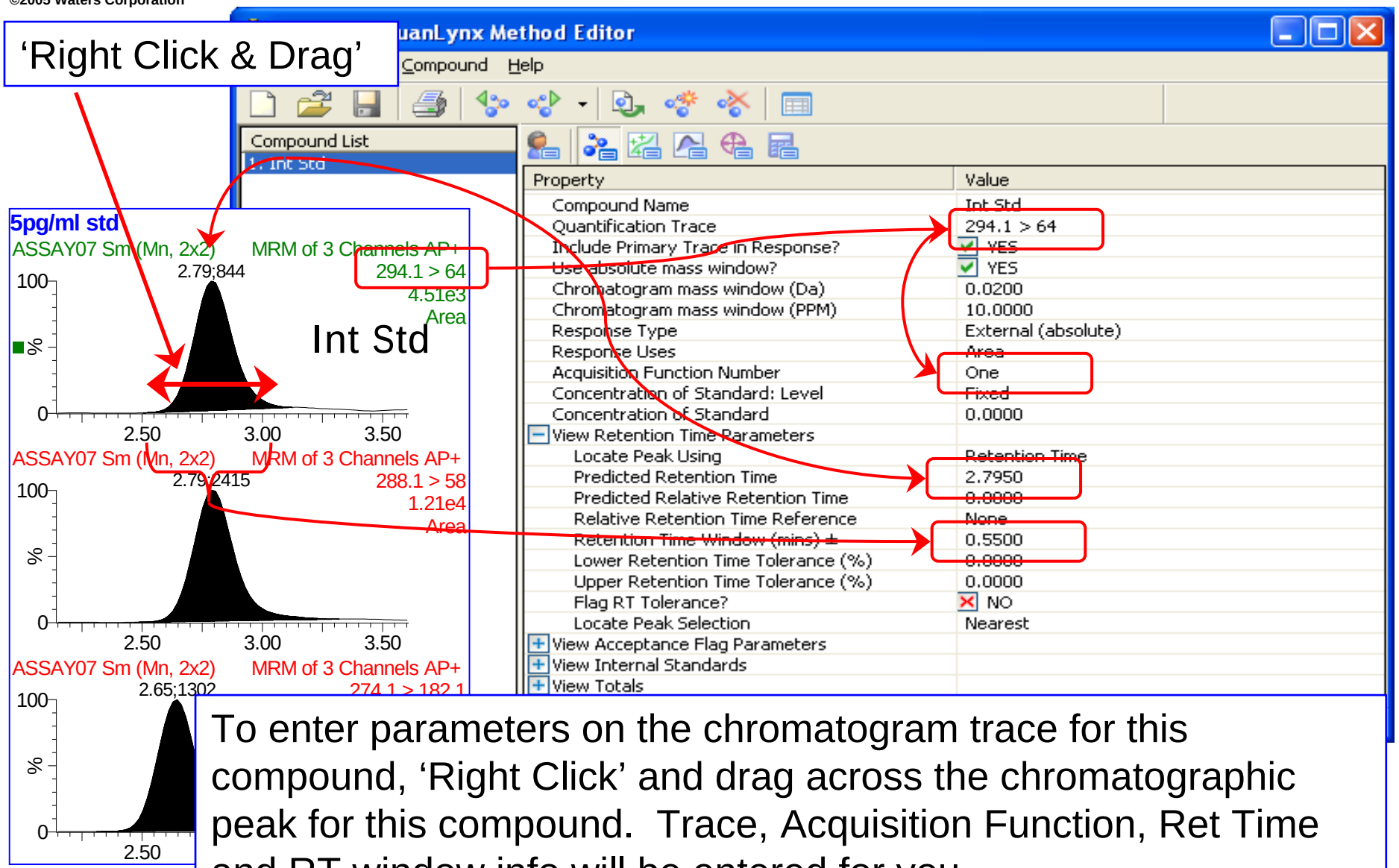



To control the parameters that are displayed, 'Right Click' on the display. A window similar to the one at the right will appear and you can select which parameters you want displayed

# 3. Build Quantitation Method



‘Right Click & Drag’



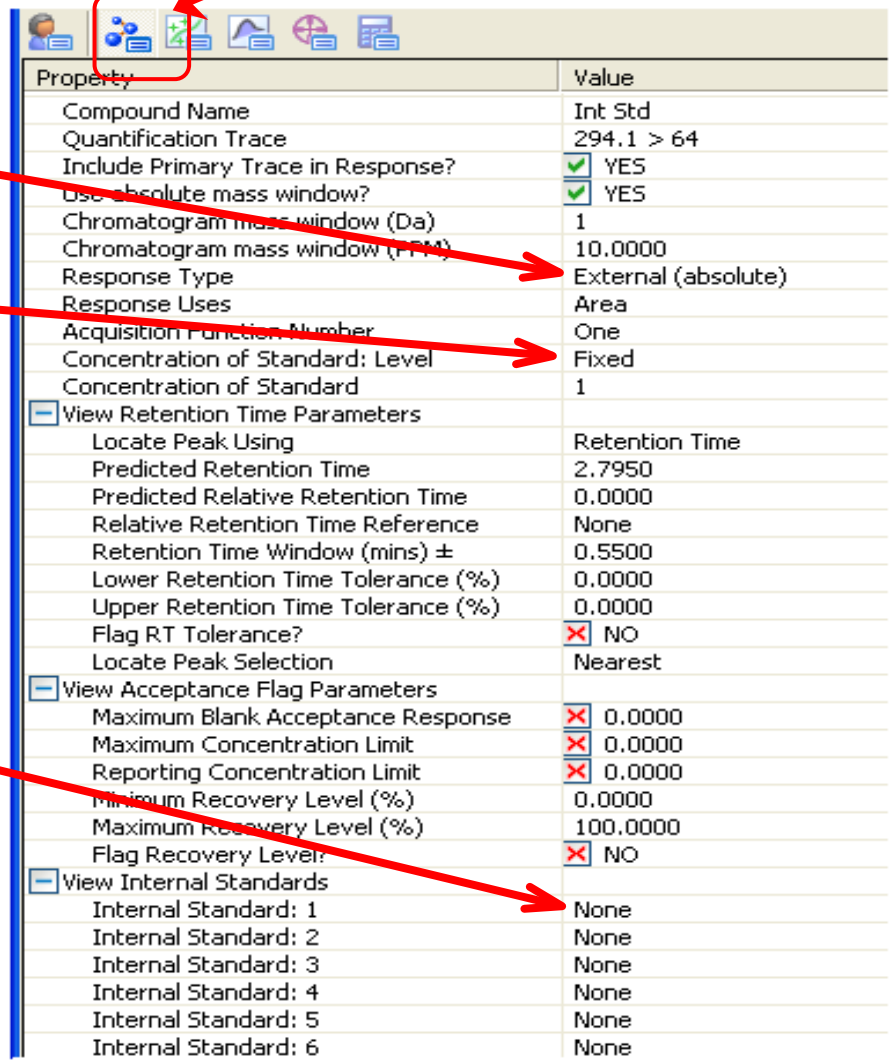

To enter parameters on the chromatogram trace for this compound, ‘Right Click’ and drag across the chromatographic peak for this compound. Trace, Acquisition Function, Ret Time and RT window info will be entered for you.

# 3. Quant Method Editor – Enter Compound Properties (Internal Standard)



- ***Response Type***
  - –Set to “External (absolute)”
- ***Concentration of Standard***
  - –Set Level to “Fixed”
  - – Set Concentration to “1”
- ***Internal Standards***
  - –Set to “None” for the internal standard

Compound Properties

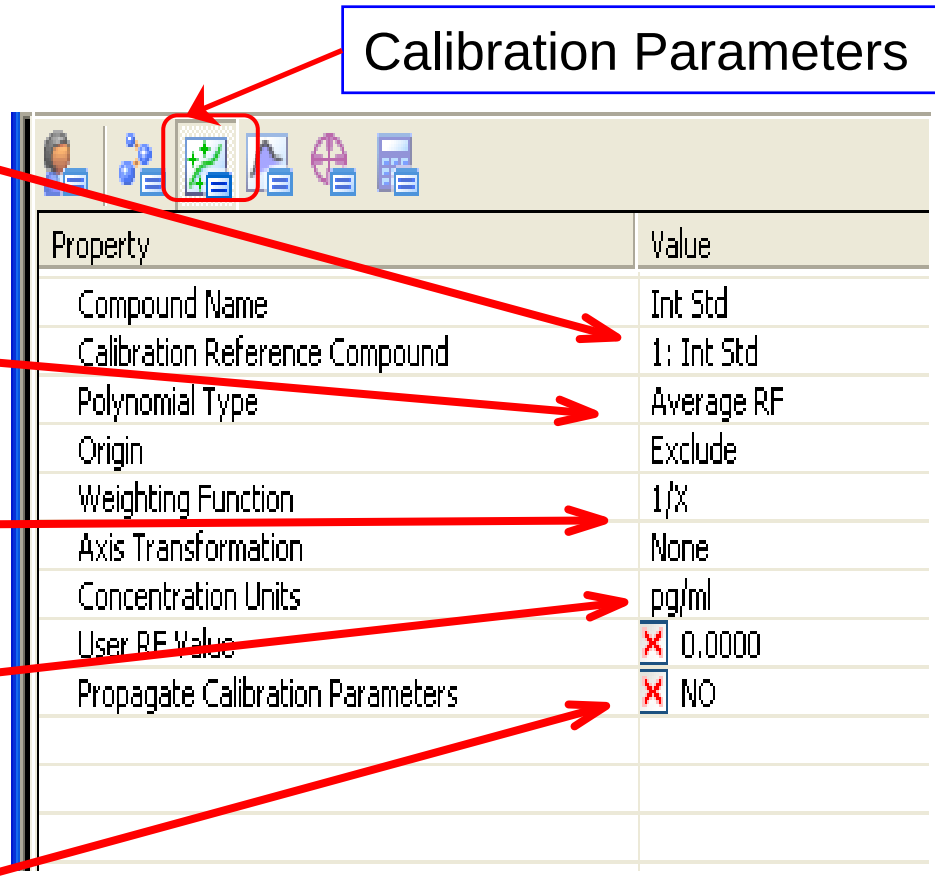


| Property | Value |
|---|---|
| Compound Name | Int Std |
| Quantification Trace | 294.1 > 64 |
| Include Primary Trace in Response? | YES |
| Use absolute mass window? | YES |
| Chromatogram mass window (Da) | 1 |
| Chromatogram mass window (PPM) | 10.0000 |
| Response Type | External (absolute) |
| Response Uses | Area |
| Acquisition Function Number | One |
| Concentration of Standard: Level | Fixed |
| Concentration of Standard | 1 |
| View Retention Time Parameters | |
| Locate Peak Using | Retention Time |
| Predicted Retention Time | 2.7950 |
| Predicted Relative Retention Time | 0.0000 |
| Relative Retention Time Reference | None |
| Retention Time Window (mins) ± | 0.5500 |
| Lower Retention Time Tolerance (%) | 0.0000 |
| Upper Retention Time Tolerance (%) | 0.0000 |
| Flag RT Tolerance? | NO |
| Locate Peak Selection | Nearest |
| View Acceptance Flag Parameters | |
| Maximum Blank Acceptance Response | 0.0000 |
| Maximum Concentration Limit | 0.0000 |
| Reporting Concentration Limit | 0.0000 |
| Minimum Recovery Level (%) | 0.0000 |
| Maximum Recovery Level (%) | 100.0000 |
| Flag Recovery Level? | NO |
| View Internal Standards | |
| Internal Standard: 1 | None |
| Internal Standard: 2 | None |
| Internal Standard: 3 | None |
| Internal Standard: 4 | None |
| Internal Standard: 5 | None |
| Internal Standard: 6 | None |

# 3. Quant Method Editor – Enter Calibration Parameters (Internal Standard)



- ***Calibration Reference Compound***
  - –Select the reference compound to match the current compound
- ***Polynomial Type***
  - –Select “Average RF”
- ***Origin, Weighting Function, Axis Transformation***
  - –These settings are not applied if the Polynomial Type is “Average RF” (which is the case for the internal standard)
- ***Concentration Units***
  - –Type concentration unit of internal Standard (in this example it is pg/ml)
- ***Propagate Calibration Parameters***
  - –When using an Internal Standard, the Propagate function is disabled
  - –Click on the box until a red “X” appears. The Value should change to “No”

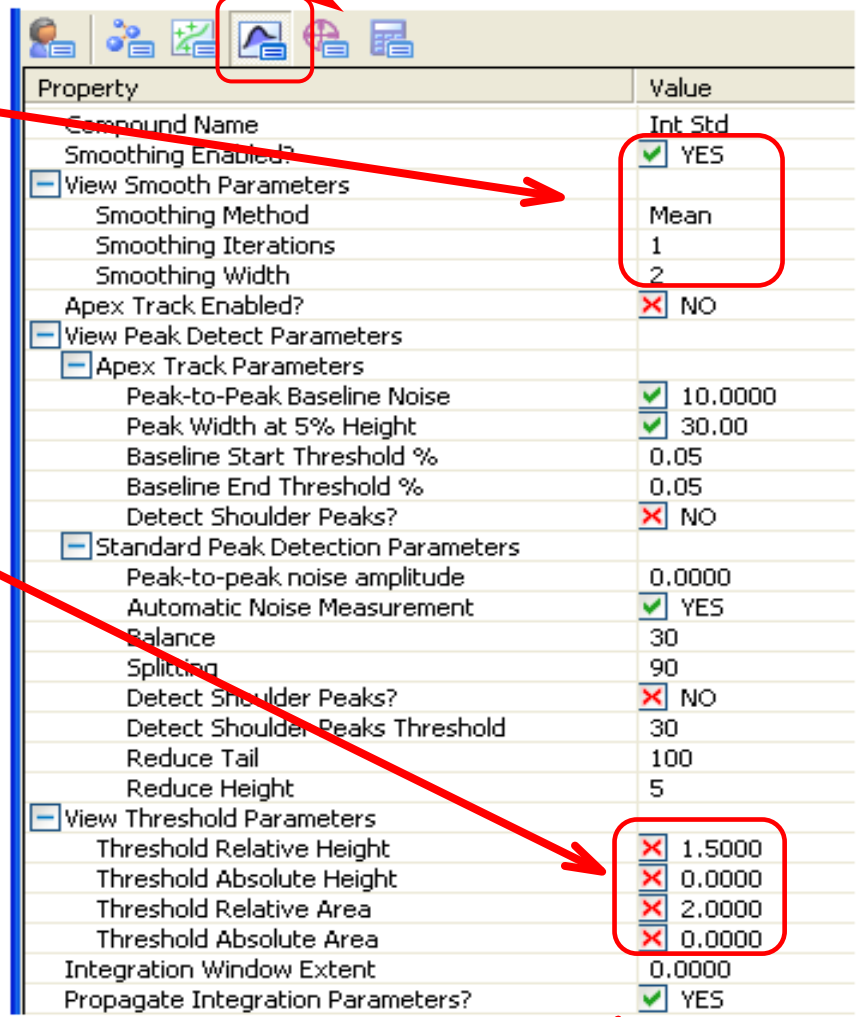


# 3. Quant Method Editor – Enter Integration Properties (Internal Standard and Analyte)



- ***Smoothing***
  - –To apply smoothing, click on the box until a green “Check” appears. The Value should change to “Yes”
  - –Select the appropriate Smoothing Method, and enter the Smoothing Iterations and Width determined from the Peak Integration performed in the Chromatogram Window
- ***Threshold Parameters***
  - –To apply threshold, click on the box next to the appropriate threshold parameter until a green “Check” appears. Enter the threshold value to be applied
- ***Propagate Integration Parameters***
  - –Under most conditions, the integration parameters can be propagated for all compounds
  - –To Propagate, click on the box until a green “Check” appears. The Value should change to “Yes”

Integration Parameters

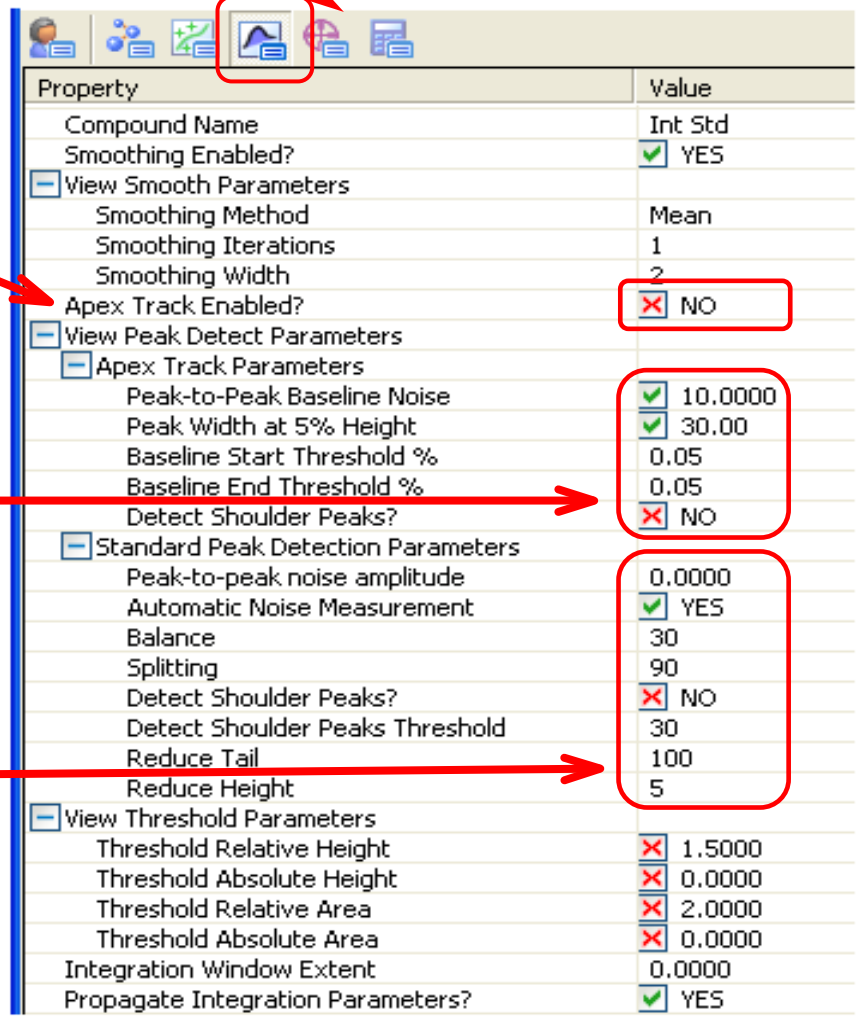


# 3. Quant Method Editor – Enter Integration Properties (Internal Standard and Analyte)



- ***Peak Detect Parameters***
  - –To enable Apex Track, click on the box until a green "Check" appears. The Value should change to "Yes" (In this example Apex Track is disabled)
  - – Apex Track Parameters: Typical setting are shown. These parameters are applied only when Apex Track is enabled
  - – Standard Peak Detection Parameters: Typical settings are shown. These parameters are applied when Apex Track is disabled

