Agilent MassHunter Quantitative Data Analysis

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MassHunter Quantitation: Batch Table, Compound Information Setup, Calibration Curve and Globals Settings

MassHunter Quantitative Software Review and Quant Method Optimization

Topics

- Brief Review
- Batch Table Navigation
- Compound Information
  - Data review and manual integration
- Calibration Curve
- Working in Compounds At a Glance
- Method Editor Globals Setting
- Tip: Tips are labeled throughout the presentation

Review

Three major views in MassHunter Quantitative Analysis.

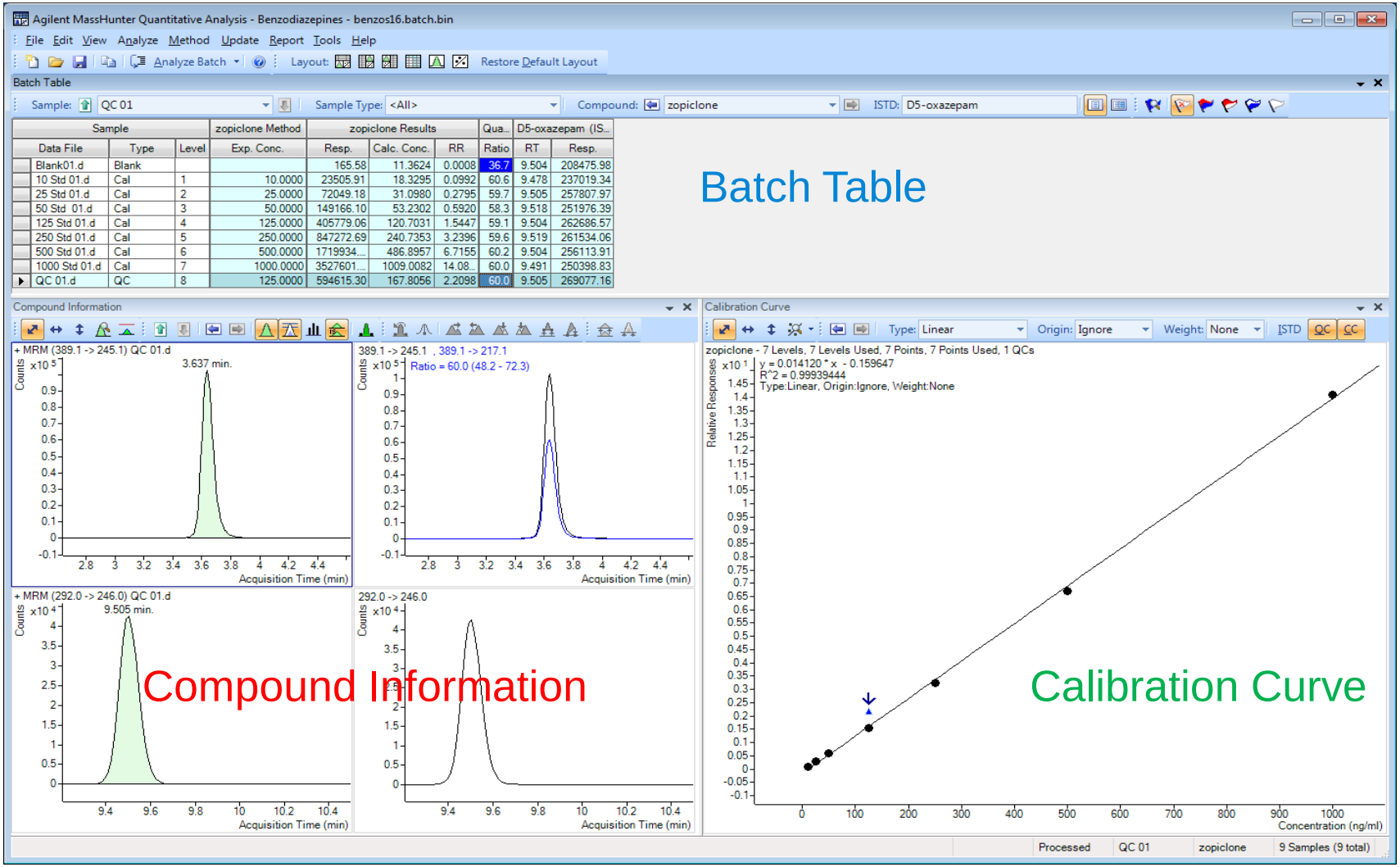
- Batch At a Glance
- Method Editor
- Compounds At a Glance

Handling MS/MS data QQQ and QTOF.

Handling accurate mass data TOF and QTOF.

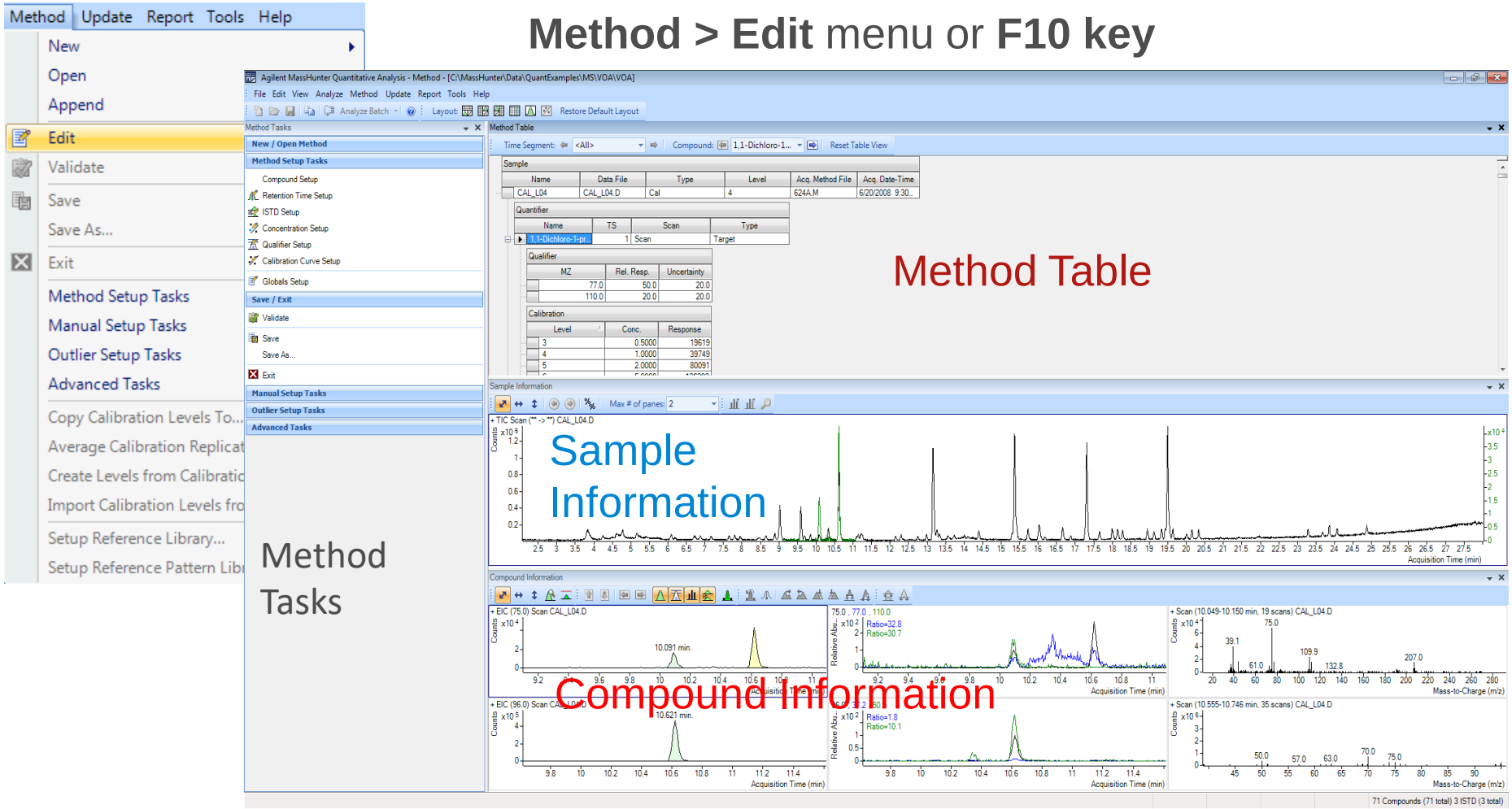
MassHunter Quantitative Software

Batch-at-a-Glance View



MassHunter Quantitative Software

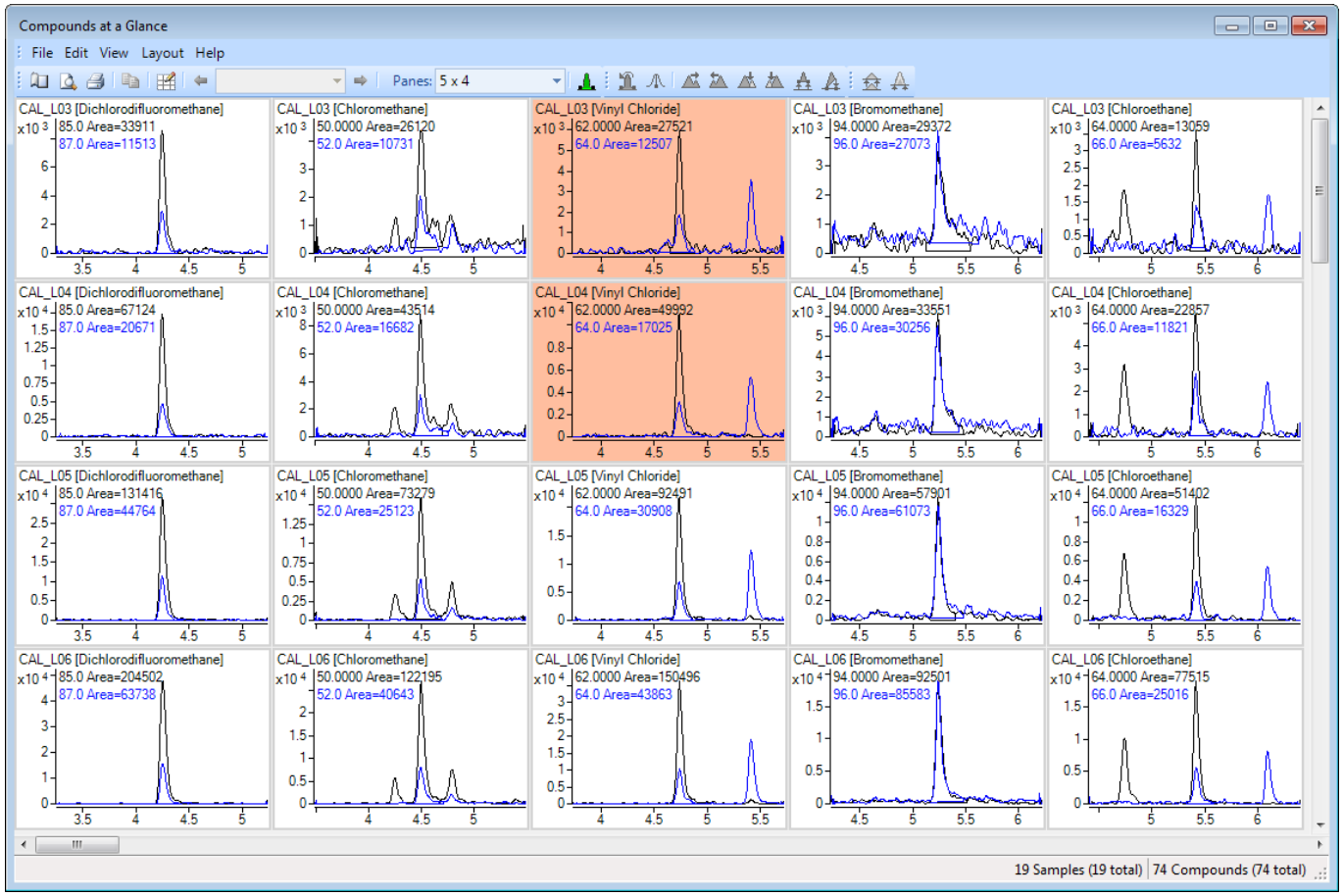
Method Editor View



MassHunter Quantitative Software

Compounds at a Glance View

View > Compounds-at-a-Glance



Editing a Quantitation Method for TOF
Mass Extraction Setup

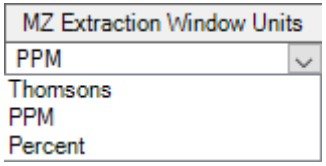
Method > Edit > Advanced Tasks > Mass Extraction Setup

Allows for a mass range for the extraction of the accurate mass (MZ).

Quantifier

Name	TS	Scan	Type	Extract Left m/z	MZ	Extract Right m/z	MZ Extraction Window Units
Sulfadimethoxine	1	Scan	ISTD	20.0000	311.0808	20.0000	PPM
Caffeine-DAD	1	Scan	Target	20.0000	195.0876	20.0000	PPM
Sulfadimethoxin...	1	Scan	ISTD	20.0000	311.0808	20.0000	PPM

Available MZ Extraction Window Units



Editing a Quantitation Method for QTOF

Compound Setup & Mass Extraction Setup

QTOF is a combination of MS/MS and accurate mass data

Quantifier

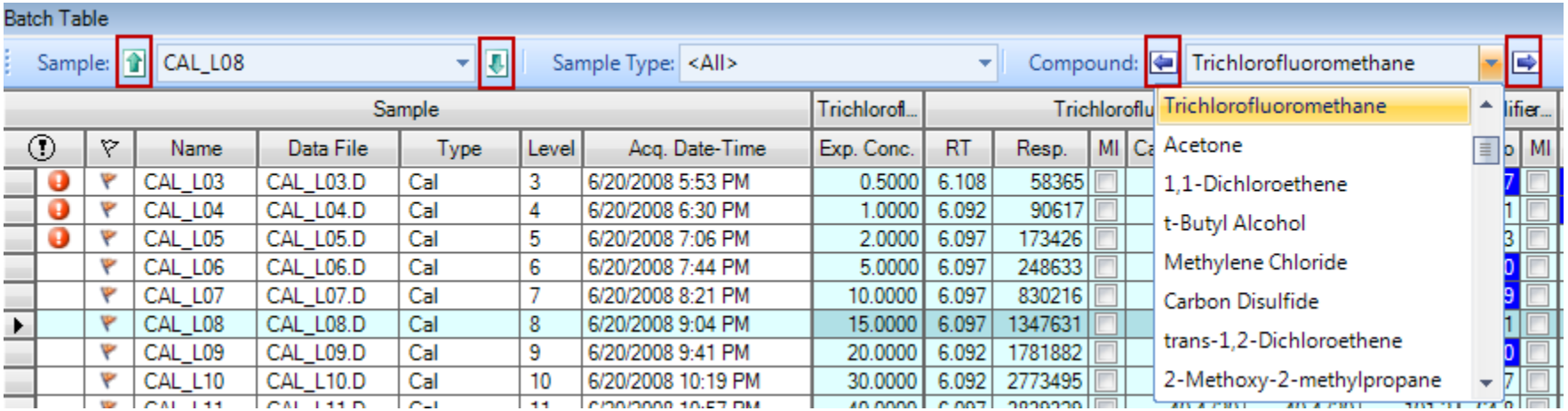
Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Ion Polarity	Criteria
Sulfamethizole	1	271.0318 -> 156.0114	Product Ion	Target	271.0318	156.0114	0.620	Positive	Greatest Response
Sulfachloropyridazine	1	285.0208 -> 156.0114	Product Ion	Target	285.0208	156.0114	0.890	Positive	Greatest Response
Sulfamethazine	1	279.0910 -> 186.0332, 156.0114, 124.0869	Product Ion	Target	279.0910	186.0332	2.030	Positive	Greatest Response
Sulfadimethoxine	3	311.0809 -> 156.0768, 218.0230, 245.1030	Product Ion	Target	311.0809	156.0768	2.950	Positive	Greatest Response
Sulfamethoxazole	2	254.0594 -> 156.0114	Product Ion	ISTD	254.0594	156.0114	0.940	Positive	Greatest Response

Name – Compound name
TS – time segments may be multiple
Transition – Precursor ion →Product ion scan
Scan – Product Ion
Type – Target, ISTD, Surrogate or Matrix Spike
Precursor Ion – mass of the ion
Product Ion – mass of the ion for the target ion to monitor
RT – retention time of compound of interest
Ion Polarity – usually positive
Criteria – Close RT, Close RT with Qualifiers, Greatest Response or Greatest Q-Value

Tip: Enter the Precursor Ion and the Product Ion—Transition auto populates.

Batch Table

Navigation



Click in a sample row to display data on that sample

Click on **Next** and **Previous** icons to move through a batch or use Hotkeys:

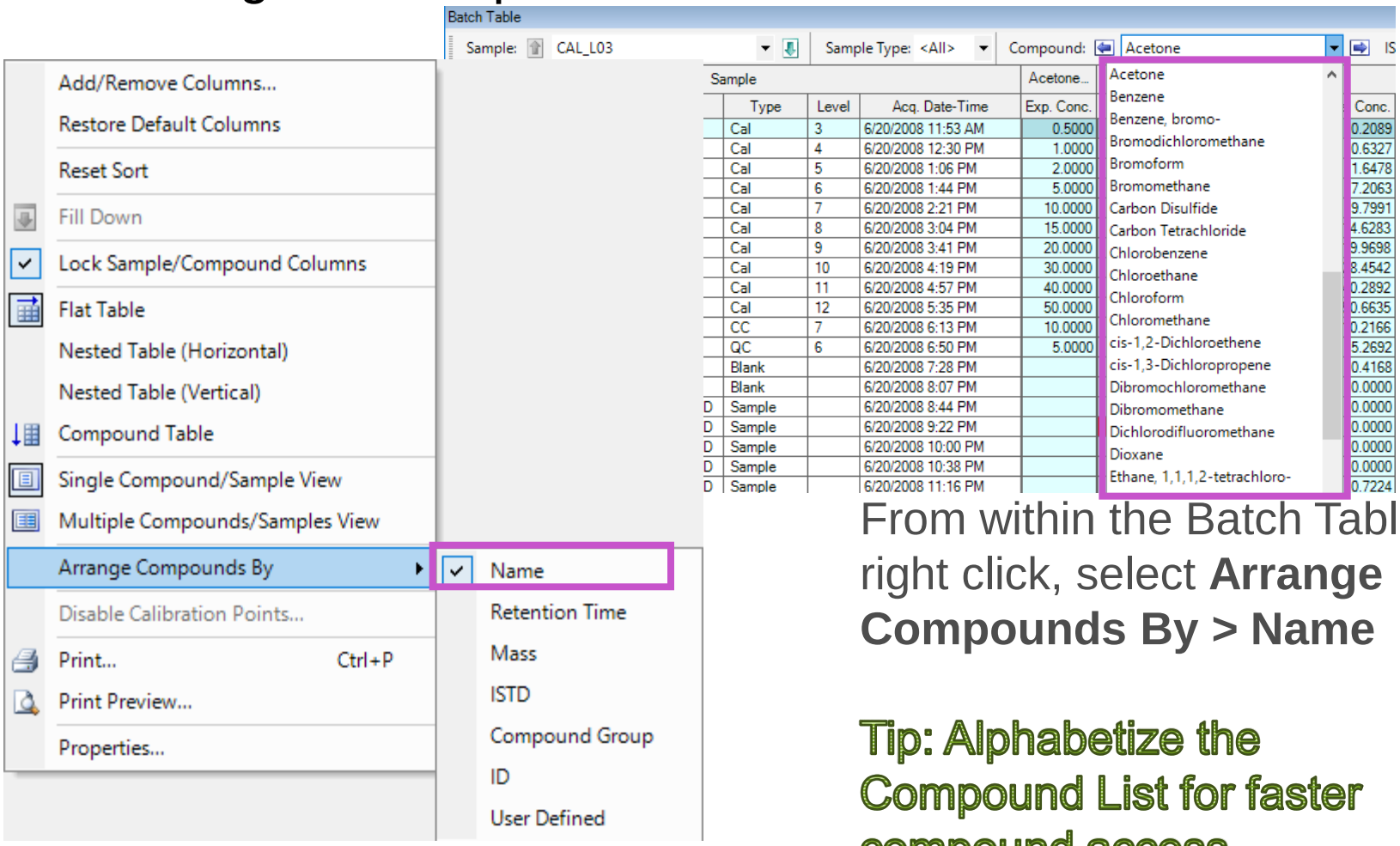
Next Sample = Alt + Down Next Compound = Alt + Right

Previous Sample= Alt + Up Previous Compound = Alt + Left

Compound list are frequently long, try the **Compound** drop down list to go directly to the desired compound.

Batch Table

Alphabetizing the Compound List

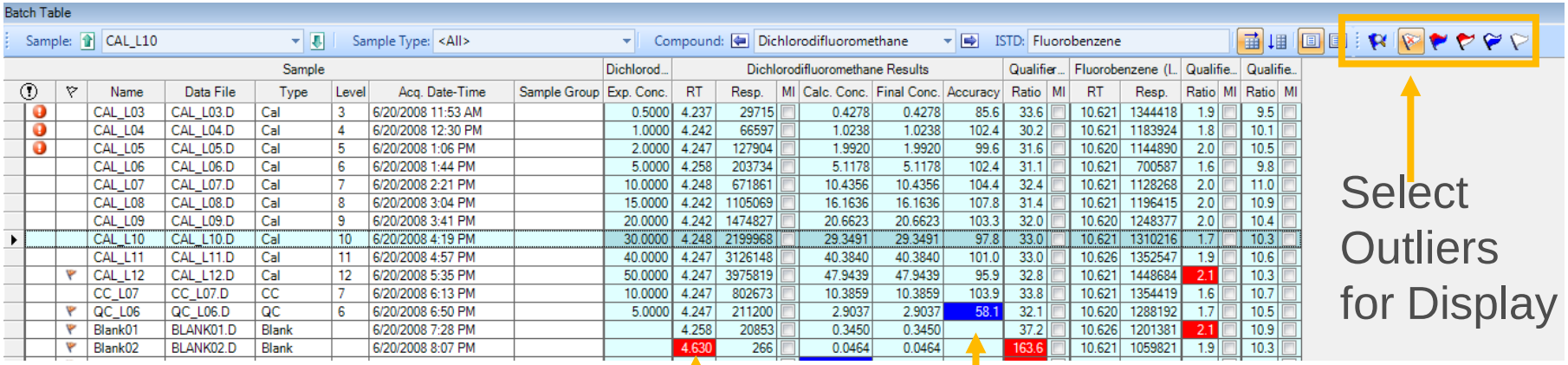


From within the Batch Table right click, select **Arrange Compounds By > Name**

Tip: Alphabetize the Compound List for faster compound access.

Batch Table

Messages and Outliers

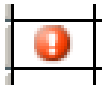


Sample							Dichlorod...	Dichlorodifluoromethane Results							Qualifier...	Fluorobenzene (I...			Qualifie...	Qualifie...	
Name	Data File	Type	Level	Acq. Date-Time	Sample Group	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
CAL_L03	CAL_L03.D	Cal	3	6/20/2008 11:53 AM		0.5000	4.237	29715		0.4278	0.4278	85.6		33.6		10.621	1344418	1.9		9.5	
CAL_L04	CAL_L04.D	Cal	4	6/20/2008 12:30 PM		1.0000	4.242	66597		1.0238	1.0238	102.4		30.2		10.621	1183924	1.8		10.1	
CAL_L05	CAL_L05.D	Cal	5	6/20/2008 1:06 PM		2.0000	4.247	127904		1.9920	1.9920	99.6		31.6		10.620	1144890	2.0		10.5	
CAL_L06	CAL_L06.D	Cal	6	6/20/2008 1:44 PM		5.0000	4.258	203734		5.1178	5.1178	102.4		31.1		10.621	700587	1.6		9.8	
CAL_L07	CAL_L07.D	Cal	7	6/20/2008 2:21 PM		10.0000	4.248	671861		10.4356	10.4356	104.4		32.4		10.621	1128268	2.0		11.0	
CAL_L08	CAL_L08.D	Cal	8	6/20/2008 3:04 PM		15.0000	4.242	1105069		16.1636	16.1636	107.8		31.4		10.621	1196415	2.0		10.9	
CAL_L09	CAL_L09.D	Cal	9	6/20/2008 3:41 PM		20.0000	4.242	1474827		20.6623	20.6623	103.3		32.0		10.620	1248377	2.0		10.4	
CAL_L10	CAL_L10.D	Cal	10	6/20/2008 4:19 PM		30.0000	4.248	2199968		29.3491	29.3491	97.8		33.0		10.621	1310216	1.7		10.3	
CAL_L11	CAL_L11.D	Cal	11	6/20/2008 4:57 PM		40.0000	4.247	3126148		40.3840	40.3840	101.0		33.0		10.626	1352547	1.9		10.6	
CAL_L12	CAL_L12.D	Cal	12	6/20/2008 5:35 PM		50.0000	4.247	3975819		47.9439	47.9439	95.9		32.8		10.621	1448684	2.1		10.3	
CC_L07	CC_L07.D	CC	7	6/20/2008 6:13 PM		10.0000	4.247	802673		10.3859	10.3859	103.9		33.8		10.621	1354419	1.6		10.7	
QC_L06	QC_L06.D	QC	6	6/20/2008 6:50 PM		5.0000	4.247	211200		2.9037	2.9037	58.1		32.1		10.620	1288192	1.7		10.5	
Blank01	BLANK01.D	Blank		6/20/2008 7:28 PM			4.258	20853		0.3450	0.3450			37.2		10.626	1201381	2.1		10.9	
Blank02	BLANK02.D	Blank		6/20/2008 8:07 PM			4.630	266		0.0464	0.0464			163.6		10.621	1059821	1.9		10.3	

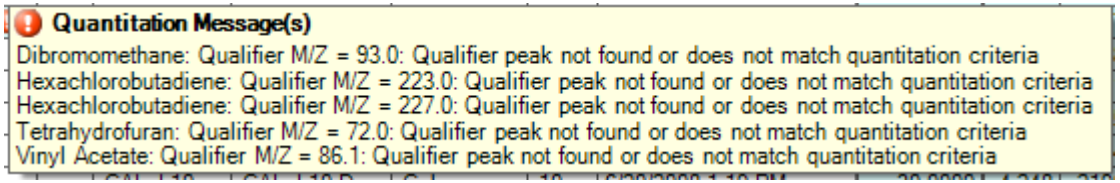
Select Outliers for Display

Red Outlier – High (above upper limit)

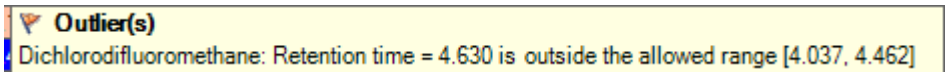
Blue Outlier – Low (below lower limit)



Messages

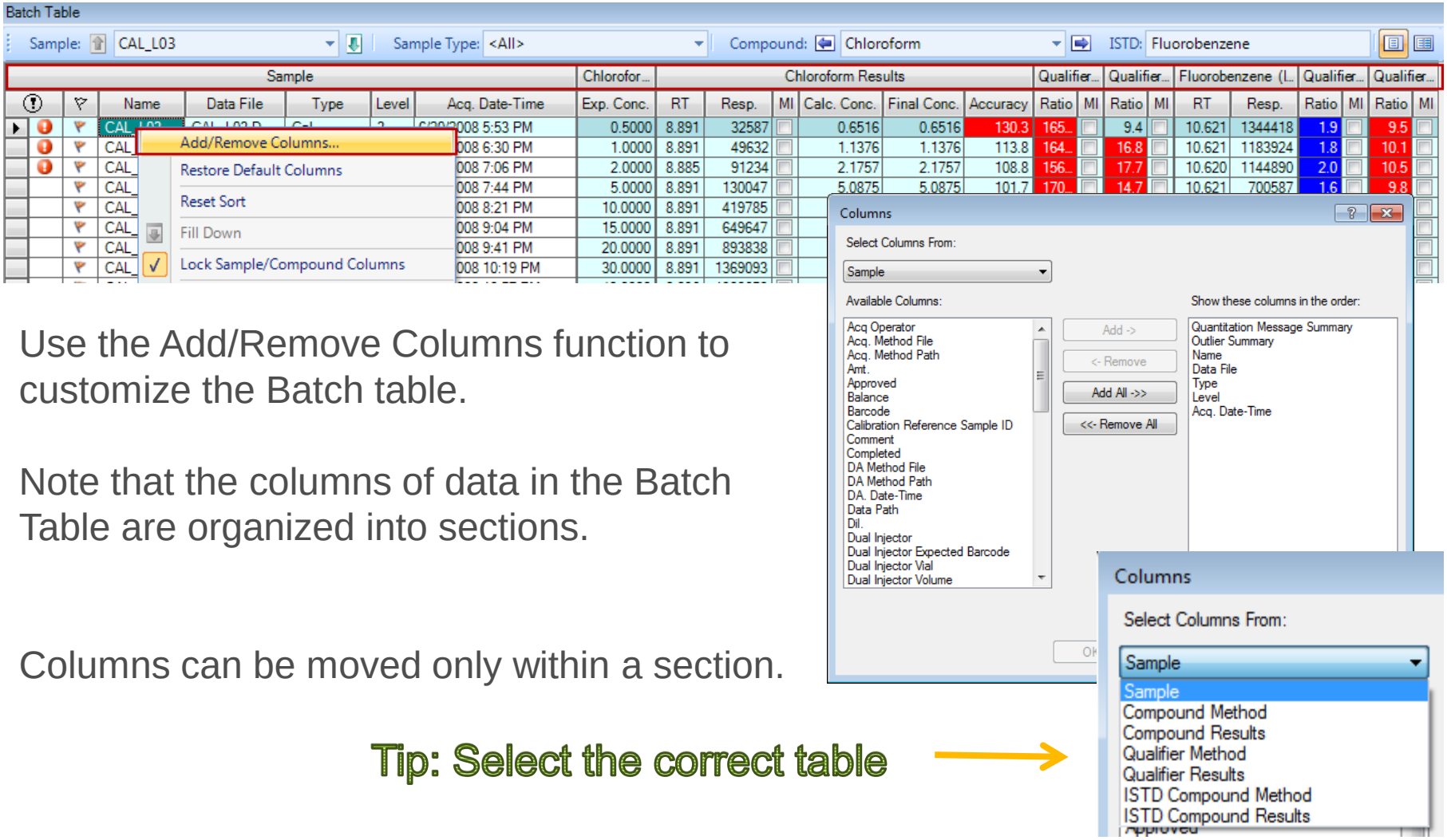


Outliers



Hover cursor over the outlier or message to display details

Batch Table Layout Modification
Add/Remove/Move Columns



Use the Add/Remove Columns function to customize the Batch table.

