

# MassHunter Quantitative Analysis Software

Data Processing may be performed in the computer lab by locating the Mass Hunter Quantitative software on the desktop



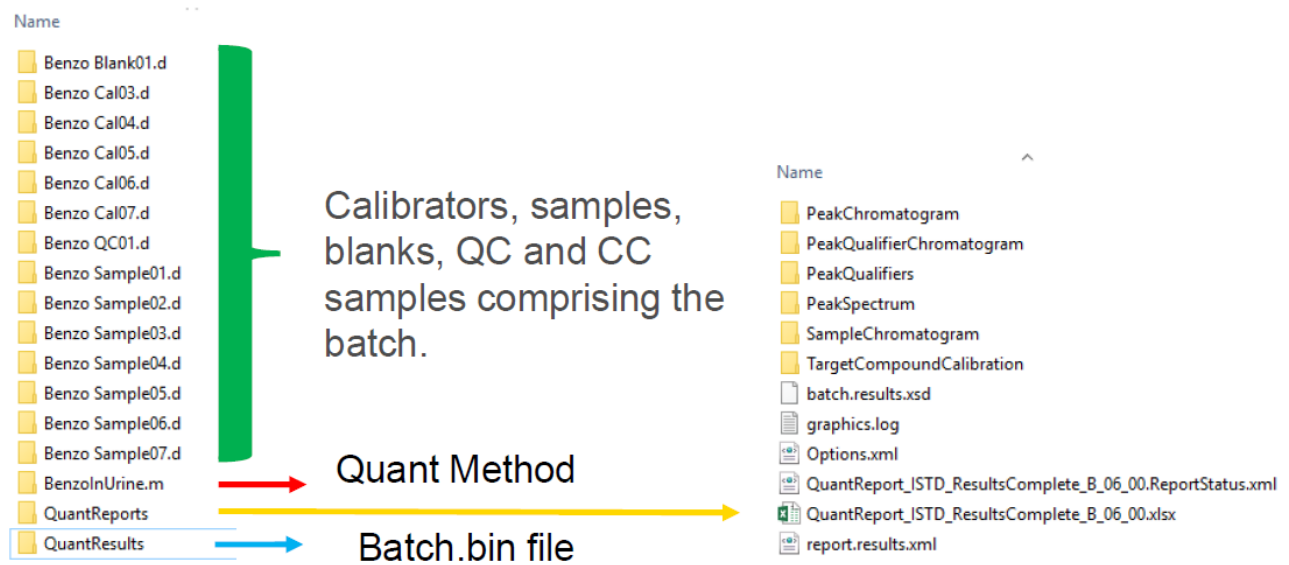
**Note:** All the data files must reside in the same directory, so put them together before creating a new batch.

**Batch:** A set of data files, including calibration solutions, samples, blank, etc. Data in Batch will operate on only one method.

**Method:** A list of target compounds with quantifier and qualifier ions specified for each compound, RT, ISTD info, integration parameters, etc.

**Results:** After analyzing the Batch, generated results (concentration, LOD, LOQ,  $R^2$ , and any other quantitative data we are looking for) are in the Batch (xml file). These results are generated and contained within the batch Table! and graphically in either the Batch view or the compounds at a glance view.