Integration Parameters

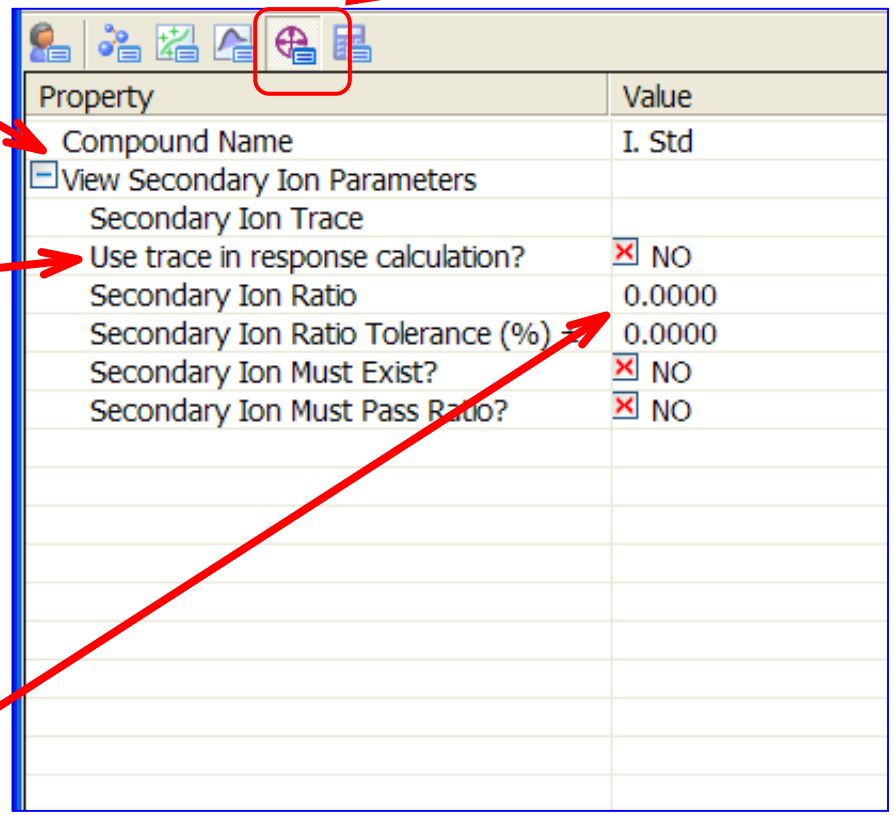


# 3. Quant Method Editor – Enter Target (Confirmatory) Ion Parameters



- ***Secondary Ion Parameters***
  - –Secondary Ion Trace: If a secondary ion is being used, then the transition is entered (in this example there is no Secondary Ion Trace)
  - –Use trace in response calculation: If enabled, then the area of the secondary trace is added to the area of the primary trace when calculating the response. To enable, click on the box until the green “Check” appears. The value should change to “Yes”
  - –Secondary Ion Ratio: Enter the ratio of the secondary ion to the primary ion. This can be calculated from the chromatogram window

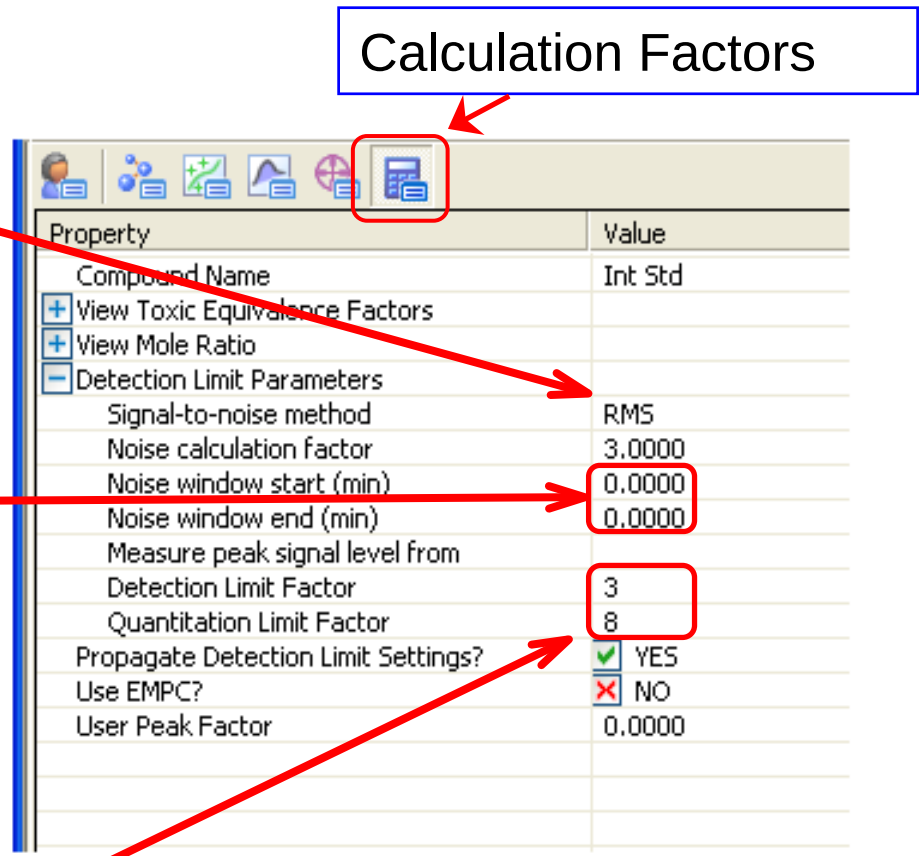
Target Ion Parameters



# 3. Quant Method Editor – Enter Calculation Factors



- ***Detection Limit Parameters***
  - –Signal-to-noise method: If RMS is used, then Noise calculation factor should be “3”; If Peak-to-peak is used, then Noise calculation factor should be “1”
  - – Noise window: Specify portion in chromatogram from which noise will be calculated. If both fields are set to “0.0000”, then the noise window will be determined automatically by the software
  - – Detection/Quantification Limit Factor: User-defined parameters. Typical Values are shown



Waters

The Quantify Trace is the trace descriptor of the chromatogram being used to quantify the compound.

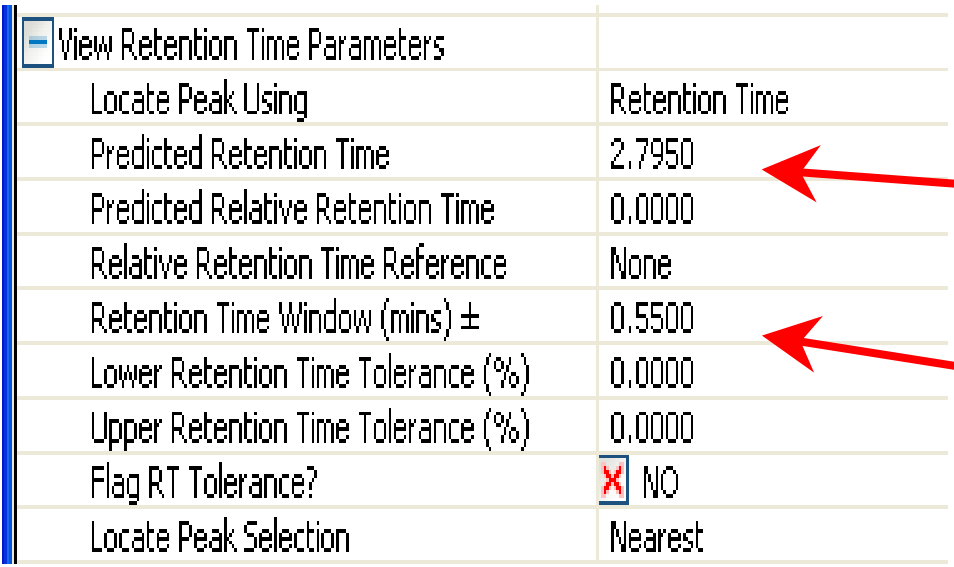


This can be:

- A single decimal number (m/z) for mass chromatograms (from SIR or Full Scan (continuum or centroid))
- Two decimal numbers separated by a ">" for an MRM function e.g. 609.2 > 195.1
- 'TIC' for total ion current chromatograms
- 'BPI' for base peak intensity chromatograms
- An1, An2, An3, or An4 for analog data
- The wavelength for DAD data.
- Ch1, Ch2 etc for SIR data to use one quantify method with multiple SIR functions. Where Ch1 is the first mass in the list, Ch2 is the second etc.

# 3. Quant Method – More on Trace & Peak Info





| View Retention Time Parameters | |
|---|---|
| Locate Peak Using | Retention Time |
| Predicted Retention Time | 2.7950 |
| Predicted Relative Retention Time | 0.0000 |
| Relative Retention Time Reference | None |
| Retention Time Window (mins) ± | 0.5500 |
| Lower Retention Time Tolerance (%) | 0.0000 |
| Upper Retention Time Tolerance (%) | 0.0000 |
| Flag RT Tolerance? | NO |
| Locate Peak Selection | Nearest |

*Peak Location and Time Window*
Parameters can be entered from the keyboard if needed.

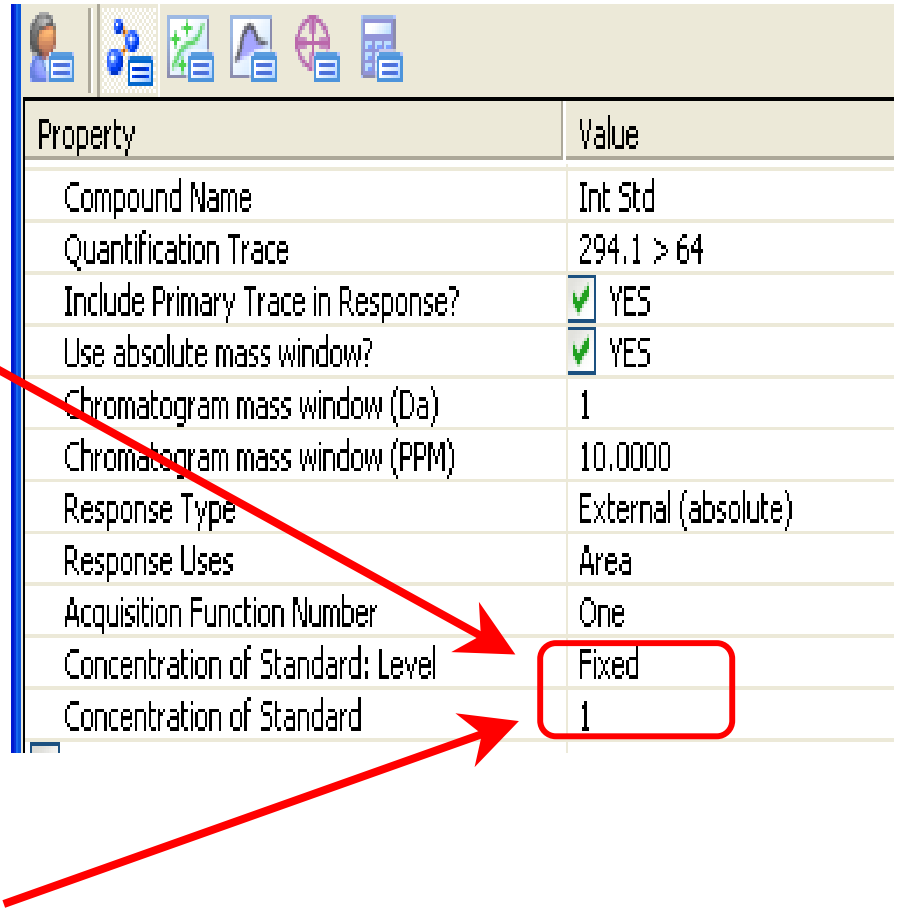
- ***Peak Location: Retention Time (RT) and Time Window***
  - –Parameters were entered during the 'right click and drag' over the peak. 'RT' is center of a time interval that the peak must appear in to be associated with this compound. 'Time Window' is the width of this interval. So for this example, the peak must appear at 2.7950±0.55 min.

# 3. Quant Method Editor – Specify Concentrations



For ‘Concentration of Standards’ select ‘Conc A’ (or ‘Conc B’, ‘Conc C’, etc.) from the drop down menu for the column in the sample list that has the concentration values for this compound.

If this compound is an internal standard you may select ‘Fixed’ (usually done for Int Std’s). If you enter ‘Fixed’, you can enter the concentration of the Int Std or since it is the same concentration in all of the samples, simply enter 1.000 (usually done for Int Std’s).



# 3. Quant Method Editor – Peak Selection



| View Retention Time Parameters | |
|---|---|
| Locate Peak Using | Retention Time |
| Predicted Retention Time | 2.7950 |
| Predicted Relative Retention Time | 0.0000 |
| Relative Retention Time Reference | None |
| Retention Time Window (mins) ± | 0.5500 |
| Lower Retention Time Tolerance (%) | 0.0000 |
| Upper Retention Time Tolerance (%) | 0.0000 |
| Flag RT Tolerance? | X NO |
| Locate Peak Selection | Nearest |

- ***Peak Selection***
  - If more than one peak is detected in the 'Time Window', this designates which peak to choose.
  - The peak **Nearest** to the entered RT,
  - the **Largest** peak,
  - the **First** peak,
  - the **Last** peak
  - or **Totals** (sum up all of the peaks).

If your compound always has the largest peak in the chromatogram, select **'Largest'**.

# 3. Adding to the Quantify Method

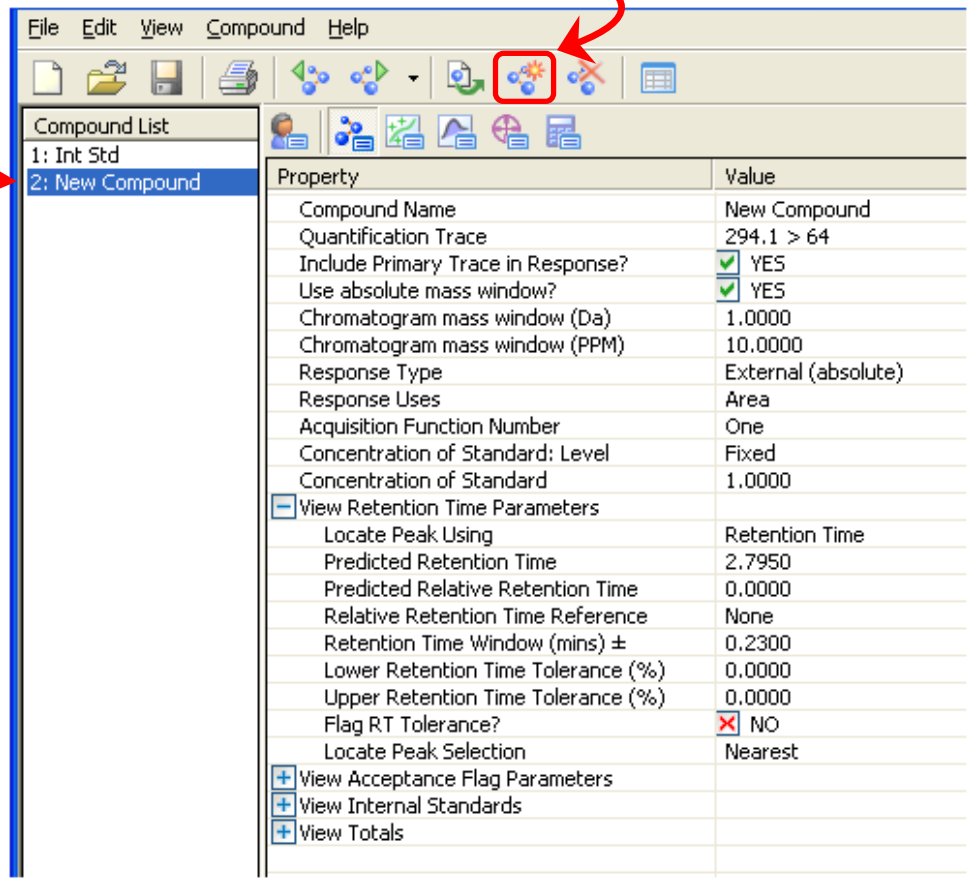


- This entire process now needs to be repeated for the two other compounds (for the Quantify.pro example).
- Things that may differ between compounds:
  - Transition (Quantify Trace)
  - Name
  - Integration Parameters
  - Internal Reference (Select Internal Standard if Used)
  - Concentration of Standards
  - Retention Time
  - Time Window
  - Response Type in General Parameters Window
  - Polynomial Type in the General Parameters Window

