QTOF Small Molecule Application Update

January 10, 2008

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Agenda

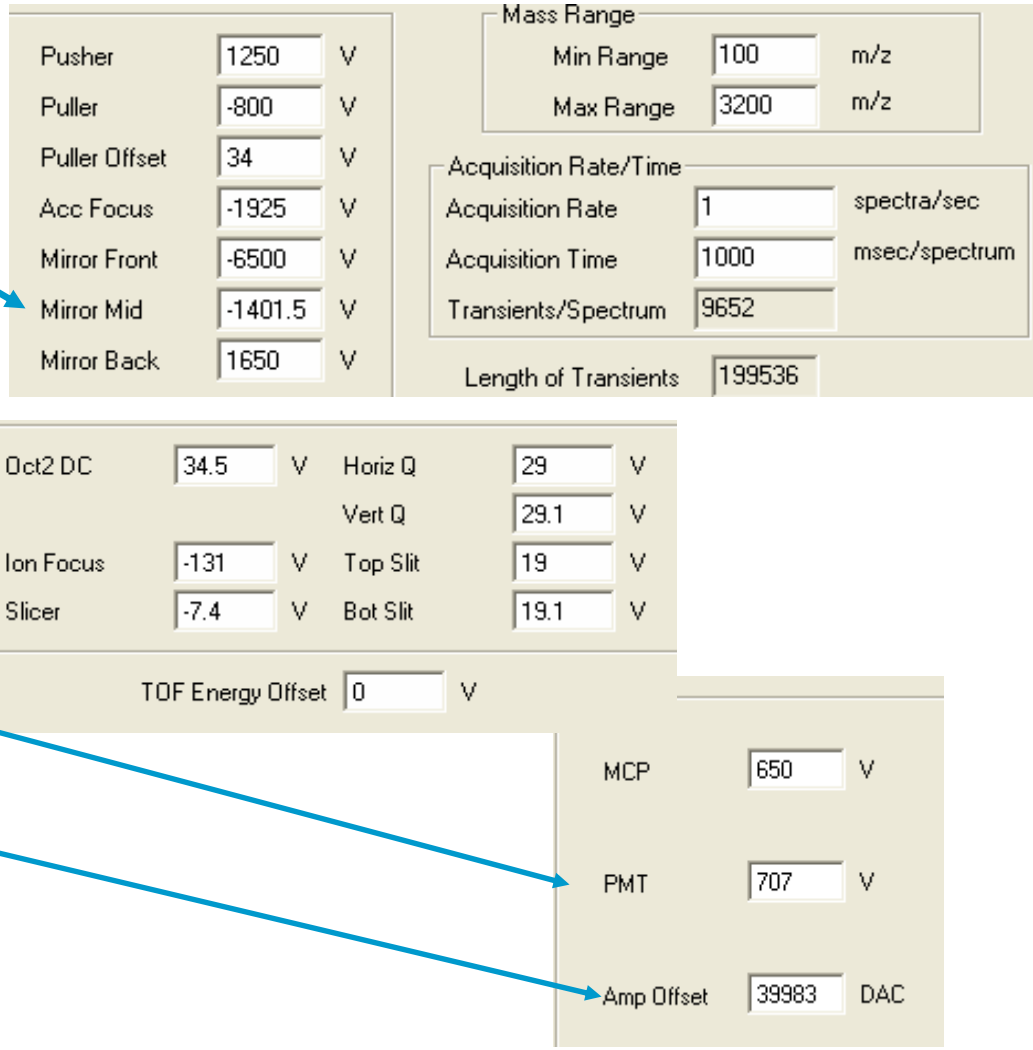
- Goal – Share Best Practices, Updates Software/Hardware
- Training
  - R1904A 4 Day Customer Training Course (Jan 14th next one)
  - Recorded Webex's: Impurity Profiling, QTOF-(August, 2007)
  - QTOF User Sponsored Email Group (QTOF@googlegroups.com)

Today's Topics:

- Upgrading TOF/QTOF to 4 GHz Acquisition Hardware (tuning)
- Answer Questions about Targeted MSMS, MFE and Excel 2003(7)
- Overview of MassHunter B.01.04 Quant
- Questions and Answer Period