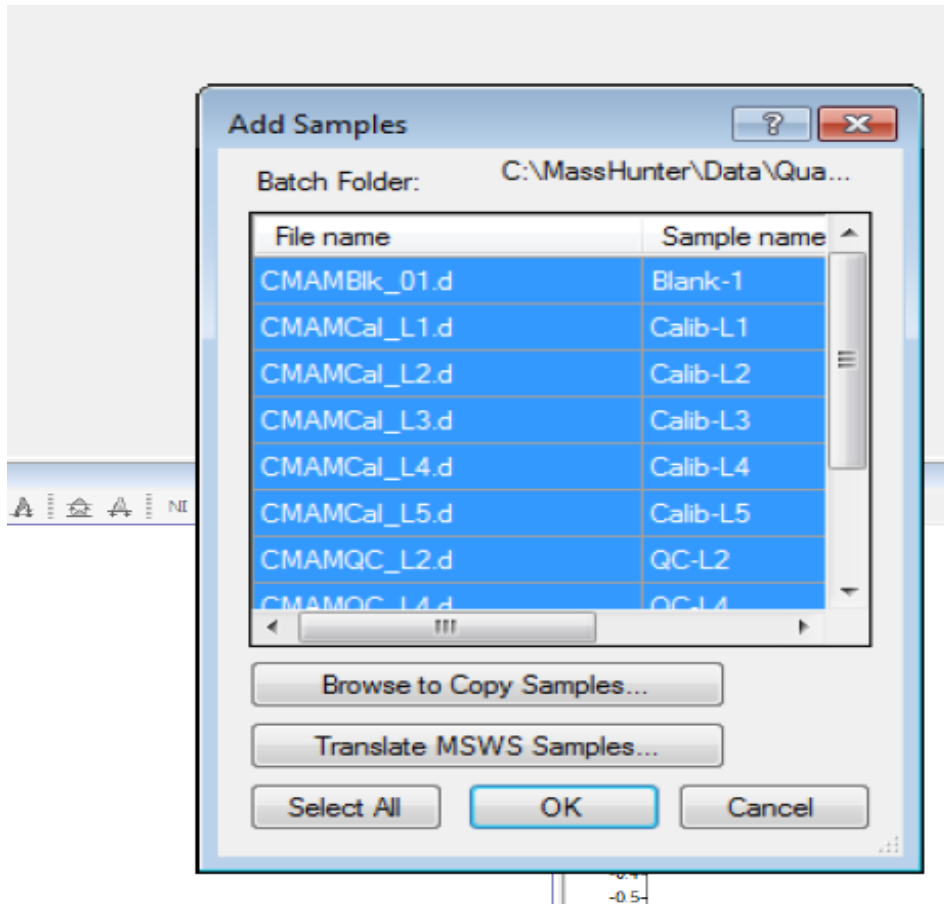
Typical files located in the data files folder:



## Creating a Batch for processing:

Click on File → new Batch → create the Batch in the directory in which the data files reside (if you tried to do the data processing with your data before, you may face some existing Batch, You shouldn't use the existing Batch because at first you chose to create new Batch) write a proper name for your batch in file name → Create.

Then you will see the add sample window, you can add all samples or some of them Ok. As seen in the picture.



You can see a table of data, in the **Type column** you need to select the type of sample by clicking on the little arrow near it and choosing cal, blank, sample etc.

In the **level column**, you need to name only calibration samples using for example L1, L2, L3 or A, B, C

You can add data files to this data table, File menu → add sample → Brows to copy new sample → navigate to where the samples are located → selecting the data that you want to save the Batch.

### Creating Method for Data Processing:

Click on method → Here you have two options, you can open the existing method (.m file) or create a new method, If you decide to create a new method you will see a menu like the picture below:

# Creating a Quantitation Method for SQ

## Method > New

### New Method from Acquired SIM Data...

Only available from MS Quantitative Analysis icon.  
Available in QQQ but not as useful.

### New Method from Acquired Scan Data... (with Library Search)

Use Library Method to identify and create compounds.  
Clean up unwanted compounds.  
Edit ions choices (Selected ions may not be the "best" ions).

### New Method from Acquired Chromatographic Data...

Used for 2D Data (UV, FID, ECD, etc.).  
Gives only Compound\_1, etc. based on RT.