# 3. Quant Method Editor – Add Compound to List

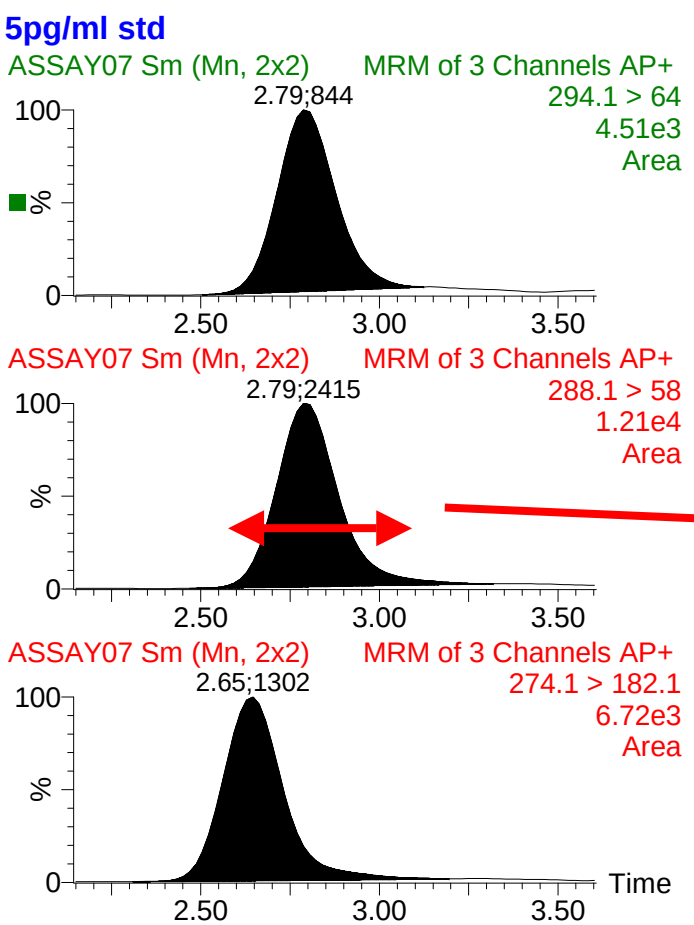


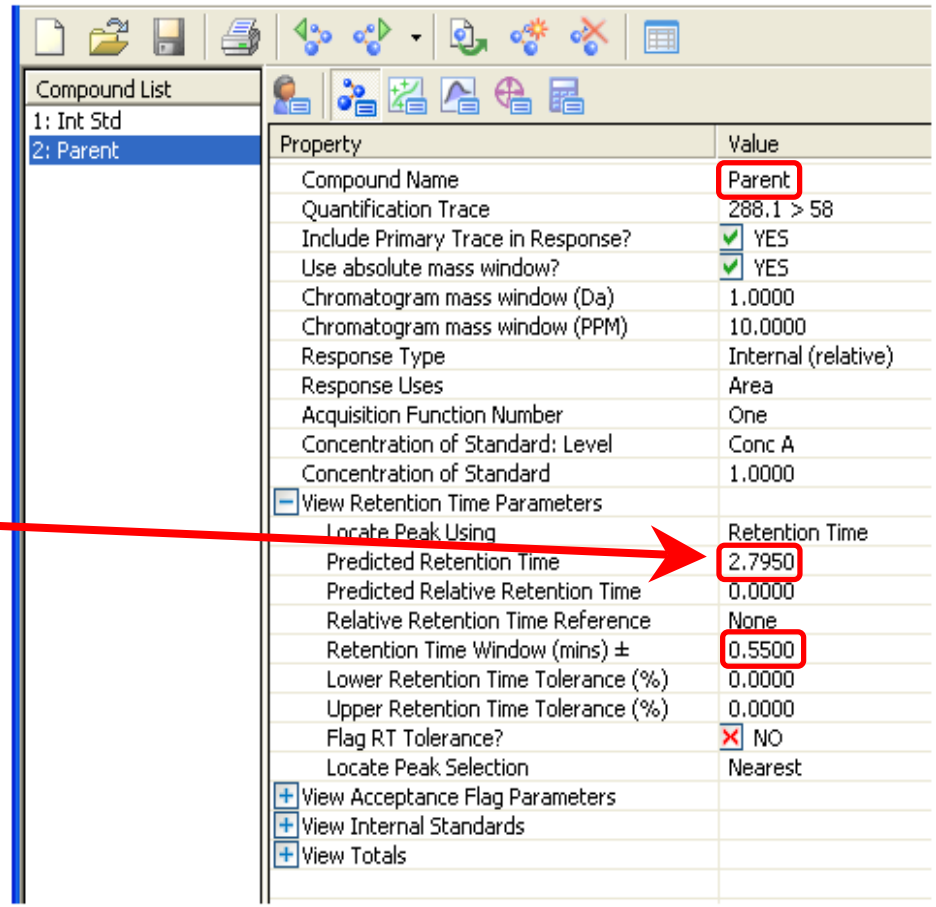
- Click ***Add New Compound Icon*** to enter the compound into the Compound List (Shown is New Compound entered into the list)
- Note: Information from the previous compound has been propagated to the new compound. Check all parameters to ensure proper quantification of the new compound



# 3. Quant Method Editor – Add Compound to List





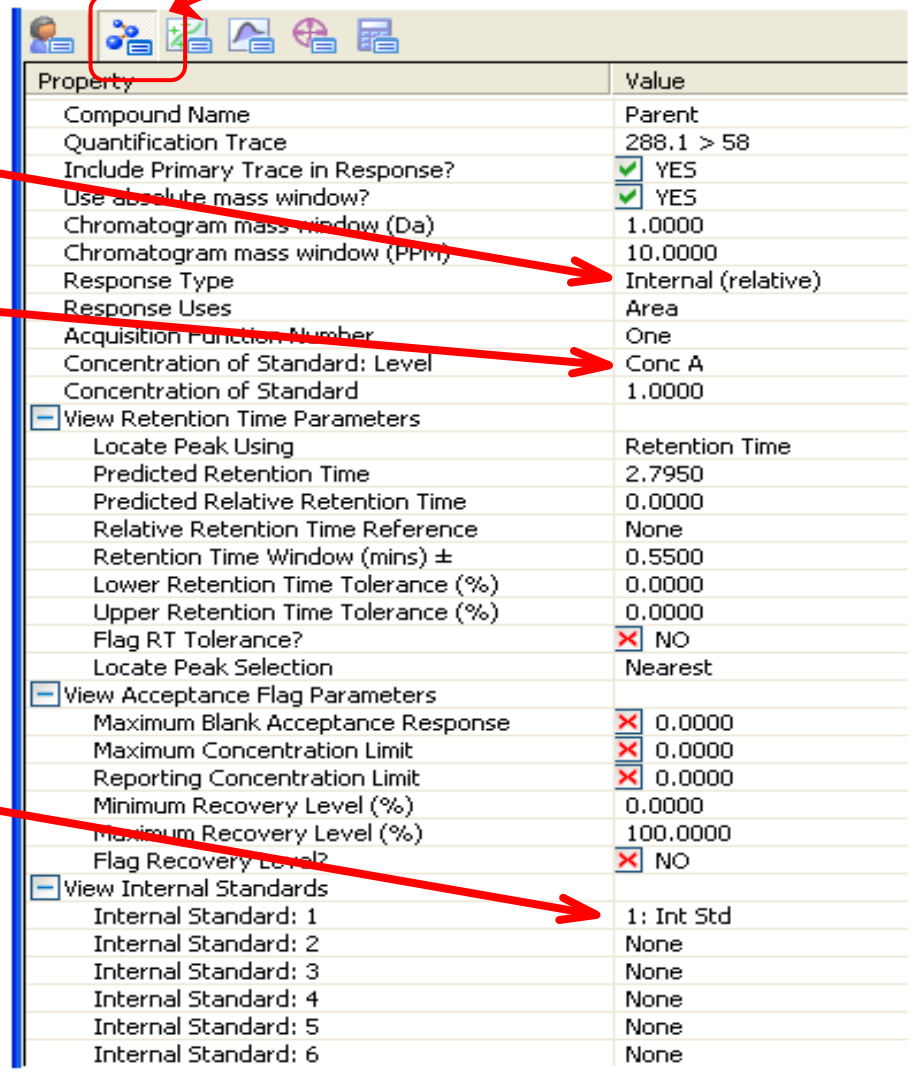

- Right Click and Drag on the chromatogram for the new compound trace
- Rename the new compound

# 3. Quant Method Editor – Enter Compound Properties (Analyte)



- ***Response Type***
  - –Set to “Internal (relative)”
- ***Concentration of Standard***
  - –Set Level to “Conc A (or B,C,…)”
- ***Internal Standards***
  - –Select the compound from the ‘drop down’ list that is the internal standard (In this case, Int Std).

Compound Properties



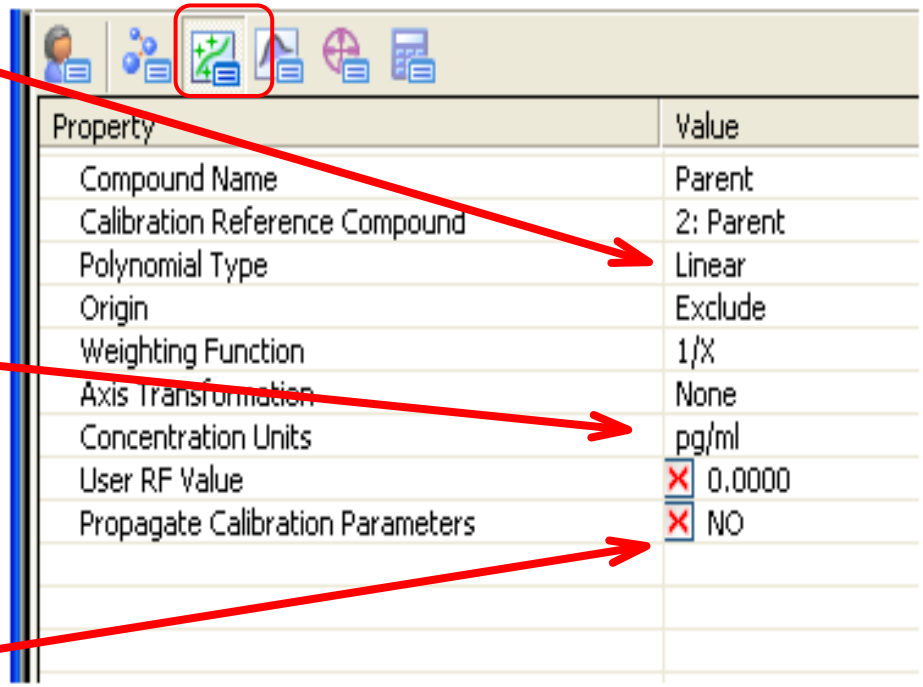
| Property | Value |
|---|---|
| Compound Name | Parent |
| Quantification Trace | 288.1 > 58 |
| Include Primary Trace in Response? | YES |
| Use absolute mass window? | YES |
| Chromatogram mass window (Da) | 1.0000 |
| Chromatogram mass window (PPM) | 10.0000 |
| Response Type | Internal (relative) |
| Response Uses | Area |
| Acquisition Function Number | One |
| Concentration of Standard: Level | Conc A |
| Concentration of Standard | 1.0000 |
| View Retention Time Parameters | |
| Locate Peak Using | Retention Time |
| Predicted Retention Time | 2.7950 |
| Predicted Relative Retention Time | 0.0000 |
| Relative Retention Time Reference | None |
| Retention Time Window (mins) ± | 0.5500 |
| Lower Retention Time Tolerance (%) | 0.0000 |
| Upper Retention Time Tolerance (%) | 0.0000 |
| Flag RT Tolerance? | NO |
| Locate Peak Selection | Nearest |
| View Acceptance Flag Parameters | |
| Maximum Blank Acceptance Response | 0.0000 |
| Maximum Concentration Limit | 0.0000 |
| Reporting Concentration Limit | 0.0000 |
| Minimum Recovery Level (%) | 0.0000 |
| Maximum Recovery Level (%) | 100.0000 |
| Flag Recovery Level? | NO |
| View Internal Standards | |
| Internal Standard: 1 | 1: Int Std |
| Internal Standard: 2 | None |
| Internal Standard: 3 | None |
| Internal Standard: 4 | None |
| Internal Standard: 5 | None |
| Internal Standard: 6 | None |

# 3. Quant Method Editor – Enter Calibration Parameters (Analyte)



Calibration Parameters

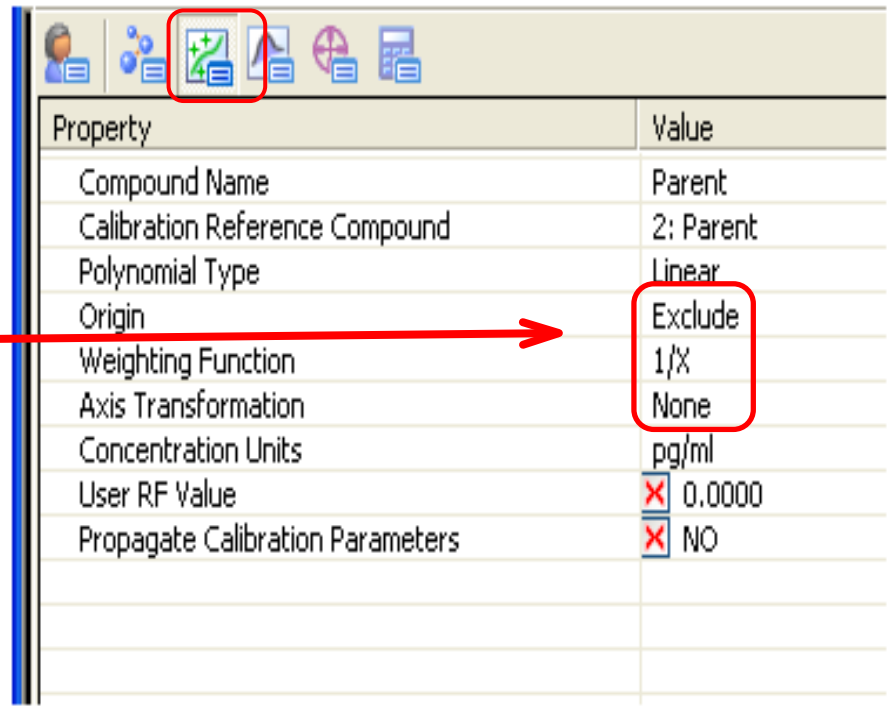
- ***Polynomial Type***
  - –For Analytes, “Linear” is the typical polynomial type. Other options include “Quadratic, Cubic, Quartic”
- ***Concentration Units***
  - –Type concentration unit of internal Standard (in this example it is pg/ml)
- ***Propagate Calibration Parameters***
  - –When using an Internal Standard, the Propagate function is disabled
  - –Click on the box until a red “X” appears. The Value should change to “No”



# 3. Quant Method Editor – Enter Calibration Parameters (Analyte)

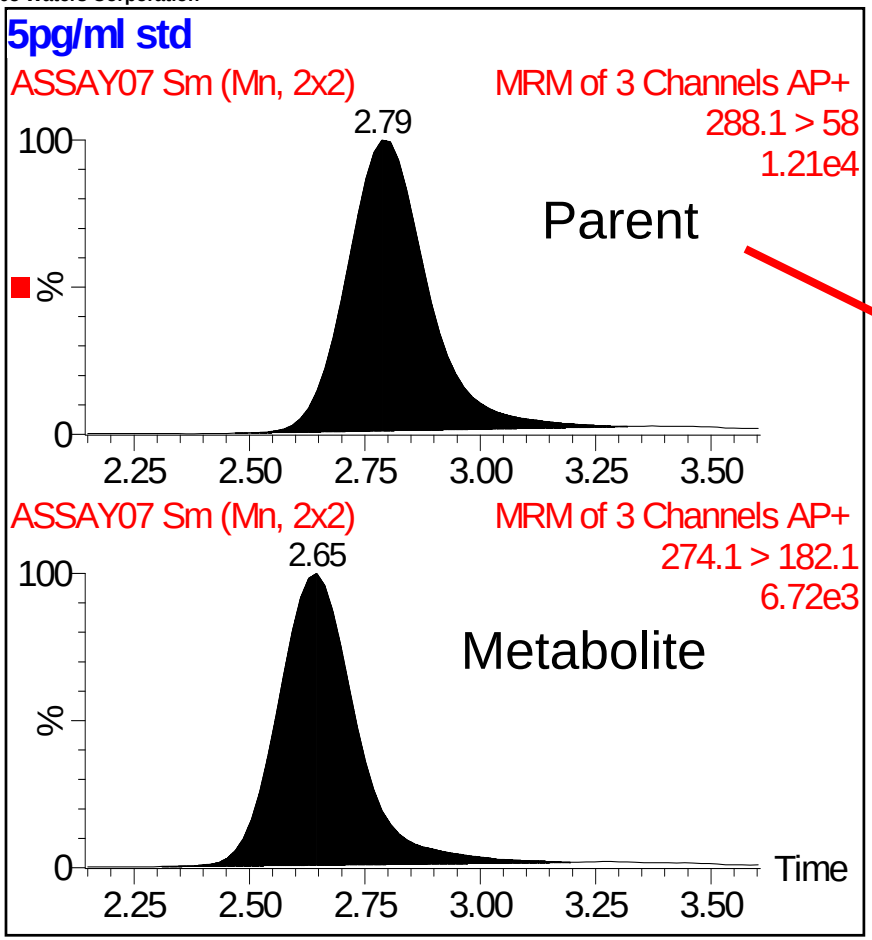


- ***Origin, Weighting Function, Axis Transformation***
  - –Origin: Typically the origin is excluded as a point of the calibration curve
  - –Weighting Function: A weighting factor is appropriate for a calibration range that is greater than an order of magnitude. For this example, a weighting of "1/X" will be used
  - –Axis Transformation: When the weighting function is used, the Axis Transformation function is not applied.