Note that the columns of data in the Batch Table are organized into sections.

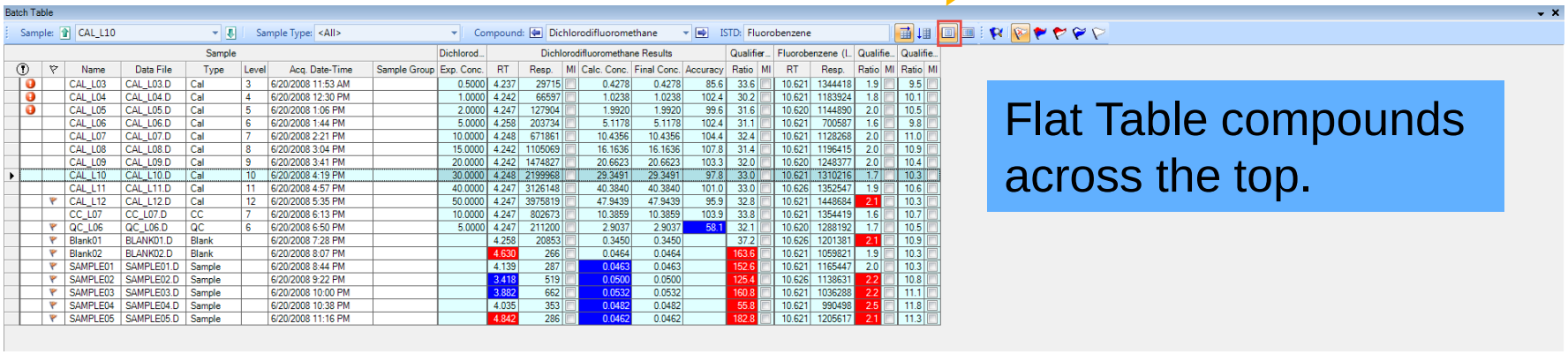
Columns can be moved only within a section.

Tip: Select the correct table

Batch Table Layout Modification

Single and Multiple Compound Modes

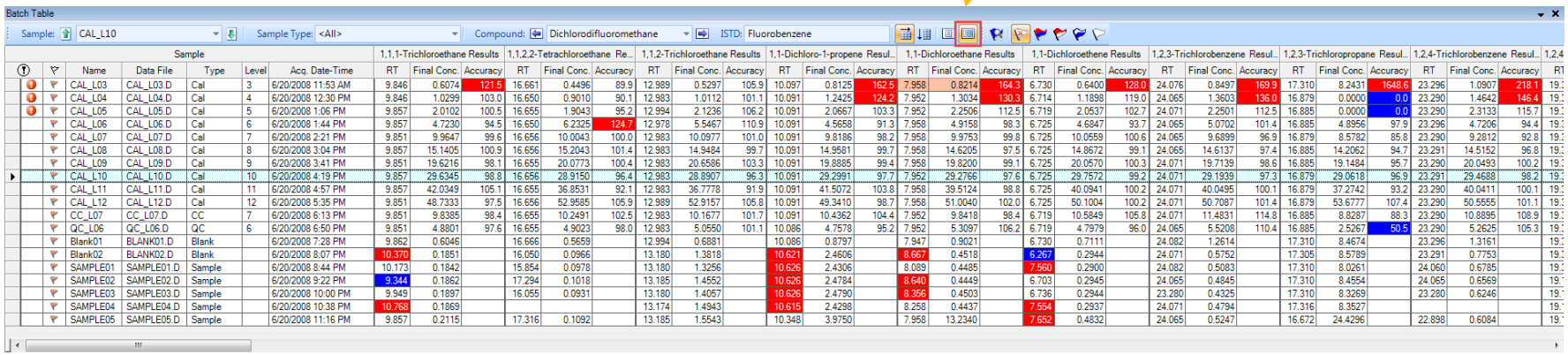
Single Compound Mode



Sample							Dichlorod...	Dichlorodifluoromethane Results						Qualifier...	Fluorobenzene (I...		Qualifie...	Qualifie...
Name	Data File	Type	Level	Acq. Date-Time	Sample Group	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
CAL_L03	CAL_L03.D	Cal	3	6/20/2008 11:53 AM		0.5000	4.237	29715		0.4278	0.4278	85.6	33.6		10.621	1344418	1.9		9.5	
CAL_L04	CAL_L04.D	Cal	4	6/20/2008 12:30 PM		1.0000	4.242	66597		1.0238	1.0238	102.4	30.2		10.621	1183924	1.8		10.1	
CAL_L05	CAL_L05.D	Cal	5	6/20/2008 1:06 PM		2.0000	4.247	127904		1.9920	1.9920	99.6	31.6		10.620	1144890	2.0		10.5	
CAL_L06	CAL_L06.D	Cal	6	6/20/2008 1:44 PM		5.0000	4.258	203734		5.1178	5.1178	102.4	31.1		10.621	700587	1.6		9.8	
CAL_L07	CAL_L07.D	Cal	7	6/20/2008 2:21 PM		10.0000	4.248	671861		10.4356	10.4356	104.4	32.4		10.621	1128268	2.0		11.0	
CAL_L08	CAL_L08.D	Cal	8	6/20/2008 3:04 PM		15.0000	4.242	1105069		16.1636	16.1636	107.8	31.4		10.621	1196415	2.0		10.9	
CAL_L09	CAL_L09.D	Cal	9	6/20/2008 3:41 PM		20.0000	4.242	1474827		20.6623	20.6623	103.3	32.0		10.620	1248377	2.0		10.4	
CAL_L10	CAL_L10.D	Cal	10	6/20/2008 4:19 PM		30.0000	4.248	2199968		29.3491	29.3491	97.8	33.0		10.621	1310216	1.7		10.3	
CAL_L11	CAL_L11.D	Cal	11	6/20/2008 4:57 PM		40.0000	4.247	3126148		40.3840	40.3840	101.0	33.0		10.626	1352547	1.9		10.6	
CAL_L12	CAL_L12.D	Cal	12	6/20/2008 5:35 PM		50.0000	4.247	3975819		47.9439	47.9439	95.9	32.8		10.621	1448684	2.1		10.3	
CC_L07	CC_L07.D	CC	7	6/20/2008 6:13 PM		10.0000	4.247	802673		10.3859	10.3859	103.9	33.8		10.621	1354419	1.6		10.7	
QC_L06	QC_L06.D	QC	6	6/20/2008 6:50 PM		5.0000	4.247	211200		2.9037	2.9037	58.1	32.1		10.620	1288192	1.7		10.5	
Blank01	BLANK01.D	Blank		6/20/2008 7:28 PM			4.258	20853		0.3450	0.3450		37.2		10.626	1201381	2.1		10.9	
Blank02	BLANK02.D	Blank		6/20/2008 8:07 PM			4.630	266		0.0464	0.0464		163.6		10.621	1059821	1.9		10.3	
SAMPLE01	SAMPLE01.D	Sample		6/20/2008 8:44 PM			4.139	287		0.0463	0.0463		152.6		10.621	1165447	2.0		10.3	
SAMPLE02	SAMPLE02.D	Sample		6/20/2008 9:22 PM			3.418	519		0.0500	0.0500		125.4		10.626	1138631	2.2		10.8	
SAMPLE03	SAMPLE03.D	Sample		6/20/2008 10:00 PM			3.882	662		0.0532	0.0532		160.8		10.621	1036288	2.2		11.1	
SAMPLE04	SAMPLE04.D	Sample		6/20/2008 10:38 PM			4.035	353		0.0482	0.0482		55.8		10.621	990498	2.5		11.8	
SAMPLE05	SAMPLE05.D	Sample		6/20/2008 11:16 PM			4.842	286		0.0462	0.0462		182.8		10.621	1205617	2.1		11.3	

Flat Table compounds across the top.

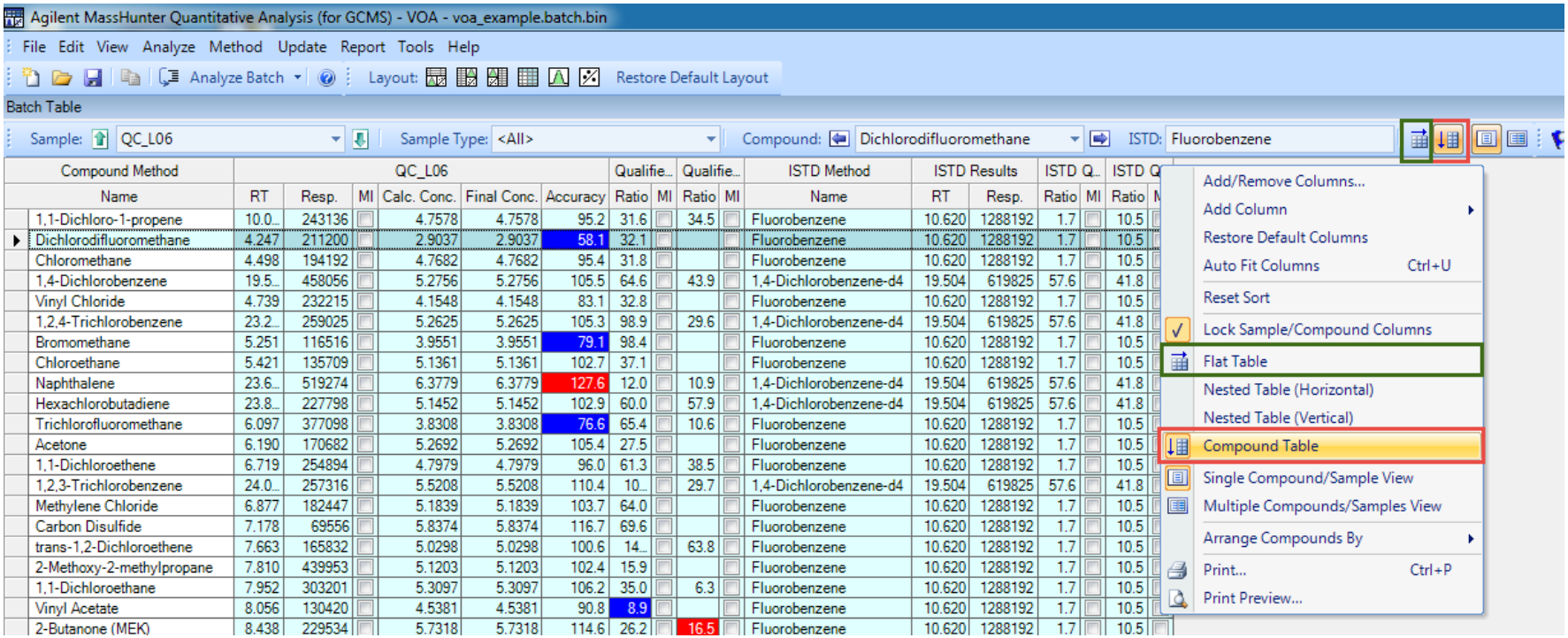
Multiple Compound Mode



Sample					1,1,1-Trichloroethane Results			1,1,2,2-Tetrachloroethane Re...			1,1,2-Trichloroethane Results			1,1-Dichloro-1-propene Resul...			1,1-Dichloroethane Results			1,1-Dichloroethene Results			1,2,3-Trichlorobenzene Resul...			1,2,3-Trichloropropane Resul...			1,2,4-Trichlorobenzene Resul...			1,2,4
Name	Data File	Type	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT
CAL_L03	CAL_L03.D	Cal	3	6/20/2008 11:53 AM	9.846	0.6074	121.5	16.661	0.4496	89.9	12.989	0.5297	105.9	10.097	0.8125	162.5	7.958	0.8214	164.3	6.730	0.6400	128.0	24.076	0.8497	169.9	17.310	8.2431	1648.6	23.296	1.0907	218.1	19.
CAL_L04	CAL_L04.D	Cal	4	6/20/2008 12:30 PM	9.846	1.0299	103.0	16.650	0.9010	90.1	12.983	1.0112	101.1	10.091	1.2425	124.2	7.952	1.3034	130.3	6.714	1.1898	119.0	24.065	1.3603	136.0	16.879	0.0000	0.0	23.290	1.4642	146.4	19.
CAL_L05	CAL_L05.D	Cal	5	6/20/2008 1:06 PM	9.857	2.0102	100.5	16.655	1.9043	95.2	12.994	2.1236	106.2	10.091	2.0667	103.3	7.952	2.2506	112.5	6.719	2.0537	102.7	24.071	2.2501	112.5	16.885	0.0000	0.0	23.290	2.3133	115.7	19.
CAL_L06	CAL_L06.D	Cal	6	6/20/2008 1:44 PM	9.857	4.7230	94.5	16.650	6.2325	124.7	12.978	5.5467	110.9	10.091	4.5658	91.3	7.958	4.9158	98.3	6.725	4.6847	93.7	24.065	5.0702	101.4	16.885	4.8956	97.9	23.296	4.7206	94.4	19.
CAL_L07	CAL_L07.D	Cal	7	6/20/2008 2:21 PM	9.851	9.9647	99.6	16.656	10.0043	100.0	12.983	10.0977	101.0	10.091	9.8186	98.2	7.958	9.9753	99.8	6.725	10.0559	100.6	24.065	9.6899	96.9	16.879	8.5782	85.8	23.290	9.2812	92.8	19.
CAL_L08	CAL_L08.D	Cal	8	6/20/2008 3:04 PM	9.857	15.1405	100.9	16.656	15.2043	101.4	12.983	14.9484	99.7	10.091	14.9581	99.7	7.958	14.6205	97.5	6.725	14.8672	99.1	24.065	14.6137	97.4	16.885	14.2062	94.7	23.291	14.5152	96.8	19.
CAL_L09	CAL_L09.D	Cal	9	6/20/2008 3:41 PM	9.851	19.6216	98.1	16.655	20.0773	100.4	12.983	20.6586	103.3	10.091	19.8885	99.4	7.958	19.8200	99.1	6.725	20.0570	100.3	24.071	19.7139	98.6	16.885	19.1484	95.7	23.290	20.0493	100.2	19.
CAL_L10	CAL_L10.D	Cal	10	6/20/2008 4:19 PM	9.857	29.6345	98.8	16.656	28.9150	96.4	12.983	28.8907	96.3	10.091	29.2991	97.7	7.952	29.2766	97.6	6.725	29.7572	99.2	24.071	29.1939	97.3	16.879	29.0618	96.9	23.291	29.4688	98.2	19.
CAL_L11	CAL_L11.D	Cal	11	6/20/2008 4:57 PM	9.857	42.0349	105.1	16.655	36.8531	92.1	12.983	36.7778	91.9	10.091	41.5072	103.8	7.958	39.5124	98.8	6.725	40.0941	100.2	24.071	40.0495	100.1	16.879	37.2742	93.2	23.290	40.0411	100.1	19.
CAL_L12	CAL_L12.D	Cal	12	6/20/2008 5:35 PM	9.851	48.7333	97.5	16.656	52.9585	105.9	12.989	52.9157	105.8	10.091	49.3410	98.7	7.958	51.0040	102.0	6.725	50.1004	100.2	24.071	50.7087	101.4	16.879	53.6777	107.4	23.290	50.5555	101.1	19.
CC_L07	CC_L07.D	CC	7	6/20/2008 6:13 PM	9.851	9.8385	98.4	16.655	10.2491	102.5	12.983	10.1677	101.7	10.091	10.4362	104.4	7.952	9.8418	98.4	6.719	10.5849	105.8	24.071	11.4831	114.8	16.885	8.8287	88.3	23.290	10.8895	108.9	19.
QC_L06	QC_L06.D	QC	6	6/20/2008 6:50 PM	9.851	4.8801	97.6	16.655	4.9023	98.0	12.983	5.0550	101.1	10.086	4.7578	95.2	7.952	5.3097	106.2	6.719	4.7979	96.0	24.065	5.5208	110.4	16.885	2.5267	50.5	23.290	5.2625	105.3	19.
Blank01	BLANK01.D	Blank		6/20/2008 7:28 PM	9.862	0.6046		16.666	0.5659		12.994	0.6881		10.086	0.8797		7.947	0.9021		6.730	0.7111		24.082	1.2614		17.310	8.4674		23.296	1.3161		19.
Blank02	BLANK02.D	Blank		6/20/2008 8:07 PM	10.370	0.1851		16.050	0.0966		13.180	1.3818		10.621	2.4606		8.667	0.4518		6.267	0.2944		24.071	0.5752		17.305	8.5789		23.291	0.7753		19.
SAMPLE01	SAMPLE01.D	Sample		6/20/2008 8:44 PM	10.173	0.1842		15.854	0.0978		13.180	1.3256		10.626	2.4306		8.089	0.4485		7.560	0.2900		24.082	0.5083		17.310	8.0261		24.060	0.6785		19.
SAMPLE02	SAMPLE02.D	Sample		6/20/2008 9:22 PM	9.344	0.1862		17.294	0.1018		13.185	1.4552		10.626	2.4784		8.640	0.4449		6.703	0.2945		24.065	0.4845		17.310	8.4554		24.065	0.6569		19.
SAMPLE03	SAMPLE03.D	Sample		6/20/2008 10:00 PM	9.949	0.1897		16.055	0.0931		13.180	1.4057		10.626	2.4790		8.356	0.4503		6.736	0.2944		23.280	0.4325		17.310	8.3269		23.280	0.6246		19.
SAMPLE04	SAMPLE04.D	Sample		6/20/2008 10:38 PM	10.768	0.1869					13.174	1.4943		10.615	2.4298		8.258	0.4437		7.554	0.2937		24.071	0.4794		17.316	8.3527					19.
SAMPLE05	SAMPLE05.D	Sample		6/20/2008 11:16 PM	9.857	0.2115		17.316	0.1092		13.185	1.5543		10.348	3.9750		7.958	13.2340		7.652	0.4832		24.065	0.5247		16.672	24.4296		22.898	0.6084		19.

Batch Table Layout Modification

Compound Table Modes

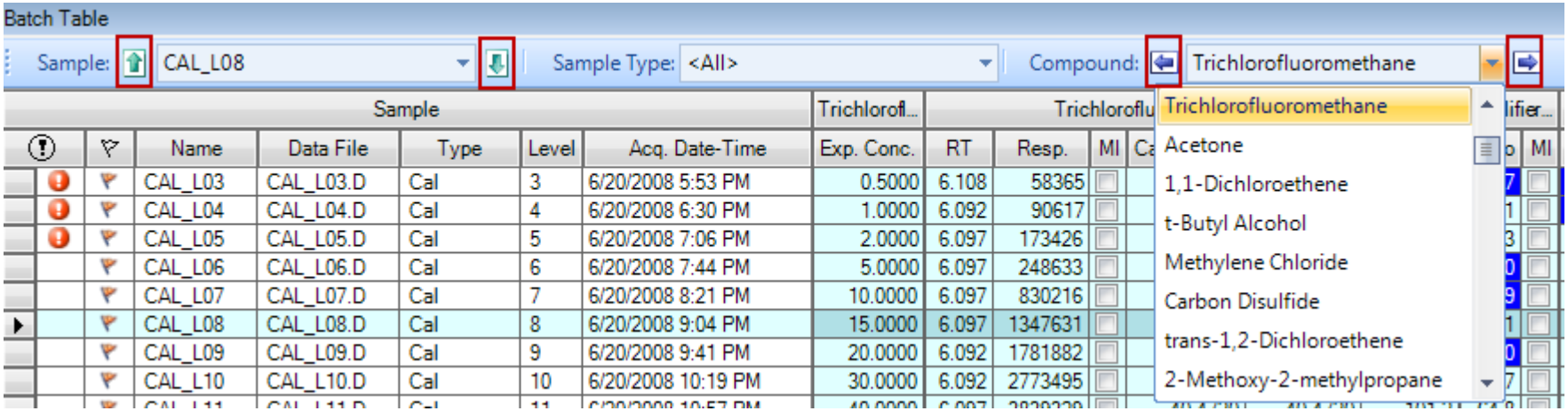


Compound Method	QC_L06						Qualifie...		Qualifie...		ISTD Method	ISTD Results		ISTD Q...		ISTD Q
Name	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	Name	RT	Resp.	Ratio	MI	Ratio
1,1-Dichloro-1-propene	10.0...	243136		4.7578	4.7578	95.2	31.6		34.5		Fluorobenzene	10.620	1288192	1.7		10.5
Dichlorodifluoromethane	4.247	211200		2.9037	2.9037	58.1	32.1				Fluorobenzene	10.620	1288192	1.7		10.5
Chloromethane	4.498	194192		4.7682	4.7682	95.4	31.8				Fluorobenzene	10.620	1288192	1.7		10.5
1,4-Dichlorobenzene	19.5...	458056		5.2756	5.2756	105.5	64.6		43.9		1,4-Dichlorobenzene-d4	19.504	619825	57.6		41.8
Vinyl Chloride	4.739	232215		4.1548	4.1548	83.1	32.8				Fluorobenzene	10.620	1288192	1.7		10.5
1,2,4-Trichlorobenzene	23.2...	259025		5.2625	5.2625	105.3	98.9		29.6		1,4-Dichlorobenzene-d4	19.504	619825	57.6		41.8
Bromomethane	5.251	116516		3.9551	3.9551	79.1	98.4				Fluorobenzene	10.620	1288192	1.7		10.5
Chloroethane	5.421	135709		5.1361	5.1361	102.7	37.1				Fluorobenzene	10.620	1288192	1.7		10.5
Naphthalene	23.6...	519274		6.3779	6.3779	127.6	12.0		10.9		1,4-Dichlorobenzene-d4	19.504	619825	57.6		41.8
Hexachlorobutadiene	23.8...	227798		5.1452	5.1452	102.9	60.0		57.9		1,4-Dichlorobenzene-d4	19.504	619825	57.6		41.8
Trichlorofluoromethane	6.097	377098		3.8308	3.8308	76.6	65.4		10.6		Fluorobenzene	10.620	1288192	1.7		10.5
Acetone	6.190	170682		5.2692	5.2692	105.4	27.5				Fluorobenzene	10.620	1288192	1.7		10.5
1,1-Dichloroethene	6.719	254894		4.7979	4.7979	96.0	61.3		38.5		Fluorobenzene	10.620	1288192	1.7		10.5
1,2,3-Trichlorobenzene	24.0...	257316		5.5208	5.5208	110.4	10...		29.7		1,4-Dichlorobenzene-d4	19.504	619825	57.6		41.8
Methylene Chloride	6.877	182447		5.1839	5.1839	103.7	64.0				Fluorobenzene	10.620	1288192	1.7		10.5
Carbon Disulfide	7.178	69556		5.8374	5.8374	116.7	69.6				Fluorobenzene	10.620	1288192	1.7		10.5
trans-1,2-Dichloroethene	7.663	165832		5.0298	5.0298	100.6	14...		63.8		Fluorobenzene	10.620	1288192	1.7		10.5
2-Methoxy-2-methylpropane	7.810	439953		5.1203	5.1203	102.4	15.9				Fluorobenzene	10.620	1288192	1.7		10.5
1,1-Dichloroethane	7.952	303201		5.3097	5.3097	106.2	35.0		6.3		Fluorobenzene	10.620	1288192	1.7		10.5
Vinyl Acetate	8.056	130420		4.5381	4.5381	90.8	8.9				Fluorobenzene	10.620	1288192	1.7		10.5
2-Butanone (MEK)	8.438	229534		5.7318	5.7318	114.6	26.2		16.5		Fluorobenzene	10.620	1288192	1.7		10.5

Compound Table lists by compound rather than sample.