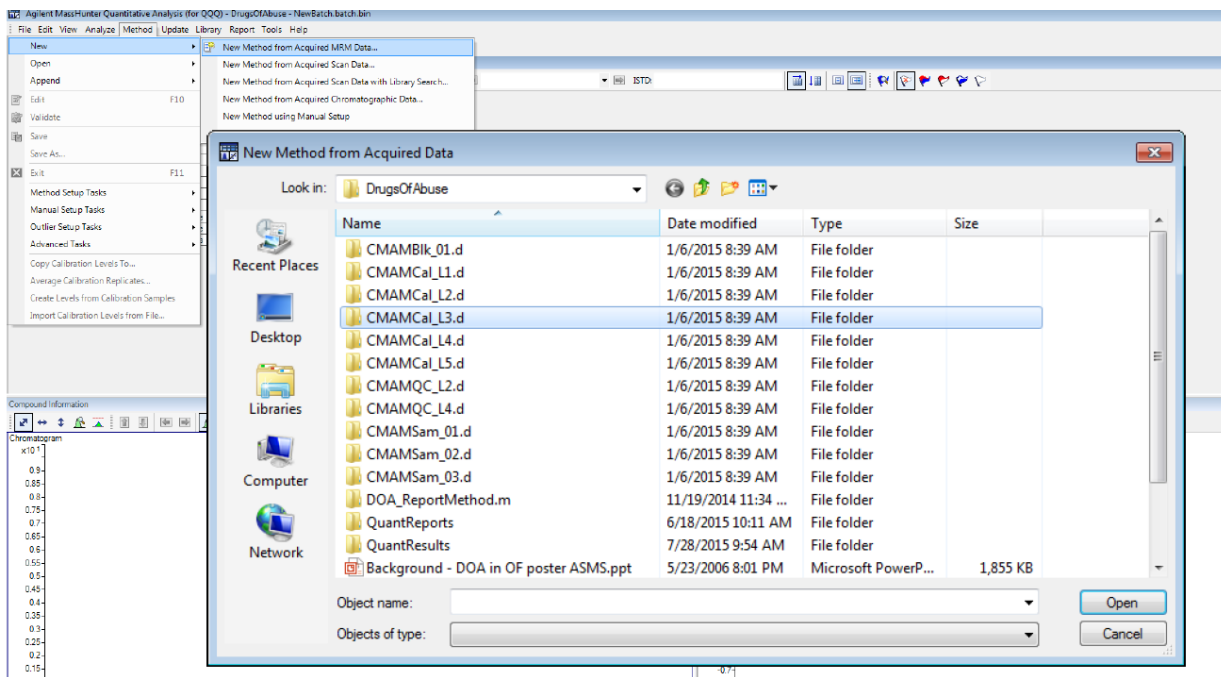
### New Method using Manual Setup

Manually enter compound data (RT, concentration, masses, etc.).  
Can be tedious for large number of compounds.

### New Method from File...

From CEF file generated in Qualitative Analysis.  
From a small user generated library.

**Tip: Avoid using Manual Setup**



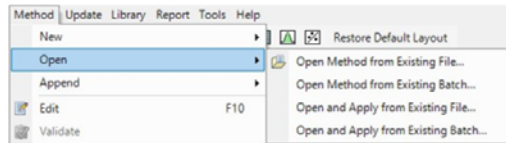
*If you want to open the existing Batch you will face some options like the picture.*

## Using an Existing Method

**Method > Open from Existing File...**

**Method > Open from Existing Batch...**

The quantitation database is saved in the method and in the batch file and can be recalled from either location.



Opening and applying method from existing file or batch does not automatically enter the Method Editor—stays at the Batch Table.

Go to Method menu → new → from acquired data with library search → IN Library method select New → Library search tab → choose the NIST as library → save and exit (**it will ask for directory and saving the method, a) choose the data file directory and name it like the batch name or b) save it in Acquisition method or c) in a separate DaMethod folder in you D drive**) After that brows → choose only one of the data not all of them (the high concentration one is recommended) → set the number of qualifiers equal to 2 → ok.

On the left side you will see the Method setup task menu:

Compound setup, RT setup, ISTD setup, Concentration setup, Qualifier setup, and .... Step through each of them one by one to develop and validate the quantitation of the database.

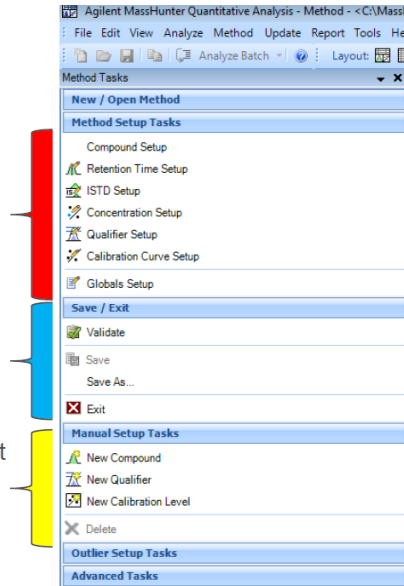
## -Method Tasks

Method Tasks organizes the method actions and parameters into related sections.

Main method editing functions.

Validate content, save and apply Quant method.

Use when you cannot add compounds or qualifiers automatically.