# 3. Quantification Method Editor – Relative R.T.






| Property | Value |
|---|---|
| Compound Name | Metabolite |
| Quantification Trace | 274.1 > 182.1 |
| Include Primary Trace in Response? | YES |
| Use absolute mass window? | YES |
| Chromatogram mass window (Da) | 1.0000 |
| Chromatogram mass window (PPM) | 10.0000 |
| Response Type | Internal (relative) |
| Response Uses | Area |
| Acquisition Function Number | One |
| Concentration of Standard: Level | Conc A |
| Concentration of Standard | 1.0000 |
| View Retention Time Parameters | |
| Locate Peak Using | Relative Retention Time |
| Predicted Retention Time | 2.65 |
| Predicted Relative Retention Time | .950 |
| Relative Retention Time Reference | 2: Parent |
| Retention Time Window (mins) ± | 0.3200 |
| Lower Retention Time Tolerance (%) | 0.0000 |
| Upper Retention Time Tolerance (%) | 0.0000 |
| Flag RT Tolerance? | NO |
| Locate Peak Selection | Nearest |
| View Acceptance Flag Parameters | |
| View Internal Standards | |
| View Totals | |

R.T. of Metabolite Relative to Parent
(2.65) / (2.79) = 0.950

Example of Use of Relative Retention Time

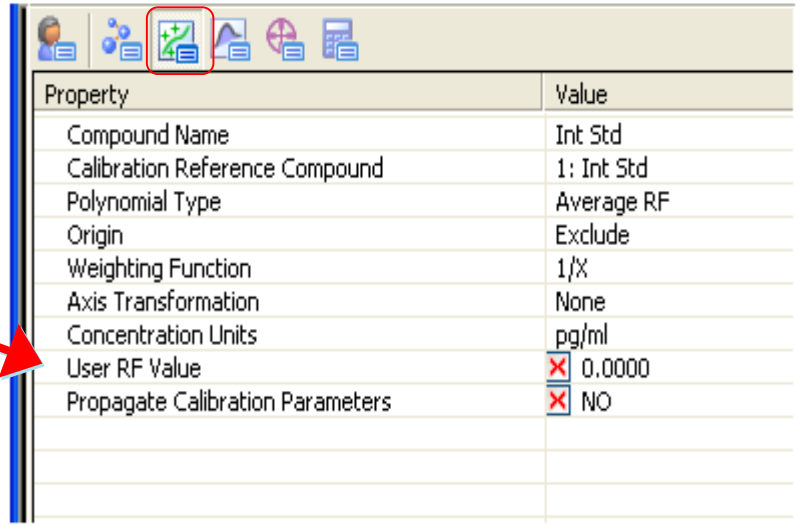
# 3. Quant Method Editor-User RF/Peak



Minor Points About the Method Editor.

## User RF Value

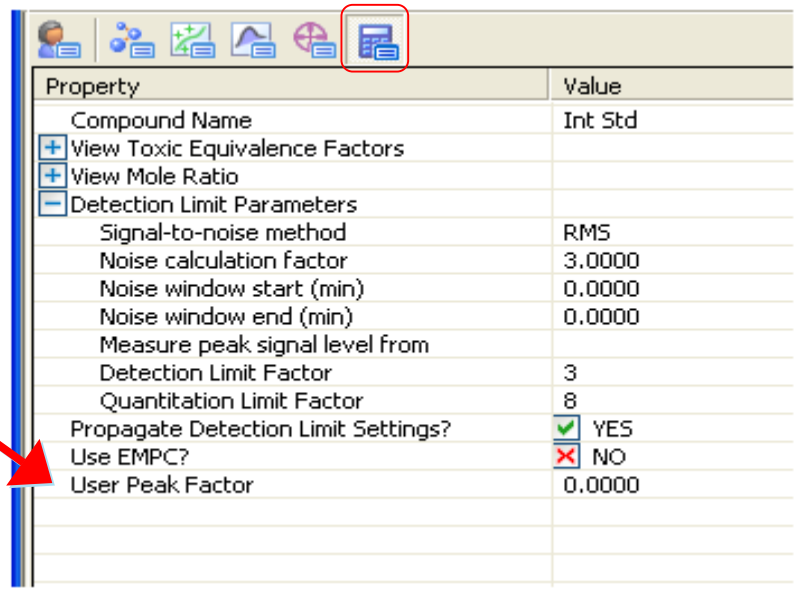
If no calibration curve is available, then divide the peak area (response) by this factor to calculate the conc.'s.



| Property | Value |
|---|---|
| Compound Name | Int Std |
| Calibration Reference Compound | 1: Int Std |
| Polynomial Type | Average RF |
| Origin | Exclude |
| Weighting Function | 1/X |
| Axis Transformation | None |
| Concentration Units | pg/ml |
| User RF Value | 0.0000 |
| Propagate Calibration Parameters | NO |

## User Peak Factor

All concentrations calculated will be multiplied by this factor.



| Property | Value |
|---|---|
| Compound Name | Int Std |
| View Toxic Equivalence Factors | |
| View Mole Ratio | |
| Detection Limit Parameters | |
| Signal-to-noise method | RMS |
| Noise calculation factor | 3.0000 |
| Noise window start (min) | 0.0000 |
| Noise window end (min) | 0.0000 |
| Measure peak signal level from | |
| Detection Limit Factor | 3 |
| Quantitation Limit Factor | 8 |
| Propagate Detection Limit Settings? | YES |
| Use EMPC? | NO |
| User Peak Factor | 0.0000 |

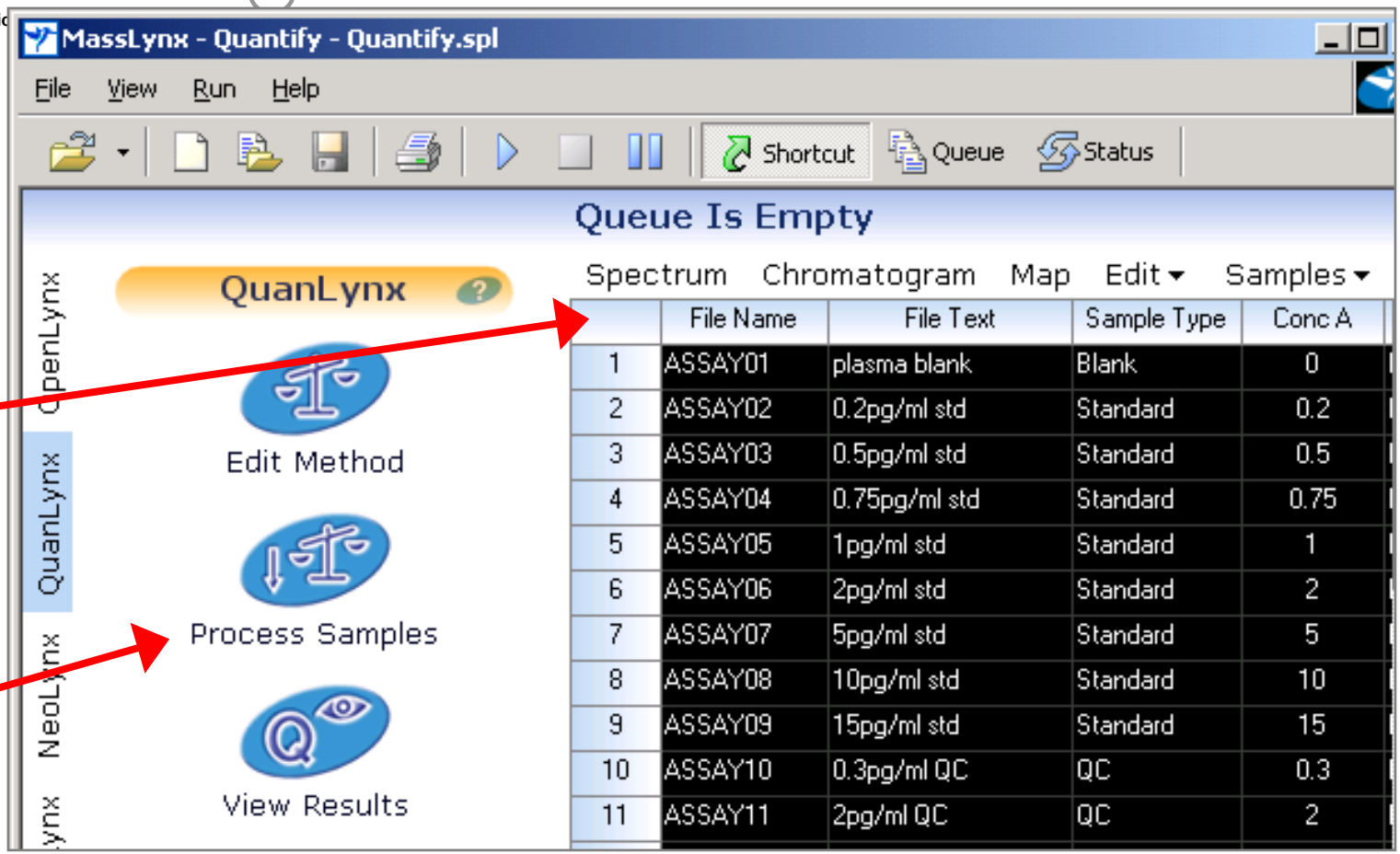
# 4. Processing Samples



- Once the entire method is built, it's time to process the samples.
- Highlight the samples to quantitate. If the entire sample list is to be processed, click on the upper left box to activate the entire sample list
- Click on *QuanLynx, Process Samples*.

# 4. Processing Samples – QuanLynx





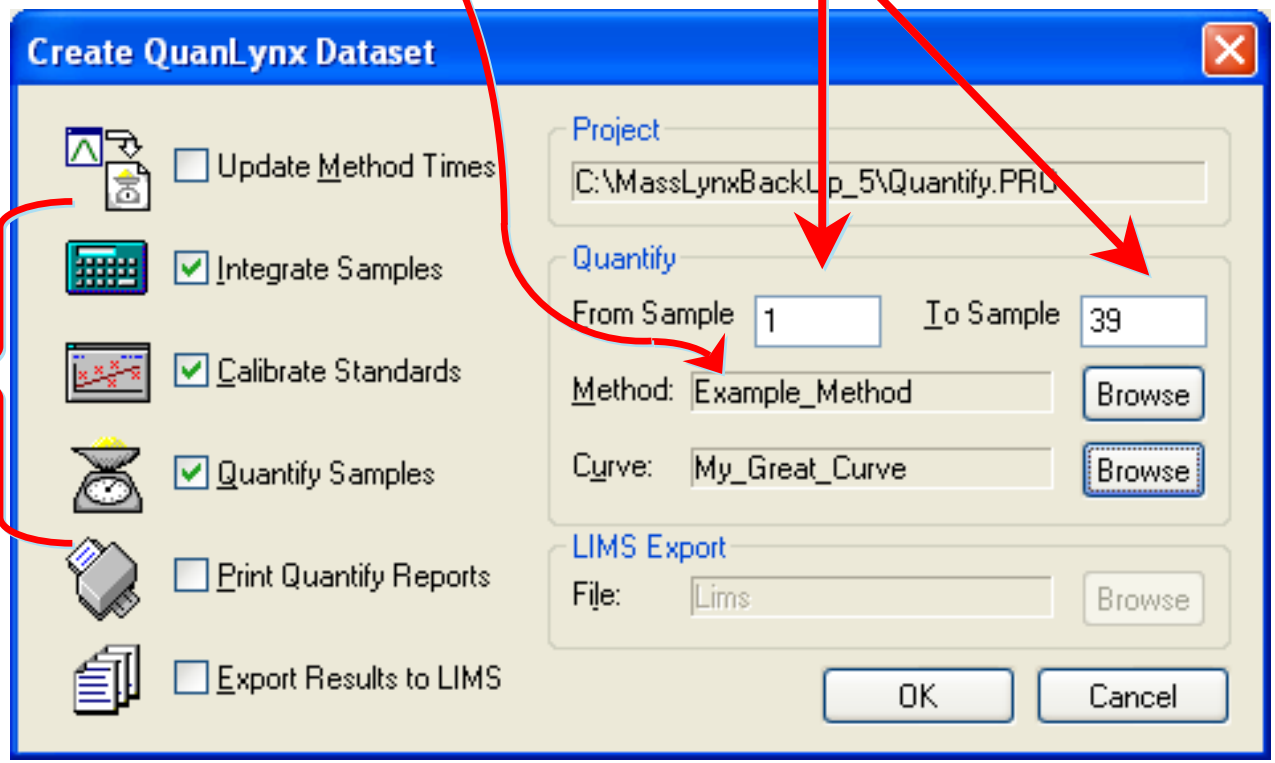

Highlight the samples you wish to quantitate and then select ‘Process Samples’

This window appears to confirm the specifics prior to processing samples. Double-check that the:

1) Designated samples are correct
2) That it is using the correct method.

For complete quantitation set this window to:

1) Integrate the chromatograms
2) Create a calibration curve
3) Calc the concentrations in each sample



# 5. Reviewing Results



After samples are done processing, a ***"Quantify"*** box will appear on the lower tool bar. Double click to bring up the results.

***OR***

Using the main toolbar, click on the ***View Results*** button.

# 5. Sample of QuanLynx Quantification Results



From the View drop down menu you set this window to show (or not show):

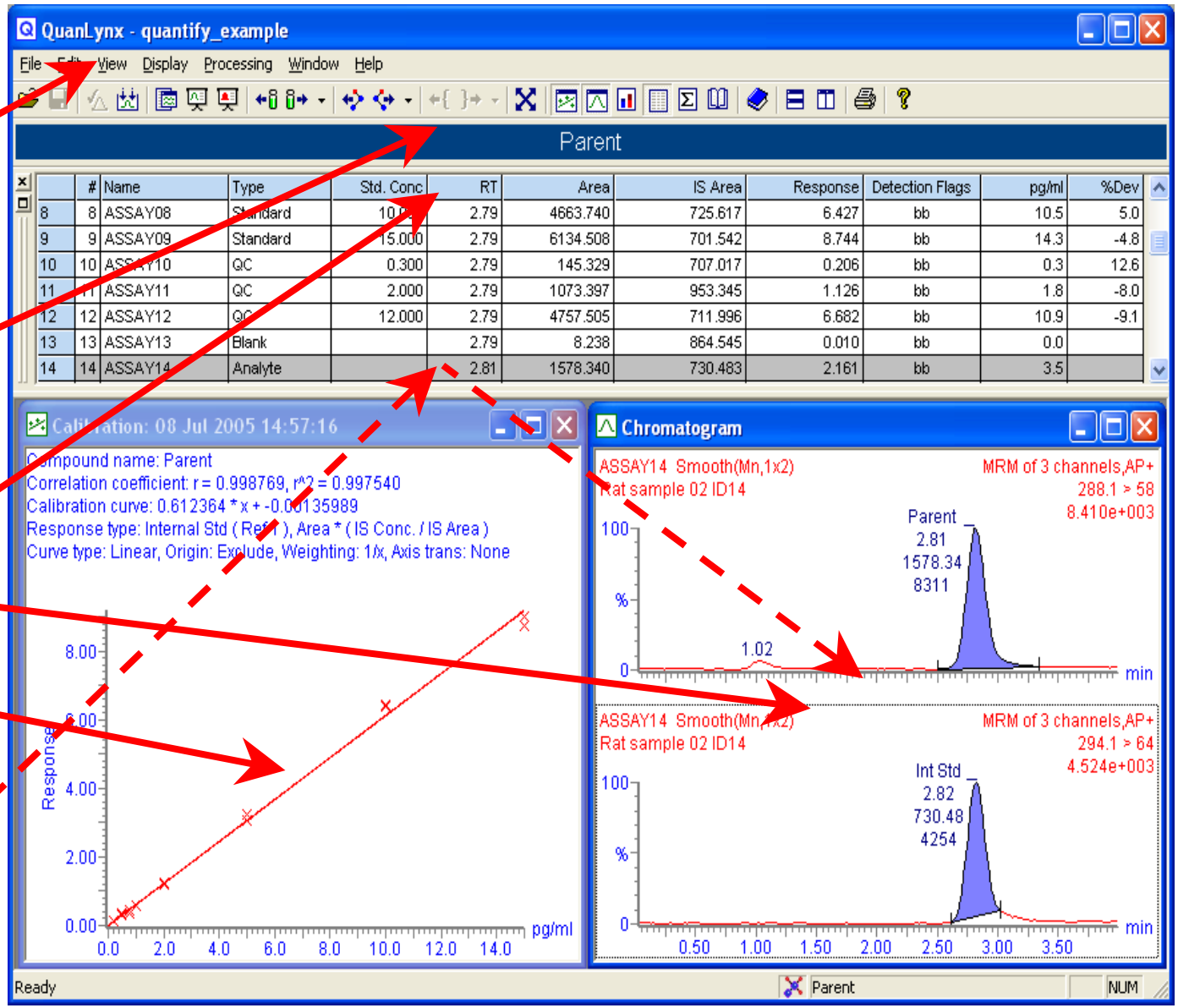
Information Bar (name of compound)