Select **Flat Table** to see list by sample.

Batch Table
Compounds Groups



Sample							Trichlorofl...	Trichloroflu				
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Ca	MI
		CAL_L03	CAL_L03.D	Cal	3	6/20/2008 5:53 PM	0.5000	6.108	58365			
		CAL_L04	CAL_L04.D	Cal	4	6/20/2008 6:30 PM	1.0000	6.092	90617			
		CAL_L05	CAL_L05.D	Cal	5	6/20/2008 7:06 PM	2.0000	6.097	173426			
		CAL_L06	CAL_L06.D	Cal	6	6/20/2008 7:44 PM	5.0000	6.097	248633			
		CAL_L07	CAL_L07.D	Cal	7	6/20/2008 8:21 PM	10.0000	6.097	830216			
▶		CAL_L08	CAL_L08.D	Cal	8	6/20/2008 9:04 PM	15.0000	6.097	1347631			
		CAL_L09	CAL_L09.D	Cal	9	6/20/2008 9:41 PM	20.0000	6.092	1781882			
		CAL_L10	CAL_L10.D	Cal	10	6/20/2008 10:19 PM	30.0000	6.092	2773495			

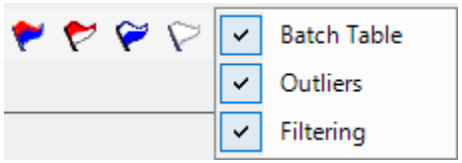
It may be useful to group compounds to organize Batch Table.

Look at parent compound and metabolites.

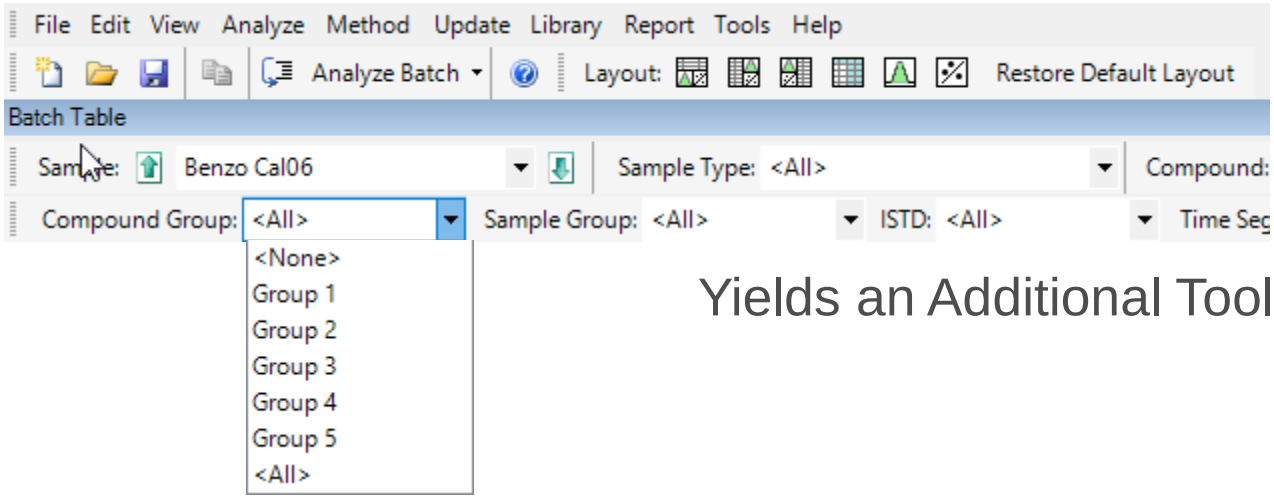
Group hydrocarbons to Group 1, aromatics to Group 2 and so forth.

Compound groups are generated in the Method Editor.

Batch Table
Compounds Groups



Activated with a right click to the right of the ‘flags’ or **View > Toolbars > Filtering**.

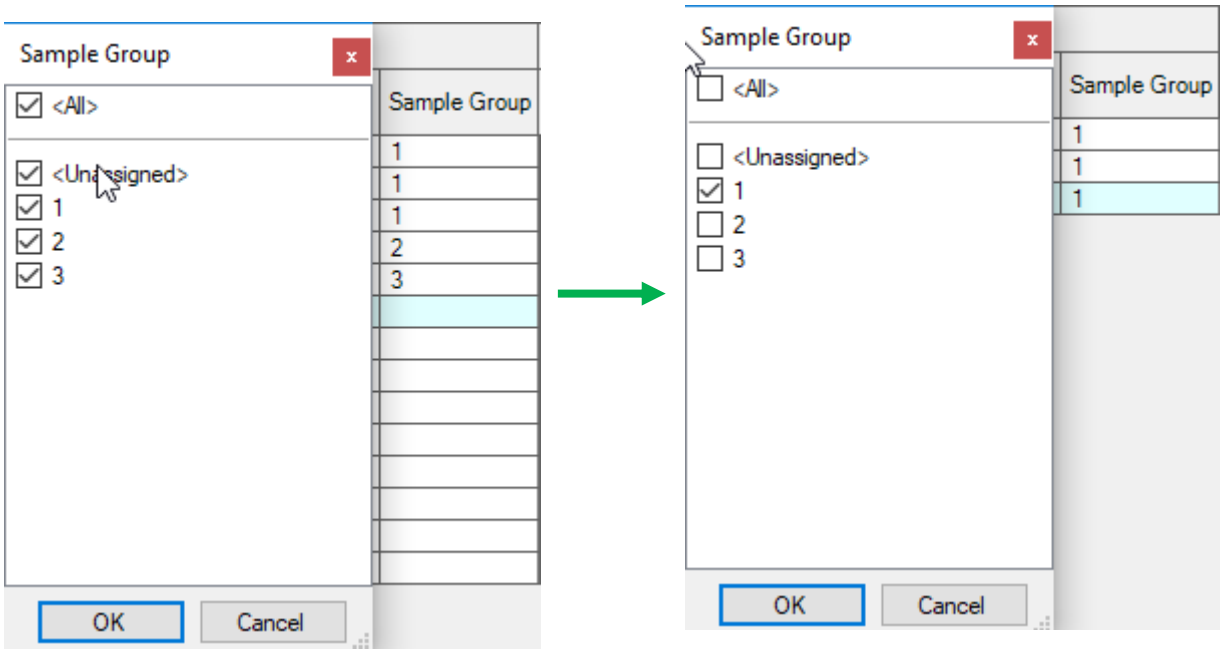


Yields an Additional Toolbar.

Batch Table
Samples Groups

Sample Groups are specified in the Batch Table through **Add/Remove Columns.**

Each Sample is assigned to a group, then only samples specific to a group are displayed and evaluated.



Samples groups are distinctively different from compound groups.

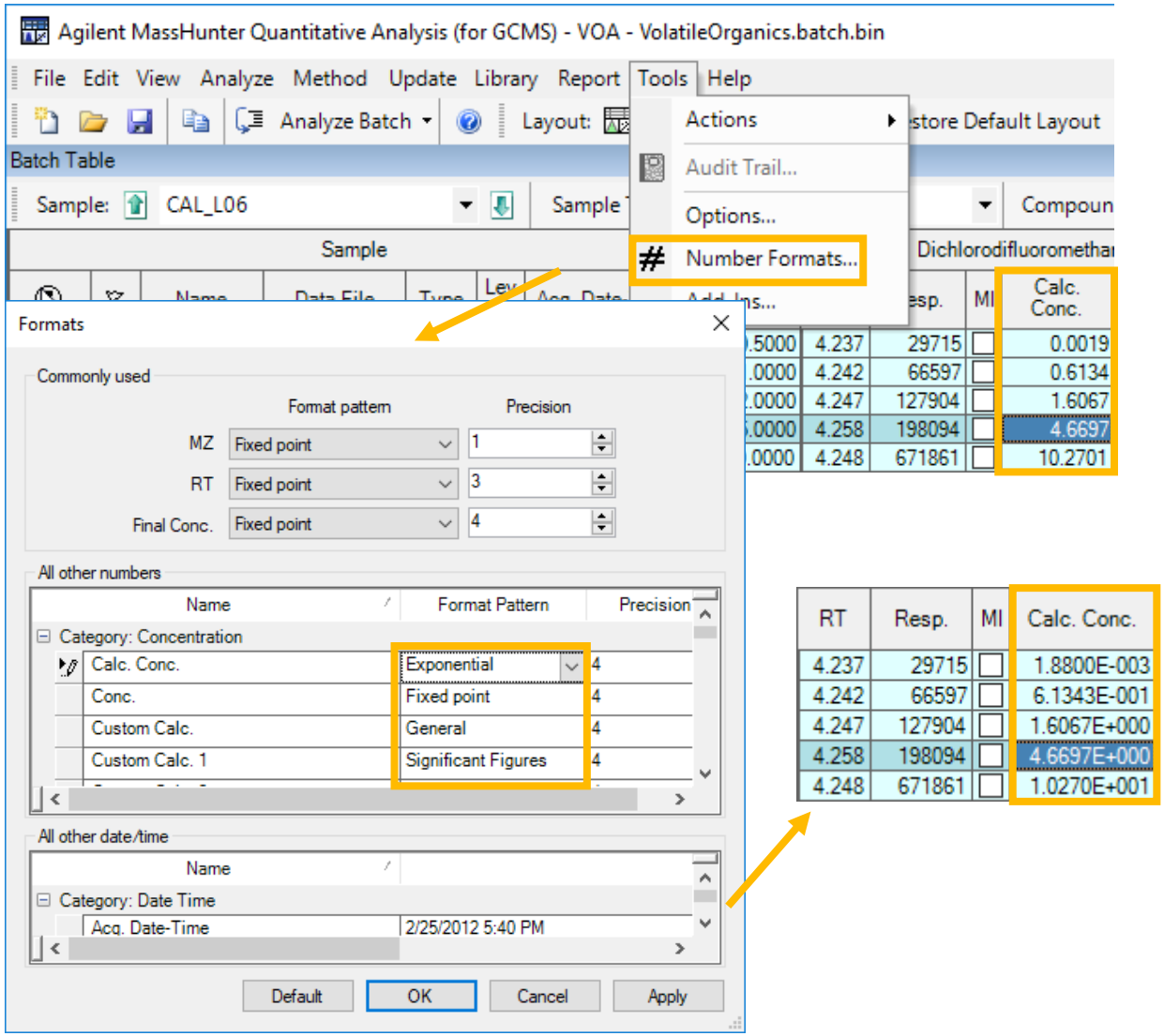
Batch Table Layout Modification
Format Columns

How to change the number of decimal places.

Alter number formats

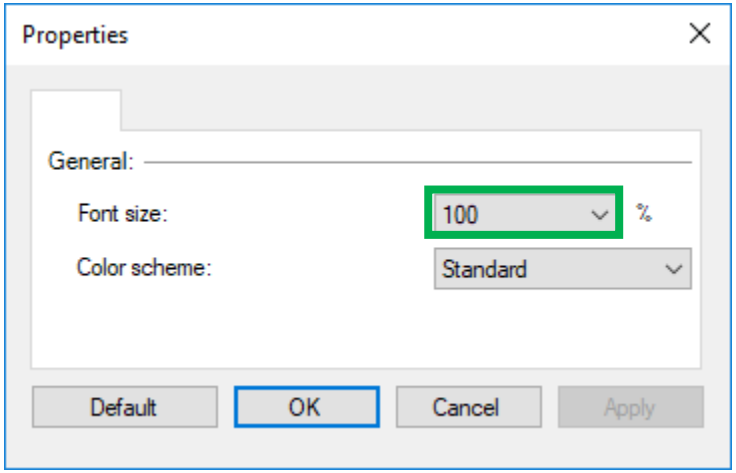
1) Exponential (scientific notation)
2) Fixed point
3) General
4) Significant Figures

Alter Date formats



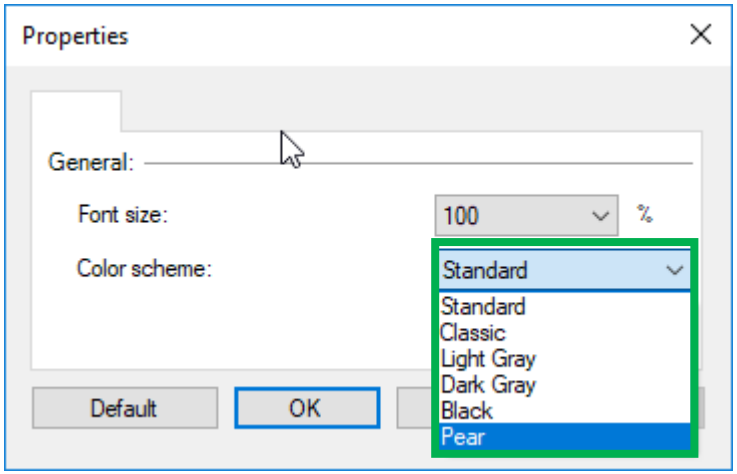
Batch Table Layout Modification
Context Menu Properties

Change the Font size

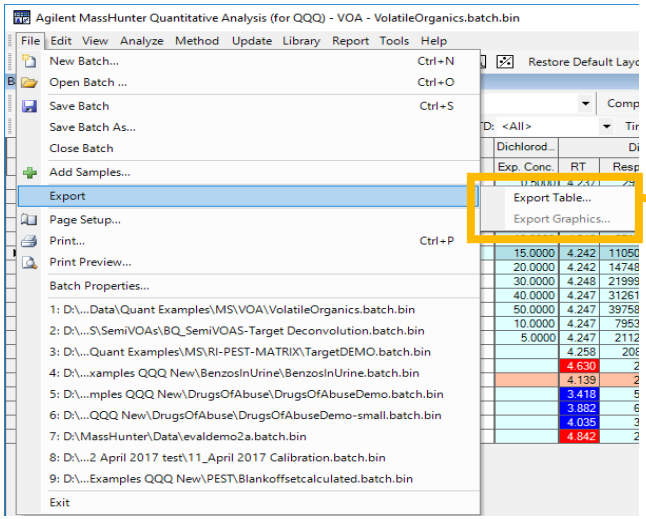


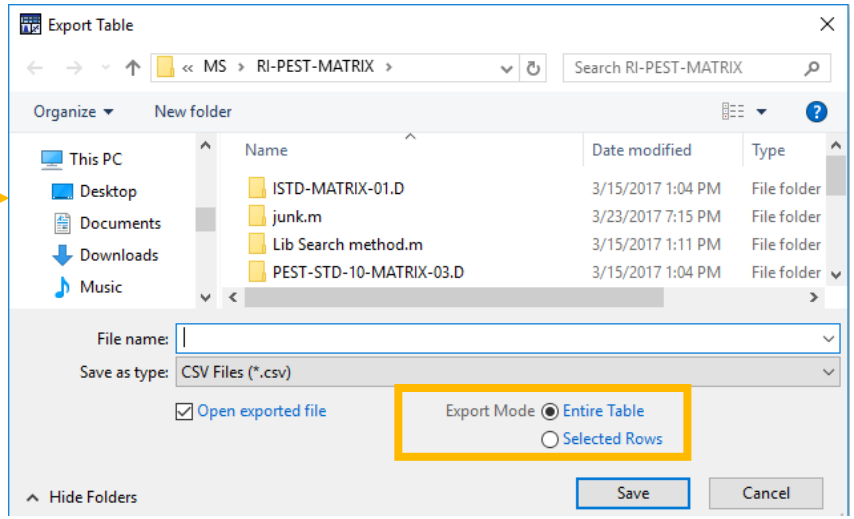
NEW FEATURE!

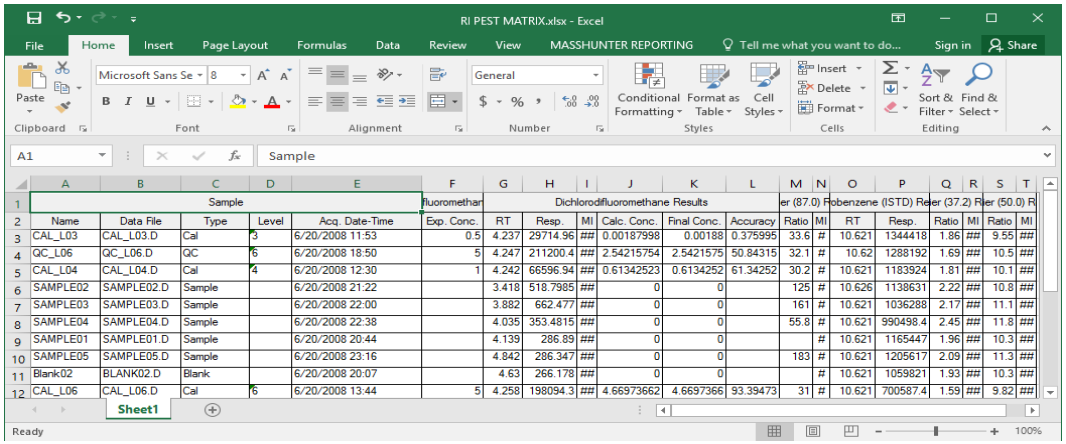
Change the Color scheme



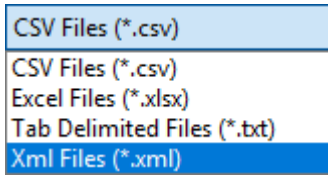
Export Batch Table







NEW FEATURE!



Tip: Popular option to Excel based reports.

Easy to layout columns and format data, then export into Excel.

Let’s take a moment for questions on Batch Table Navigation.

Time for a demo on Batch Table Navigation

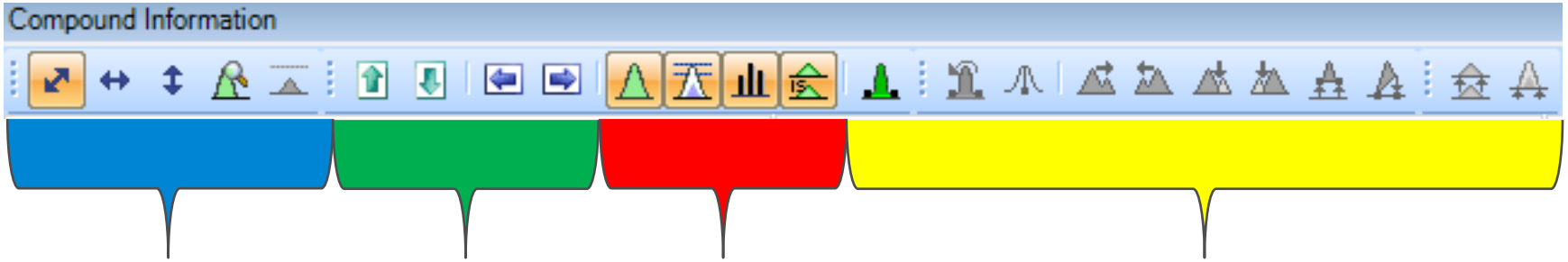
Next up:

Compound Information

Compound Information

Display and access one compound in one sample at a time.

Compound Information - Toolbar



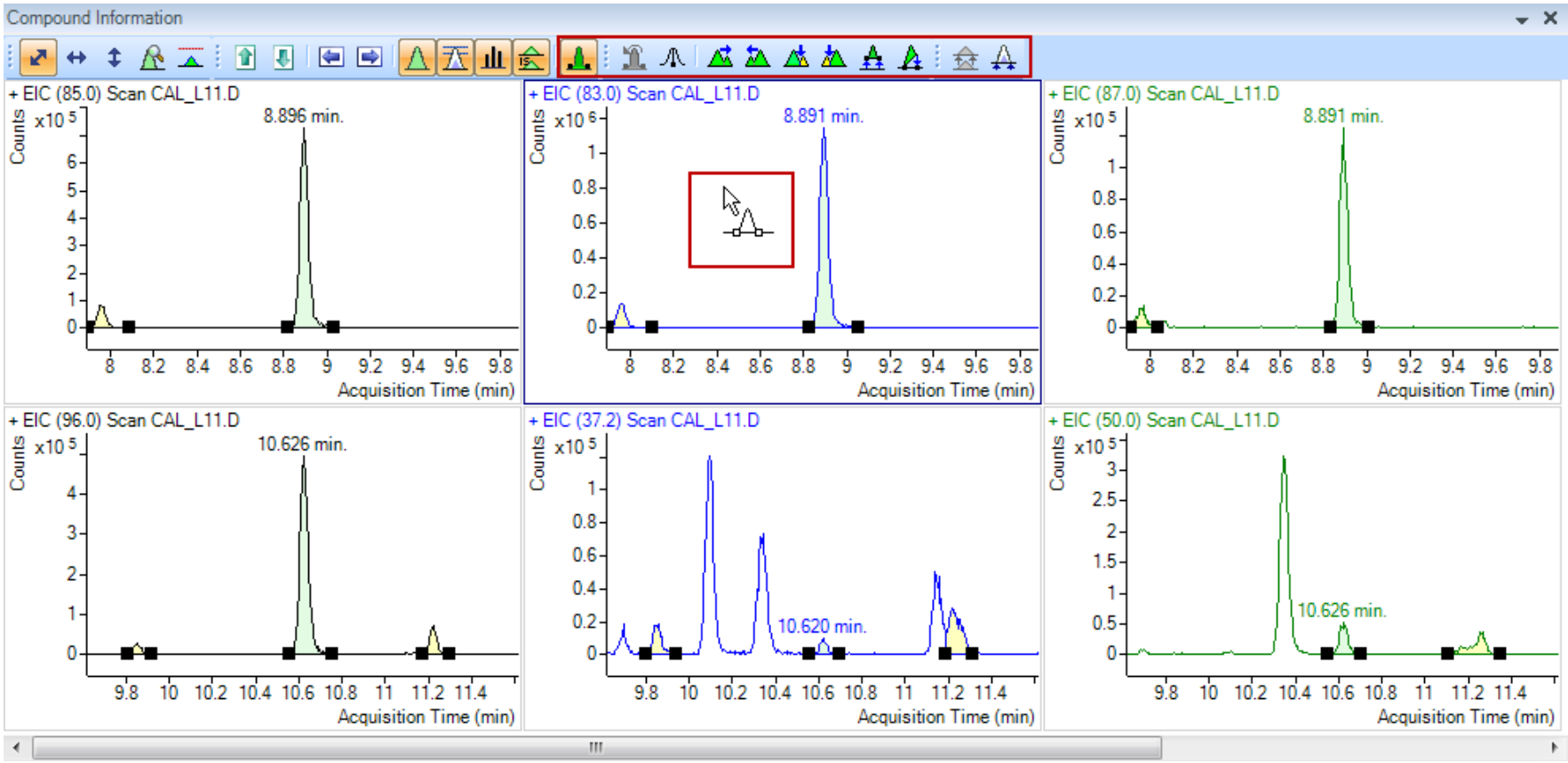
Un-zooms, Peak scaling, Auto Scales x and y axes

Sample and Compound Navigation Next / Pervious

Display/Hide Target, Qualifiers, Spectra, ISTD

Manual Integration Functions

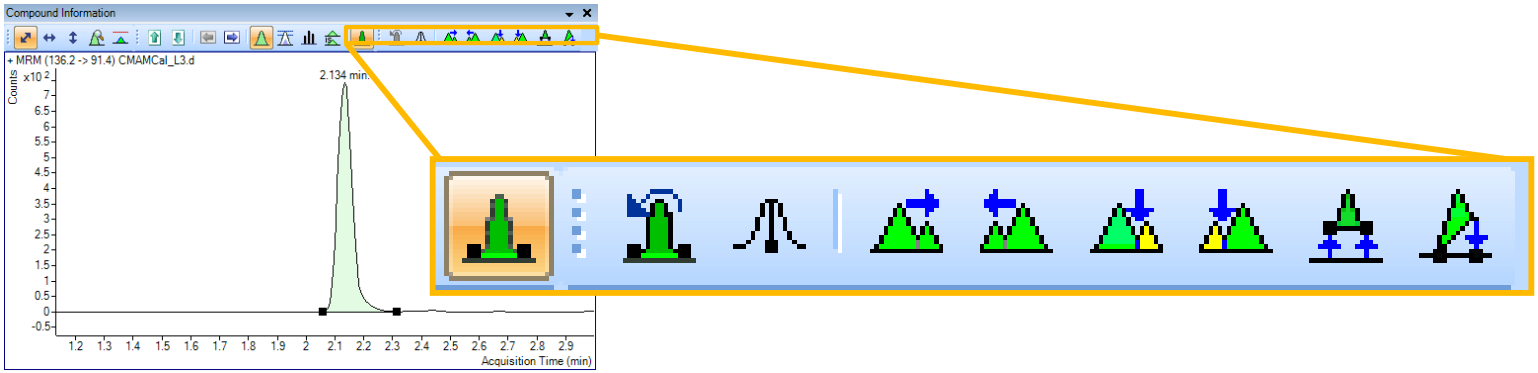
Manual Integrations and MI Toolbar



Click on the Manual Integration icon to place Compound Information in Manual Integration mode.

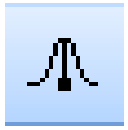
Each signal is placed in its own window (no overlay of qualifiers).

Manual Integration Toolbar

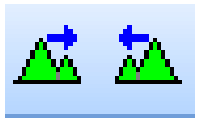


Enable Manual Integration – this tool activates the manual integration toolbar.

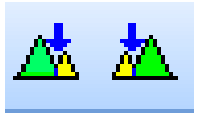
Clear Manual Integration – this tool becomes active once a manual integration is present.



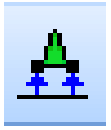
Zero Peak – this tool sets start and stop to the same value.



Merge Right/Left Peak – this tool merges the peak to the right/left of the selected peak.



Split Peak and Pick Right/Left – this tool splits the peak and then selects the right or left peak.

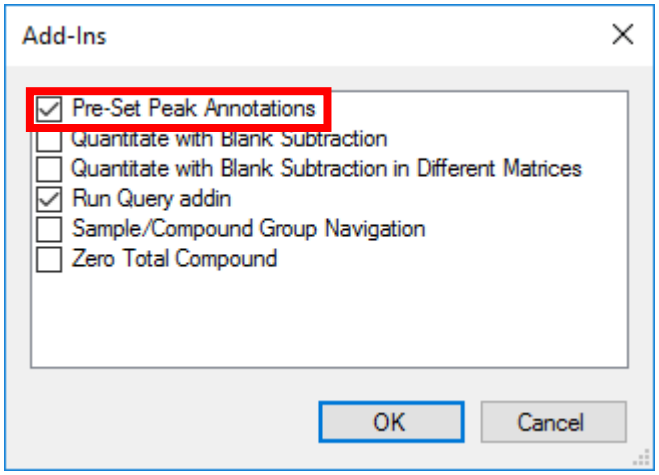


Snap Baseline – this tool places start/stop integration points on the baseline.

Drop Baseline – this tool finds the lowest end of the peak, then drops the baseline on the other side of the peak from it, thereby creating a flat baseline that avoids negative area.