### Compound setup:

Quantifier							
Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria
BHC beta isomer	1	Scan	Target	219.0	14.327	Positive	Close RT
BHC delta isomer	1	Scan	Target	181.0	15.693	Positive	Close RT
Disulfoton	1	Scan	Target	88.0	15.688	Positive	Close RT
▶ Methyl parathion	1	Scan	Target	263.0	17.726	Positive	Close RT

Here you can add more compound manually, or change the name of compound if you want, or delete some of those compounds with low matching percent (you can add match percent column to this table by right click and add/remove column option).

### Retention Time setup:

Quantifier							
Name	TS	Scan	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units
▶ BHC beta isomer	1	Scan	Target	14.327	0.164	0.164	Minutes
BHC delta isomer	1	Scan	Target	15.693	0.164	0.164	Minutes
Disulfoton	1	Scan	Target	15.688	0.164	0.164	Minutes
Methyl parathion	1	Scan	Target	17.726	0.164	0.164	Minutes
Endosulfan (alph...	1	Scan	Target	23.619	0.164	0.164	Minutes

There are Left and Right Delta windows, they show how you want to go in the left or right side of this retention time. Recommended delta is 0.2 (set the first one on 0.2, right click on it and use Fill Down option).

## ISTD setup:

Quantifier							
Name	TS	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Reference Flag
4,4'-Dibromodifluorobiphenyl	1	Scan	ISTD	<None>	<input checked="" type="checkbox"/>	0.4970	<input type="checkbox"/>
Sulfotep	1	Scan	Target	4,4'-Dibromooctafluorobiphenyl	<input type="checkbox"/>		<input type="checkbox"/>
Phorate	1	Scan	Target	4,4'-Dibromooctafluorobiphenyl	<input type="checkbox"/>		<input type="checkbox"/>
BHC alpha isomer	1	Scan	Target	4,4'-Dibromooctafluorobiphenyl	<input type="checkbox"/>		<input type="checkbox"/>
Pentachloroanisole	1	Scan	Target	4,4'-Dibromooctafluorobiphenyl	<input type="checkbox"/>		<input type="checkbox"/>

Check the ISTD flag, fill the ISTD concentration, and set the ISTD name for all the other target compounds.

## Concentration setup:

Defines the concentration of each compound in the standards.

Agilent MassHunter Quantitative Analysis - Method - <C:\MassHunter\Data\QuantExamples\MS\VOA\QuantResults\VolatilesOrganics.batch.bin>

Method Table

Name	TS	Scan	Type	Units
Dichlorodifluoro...	1	Scan	Target	ng/ml

Level	Conc.	Response	Enable
3	0.5000	29715	<input checked="" type="checkbox"/>
4	1.0000	65597	<input checked="" type="checkbox"/>
5	2.0000	127904	<input checked="" type="checkbox"/>
6	5.0000	198094	<input checked="" type="checkbox"/>
6	5.0000	211200	<input checked="" type="checkbox"/>
7	10.0000	671861	<input checked="" type="checkbox"/>
7	10.0000	795390	<input checked="" type="checkbox"/>
8	15.0000	1105065	<input checked="" type="checkbox"/>
9	20.0000	1474827	<input checked="" type="checkbox"/>
10	30.0000	2199968	<input checked="" type="checkbox"/>
11	40.0000	3126148	<input checked="" type="checkbox"/>
12	50.0000	3975819	<input checked="" type="checkbox"/>

If Unchecked a level can be disabled and not appear in the calibration curve.

Level in Method must match Level in Batch table!