Summary Table

Chromatograms

Calibration Curves

'Double Click' on a Sample in the list to see its chromatograms

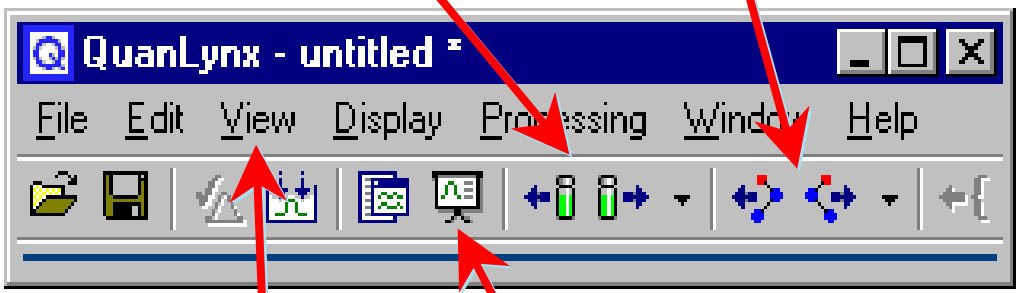


# 5. Some Features of the Menu of the QuanLynx Quantification Results Viewer

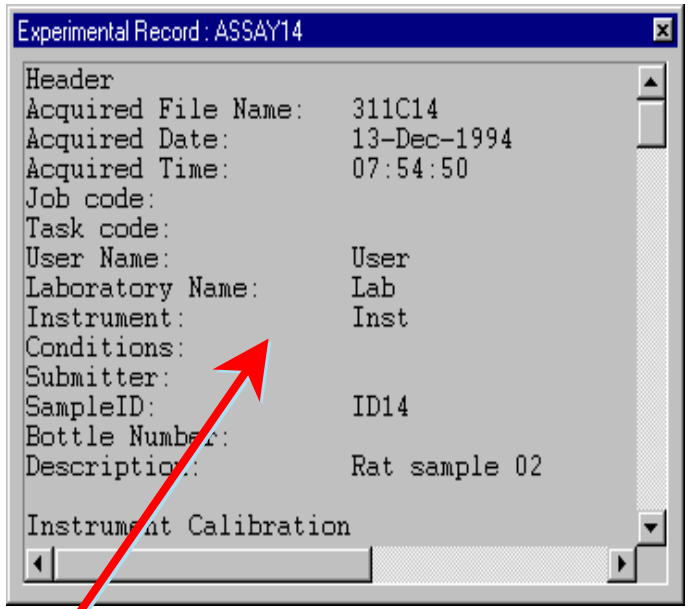


Step Through the Different Samples

Step Through the Different Compounds



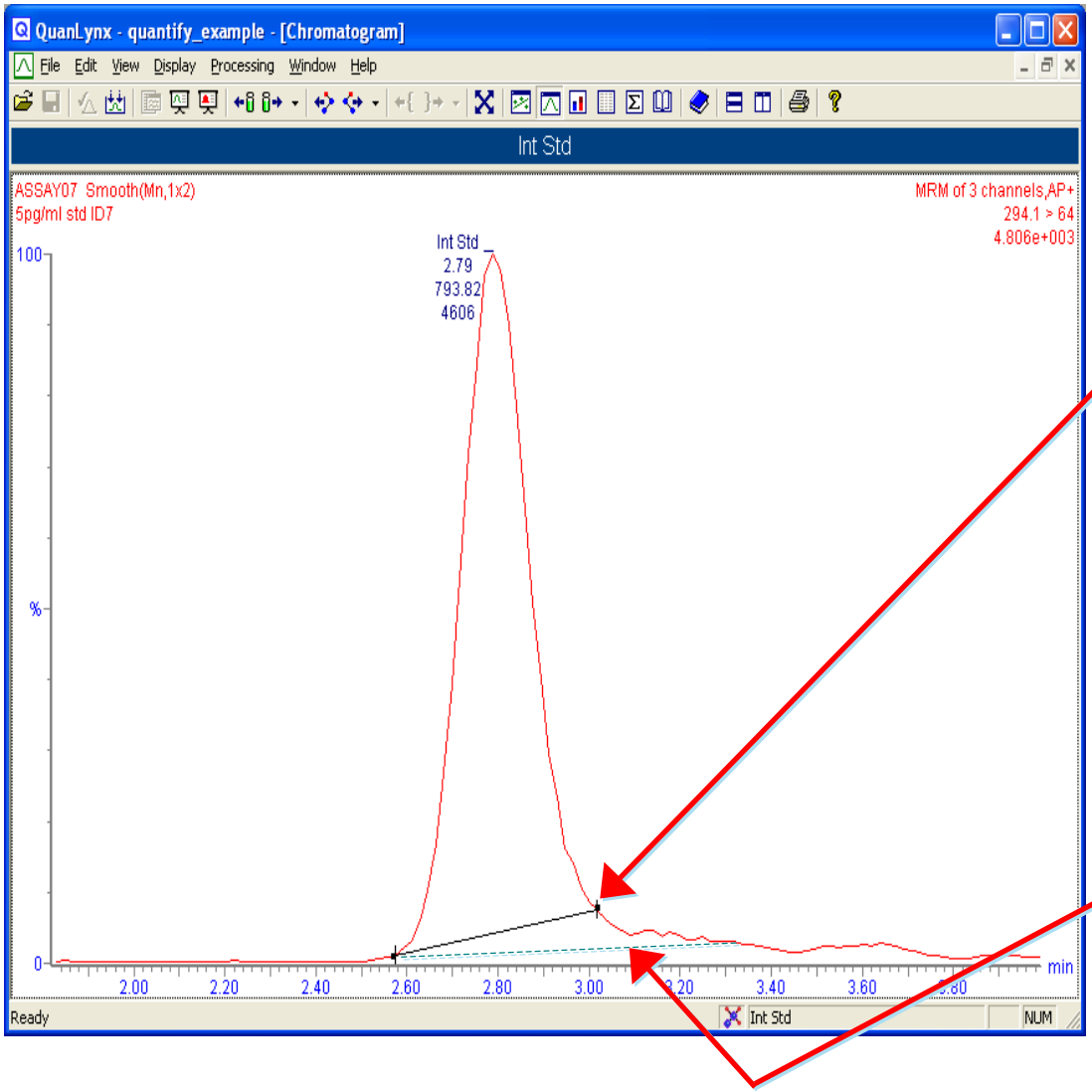

Turn On or Off the Slide Show




Experimental Record Can Be Displayed Using Menu Item

# 5. Manually Adjusting Integrations



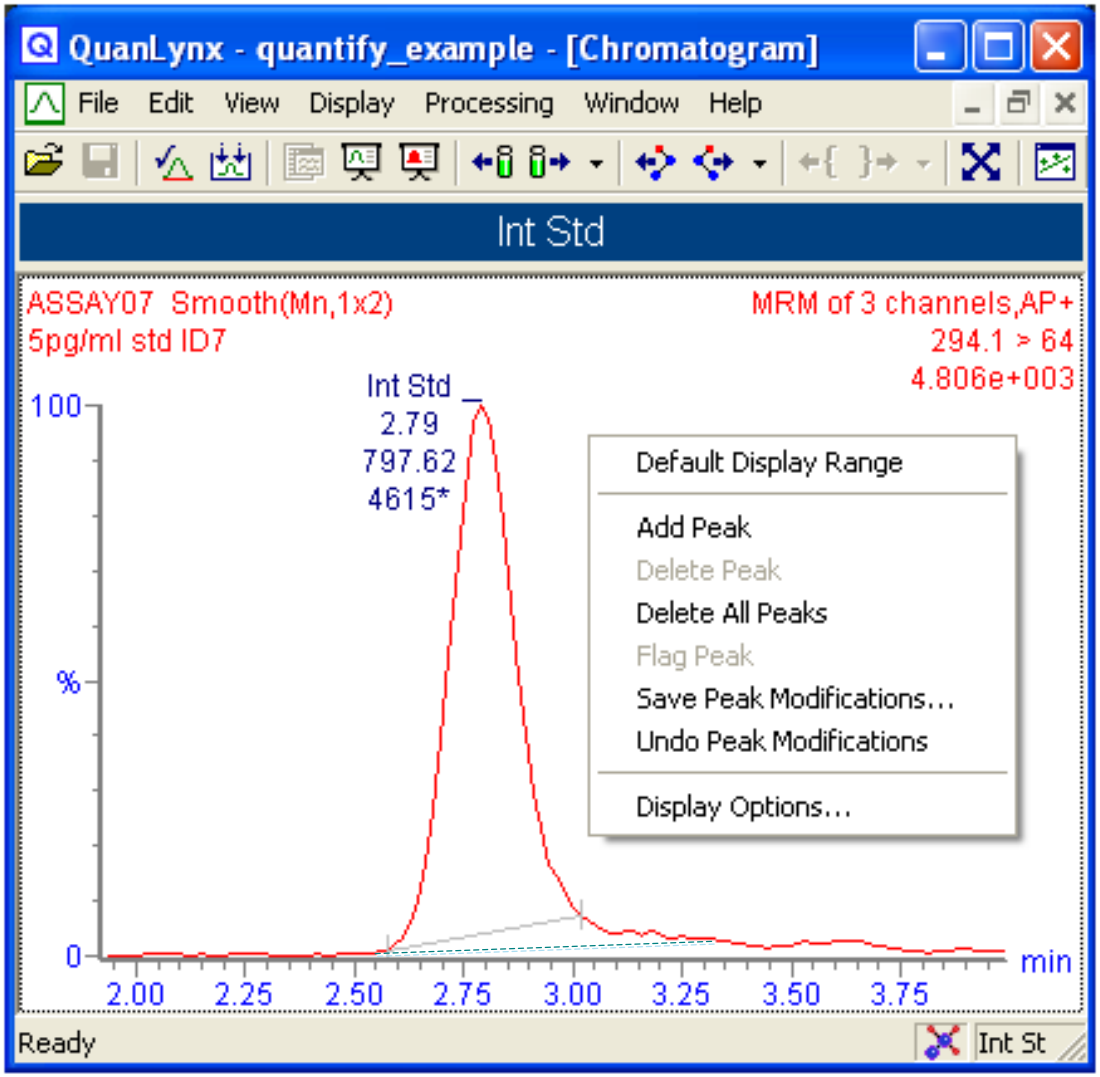



Click on the Baseline & 'Grab' the End Point using the Pointer and Left Mouse Button & Move the End Point to the desired spot.

A faint line will show the position of the original baseline. Reports will now show that this baseline was manually adjusted.

# 5. Manually Adjusting Integrations



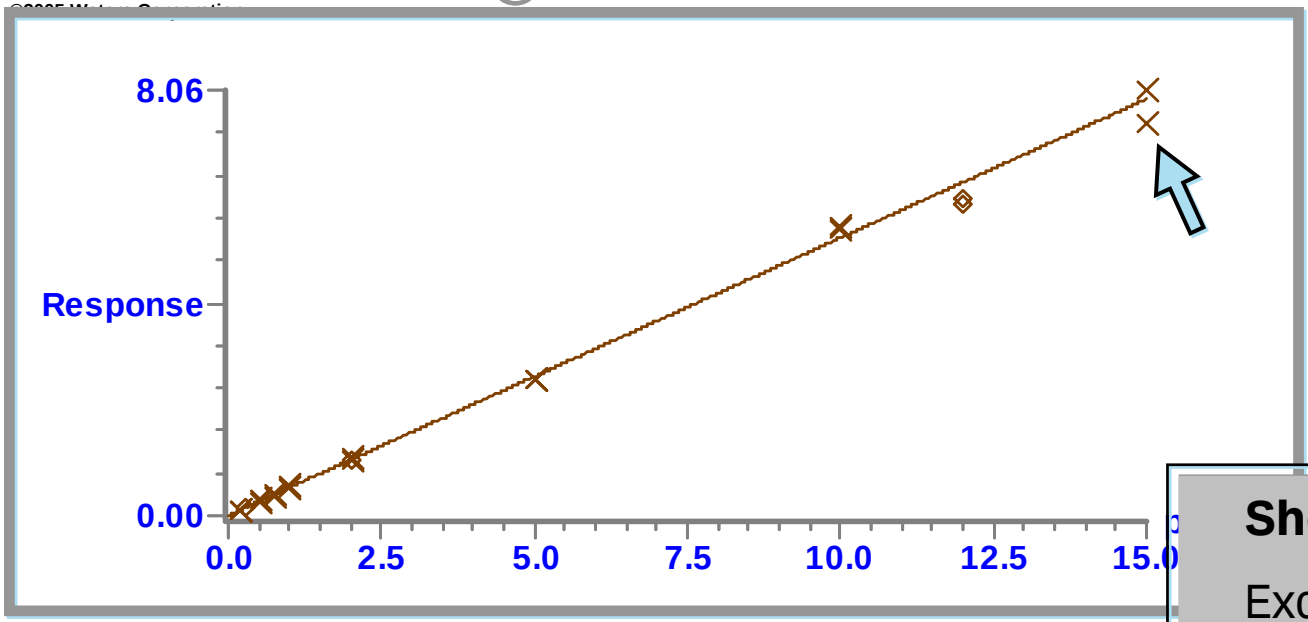



'Right Click' in the window and the 'pop up menu' shown to left will appear.

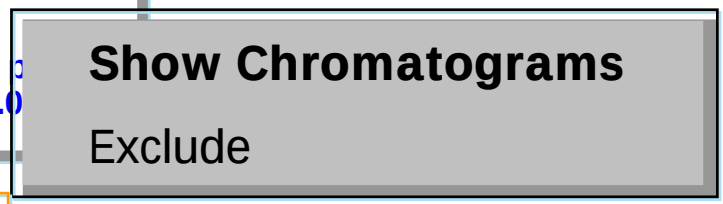
Select 'Save Peak Mod' from this menu to 'accept' and 'save' the changed baseline.

Go to another chromatogram to keep the original baseline.

# 5. Editing Calibration Curves




To remove a point from the calibration, ‘Right Click’ on the point. The dialog box shown below should appear.




You can ‘Left Click’ and ‘rubber band’ zoom in on a region of the curve to get a closer look at small regions of the curve. Use the  button at the top to ‘Unexpand’.

Note you can also ‘Left Click’ and ‘rubber band’ zoom in on a regions of the chromatograms.

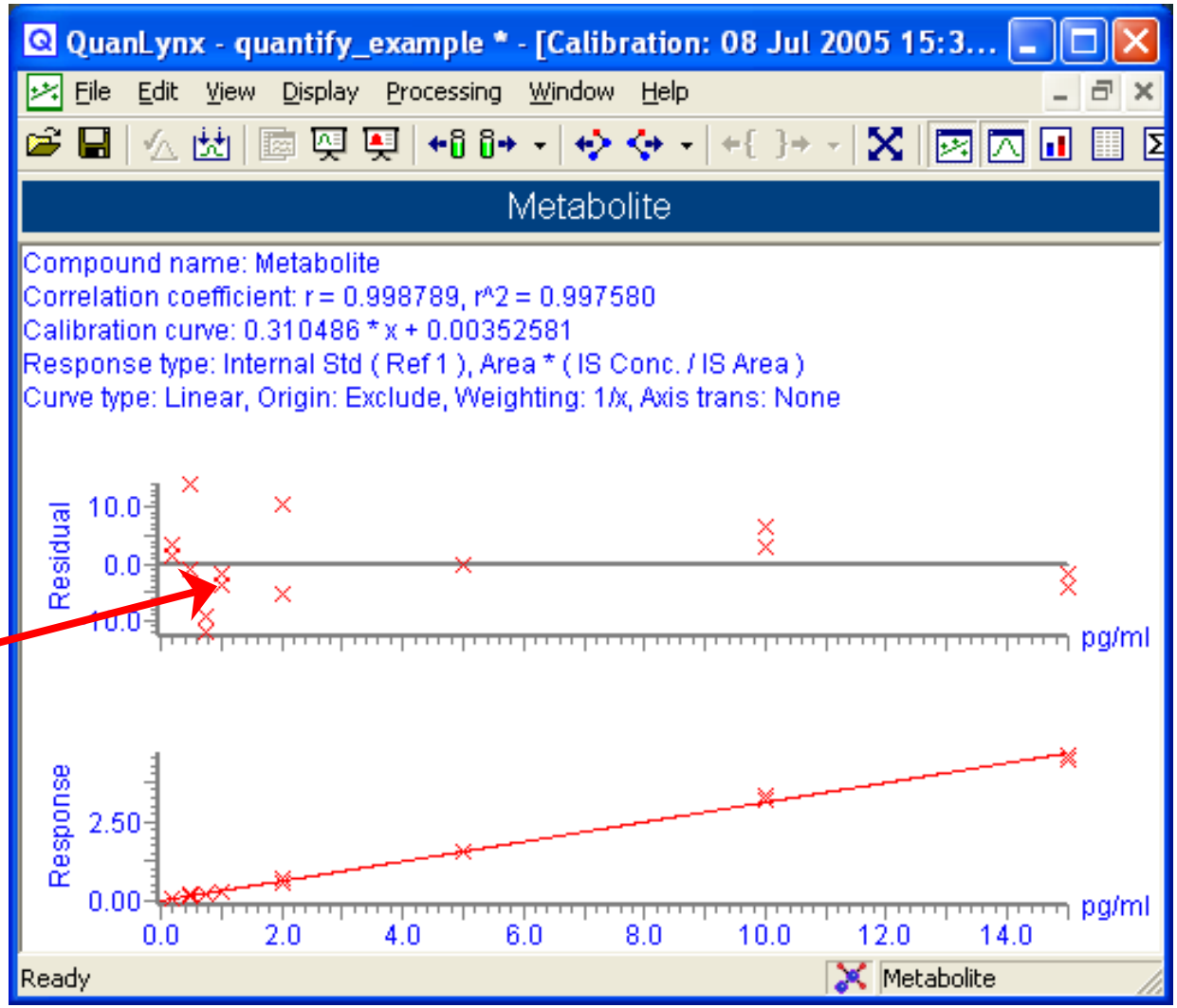
‘Left Click’ on ‘Exclude’ and the point will be removed from the curve. Reverse the process to put the point back into the curve.

# Excluding Points from the Calibration



If you have the residuals displayed in your calibration window, you can also remove a point from the calibration by ‘Right Clicking’ on the ‘bad’ point.

‘Right Click’ on the point you wish to exclude here.