Compound Information
Peak Annotation

Activated from **Tools > Add-Ins…**



NI – The peak was not integrated at all by the computer software.
LT – The peak in question was inappropriately integrated to an area less than what it should be (e.g., Peak area was cut).
GT - The peak in question was inappropriately integrated to an area greater than what it should be (e.g., Peak Tailing).

BA – The baseline had to be adjusted correctly by the analyst.
CO –The analyst had to split to co-eluting peaks apart that were not (or could not be) separated by the computer system.
RT – The retention time for the peak in question has shifted from the expected retention time.
INT – There was electronic interference (e.g., Noise).

Compound Information
Peak Annotation

The editable file is located in C:\Program Files\Agilent\MassHunter\Workstation\Quant\bin\AddIns\PeakAnnotations.xml.

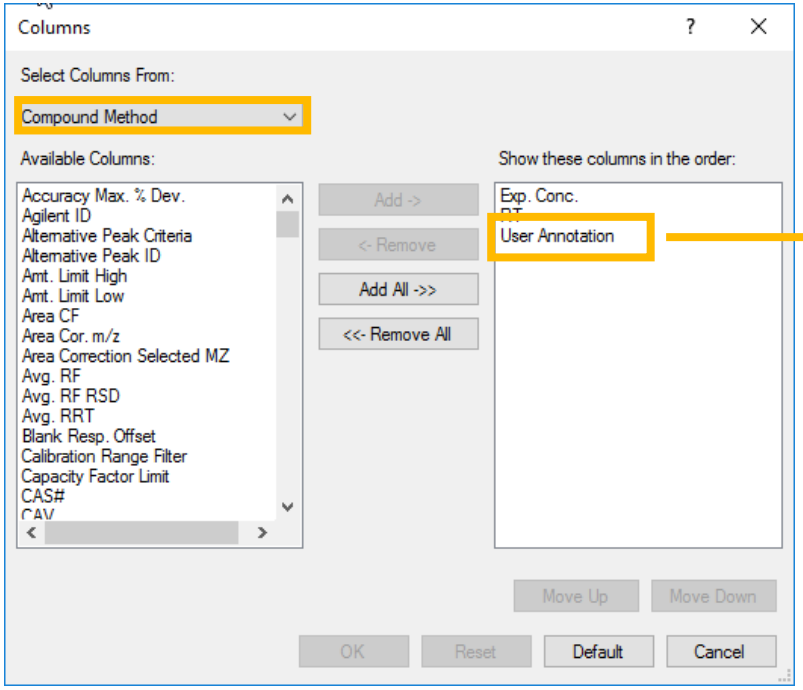
```
<?xml version="1.0" encoding="utf-8" ?>
<AnnotationButtons>
  <Button ID="NI" DisplayText="NI" Value="NI" Tooltip="The peak was not integrated at all by the computer software."/>
  <Button ID="LT" DisplayText="LT" Value="LT" Tooltip="The peak in question was inappropriately integrated to an area less than what it should be (e.g., Peak area was cut)."/>
  <Button ID="GT" DisplayText="GT" Value="GT" Tooltip="The peak in question was inappropriately integrated to an area greater than what it should be (e.g., Peak tailing)."/>
  <Button ID="BA" DisplayText="BA" Value="BA" Tooltip="The baseline had to be adjusted correctly by the analyst."/>
  <Button ID="CO" DisplayText="CO" Value="CO" Tooltip="The analyst had to split two co-eluting peaks apart that were not (or could not be) separated by the computer system."/>
  <Button ID="RT" DisplayText="RT" Value="RT" Tooltip="The retention time for the peak in question has shifted from the expected retention time."/>
  <Button ID="INT" DisplayText="INT" Value="INT" Tooltip="There was electronic interference (e.g., Noise)."/>
</AnnotationButtons>
```

Tip: Always create a back-up file before editing a configuration file.

Compound Information
Peak Annotation

The Peak Annotation can also be displayed in the Batch Table.

When the batch is saved, the values are retained.

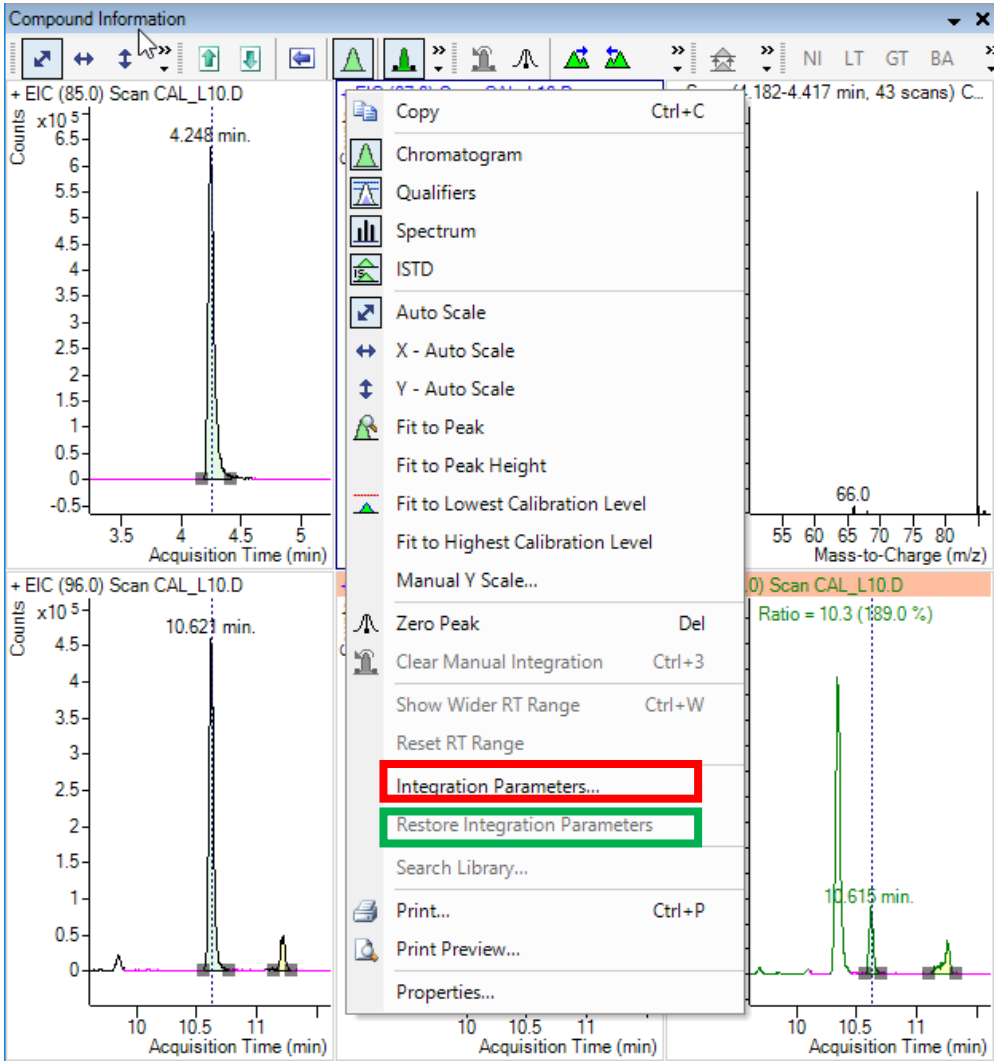


Dichlorodifluoromethane Method			Dichlorodifluoromethane Results				
Exp. Conc.	RT	User Annotation	RT	Resp.	MI	Calc. Conc.	Final Conc.
0.5000	4.249	NI	4.237	22897	☑	0.0000	0.0000
1.0000	4.249	LT	4.242	51949	☑	0.3941	0.3941
2.0000	4.249	GT	4.247	98928	☑	1.1534	1.1534
5.0000	4.249	BA	4.307	-3978	☑	0.0000	0.0000
10.0000	4.249	CO	4.248	495112	☑	7.4664	7.4664
15.0000	4.249	RT	4.406	-90142	☑	0.0000	0.0000
20.0000	4.249	INT	4.242	1149283	☑	16.0929	16.0929
30.0000	4.249		4.248	2199968	☐	29.6758	29.6758

The MI flag is checked in the Compound Results.

Compound Information

Context Menu Integration Parameters



Right click to expose the context menu.

Most of the features are toolbar icons.

Integration Parameters can be used to change the integration parameters.

Applies ONLY to this compound in this sample.

Restore Integration Parameters reverts to the method integration values.

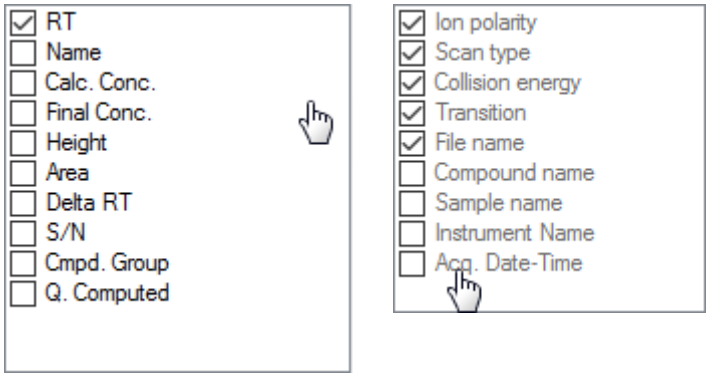
Compound Information
Context Menu Properties

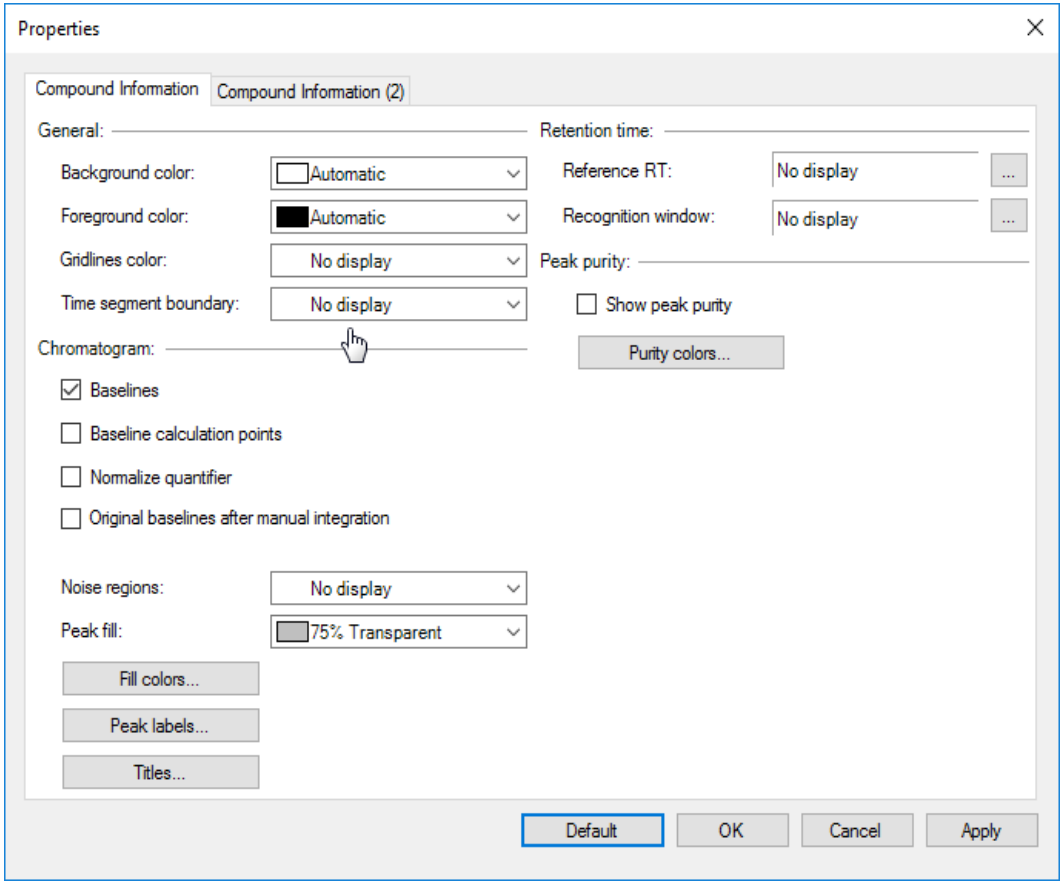
Properties allows for customization of the display.

Can change Fill colors.

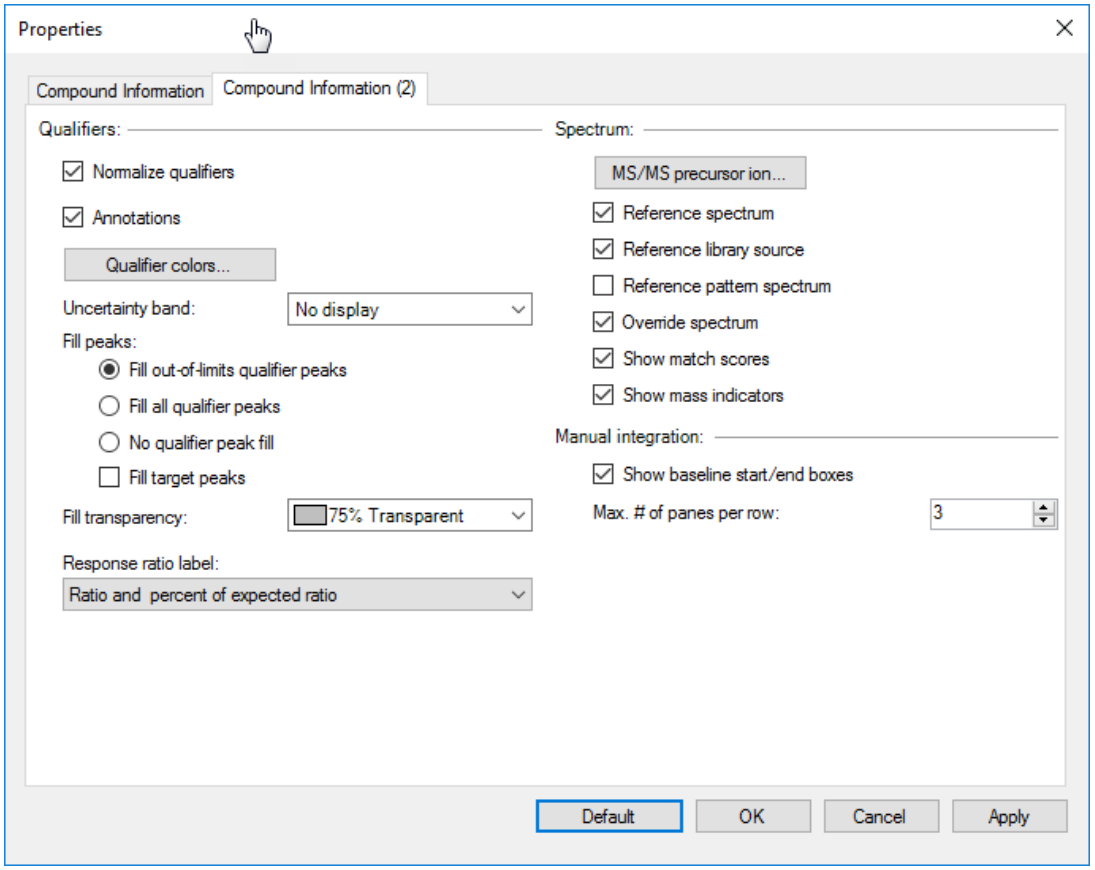
Can change Peak labels.

Can change Titles.





Compound Information
Context Menu Properties



Qualifier Properties can be changed.

Spectrum Properties can be altered.

Manual integration parameters can be varied.

Tip: Uncheck Normalize qualifiers and display the Uncertainty band for more information about the qualifiers.

Signal to Noise

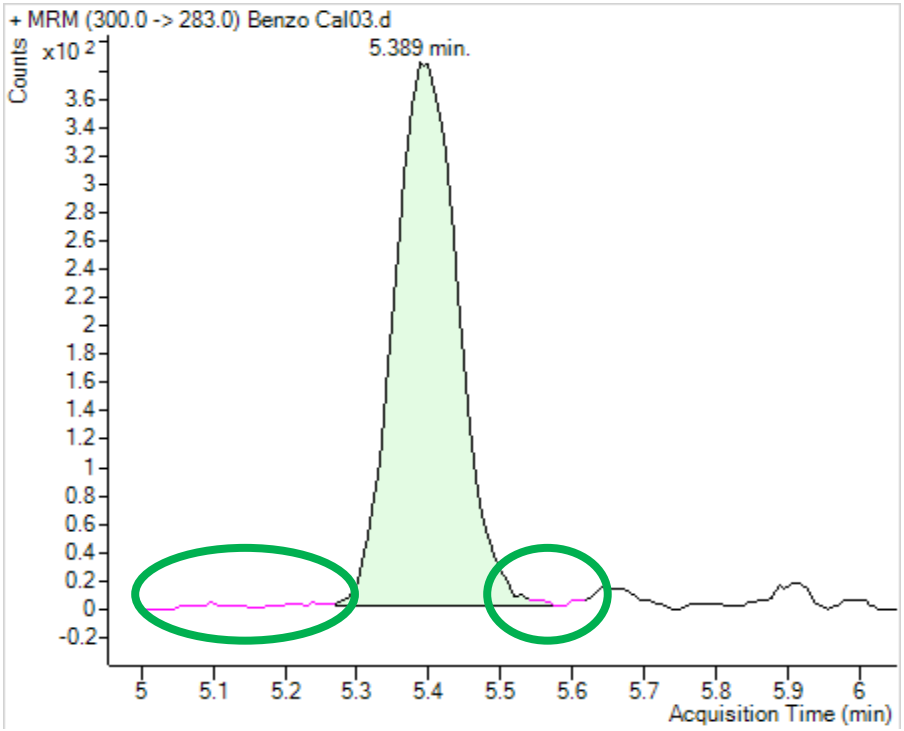
Five algorithms are available

Consult online help for more information on the algorithms. Noise regions can be automatically determined or individually specified.

Peak-to-Peak
Peak-to-Peak from Drift
ASTM
RMS
Auto-RMS

The Noise regions can be displayed in the Compound Information window under **Properties > Compound Information (1) > Baseline Calculation Points**.

In this example, 2 noise regions were determined—one before the peak and the other after.



Let’s take a moment for questions on Compound Information

Let’s take a moment for a demo on the features of Compound Information

Next up:

Calibration Curve and Curve Fit Assistant

Calibration Curve

View ISTD Responses

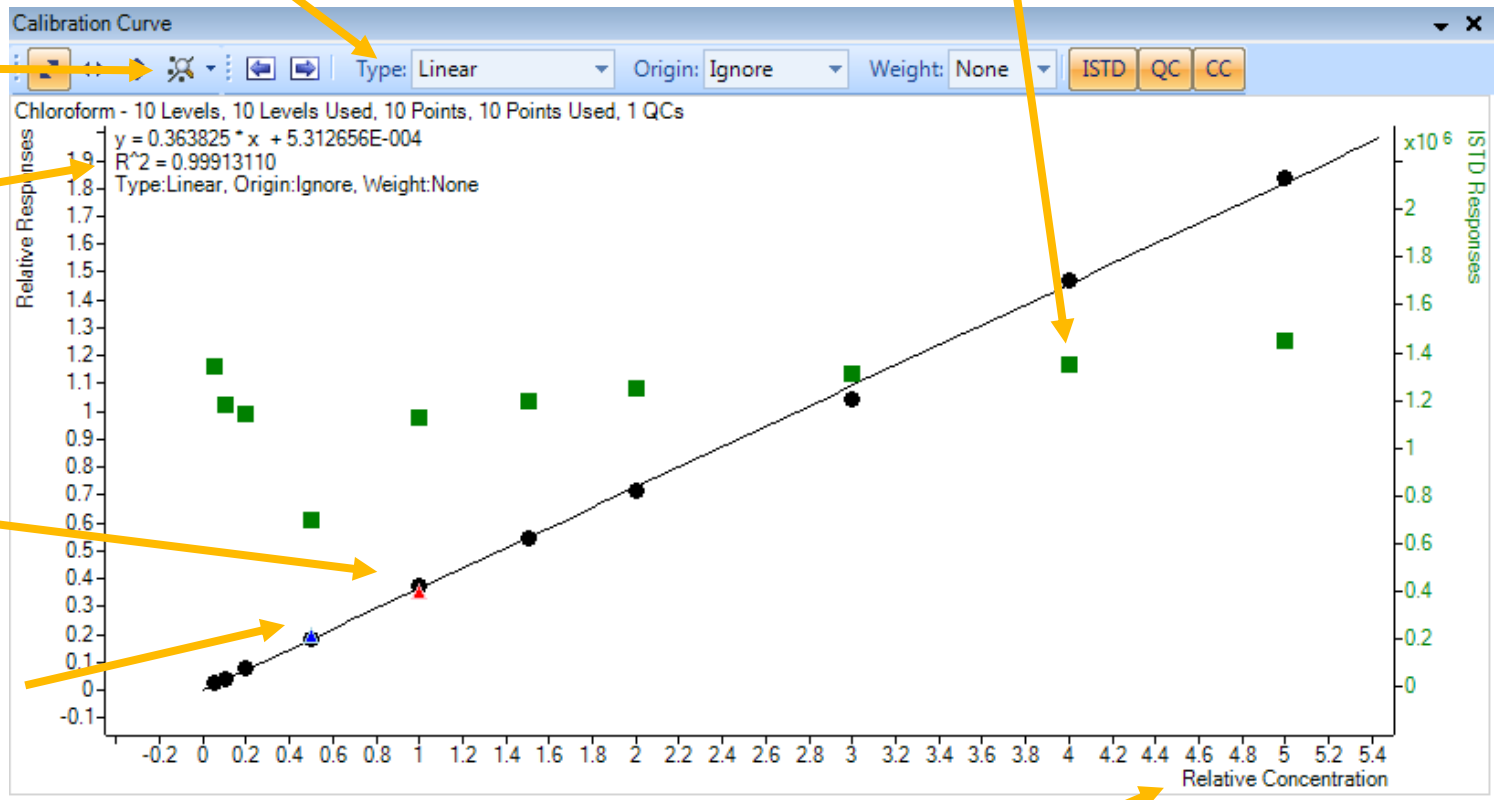
Change Curve Fit.

Fit to Levels (Scaling)

R^2

CC Levels

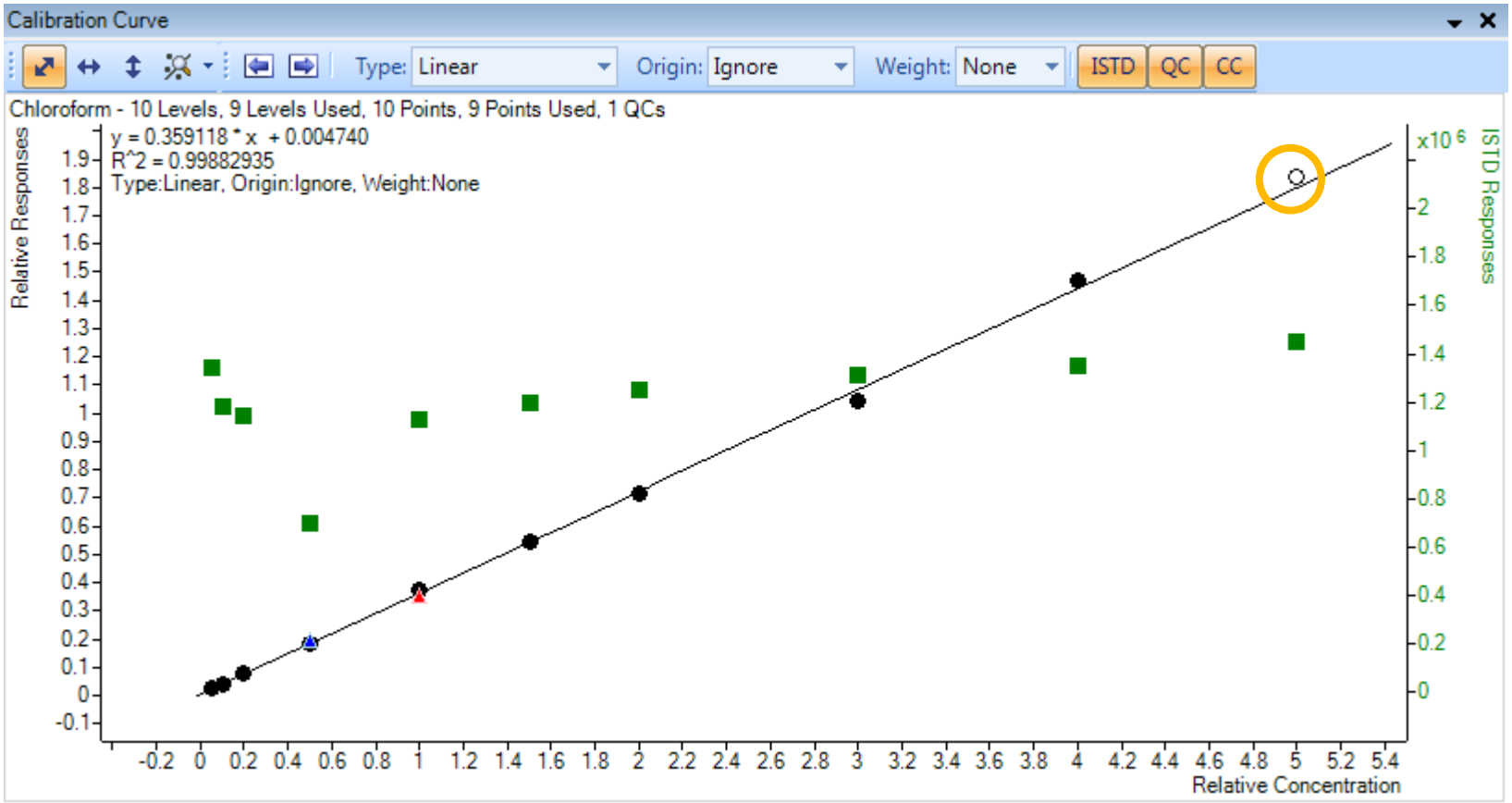
QC Samples



Concentration can be set as relative (to ISTD) or actual.