Agilent MassHunter Quantitative Analysis - VOA - VolatileOrganics.batch.bin

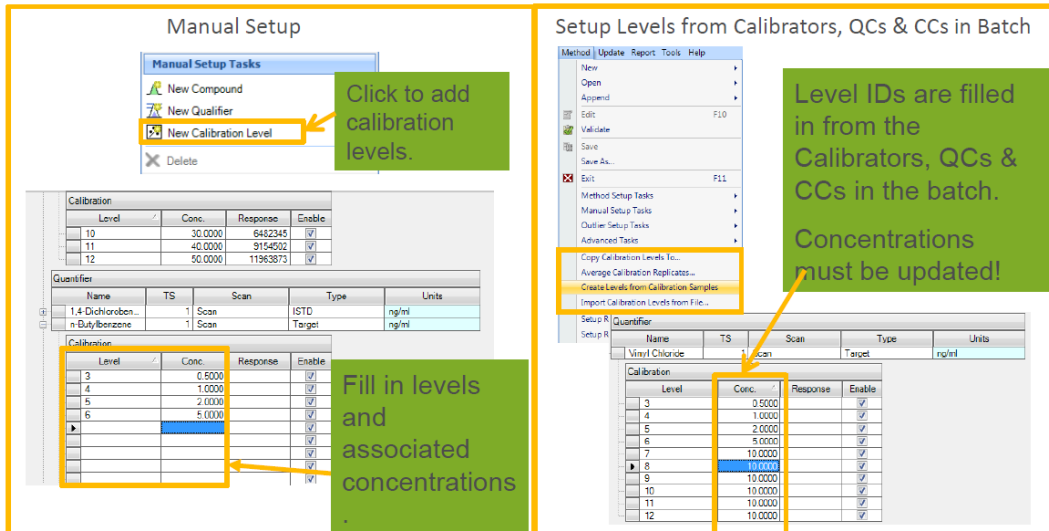
Batch Table

Name	Data File	Type	Level	Acq. Date-Time
CAL_L03	CAL_L03.D	Cal	3	6/20/2008 8:53 AM
CAL_L04	CAL_L04.D	Cal	4	6/20/2008 9:30 AM
CAL_L05	CAL_L05.D	Cal	5	6/20/2008 10:05 AM
CAL_L06	CAL_L06.D	Cal	6	6/20/2008 10:44 AM

Concentration setup bring up the calibration table with their level, concentration, response and enable Curve Setup which is a way for disabling a specific compound in calibration curve by unchecking it. Remember that Level in method must match Level in Batch table.

Now you need to create the calibration levels for all the calibration compounds (which are distinguished in Batch table). There are 2 different ways of doing this, manual setup or automatically:

Calibration levels can be created manually or automatically.



In **automatic setup** go to Method menu → create levels from calibration samples → fill the concentration column.

If you don't want to fill the concentration and have them already, you need to do this process in the first step when you were creating the data table (batch), you can add a new column like the picture and add the expected concentration there, and all those information will fill automatically in concentration setup.

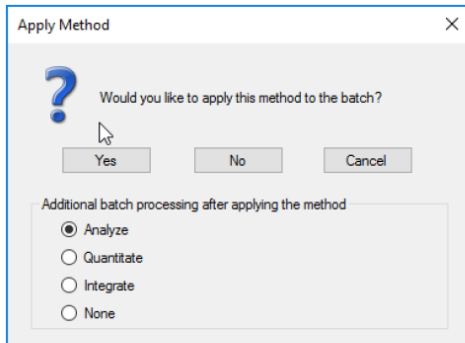
Name	Data File	Type	Level	Exp. Conc.
Benzo Cal03	Benzo Cal03.d	Cal	L1	25.0000
Benzo Cal04	Benzo Cal04.d	Cal	L2	50.0000
Benzo Cal05	Benzo Cal05.d	Cal	L3	125.0000
Benzo Cal06	Benzo Cal06.d	Cal	L4	250.0000
Benzo Cal07	Benzo Cal07.d	Cal	L5	500.0000
Benzo QC	Benzo QC01.d	QC	QC1	189.0000

Finally, if the concentrations are the same, they can be copied to other compounds too, by right click → copy calibration levels to → select all → Ok.

The next step is to **validate the method and save it**:

When you call the validation, if there is no error you can move forward, sometimes you will face some errors at the bottom of the method editor screen, the red icons must be corrected, double click on the error and check what you did wrong. Yellow icons are Warning errors. The next step is saving the method, here we are saving the method before analyzing the data, that's why the saved method does not consist of a calibration table, after saving and exiting we can analyze the Batch, and go back to the method editor and do the save as, so the method will have the calibration table within that .m file.

Exit the method, it will ask.