# 5. Right Click on the Display and Select 'Display Options' to Customize the Display:



## For Example the Chromatogram Display Can Be Changed:

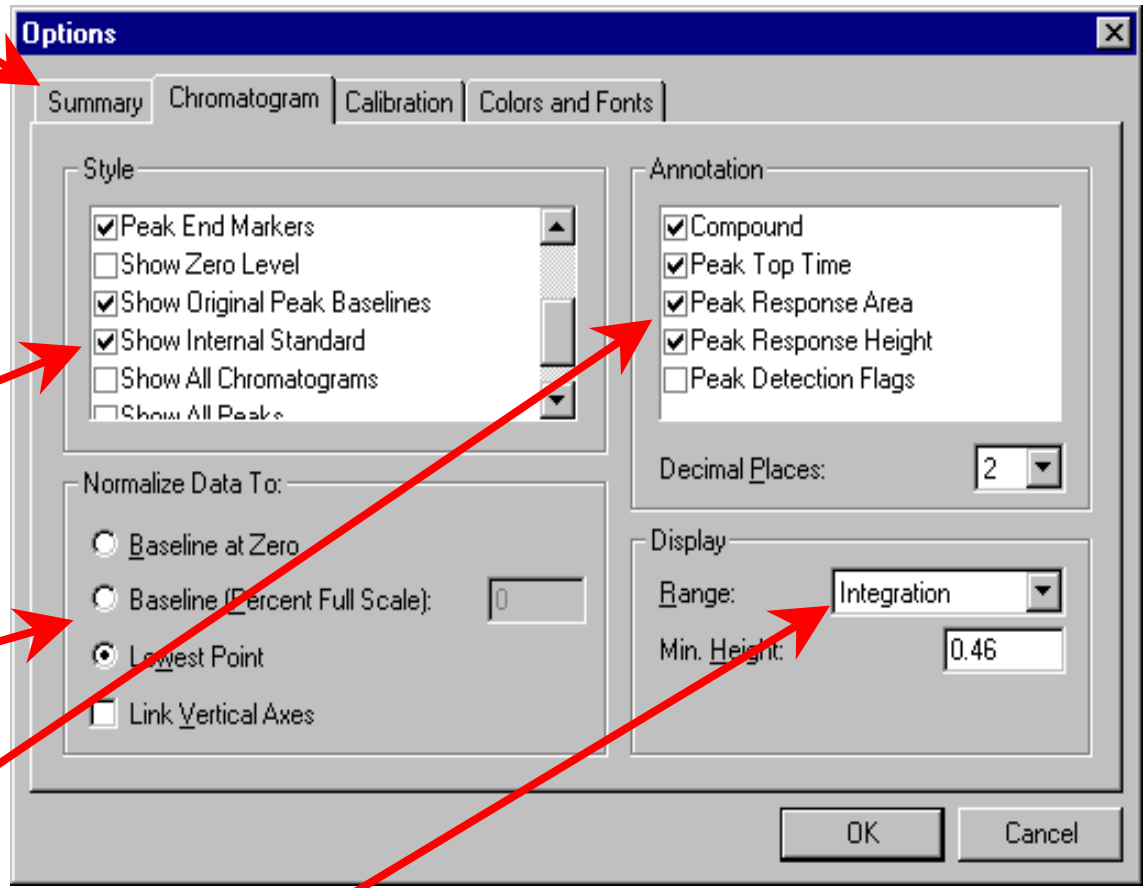
Use 'Summary' Page to Control Slide Show

Chromatogram Display Adjustments

Toggle Showing Internal Standard Chromatogram

Y Axis

Peak Annotation

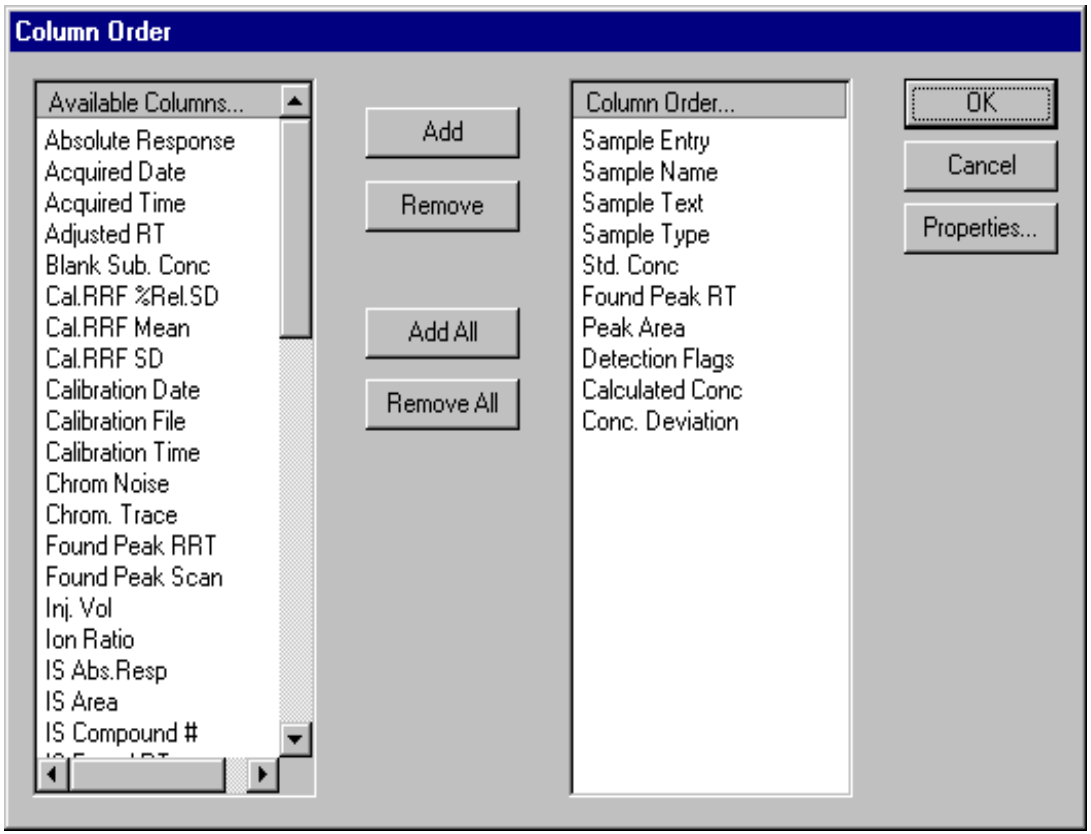



X Axis

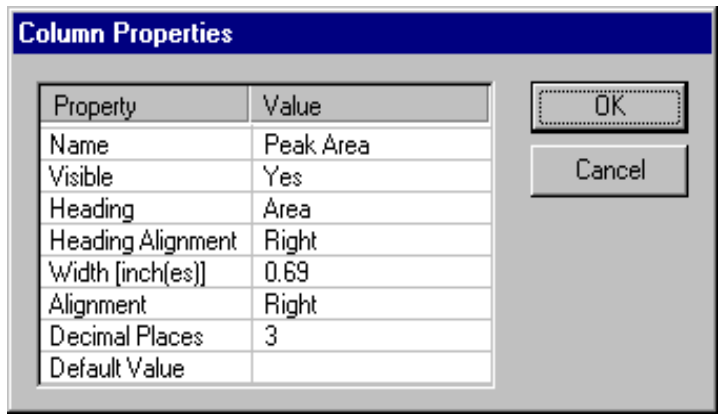
# 5. ‘Right Click’ On the Summary Table and ...



1) Click on ‘Change Column Order’ and which columns are displayed in the table can be changed.



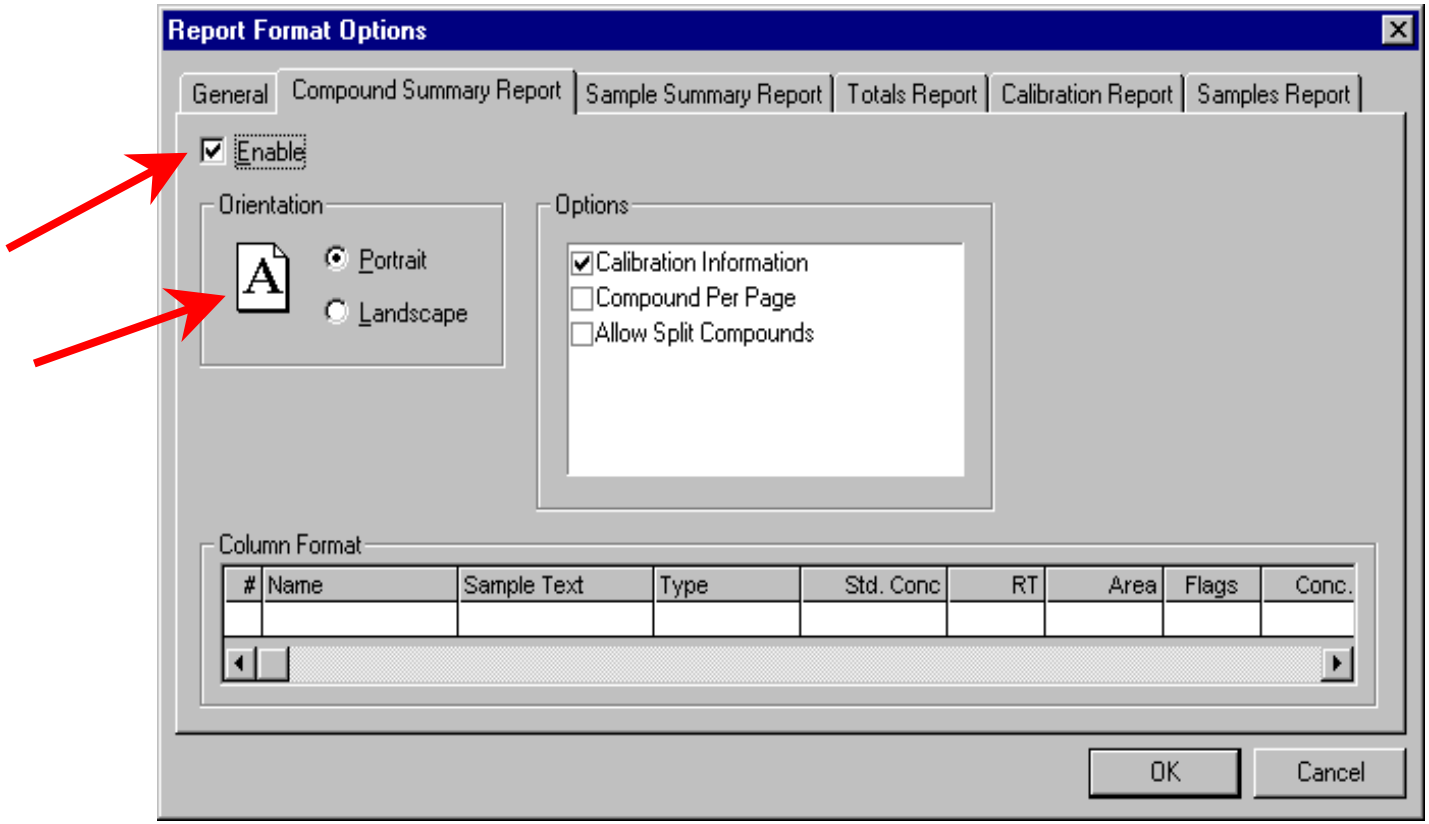

2) Click on ‘Edit Column Properties’ and the properties of which ever column you ‘right clicked’ on can be changed.



# 6. Change How the Report Will Be Printed Out



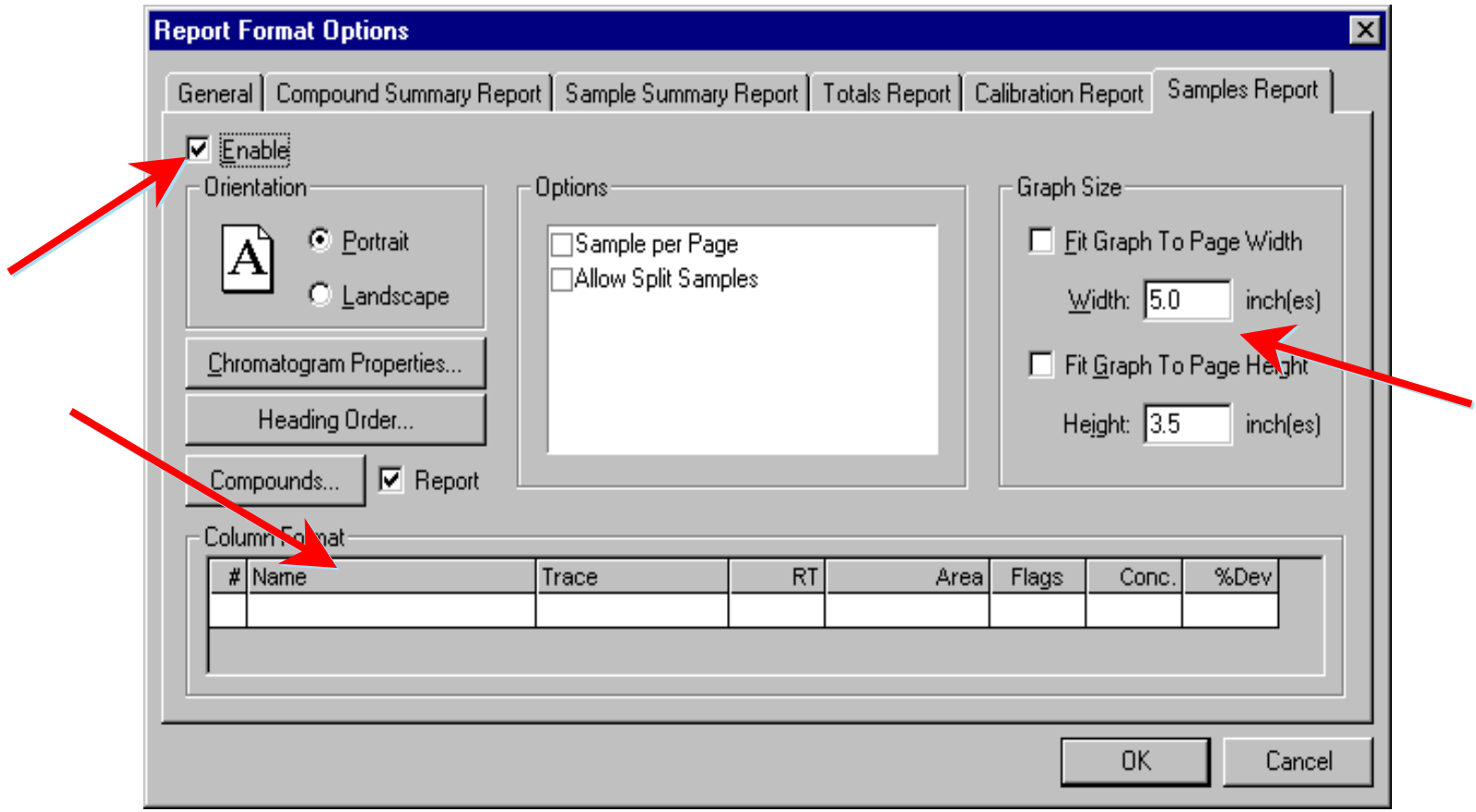
From the ‘File’ Menu, Select the ‘Report Format’ Item:




For Example the Compound & Sample Summaries Can be Formatted Differently From How They Appear on the Screen

# 6. How the Chromatograms Are Printed Out Can Also Be Adjusted






Customized Display and Report Formats Can Be Saved For Later Use

# 6. More on QuanLynx Quantification



- Printing Reports (***File, Print Report***). Besides a full report, results from a set range of samples can be printed.
- Screen and Report Format. A customized format can be saved in a *.fmt file for later use.
- The Quantify Method used with a report can be changed using (***Edit, Quantify Method***)
- Editing of Calibration Curve (***Edit, Calibration Curve***) allows excluding of specific data points. (‘Right Click’ on a point in a Calibration Curve and select ‘Exclude Point’).
- Reprocessing samples after editing Quantify Method (***Process, Calculate***)

# 6. When Finished QuanLynx Quantification Results Can Be Saved in a File



- Everything is in One File
- This file can be viewed and reports printed at a later date without reprocessing data
- This File Will Contain:
  - Compound and Sample Summaries
  - Calibration Curves
  - Chromatograms
  - Experimental Record for Each Analysis Run
  - Quantitation Method