Calibration Curve

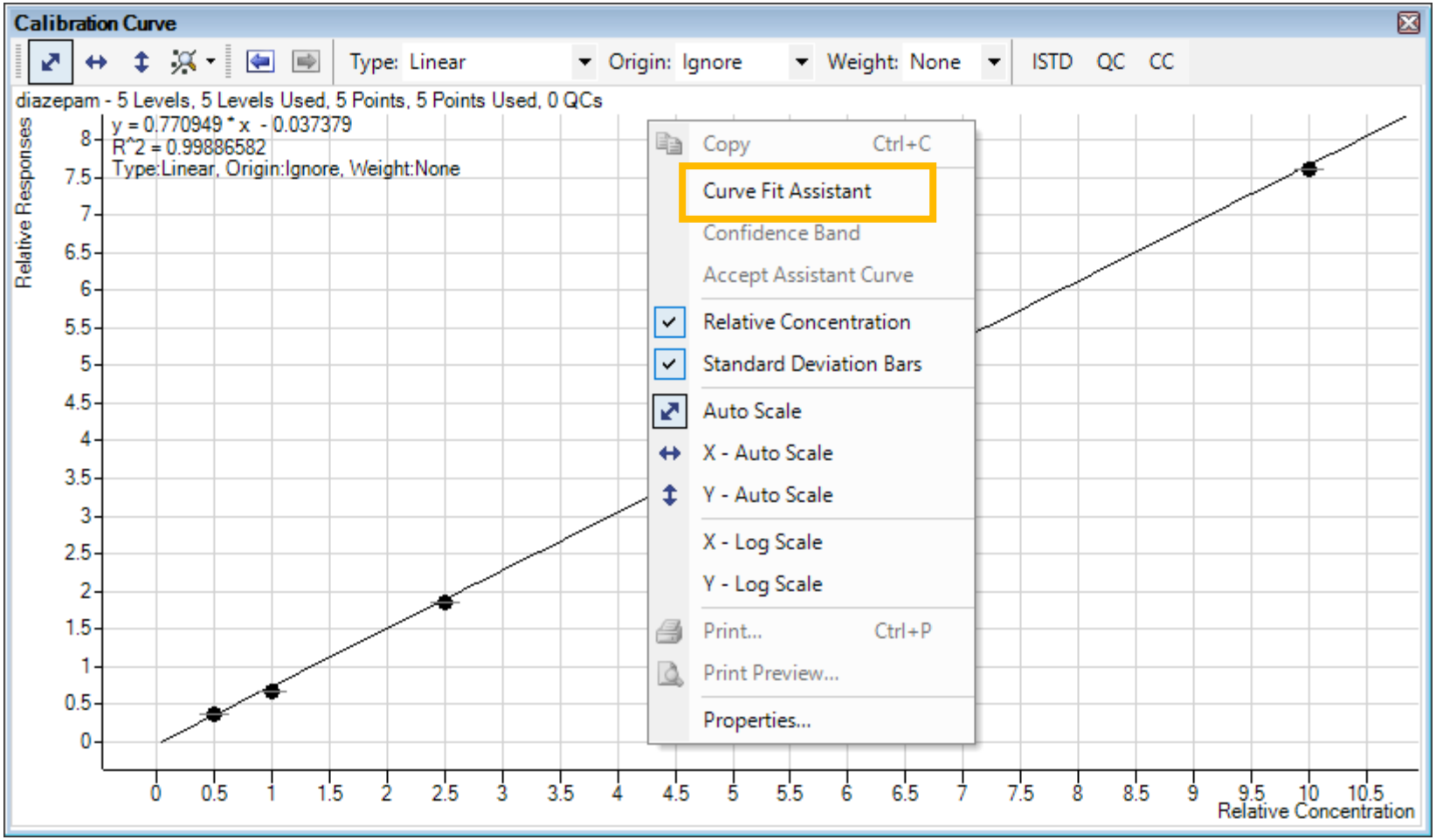
Disable calibration points



Click on **calibration point** once to disable and remove from curve.

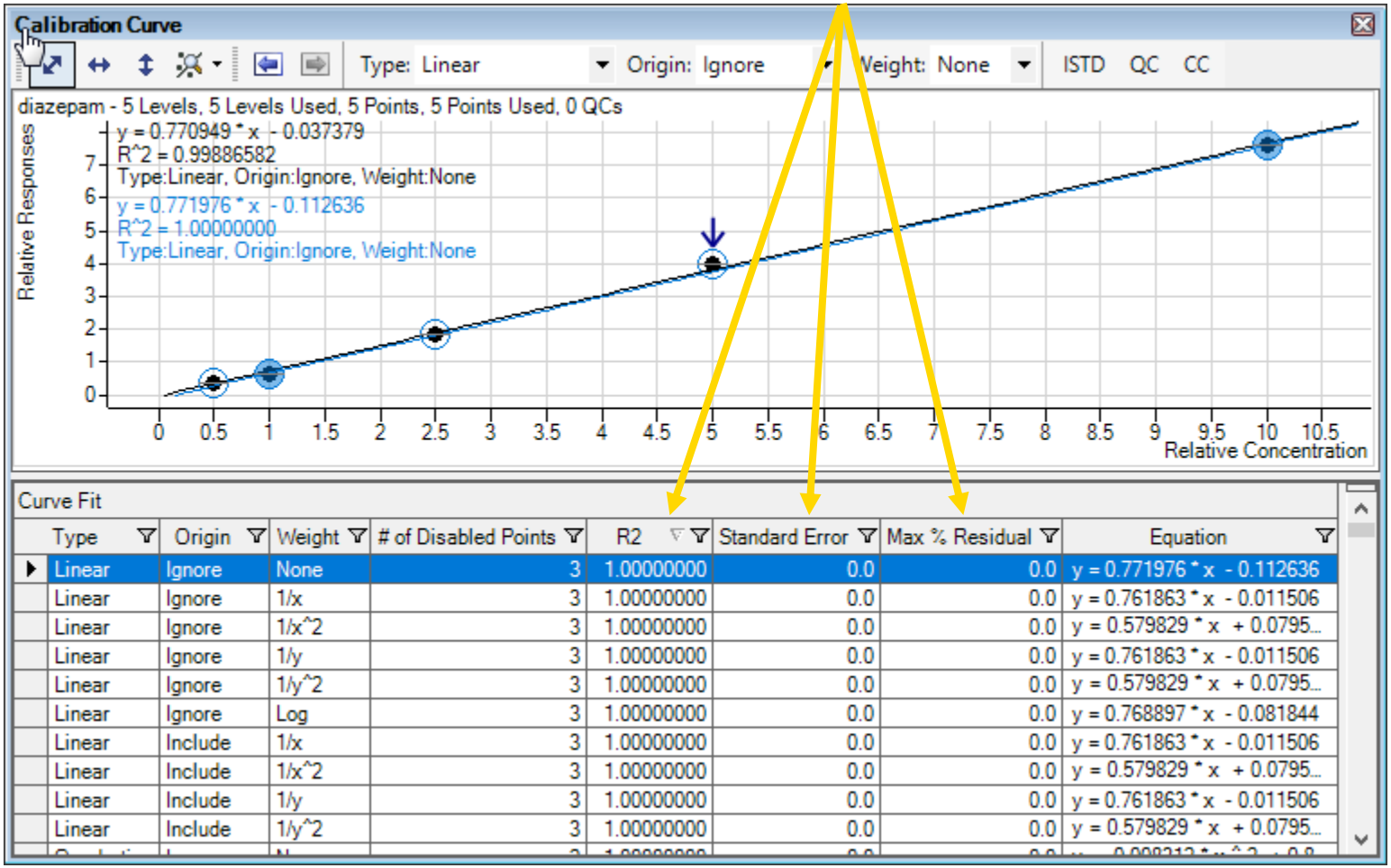
Tip: After changing the curve always reanalyze the batch.

Curve Fit Assistant



Curve Fit Assistant
Sorting Columns

Best curve fit may be ranked using by R^2, Standard Error, or Max % Residual.

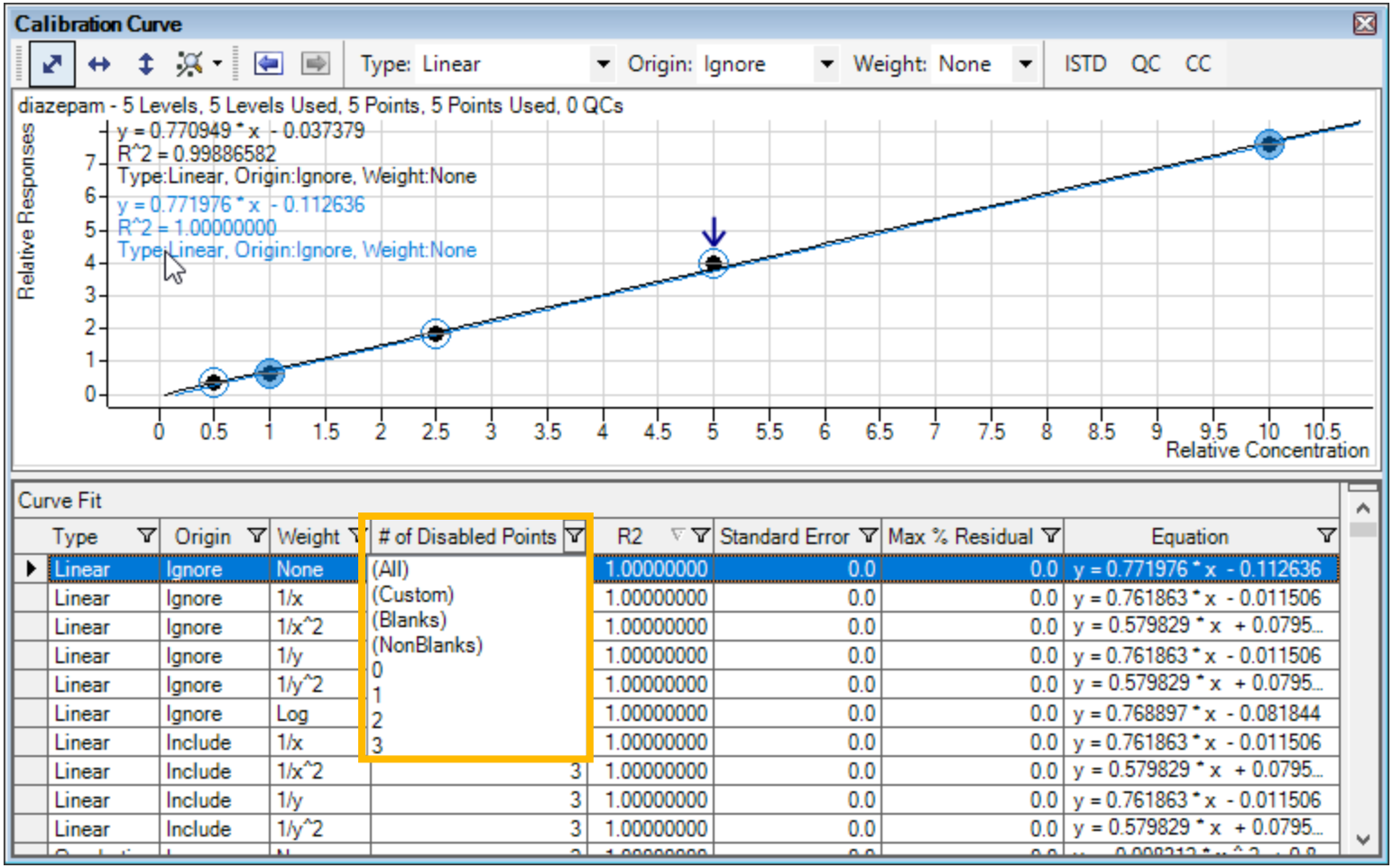


Curve Fit

Type	Origin	Weight	# of Disabled Points	R2	Standard Error	Max % Residual	Equation
Linear	Ignore	None	3	1.00000000	0.0	0.0	y = 0.771976 * x - 0.112636
Linear	Ignore	1/x	3	1.00000000	0.0	0.0	y = 0.761863 * x - 0.011506
Linear	Ignore	1/x^2	3	1.00000000	0.0	0.0	y = 0.579829 * x + 0.0795...
Linear	Ignore	1/y	3	1.00000000	0.0	0.0	y = 0.761863 * x - 0.011506
Linear	Ignore	1/y^2	3	1.00000000	0.0	0.0	y = 0.579829 * x + 0.0795...
Linear	Ignore	Log	3	1.00000000	0.0	0.0	y = 0.768897 * x - 0.081844
Linear	Include	1/x	3	1.00000000	0.0	0.0	y = 0.761863 * x - 0.011506
Linear	Include	1/x^2	3	1.00000000	0.0	0.0	y = 0.579829 * x + 0.0795...
Linear	Include	1/y	3	1.00000000	0.0	0.0	y = 0.761863 * x - 0.011506
Linear	Include	1/y^2	3	1.00000000	0.0	0.0	y = 0.579829 * x + 0.0795...

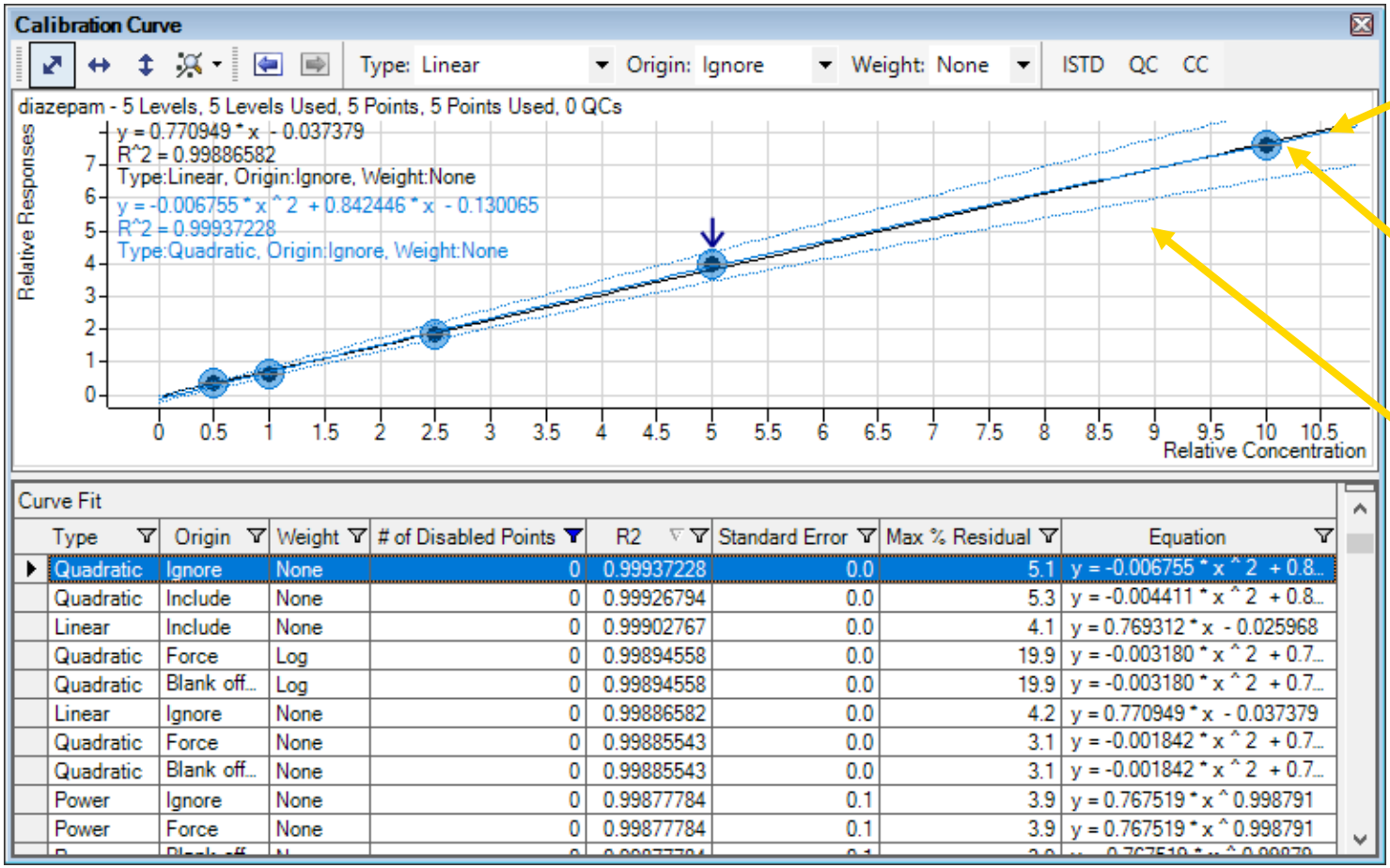
Curve Fit Assistant calculates the mathematical curves.

Curve Fit Assistant
Disabled Points



# of Disabled Points defaults to 3 but column can be filtered.

Curve Fit Assistant
Confidence Band



Curve Fit

Type	Origin	Weight	# of Disabled Points	R2	Standard Error	Max % Residual	Equation
Quadratic	Ignore	None	0	0.99937228	0.0	5.1	y = -0.006755 * x ^ 2 + 0.8...
Quadratic	Include	None	0	0.99926794	0.0	5.3	y = -0.004411 * x ^ 2 + 0.8...
Linear	Include	None	0	0.99902767	0.0	4.1	y = 0.769312 * x - 0.025968
Quadratic	Force	Log	0	0.99894558	0.0	19.9	y = -0.003180 * x ^ 2 + 0.7...
Quadratic	Blank off...	Log	0	0.99894558	0.0	19.9	y = -0.003180 * x ^ 2 + 0.7...
Linear	Ignore	None	0	0.99886582	0.0	4.2	y = 0.770949 * x - 0.037379
Quadratic	Force	None	0	0.99885543	0.0	3.1	y = -0.001842 * x ^ 2 + 0.7...
Quadratic	Blank off...	None	0	0.99885543	0.0	3.1	y = -0.001842 * x ^ 2 + 0.7...
Power	Ignore	None	0	0.99877784	0.1	3.9	y = 0.767519 * x ^ 0.998791
Power	Force	None	0	0.99877784	0.1	3.9	y = 0.767519 * x ^ 0.998791

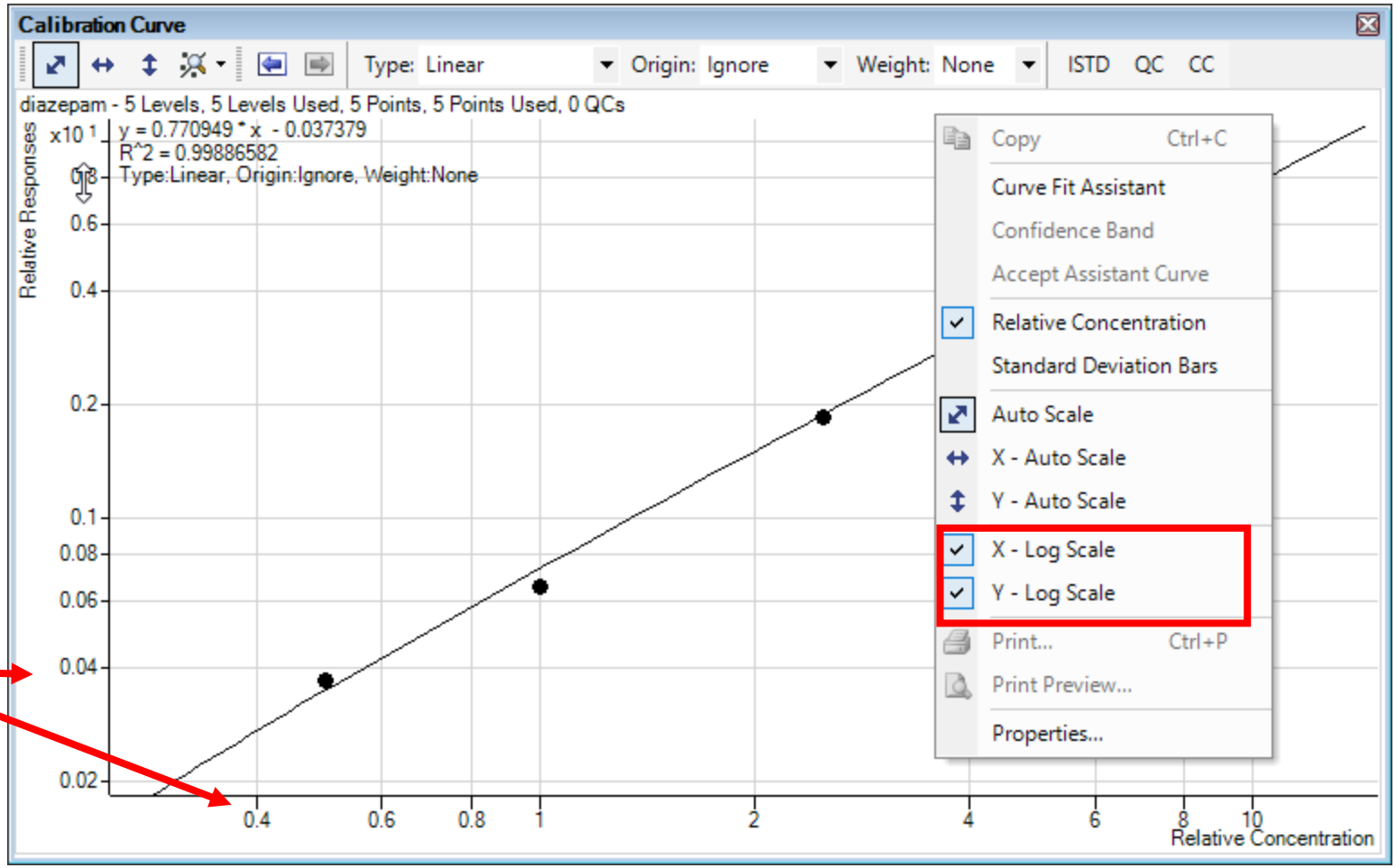
Original Fit (black)

Best Fit (blue)

Confidence Band (blue)

Calibration Curve

Log Log Plot

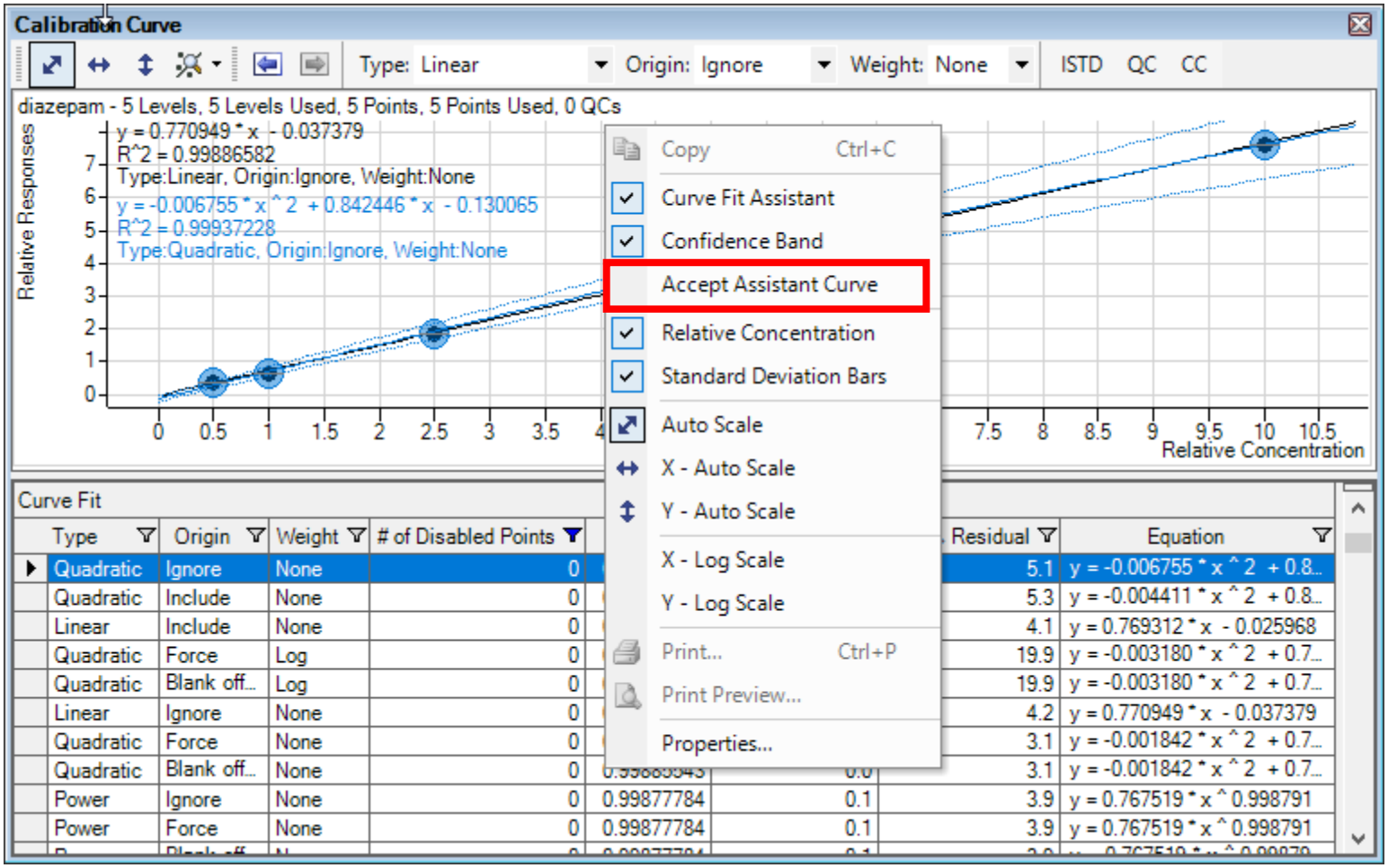


Logarithmic Scale

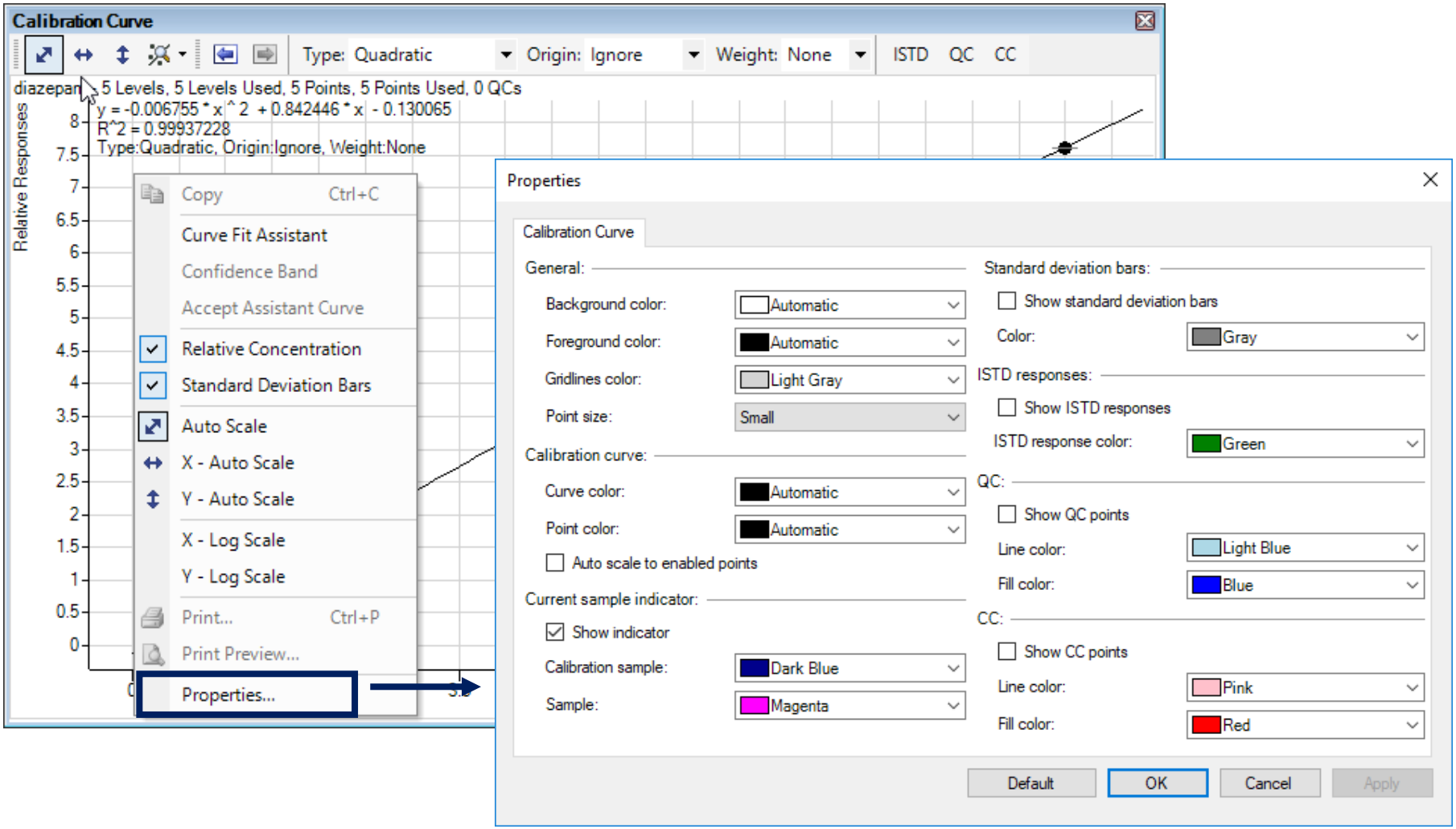
NEW FEATURE!