**Analyze** may (\*) rebuild the calibration curve and then calculates analyte concentrations.

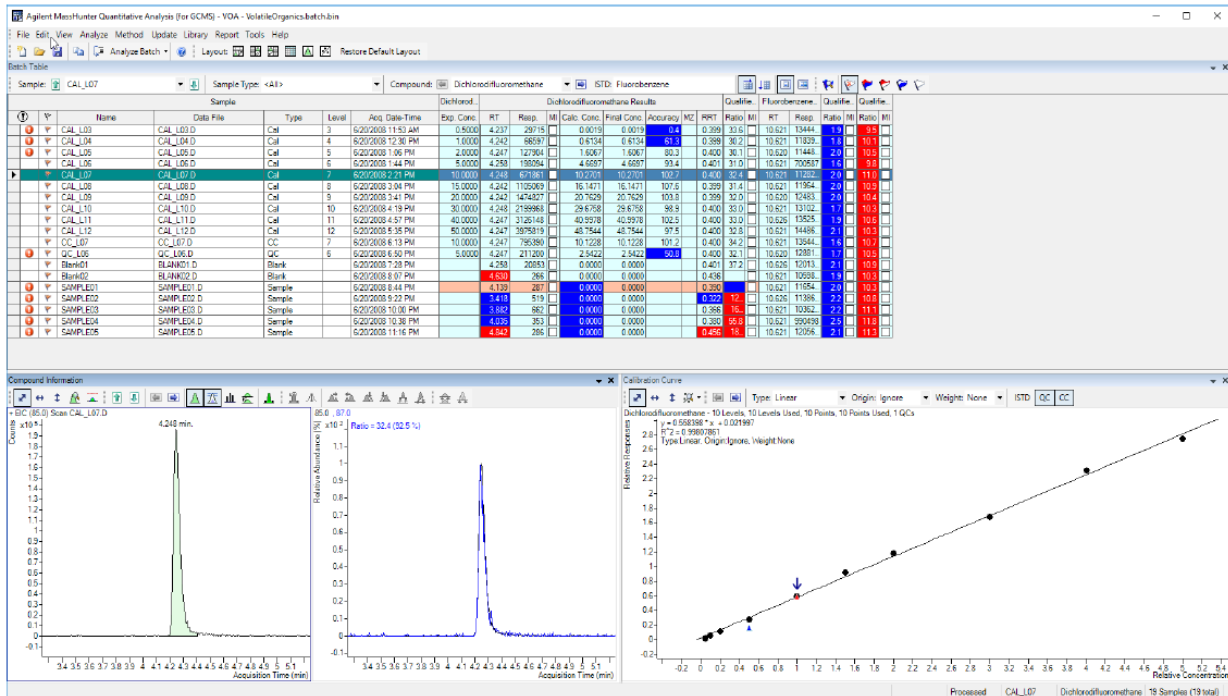
**Quantitate** does *not* rebuild the calibration curve but calculates concentrations based on the existing curve.

**Integrate** simply calculates the response for compounds in the batch. It does not use the calibration curve nor obtain final concentrations.

**\* New Feature: B.07.00 and later – Calibration Curve is cleared IF batch contains any Cals.**



Mark the Analyze icon and hit yes, and now we have analyzed the Batch.

## Analyzed Batch !!!



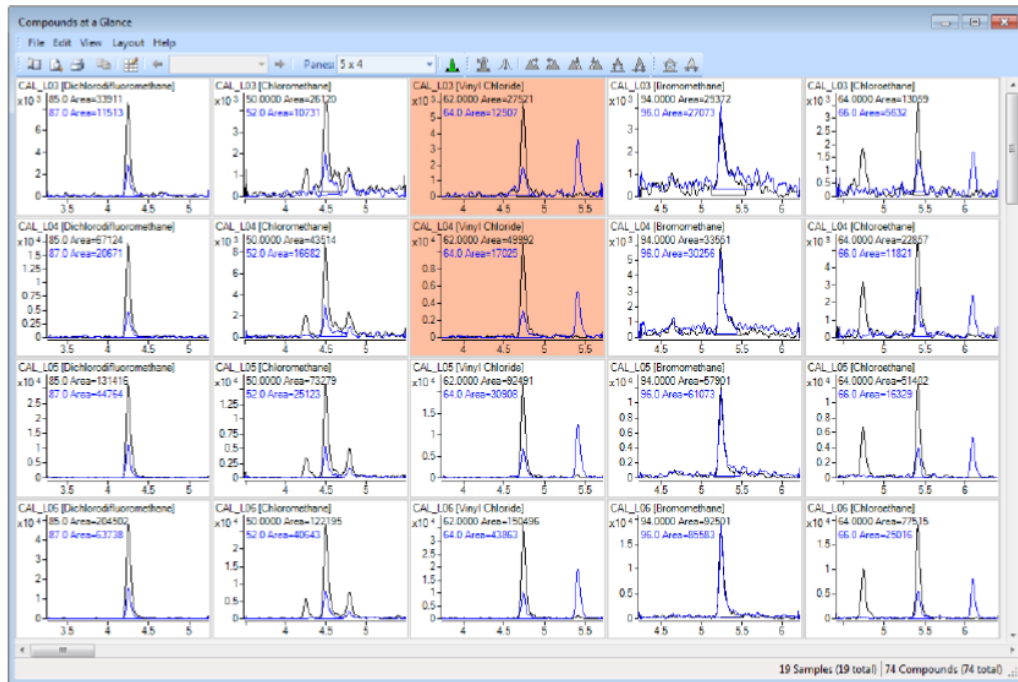
❖ **Saving a method with the calibration table information:** save the method again after analyzing the batch. Method menu → Edit → save as (to retain the calibration table) → **save it again**. Once developed, methods can be used over and over.