# Linear Least Squares Line Fitting and 1/X Weighting Factors



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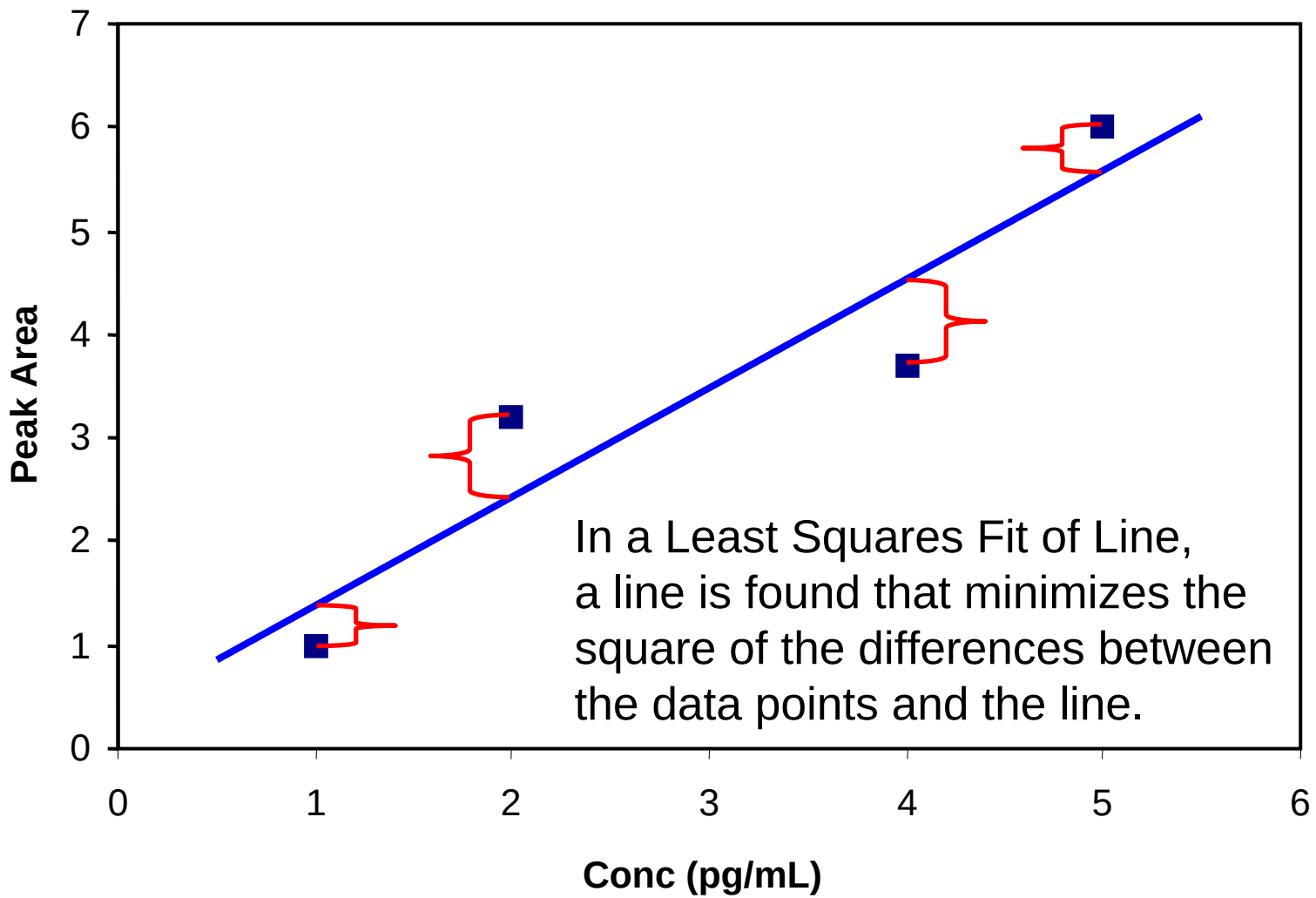
# Least Squares Fit of a Line to a Set of Data





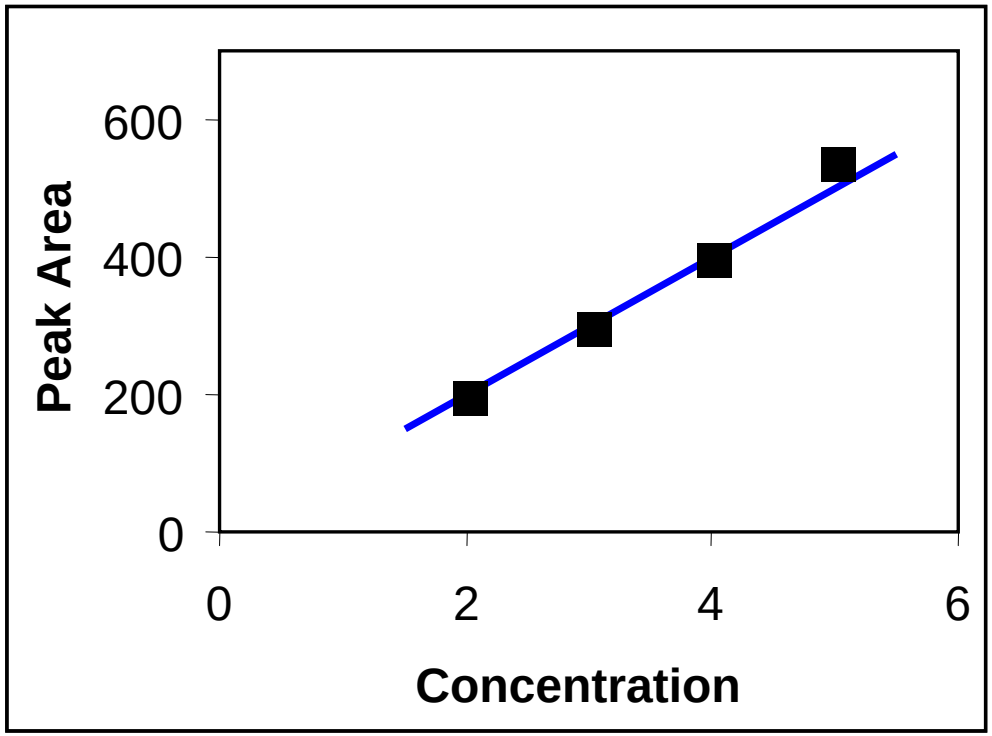
# Example of Data and a Possible Fitted Line



| Conc | Meas. | Fitted | Diff | Diff $^2$ |
|---|---|---|---|---|
| 2 | 200 | 200 | 0 | 0 |
| 3 | 300 | 300 | 0 | 0 |
| 4 | 400 | 400 | 0 | 0 |
| 5 | 540 | 500 | 40 | 1600 |

Sum of Diff $^2$ = 1600

Conc= Std Concentration
Meas=Measured Peak Area
Fitted=Peak Area from Fitted Line at given Conc
Diff= Meas – Fitted
Diff2= Diff Squared



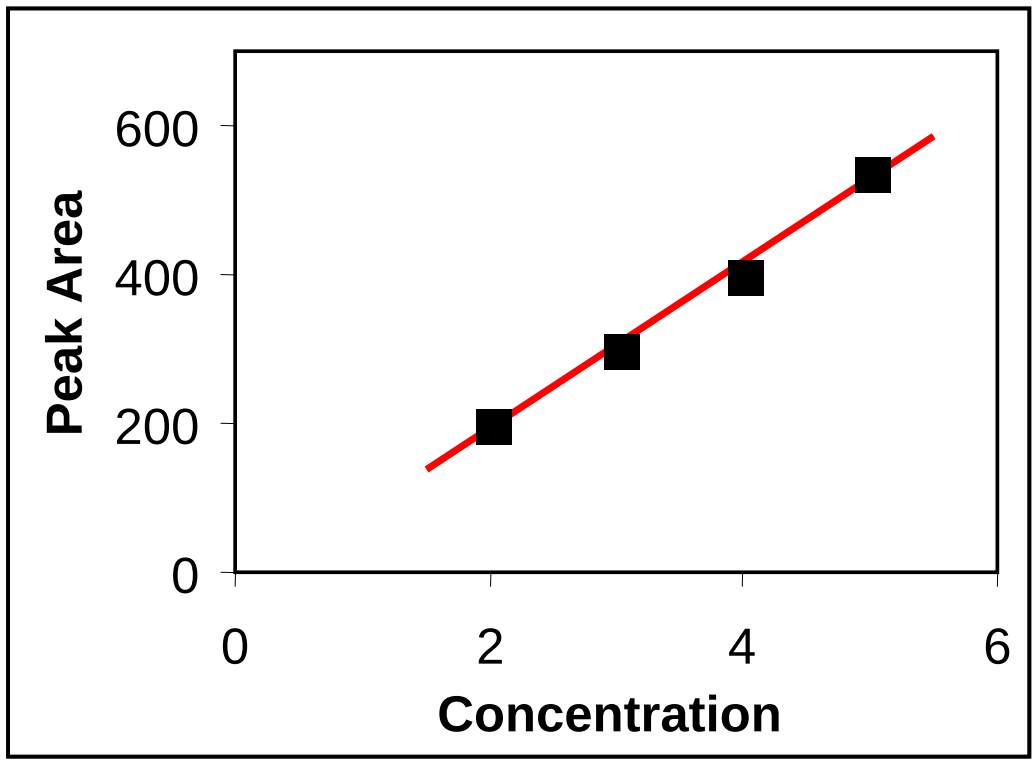
# Example of Data and a Least Squares Fitted Line



| Conc | Meas. | Fitted | Diff | Diff $^2$ |
|---|---|---|---|---|
| 2 | 200 | 192 | 8 | 64 |
| 3 | 300 | 304 | - 4 | 16 |
| 4 | 400 | 416 | - 16 | 256 |
| 5 | 540 | 528 | 12 | 144 |

Sum of Diff $^2$ = 480

Conc= Std Concentration
Meas=Measured Peak Area
Fitted=Peak Area from Fitted Line at given Conc
Diff= Meas – Fitted
Diff2= Diff Squared



# Comparison of Suggested 'Possible' Fitted Line and Least Squares Fitted Line



Using 'Possible' Fitted Line

| Conc | Meas. | Fitted | Diff | Diff $^2$ |
|---|---|---|---|---|
| 2 | 200 | 200 | 0 | 0 |
| 3 | 300 | 300 | 0 | 0 |
| 4 | 400 | 400 | 0 | 0 |
| 5 | 540 | 500 | 40 | 1600 |

Sum of Diff $^2$ = 1600

Using Linear Least Squares Fitted Line

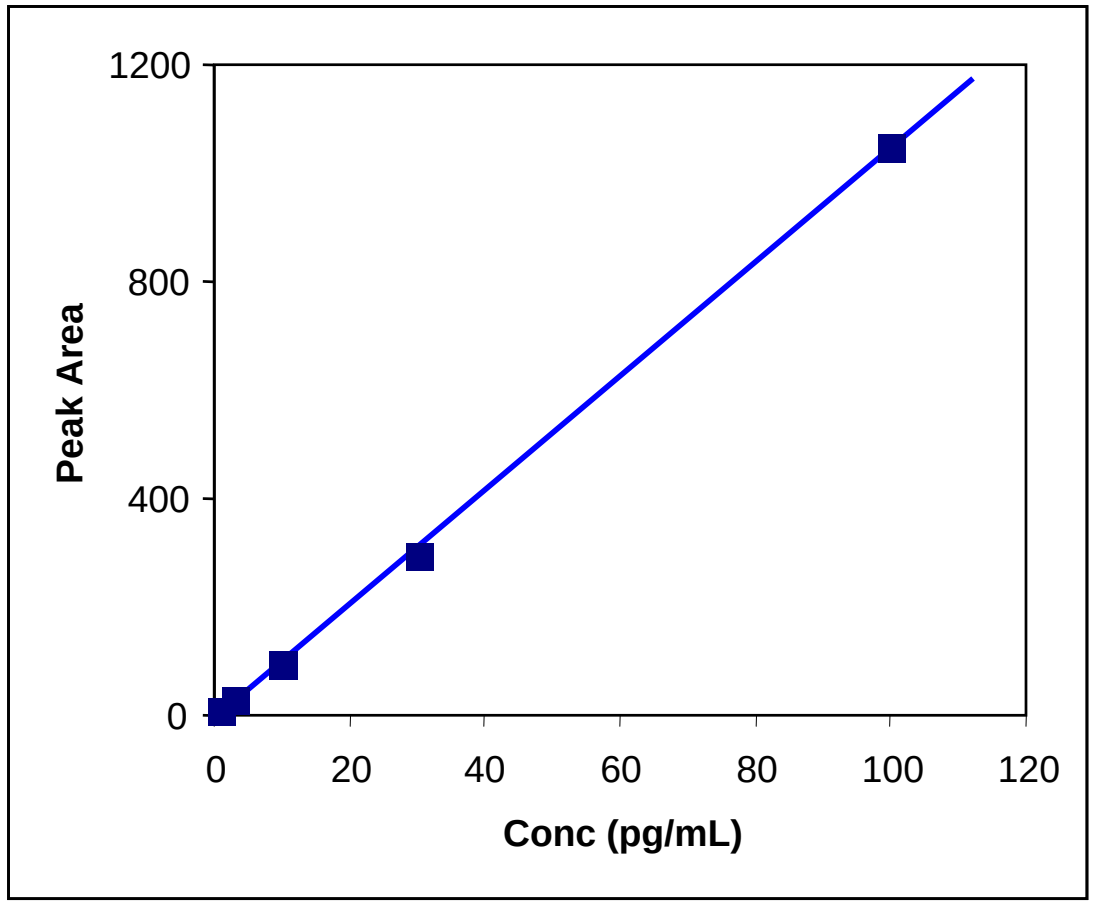
| Conc | Meas. | Fitted | Diff | Diff $^2$ |
|---|---|---|---|---|
| 2 | 200 | 192 | 8 | 64 |
| 3 | 300 | 304 | - 4 | 16 |
| 4 | 400 | 416 | - 16 | 256 |
| 5 | 540 | 528 | 12 | 144 |

Sum of Diff $^2$ = 480

# Example of Data from Analysis of Standards and Quantitation Calibration Curve

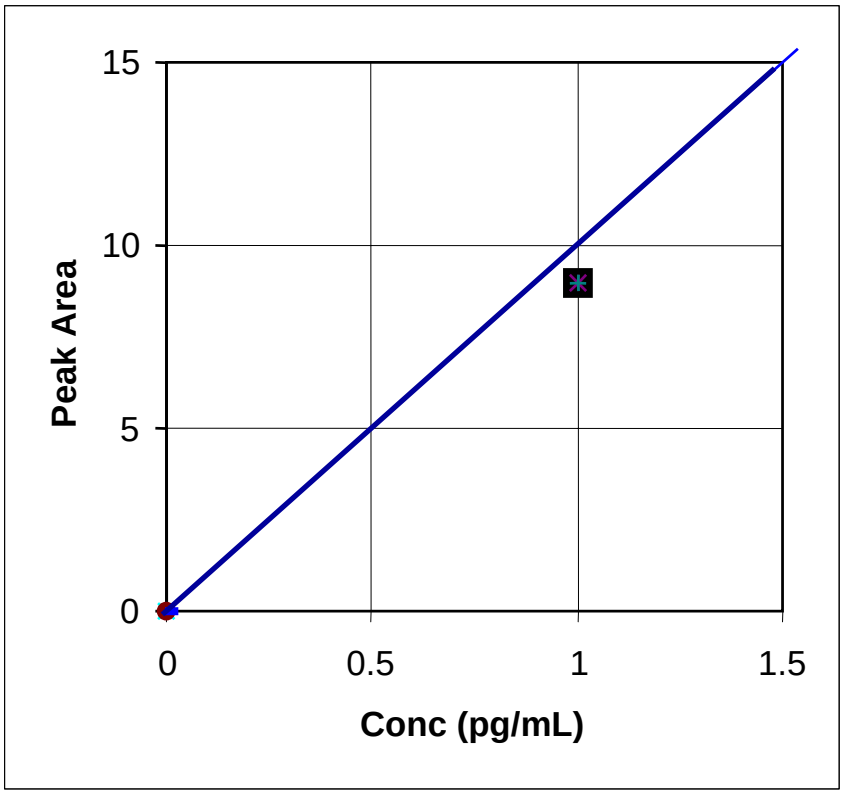


| Conc | Peak Area |
|---|---|
| 1 | 9 |
| 3 | 30 |
| 10 | 100 |
| 30 | 300 |
| 100 | 1050 |