Curve Fit Assistant

Accept Assistant Curve



Calibration Curve
Properties



Let’s take a moment for questions on Calibration Curve and Curve Fit Assistant

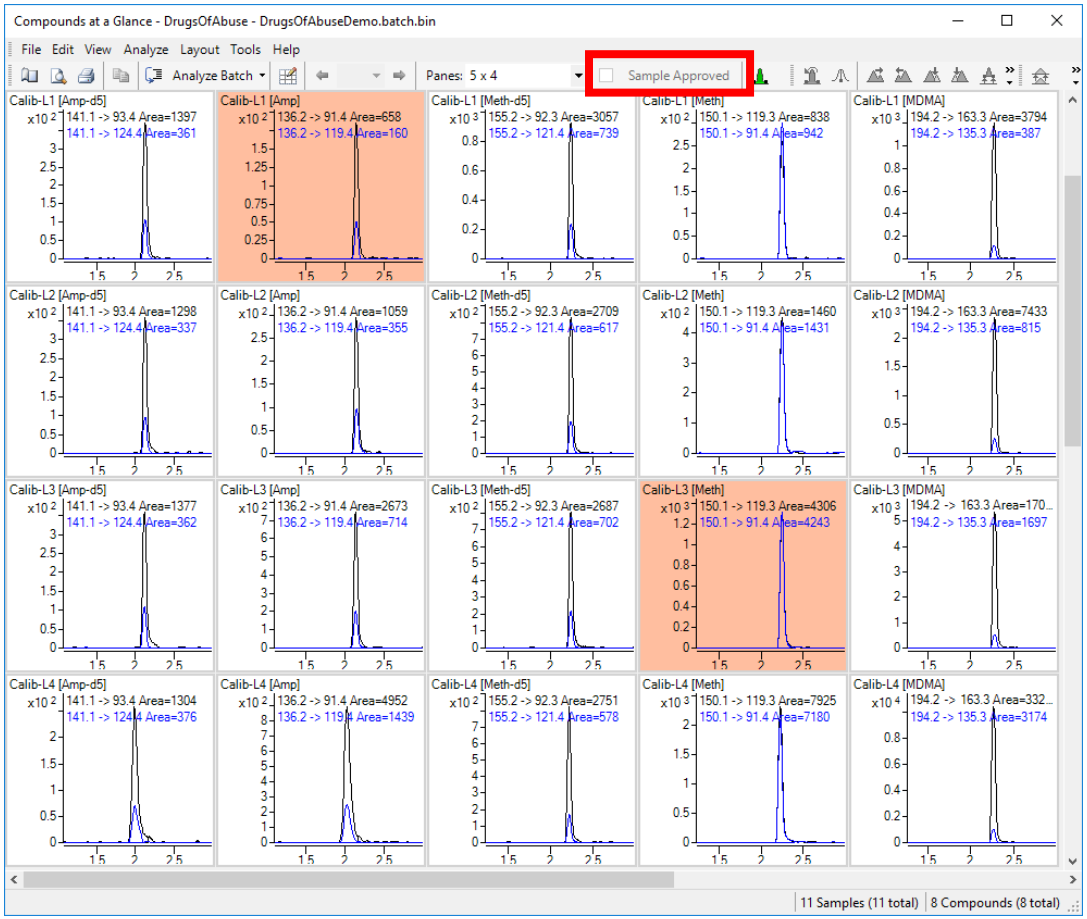
Time for a demo on Calibration Curve and Curve Fit Assistant

Next up:

Compounds at a Glance

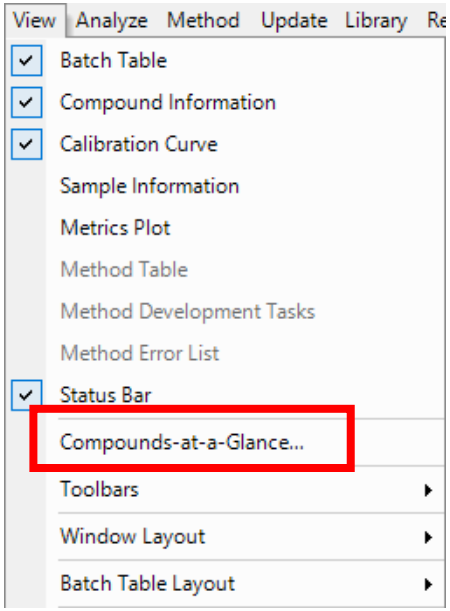
Compounds-at-a-Glance

High throughput data review environment.
View compounds across multiple samples.
View all compounds within a sample.

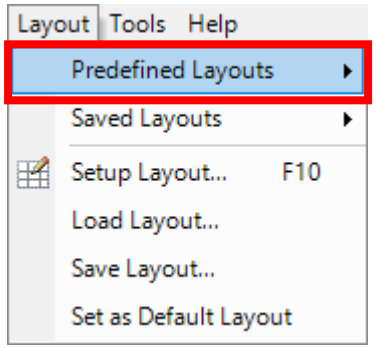


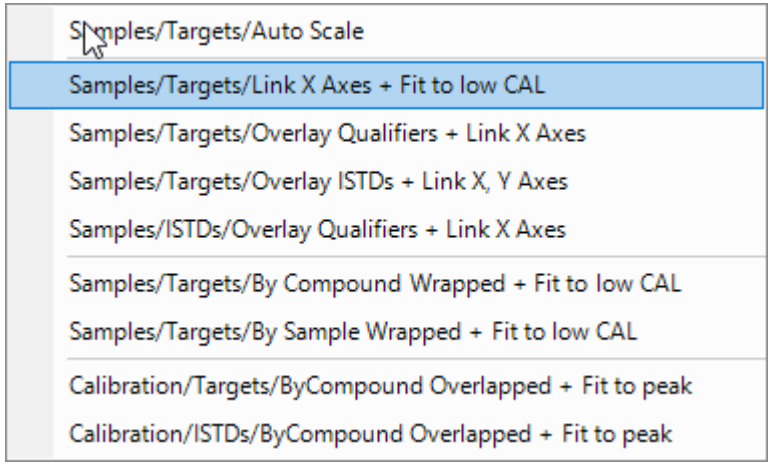
Compounds-at-a-Glance

To start, select **View > Compounds-at-a-Glance…**



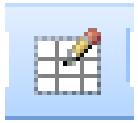
Choose **Layout > Predefined Layout**

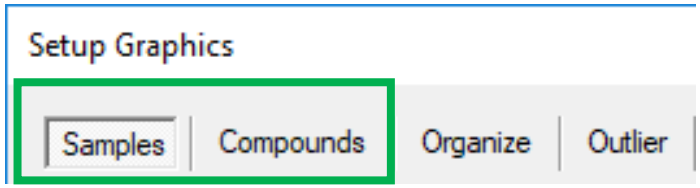




Compounds-at-a-Glance
Setup Graphics Wizard

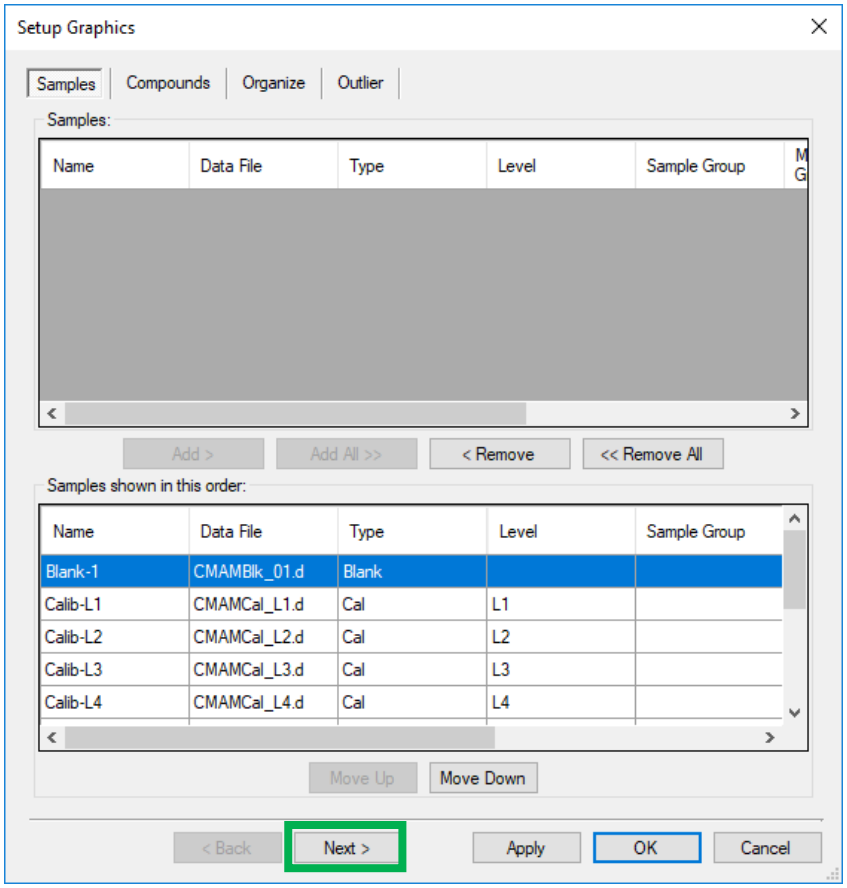
To customize select **Layout > Setup…**





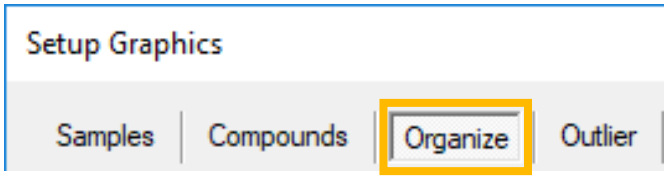
Specify **Samples** to view in Compounds-at-a-Glance then **Compounds**. By default, all samples and all compounds are selected.

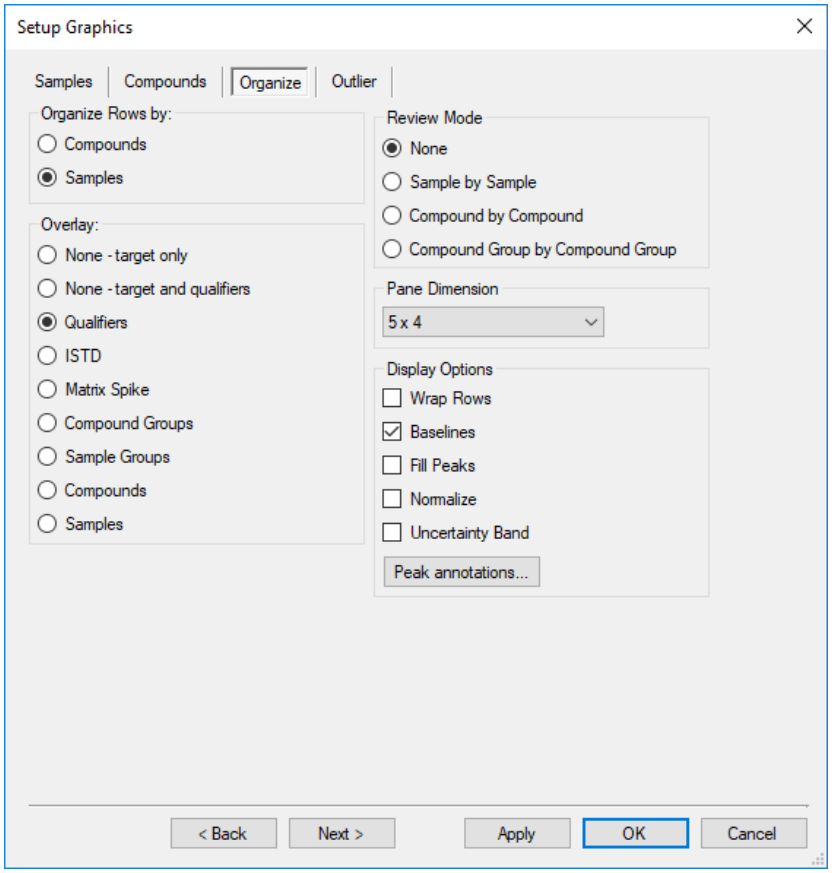
Specific samples and order can be modified.



Compounds-at-a-Glance

Setup Graphics Wizard



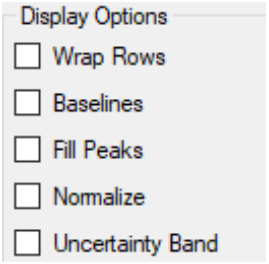


Define how to **Organize** the selected compounds and samples.

Define the Overlay mode.

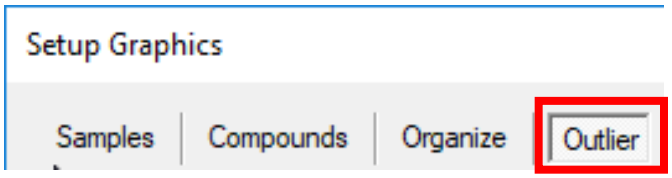
Review Mode
- Sample by Sample
- Compound by Compound
- Compound Group by Compound Group

Display Options



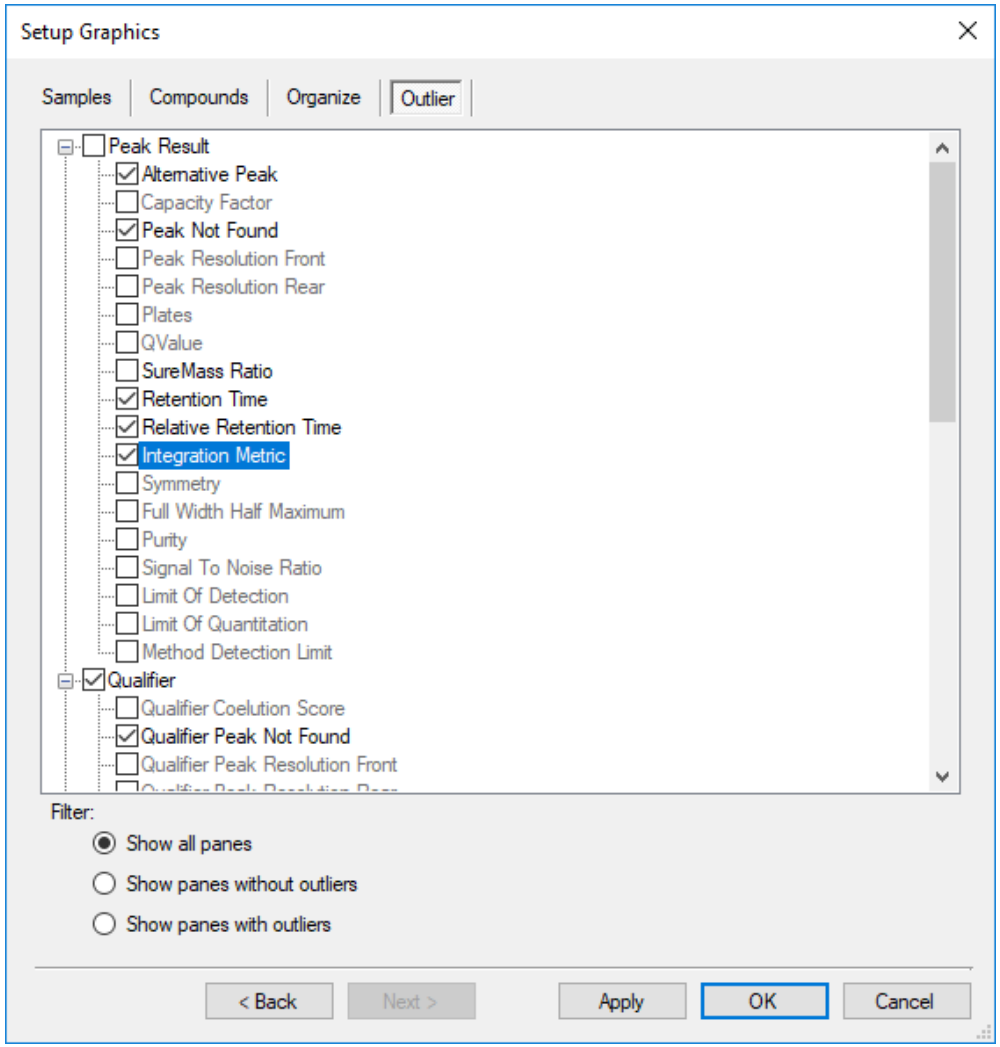
Compounds-at-a-Glance

Setup Graphics Wizard

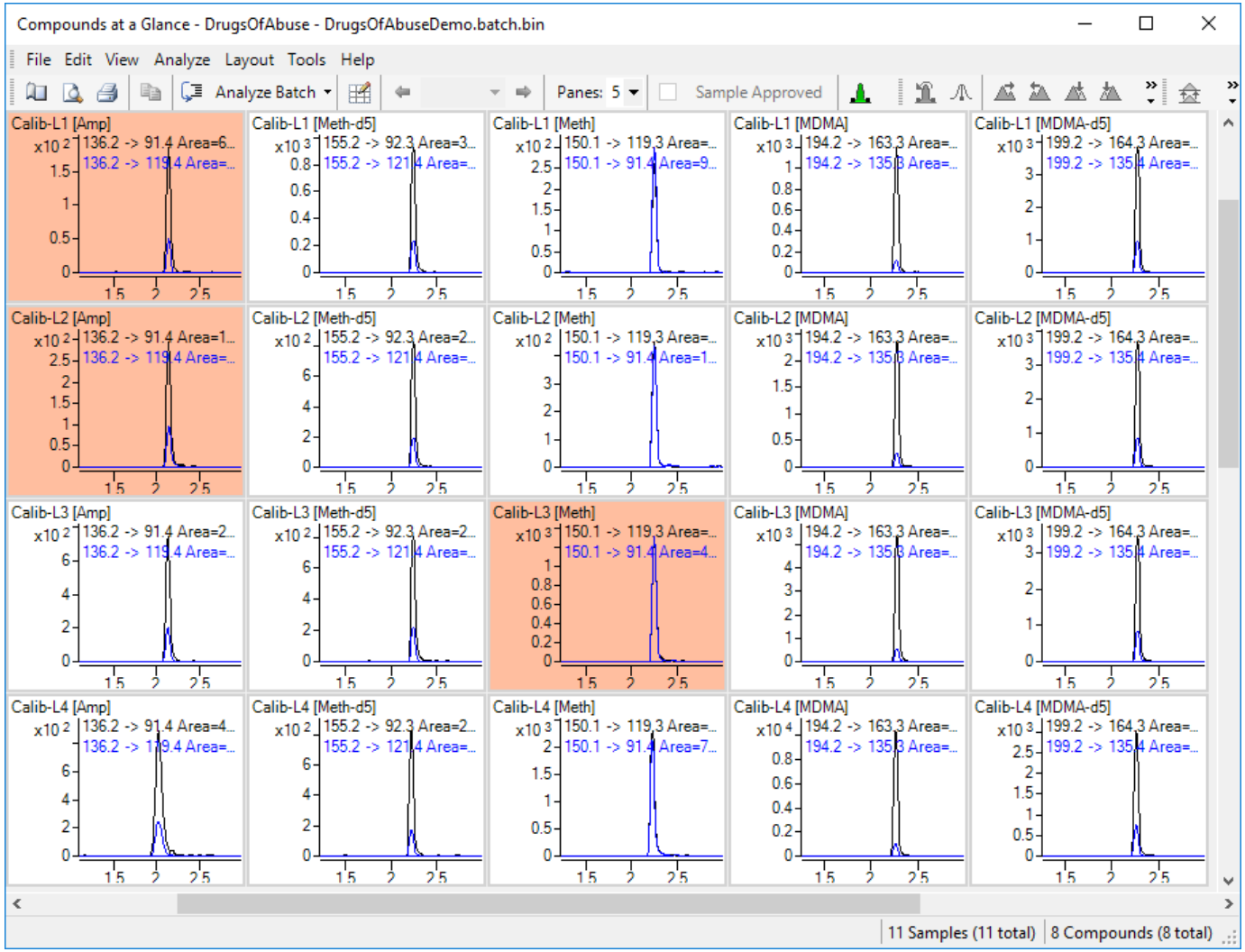


Finally, define which **Outliers** should be highlighted…

Outliers can also be filtered by
- Panes without outliers
- Panes with outliers



Compounds-at-a-Glance
Outliers



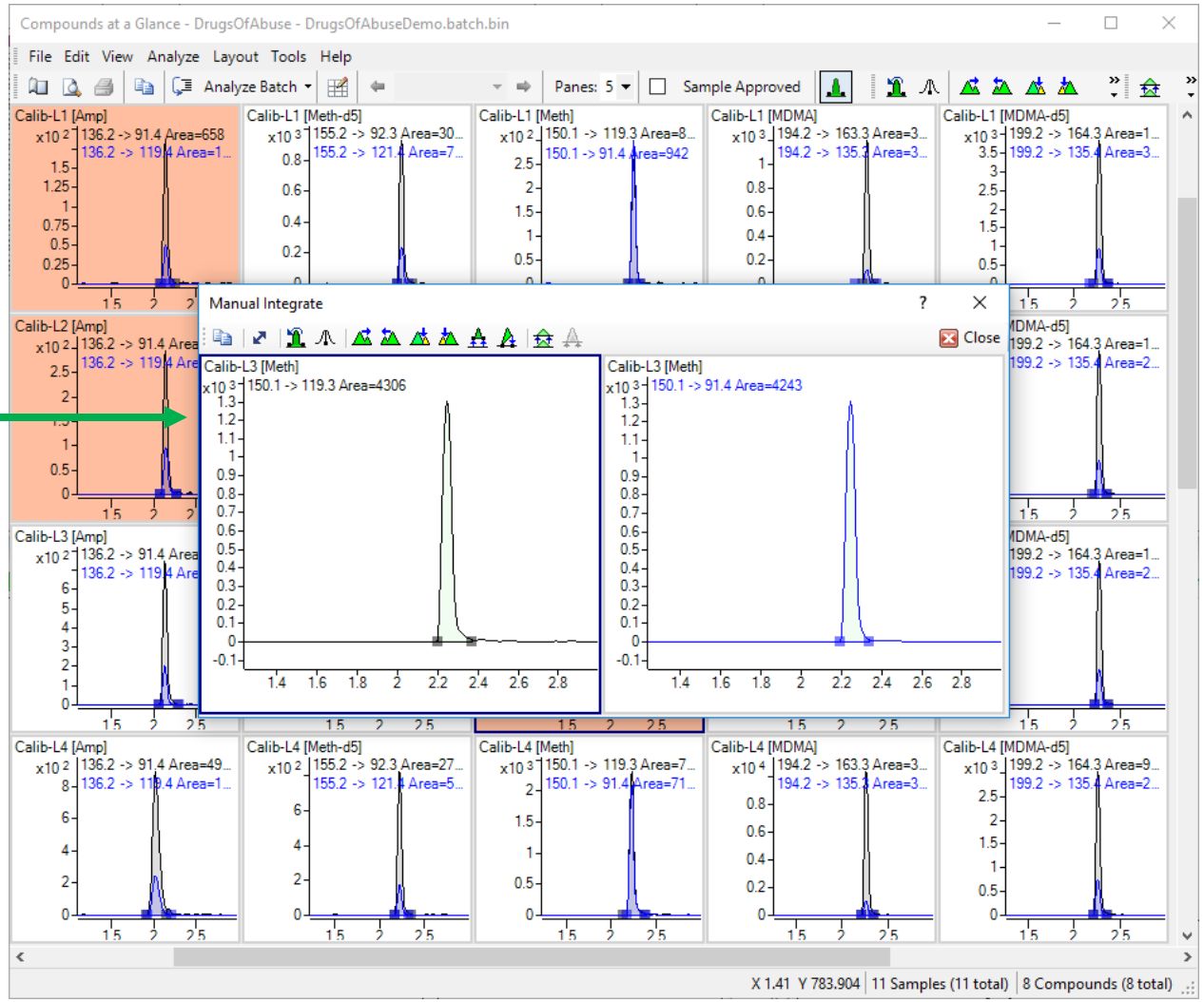
Outliers are highlighted in red.

Compounds-at-a-Glance

Manual Integration Pop Up

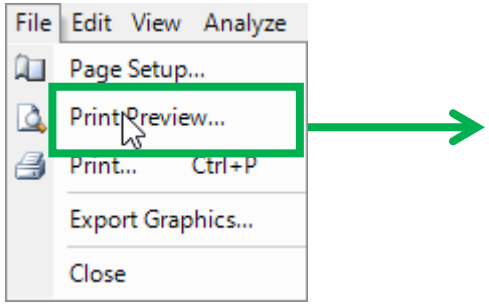
Double click for single pane access.