- ❖ Or you may want to save this method in your Acquisition method (METHODS directory in D drive), you will see after saving in Acquisition method, there will be a new sub folder called DaMethod which makes you able to copy and paste it in any other method that you want.
- ❖ Or you may want to have a separate location for all of your DaMethods in D drive too. Keep it in mind, that when you want to use a saved DaMethod in your METHODS directory, after creating a new Batch you must not create a new method, go to the Method menu → open → open and apply from existing file → select the method.
- ❖ If you need to have the top table as an Excel file, go to File menu → export → change the format as xlsx and choose the directory → save it.
- ❖ You can add or remove anything that you want (Resp., LOD, LOQ, R<sup>2</sup> ...) to this table using right-click on the table → add/removing the column.
- ❖ You can see different samples in the table using up/down arrows, and different compounds of a specific sample using left/right arrows.
- ❖ If you want to see the chromatogram in this step, go to View menu → sample information.
- ❖ By double clicking on the chromatogram, you will see the MS spectrums below the chromatogram by right Right-clicking on the MS spectrum you can do a Library search → file → open library → pick NIST → OK.
- ❖ In left down corner, in the Compound information section, using this icon  you can add the internal standard chromatogram and MS peaks. and you can do the manual integration using this icon .
- ❖ After the analyzing batch if you decide to remove some samples (like E and F which have no chromatograms) go to the Analyze menu → Replace calibration → Select all compounds but in selecting samples, select those that you want → Ok → Analyze the Batch again. (The difference between the Analyze Batch and Quantitate Batch is, that analyze will rebuild the calibration curve, the quantitate just takes the current curve and calculates results.
- ❖ For updating the Retention Time, in the top table select one of the samples (highlight it) → Method → Edit. Then go to the Update menu → you can do “update RT” or “Shift RT”.
- ❖ For getting data from both Scan and SIM, go to Method menu → Edit → Compound setup menu → right click on target compound name in table → Duplicate compounds → in the scan column, you can change the scan to SIM.
- ❖ In the calibration curve, if you want to disable one of the points, just click on it, and you will see in the table all the data will be recalculated.
- ❖ Click down below the curve → Curve fit assistant → it tries all the different possibilities for calibration curve and it has chosen what has the best R<sup>2</sup>.

In view tab → compound at a glance → you can see a nice view of all, samples in rows and compounds in columns, it is a good way to see multiple data file and compound at the same time.

## View > Compounds-at-a-Glance



You will see the main peak and 2 qualifiers, if you double click on it, you can see them separately. If you don't want to see the qualifiers, go to Layout menu → predefined layout → sample/target/auto scale (it won't show the blanks or calibrations, just the samples and targets. Also, in Layout menu you can choose to see only standards or calibrations too).

Turn on the outlier because they are off by default, there is a little icon for this purpose, or go to Layout icon → setup layout → outlier tab → select peak result and outlier and calibration and CC → apply → ok.

You can see some of them are highlighted (they are the same as those highlighted cells in the analyzed Batch table).

You can change the number of samples and compounds that you see in the panes tab.

In any compound chromatogram, by left-clicking and dragging on the X-axis, you can drag the axis forward and backward. By Right clicking and dragging, you can Zoom in. Another way of zooming in is by drawing a box around an interesting chromatogram by right click.