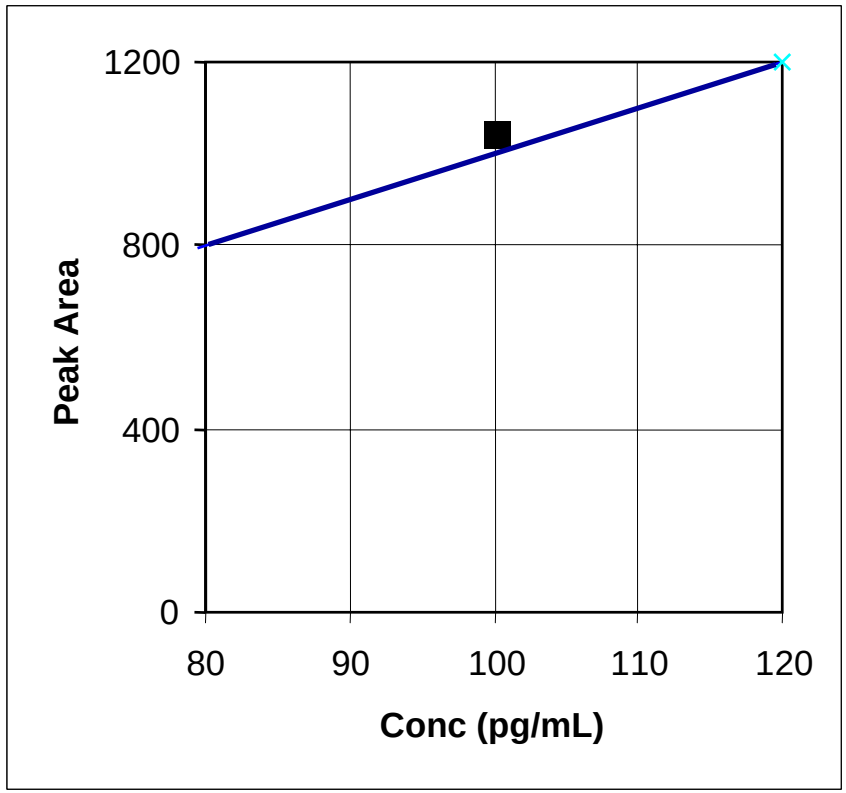


# Closer Look at 'Ideal' Fitted Line



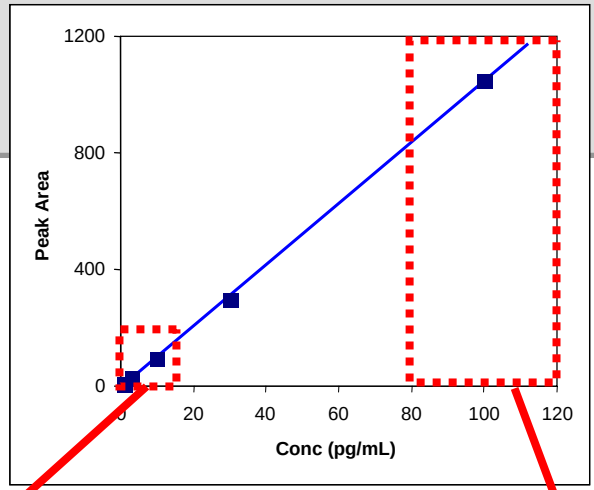


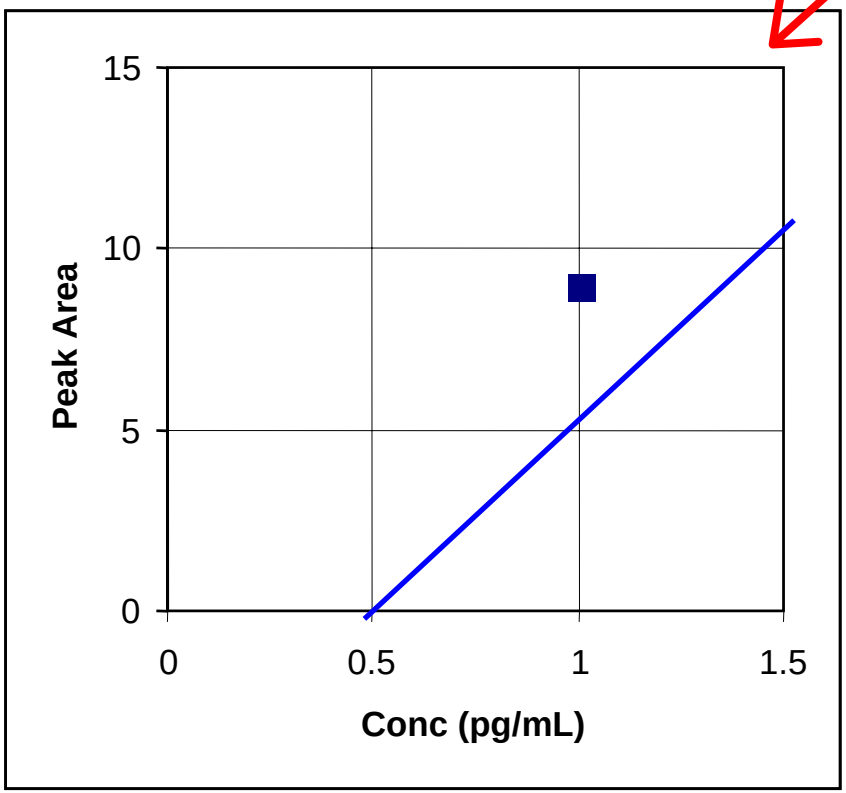

Diff = 1  Diff $^{2}$ = 1

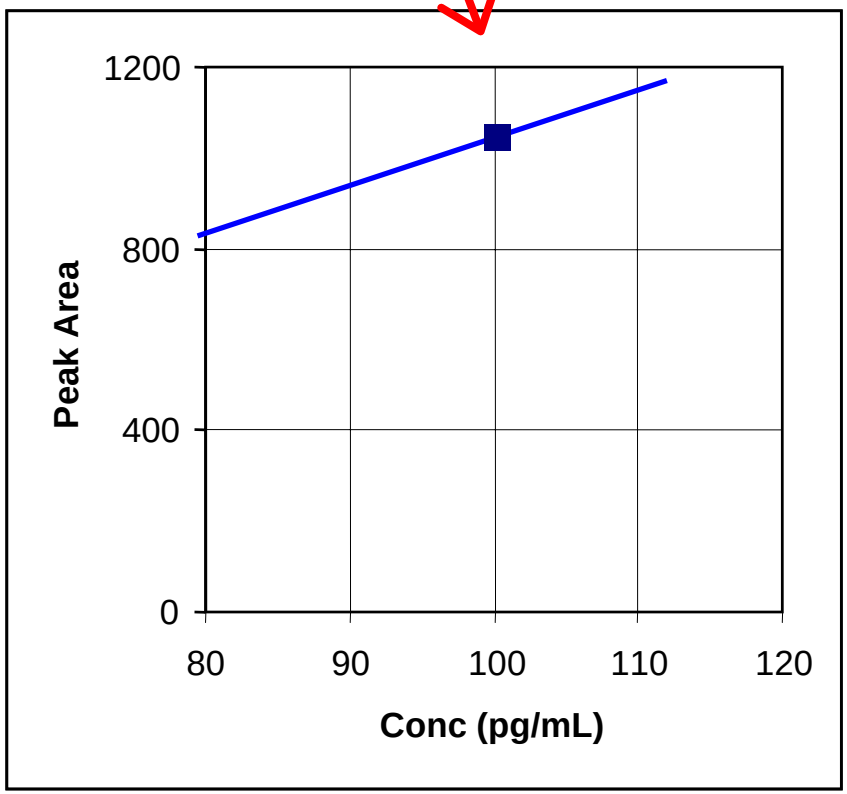



Diff = 50  Diff $^{2}$ = 2500

# Closer Look at Linear Least Squares Line Fitted with No Weighting

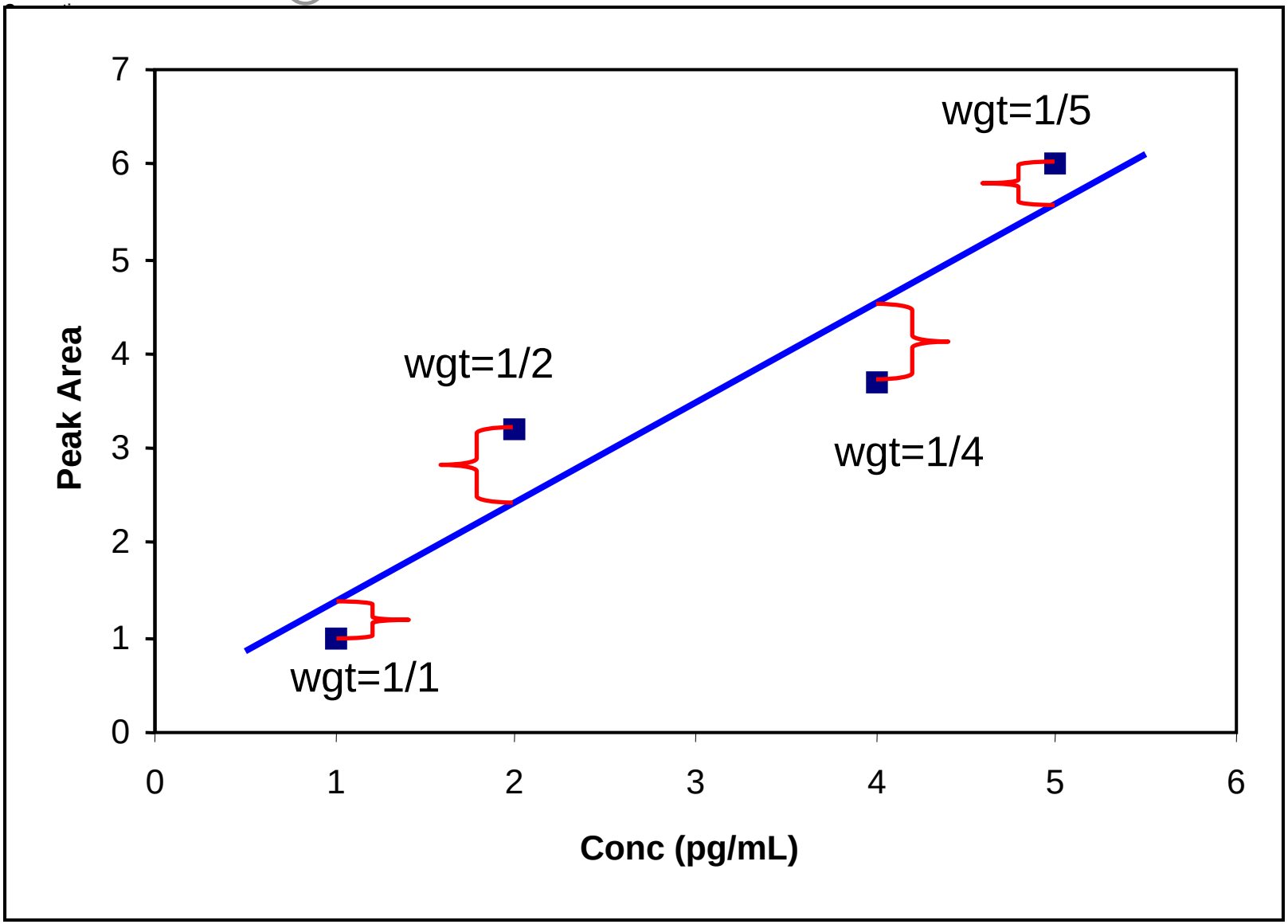


# Example of 1/X Weighting Factors

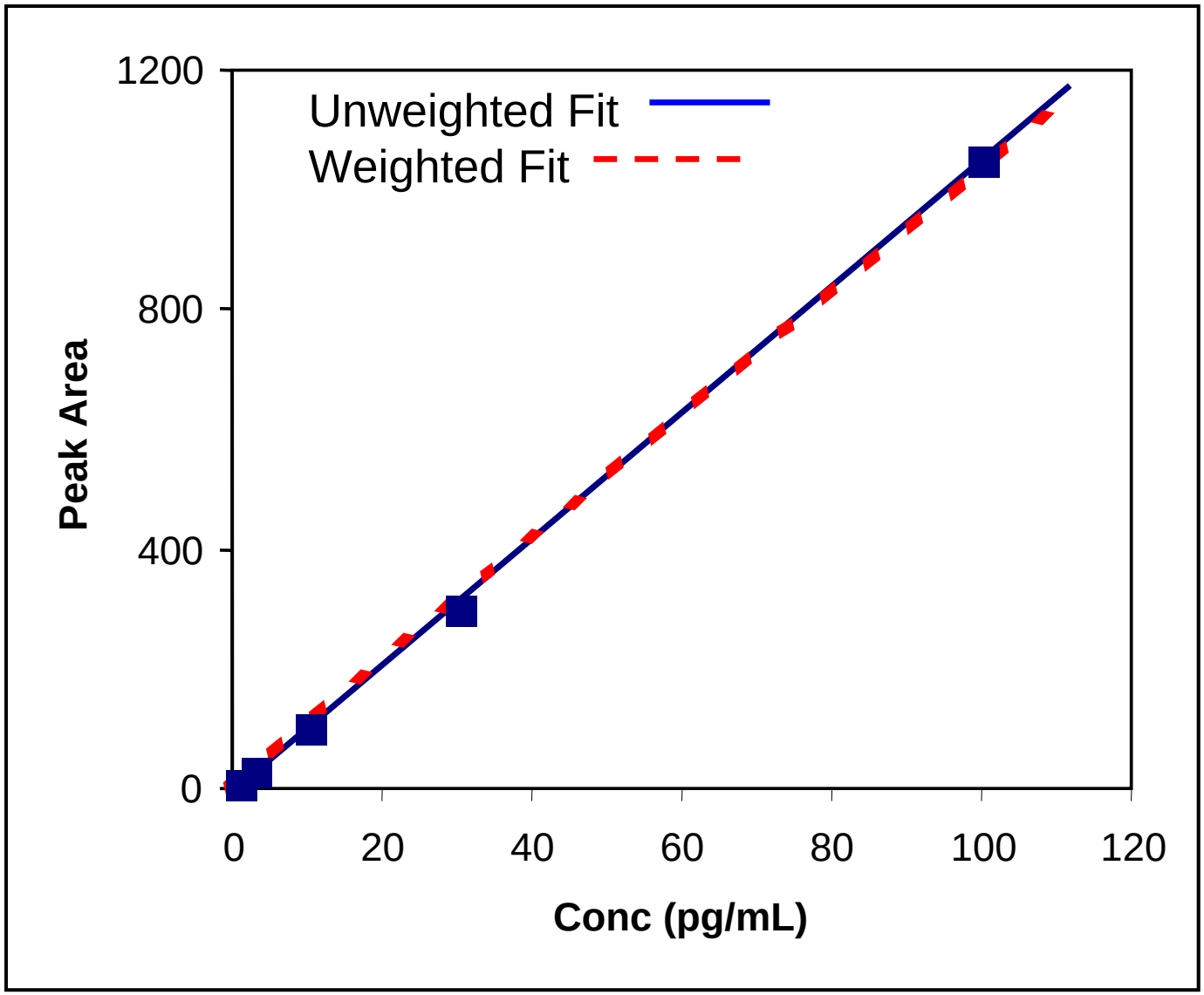




Waters

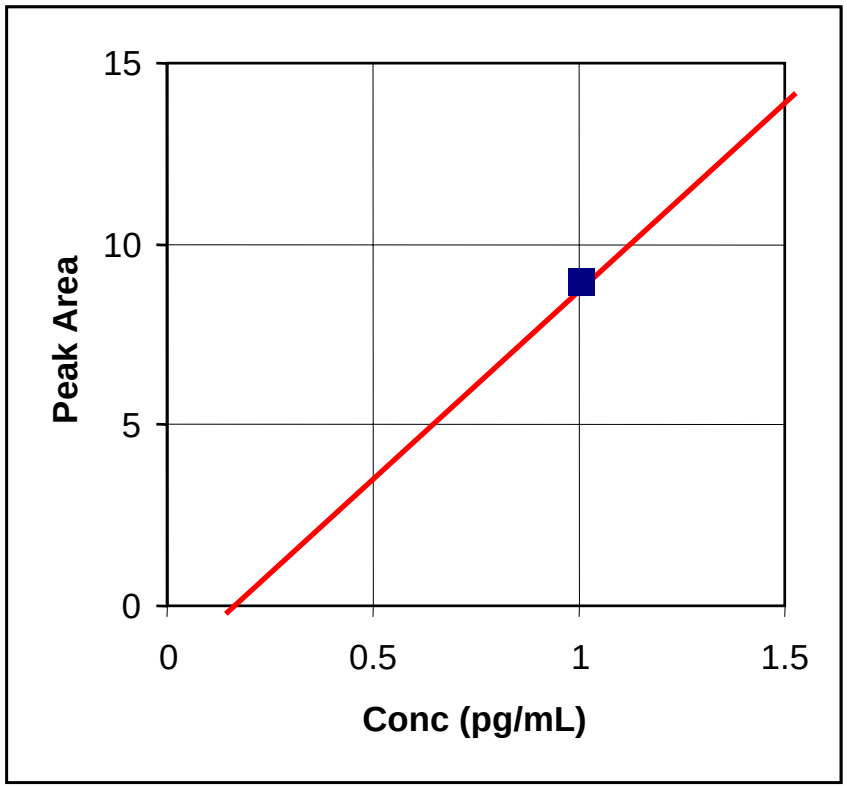
# Example of Line Fitted with No Weighting and Line Fitted with 1/X Weighting

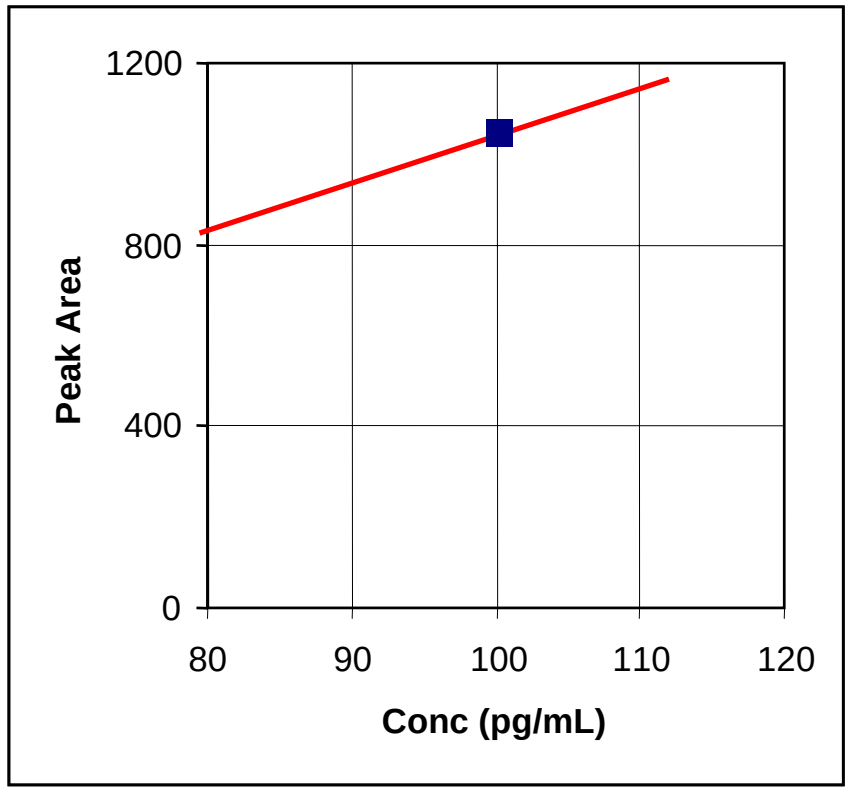




# Closer Look at Linear Least Squares Line Fitted with 1/X Weighting





# QC Samples Analyzed Using Line Fitted with No Weighting and Using Line Fitted with 1/X Weighting



| | | | Line Fitted w/ No Weighting | | | Line Fitted w/ 1/X Weighting | |
|---|---|---|---|---|---|---|---|
| Nom Conc | Peak Area | | Calc Conc | Rel Error | | Calc Conc | Rel Error |
| 1 | 10 | | 1.45 | 45.0% | | 1.12 | 12.4% |
| 3 | 30 | | 3.35 | 11.7% | | 3.05 | 1.6% |
| 10 | 100 | | 10.00 | 0.0% | | 9.78 | -2.2% |
| 30 | 300 | | 29.01 | -3.3% | | 29.01 | -3.3% |
| 100 | 1000 | | 95.53 | -4.5% | | 96.33 | -3.7% |