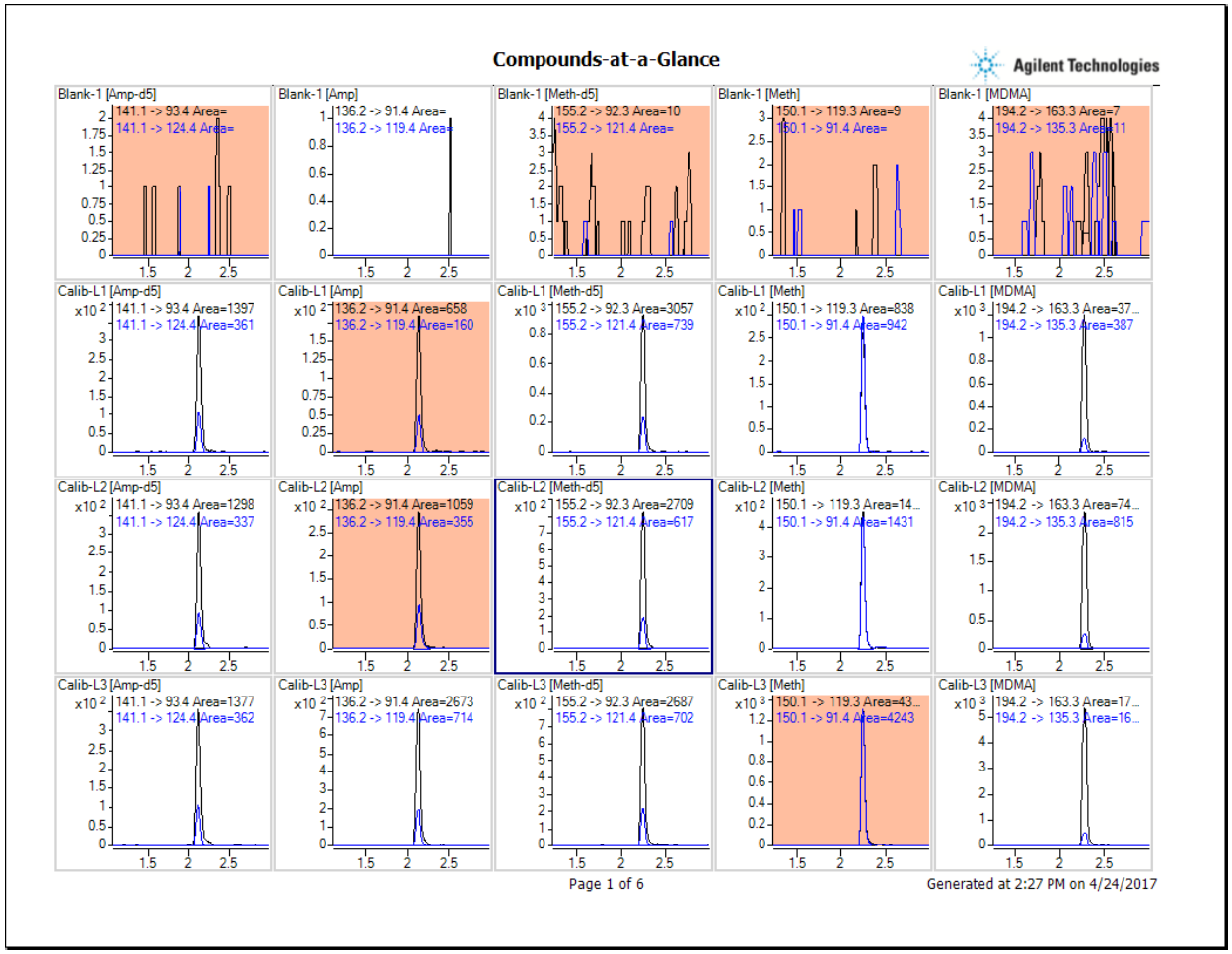
Can be accessed without manual integration being activated.



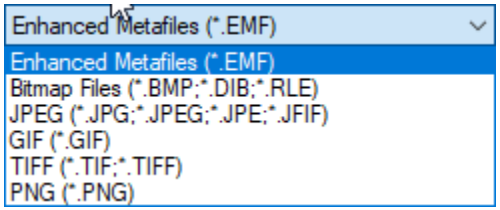
Compounds-at-a-Glance
Print Preview

Select **File** > **Print Preview** to create a chromatogram report.





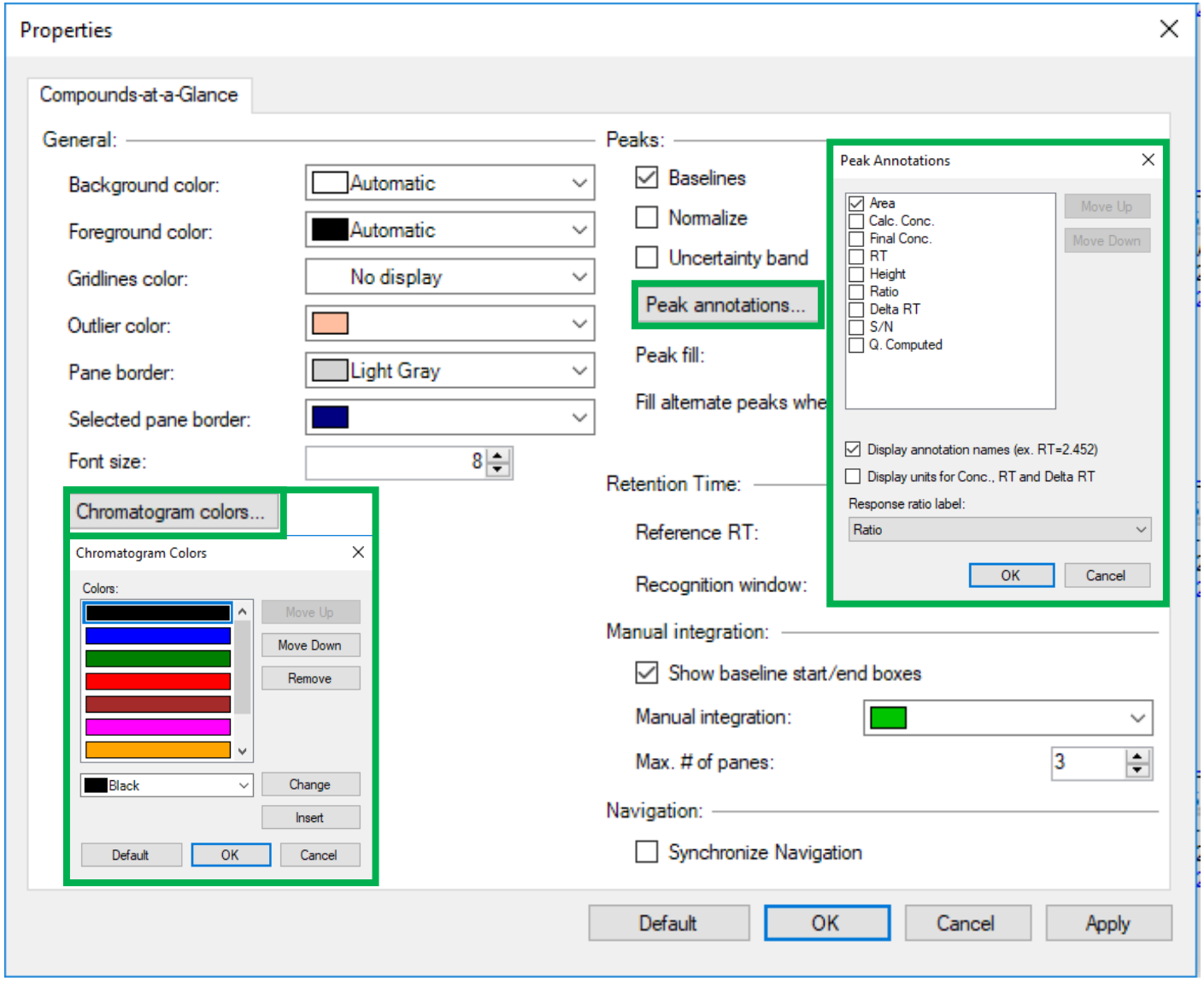
Export Graphics give a graphic image in various formats.



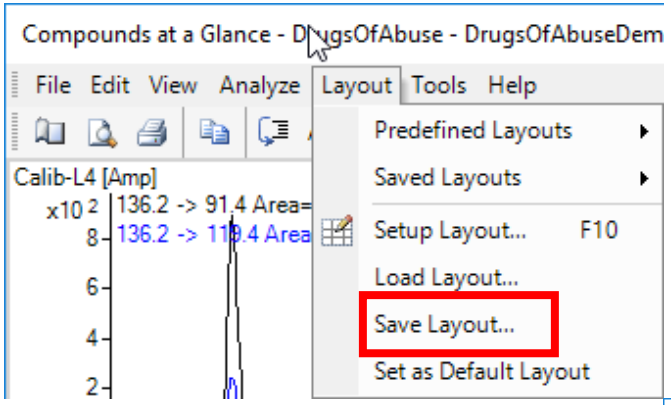
Compounds-at-a-Glance

Properties

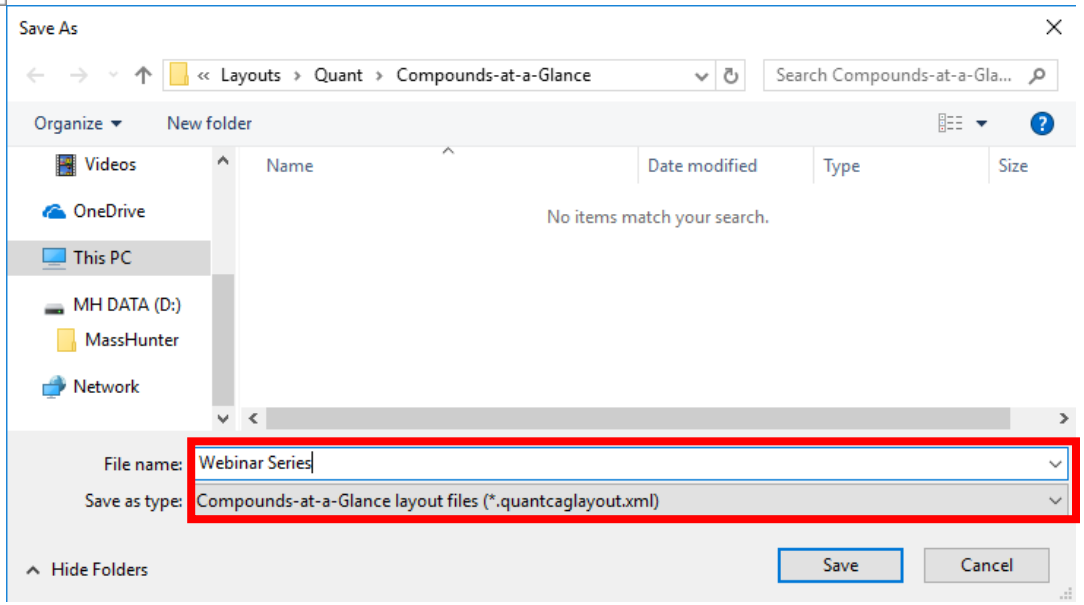
From the context menu select **Properties**



Compounds-at-a-Glance
Save Layout



Some settings in the Layout screens are not saved such as compounds, samples, etc.



Time for questions on Compounds at a Glance

Let’s watch a demo on Compounds at a Glance

Next up:

Global Settings

Globals Settings

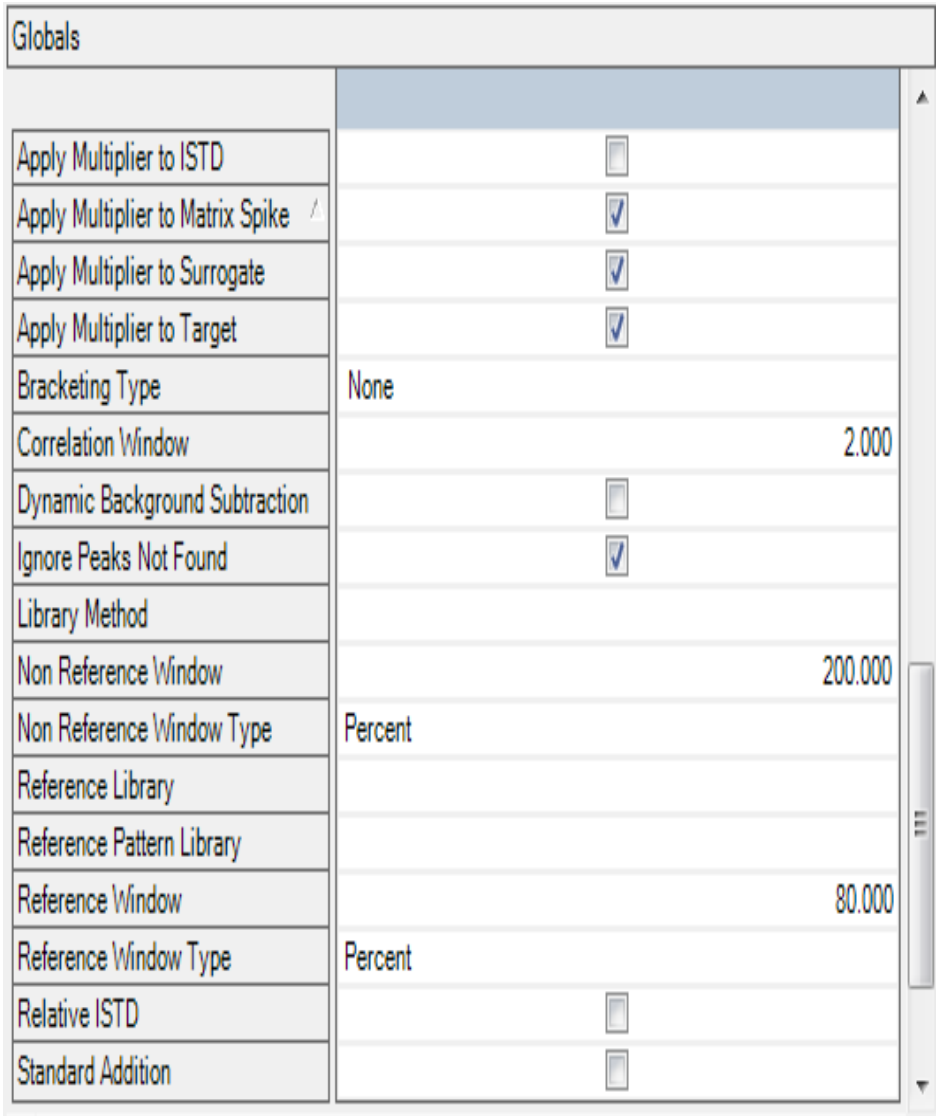
Component of Method Editor.

Global parameters are critical to proper quantitation.

Globals are method parameters.

Globals apply to the whole batch – all samples and all compounds.

Globals Settings



Apply Multiplier's

→ None or Overlapped or Sample Group

→ Associates target and qualifiers in min

→ Dynamic Background Subtraction

→ To avoid flagging target compounds that absent

→ Part of unified method

Peak identification within the extraction window

→ Spectral Reference Library (.reflibrary.xml)

→ Spectral Pattern Reference Library (.reflibrary.xml)

Peak identification within the extraction window

(ISTD with Time Reference Flag checked)

→ Semi quant relative to ISTD

→ Quantitate with Standard Addition

Globals Settings
Calculated and Final Concentration

Sample			1,2,4-Trichlorobe...		1,2,4-Trichlorobenzene Results				
Type	Level	Dil.	Exp. Conc.	Units	RT	Resp.	MI	Calc. Conc.	Final Conc.
Sample		1.0		ng/ml	23.280	1138	☐	0.6341	0.6341
Sample		1.0		ng/ml	24.071	2074	☐	0.6581	0.6581
Sample		1.0		ng/ml	23.280	2272	☐	0.6580	0.6580
Sample		1.0		ng/ml	23.280	5189	☐	0.6964	0.6964

Calculated Concentration (Calc. Conc.)—is the concentration of the compound as calculated from the calibration curve.

Final Concentration (Final Conc.)—is the concentration after the Multiplier has been applied to the Calculated Concentration.

The equations are:

Final Concentration = Calculated Concentration x Multiplier

Globals Settings
Multiplier

Multiplier = Dilution x Sample Amount Multiplier where

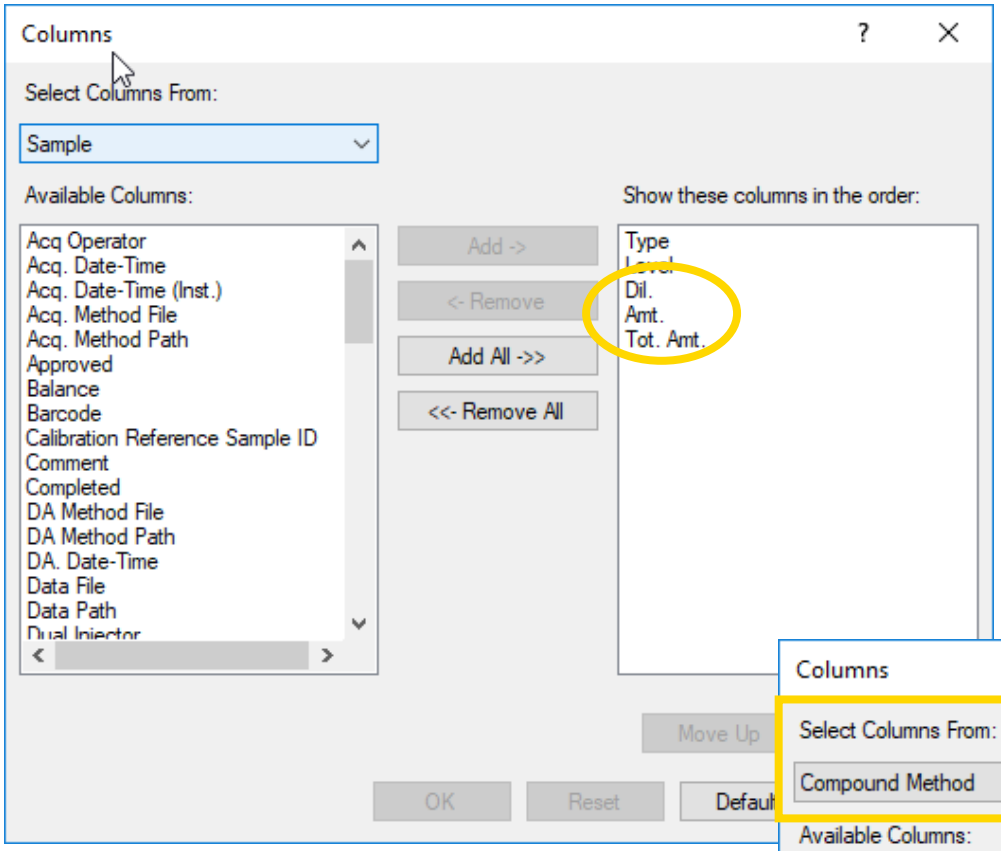
Sample Amount Multiplier = TotalAmt/Amt

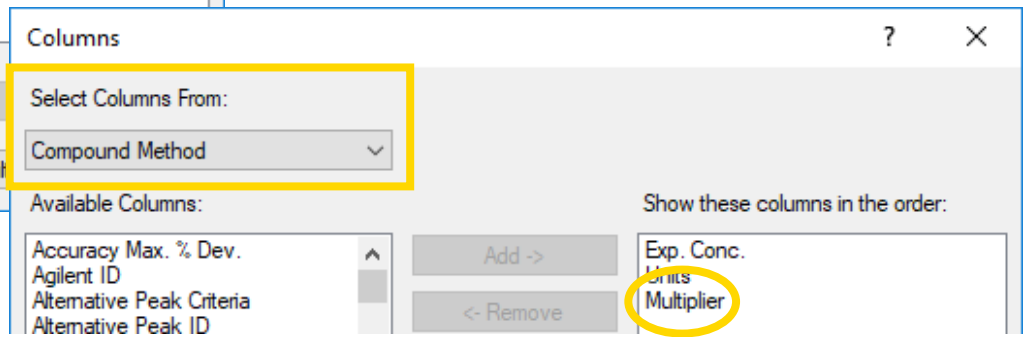
All three of the factors are columns in the Sample section of the Batch Table. By default, they are set to 1, either

explicitly (Dil.) or implicitly (Amt. and Tot. Amt.)

Sample					1,2,4-Trichlorobe...		1,2,4-Trichlorobenzene Results				
Type	Level	Dil.	Amt.	Tot. Amt.	Exp. Conc.	Units	RT	Resp.	MI	Calc. Conc.	Final Conc.
Sample		1.0				ng/ml	23.280	1138	☐	0.6341	0.6341
Sample		1.0				ng/ml	24.071	2074	☐	0.6581	0.6581
Sample		1.0				ng/ml	23.280	2272	☐	0.6580	0.6580
Sample		1.0				ng/ml	23.280	5189	☐	0.6964	0.6964

Globals Settings
Multiplier





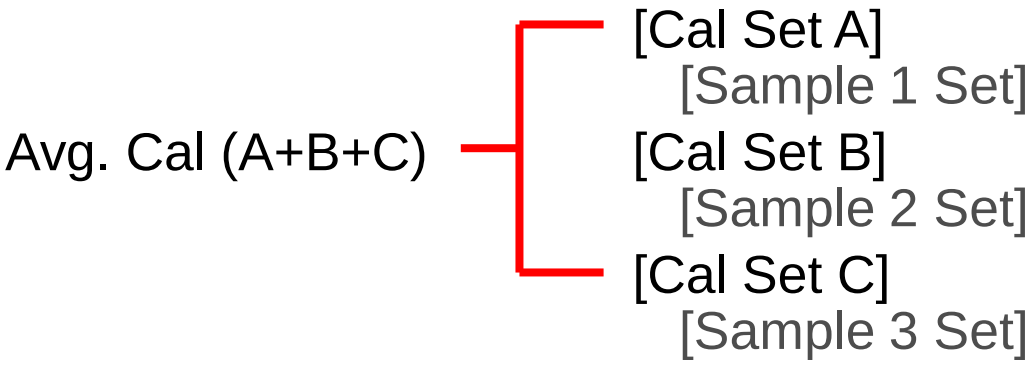
The columns (Dil., Amt., TotalAmt, and Multiplier) must be added to the Batch Table.

The Multiplier in the Compound Results represents the product applied to the compound.

Globals Settings

Bracketing type = None

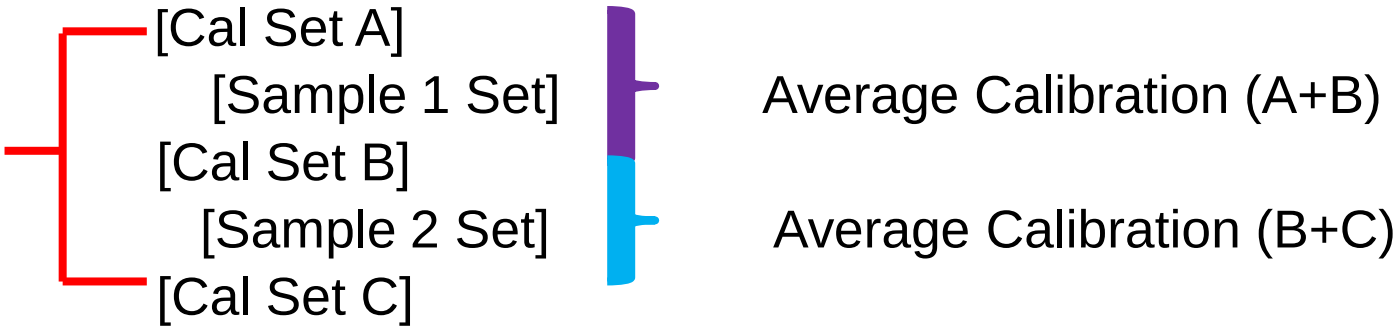
No (None) Bracketing allows the user to have sample(s) quantitated using the average of ***all*** the calibration standards in the batch without regard to order.



Globals Settings

Bracketing type = Overlapped

Overlapped Bracketing allows the user to have sample(s) quantitated using the average of the calibration standards injected both before and after the samples injection.



Globals Settings
Bracketing Type = Sample Group

Used with Stream Select (LC)

When the Bracketing Type Sample Group is implemented, only samples from a specific Sample Group (specified in the Batch Table) are considered in the formation of the calibration curve and quantitation of the samples. This feature would accommodate slight shifts in retention time and/or variations in instrument responses.

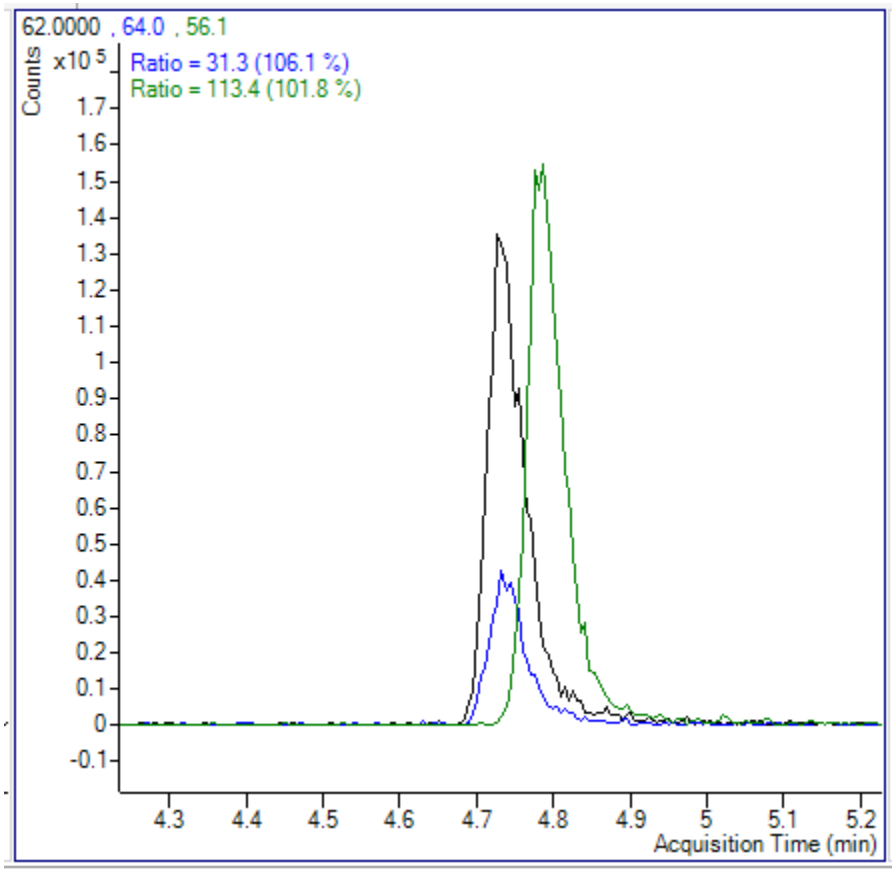
Comment	Sample Group	Info.
	Column 1	
	Column 2	
	Column 1	
	Column 2	

Added from Worklist or Batch Table.

Globals Settings

Correlation Window

Indicates the retention time relationship of target and ions to one or more qualifiers.



Tolerance of extracted ions to be considered a single peak.