For zooming out, just right-click and do Auto scale or auto-scale all panes.

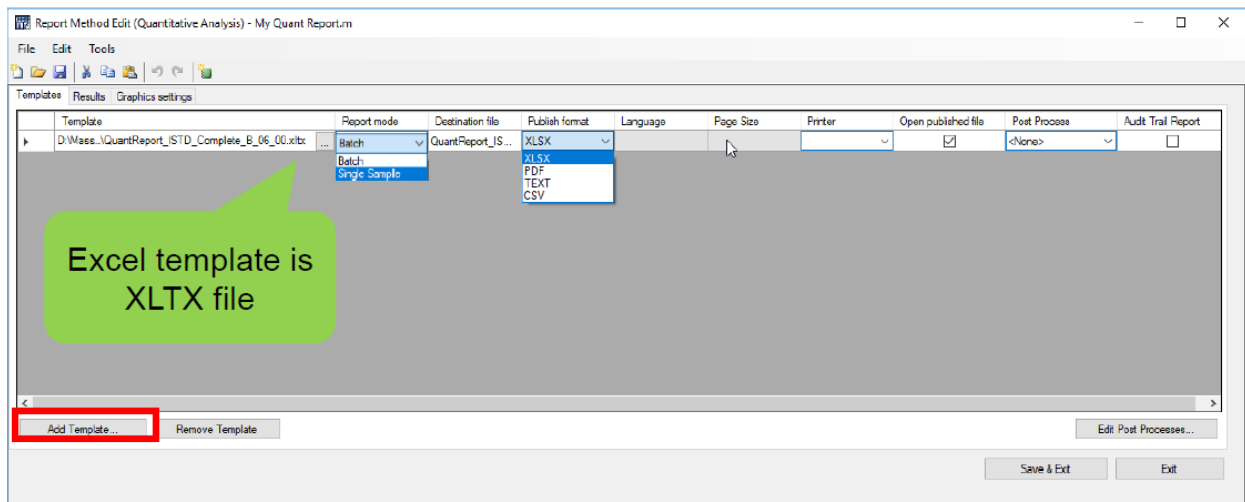
For zeroing a peak go to Tools → action → zero peaks lower LOD.

## Report generation:

We have 2 options: report generation using Excel and Report generation using PDF.

Go to Generate Report → In the “Report folder” section you need to set the location of interest (usually it has been set up in the data file directory, so do not change it).

**In Report method section → Edit tab → you will see a new window:**



### In the Template tab:

add template → Select the template that you want to use (from D drive, Mass Hunter, Report templates, Quant, PDF reporting, Gen-complete.report, open)

In report mode select what you want, the report of the whole Batch or just one sample.

In the publish format, select the format that you want, Excel or PDF.

Mark open publish the file.

### In Results tab:

## Results tab

Report Method Edit (Quantitative Analysis)

File Edit Tools

Templates Results Graphics settings

Instrument type: QQQ

Generate report results (report.results.xml):

Auto  
Generate results file only when Excel report templates are selected.

Yes  
Always generate results file

No  
Never generate results file

Upload results file

Select the correct instrument type

For Excel always select Auto or Yes

In the graphic tab:

## Graphic Settings tab.

Allows customization of the graphics output.

Report Method Edit (Quantitative Analysis) - My Quant Report.m

File Edit Tools

Templates Results Graphics settings

General:

Generate graphics files (.emf files)

Delete graphics files after report generation

Fixed range graphics:

Sample chromatograms:

Peak chromatograms:

Quantifier/Qualifier overlay chromatograms:

Spectra:

Calibration curves:

Always generate the graphics if the file contains graphic output.

Save and exit.

In the samples/compounds section choose samples → select just one of the data (it would be faster).

In the Generate section → mark open report folder after reports generated Ok.

# Report Method Edit

## PDF Reporting

PDF Reports are located D:\MassHunter\Report Templates\Quant\PDF-Reporting folder.

Run much faster for large batches.

PDF reports are built on Python code and take the form of xml files.

Only available Publish format is PDF.

On the **Results** tab, there is no need to generate the results file.

On the **Graphic Settings** tab, there is no need to Generate graphics files.

Reference: Agilent training: Quantitative Analysis Basics.