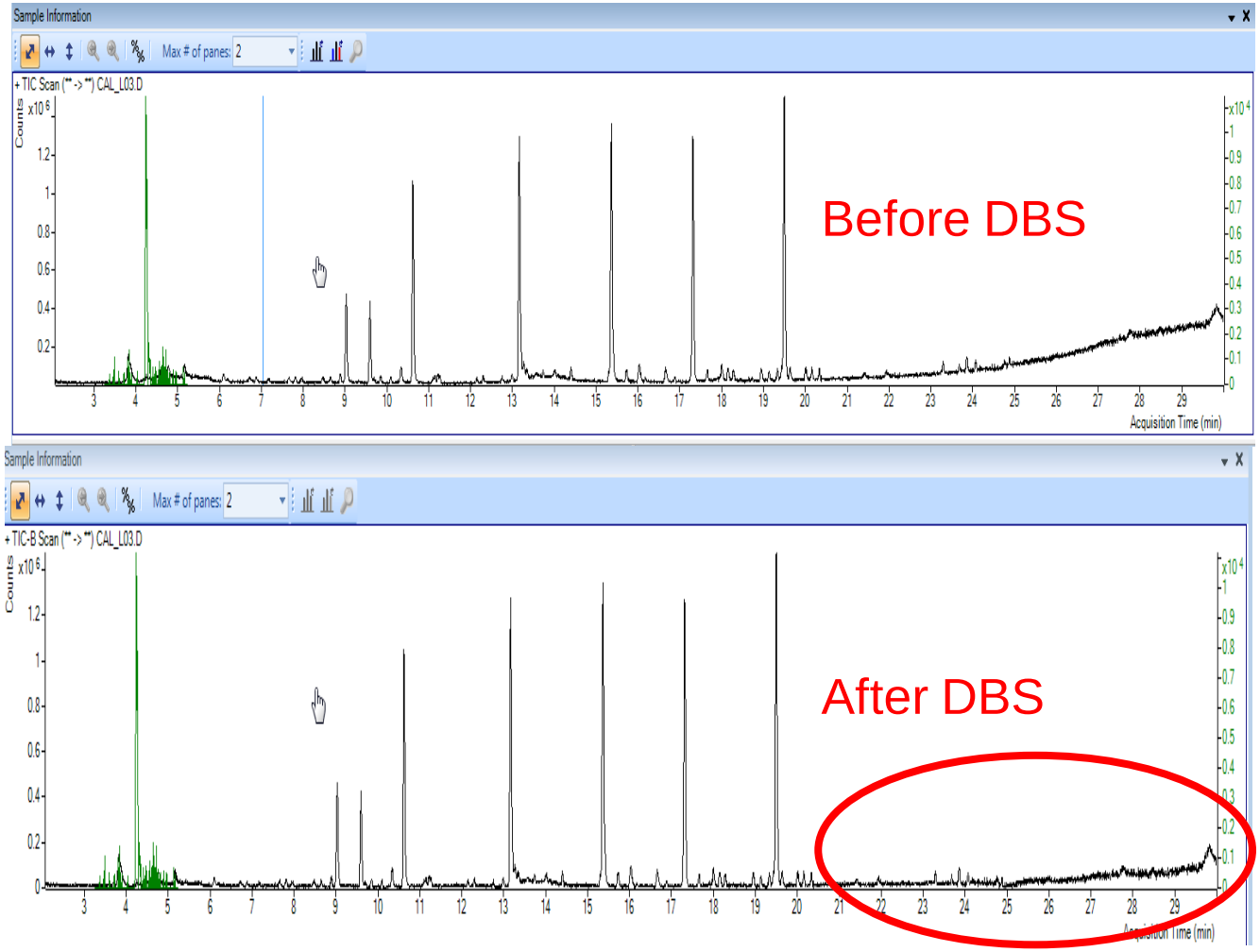
Default time of 0.5 min is rather wide.

Typically 0.01 to 0.05 min (0.6 sec to 3 sec)

Globals Settings

Dynamic Background Subtraction

Removes noise or background ions in TIC, not generally recommended.

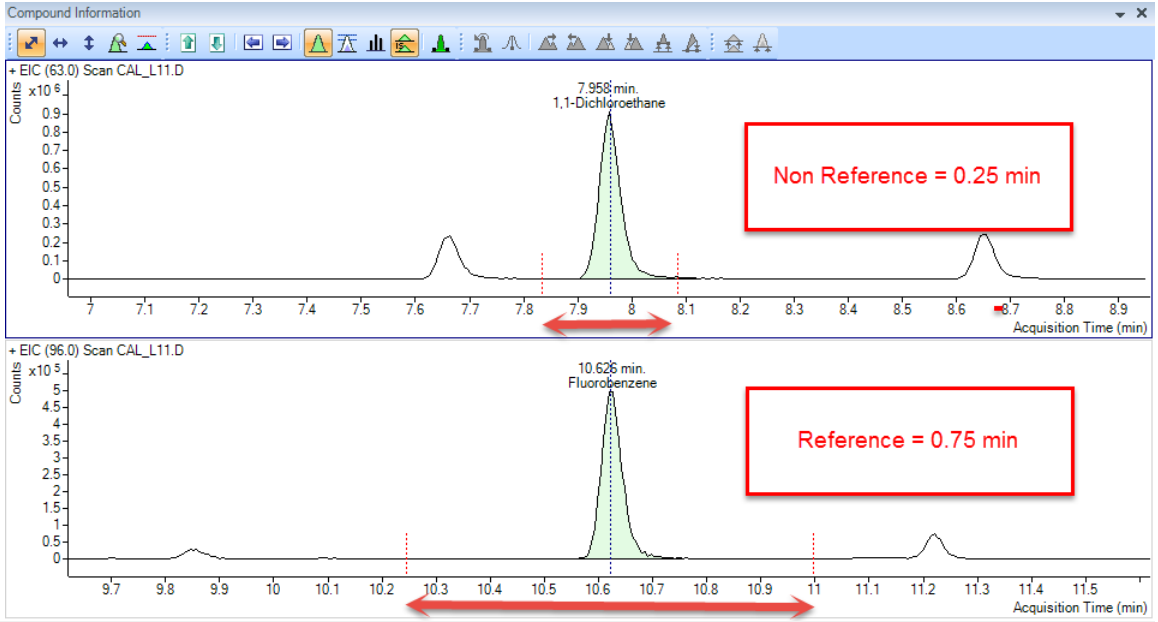


Globals Settings
Non Reference & Reference Window

Globals

Apply Multiplier to ISTD	☐
Apply Multiplier to Matrix Spike	☑
Apply Multiplier to Surrogate	☑
Apply Multiplier to Target	☑
Bracketing Type	None
CC Maximum Elapsed Time In Hours	0.000
Correlation Window	2.000
Dynamic Background Subtraction	☐
Ignore Peaks Not Found	☐
Library Method	
Non Reference Window	0.250
Non Reference Window Type	Minutes
Reference Library	
Reference Pattern Library	
Reference Window	0.750
Reference Window Type	Minutes
Relative ISTD	☐
Standard Addition	☐

Extract full signal for baseline and noise.
Restrict peak selection to smaller RT window.



Quantifier

Name	TS	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Reference Flag
Fluorobenzene	1	Scan	ISTD	<None>	☑	10.0000	☑
Chlorobenzene-d5	1	Scan	ISTD	<None>	☑	10.0000	☐
1,4-Dichlorobenzene-d4	1	Scan	ISTD	<None>	☑	10.0000	☐
1,2-Dichloroethane-d4	1	Scan	Surrogate	Fluorobenzene	☐	10.0000	☐
Toluene-D8	1	Scan	Surrogate	Chlorobenzene-d5	☐	10.0000	☐
1,1-Dichloro-1-propene	1	Scan	Target	Fluorobenzene	☐	10.0000	☐
Dichlorodifluoromethane	1	Scan	Target	Fluorobenzene	☐	10.0000	☐
Chloromethane	1	Scan	Target	Fluorobenzene	☐	10.0000	☐

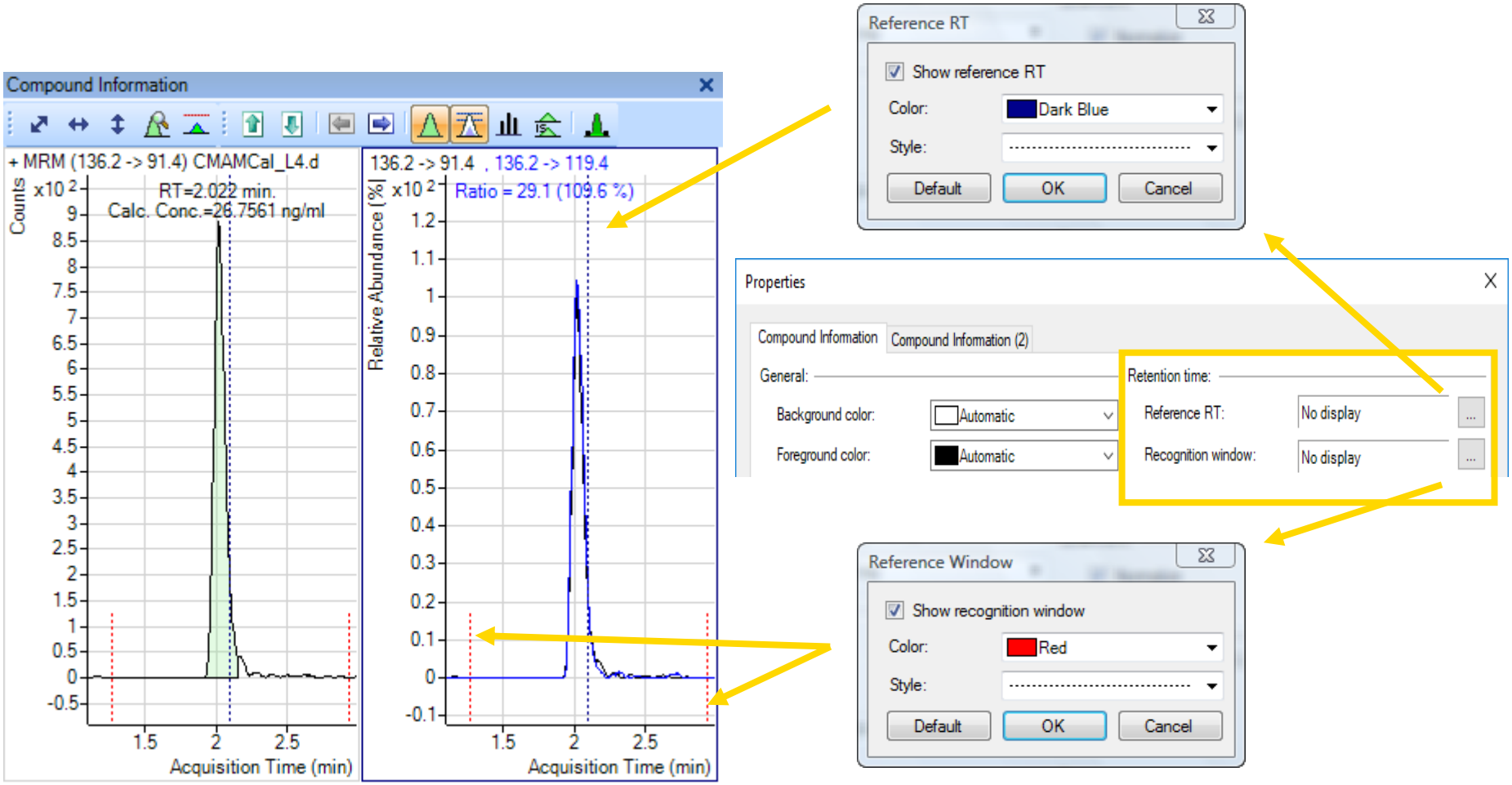
Reference = ISTD + Time Reference

Non Reference = Everything else

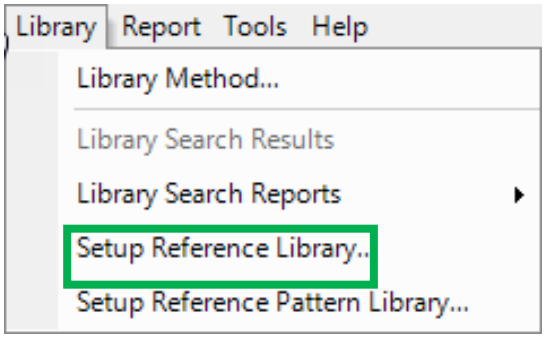
Option to Display Reference RT and Window

Compound Information > Properties > Retention time

Chose which reference points to show and in what color and style.



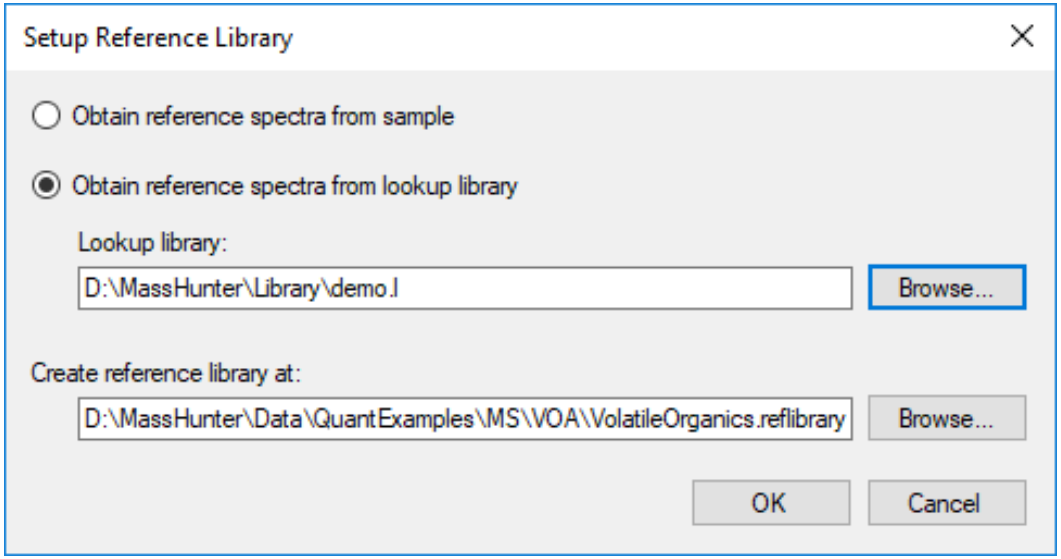
Globals Settings
Reference Library



The Reference Library is activated from **Library > Setup Reference Library…**

A reference library can be created from a calibrator or existing library and added to the quant method to aid in the identification of compounds.

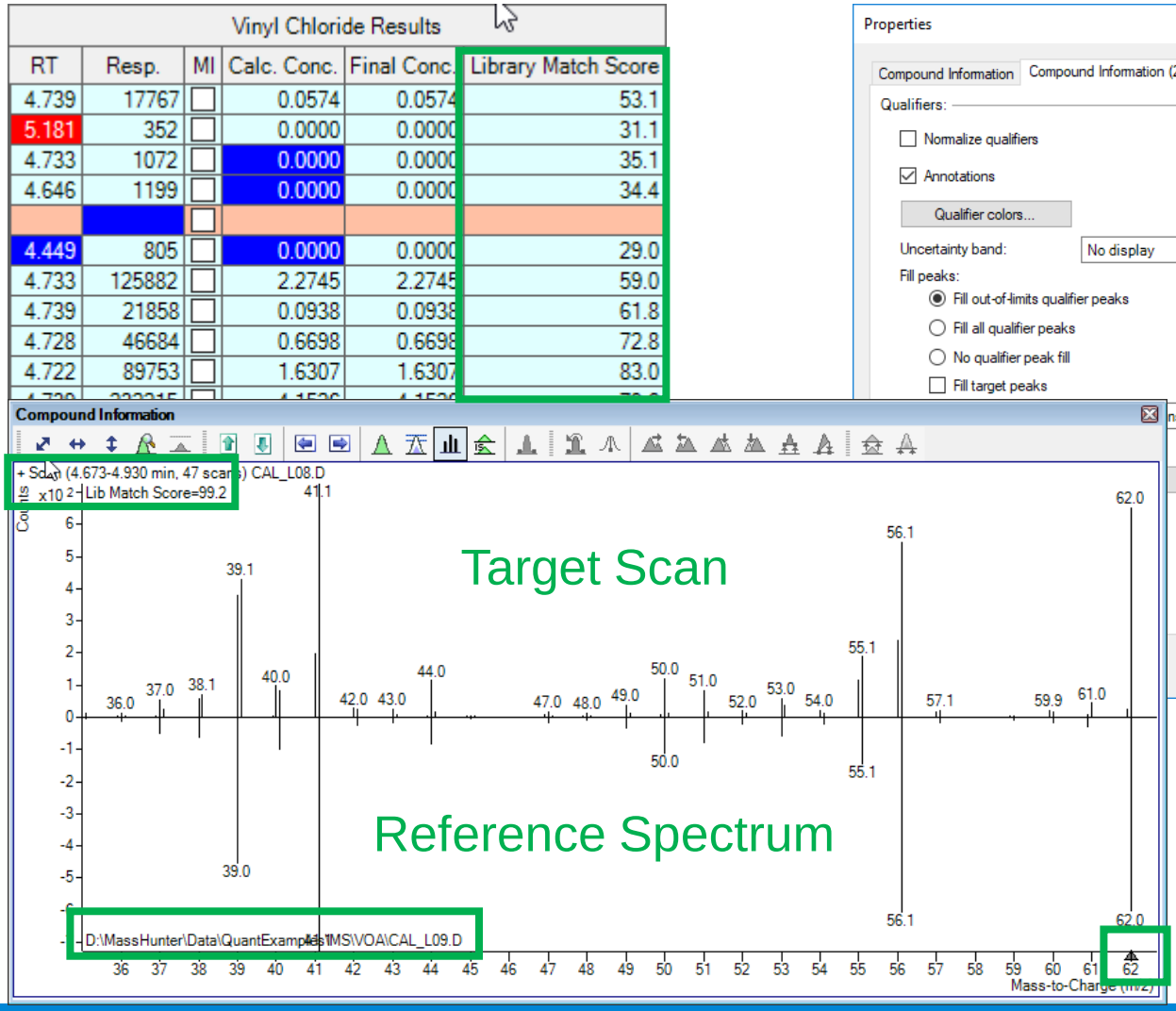
High level calibrator should be selected before entering Method Editor to obtain better quality spectra for the reference library from the sample.

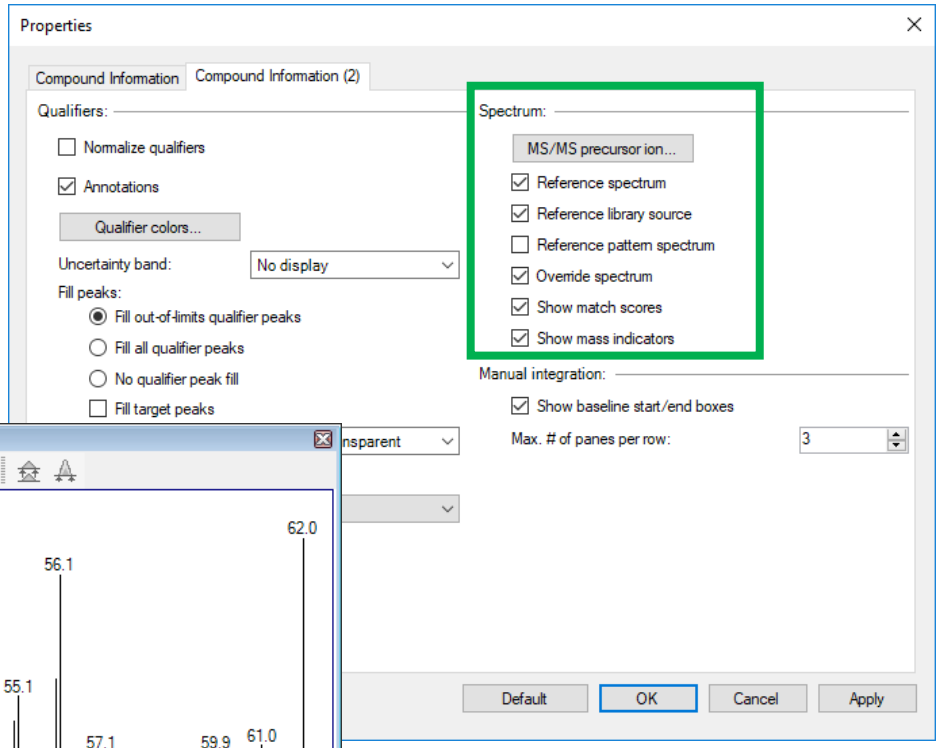


Globals Settings
Reference Library

Vinyl Chloride Results

RT	Resp.	MI	Calc. Conc.	Final Conc.	Library Match Score
4.739	17767	☐	0.0574	0.0574	53.1
5.181	352	☐	0.0000	0.0000	31.1
4.733	1072	☐	0.0000	0.0000	35.1
4.646	1199	☐	0.0000	0.0000	34.4
		☐			
4.449	805	☐	0.0000	0.0000	29.0
4.733	125882	☐	2.2745	2.2745	59.0
4.739	21858	☐	0.0938	0.0938	61.8
4.728	46684	☐	0.6698	0.6698	72.8
4.722	89753	☐	1.6307	1.6307	83.0





Library Match Score is column that can be added in the Batch Table.

Globals Settings
Relative ISTD

A method of semi quantitation.

It is a global parameter and is applied to every sample in the batch.

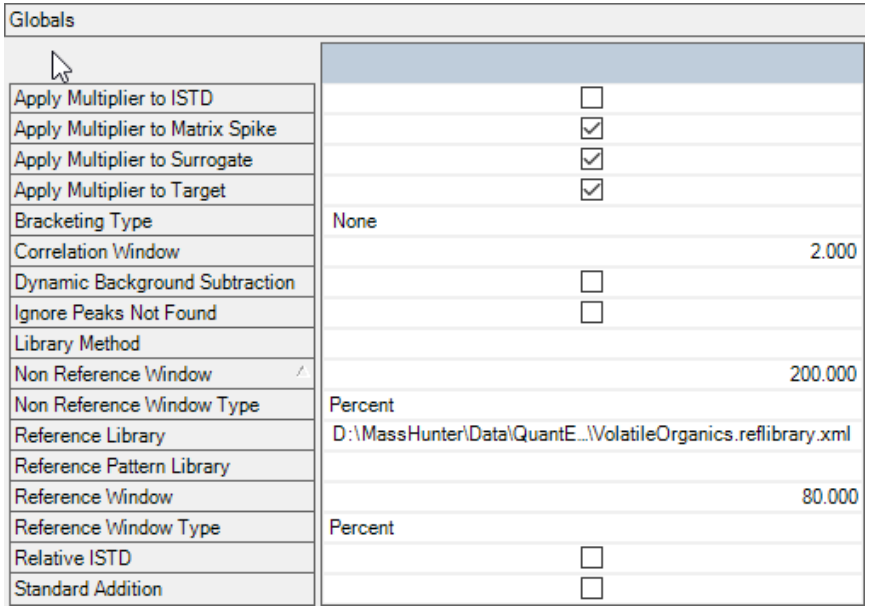
$$[Phe] = \frac{Intensity\ of\ Phe}{Intensity\ of\ D_5Phe} x\ [D_5Phe]$$

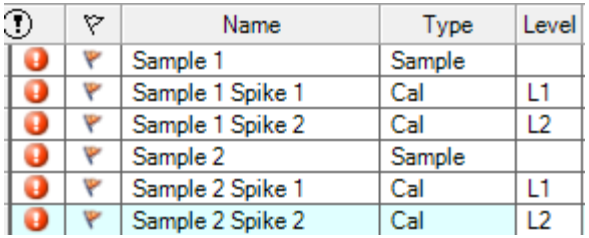
Relative ISTD is a concept where the response factor of the ISTD is used for quantitation.

$$\frac{Response}{ISTDResponse} * ISTDConcentration * ISTDRelativeMultiplier = CalculatedConcentration$$

Globals Settings
Standard Addition

Standard Addition is activated in Globals Setup in the Method Editor.
The calibration curve is Linear, Ignore Origin and No Weighting.
The non-spiked sample MUST precede the calibrators in the Batch Table.
Quant assumes that the order samples are acquired are the order in which they are analyzed.





	Name	Type	Level
	Sample 1	Sample	
	Sample 1 Spike 1	Cal	L1
	Sample 1 Spike 2	Cal	L2
	Sample 2	Sample	
	Sample 2 Spike 1	Cal	L1
	Sample 2 Spike 2	Cal	L2

Order in Batch Table is imperative.
Sample, Cal1, Cal 2….

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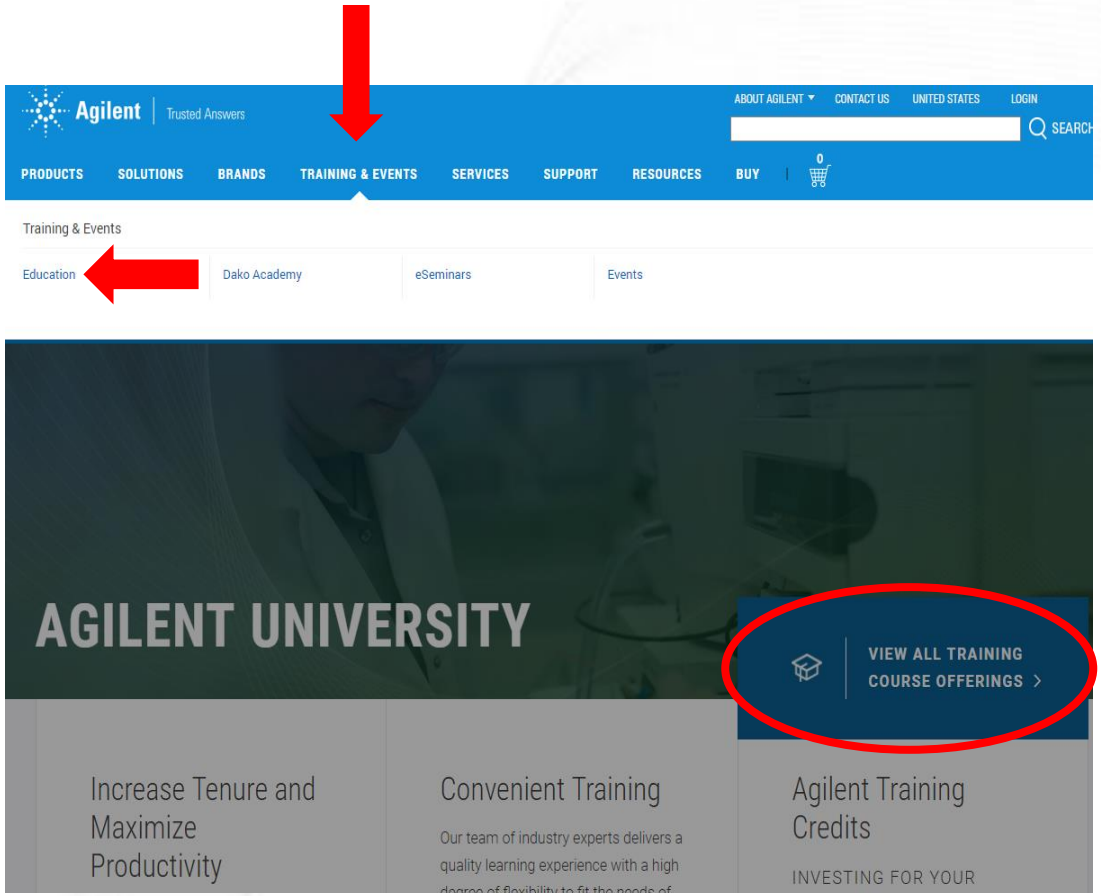

Questions on today's material…

Thank you for your attention.

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