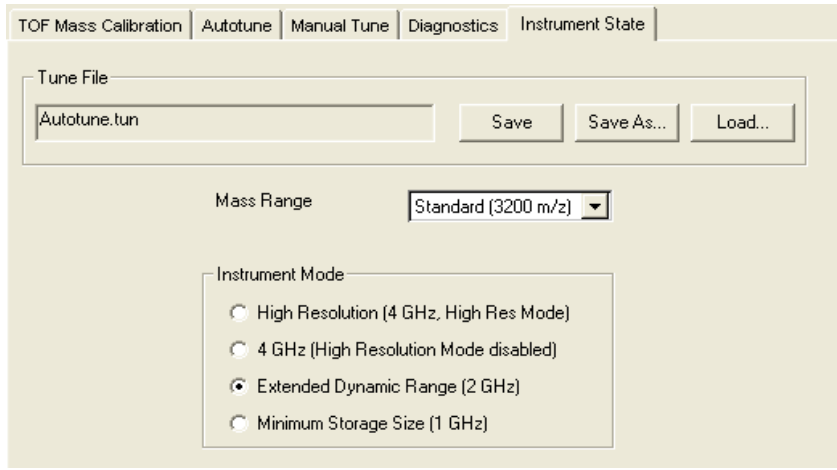
New Parameters for 4GHz Electronics

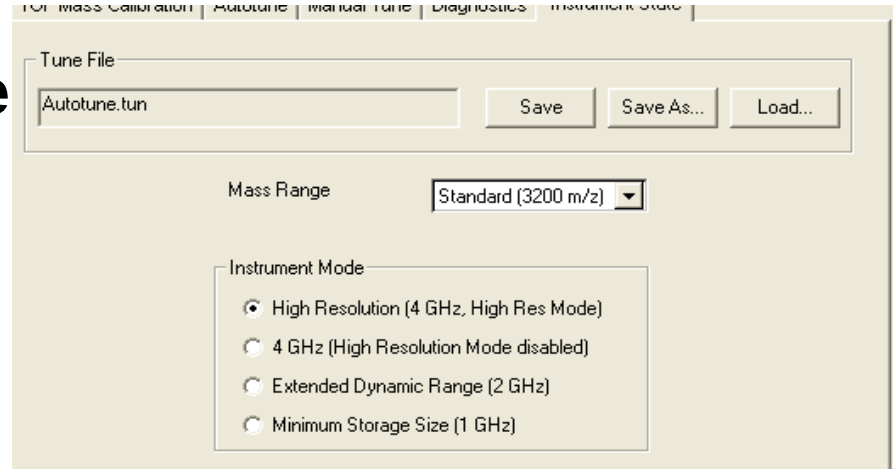
- **Mid-Mirror (0.1V) Steps**
- **Slicer**
- **Bottom/Top Slits**
- **Ion Focus**
- **PMT (Max Now 1100)**
- **Amp Offset**



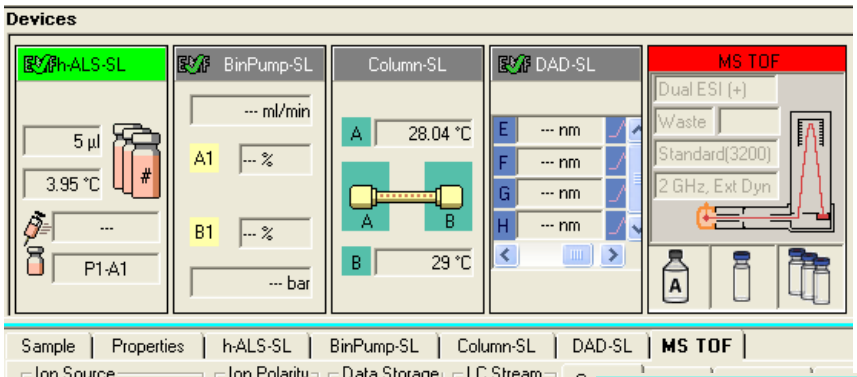
AutoTuning G6220 and G6520 Systems

- **G6220/6520 Systems Utilize New Detection Electronics**
  - Changes to Voltage on Slits, Slicer, Lens, PMT
- **6210/6510 Parameters Not Compatible with 6220/6520**
- **Initial Autotune Must Be Done in all Modes and Polarities**
- Prior to Loading B.01.03 Rename D:\MassHunter directory to D:\MassHunterold

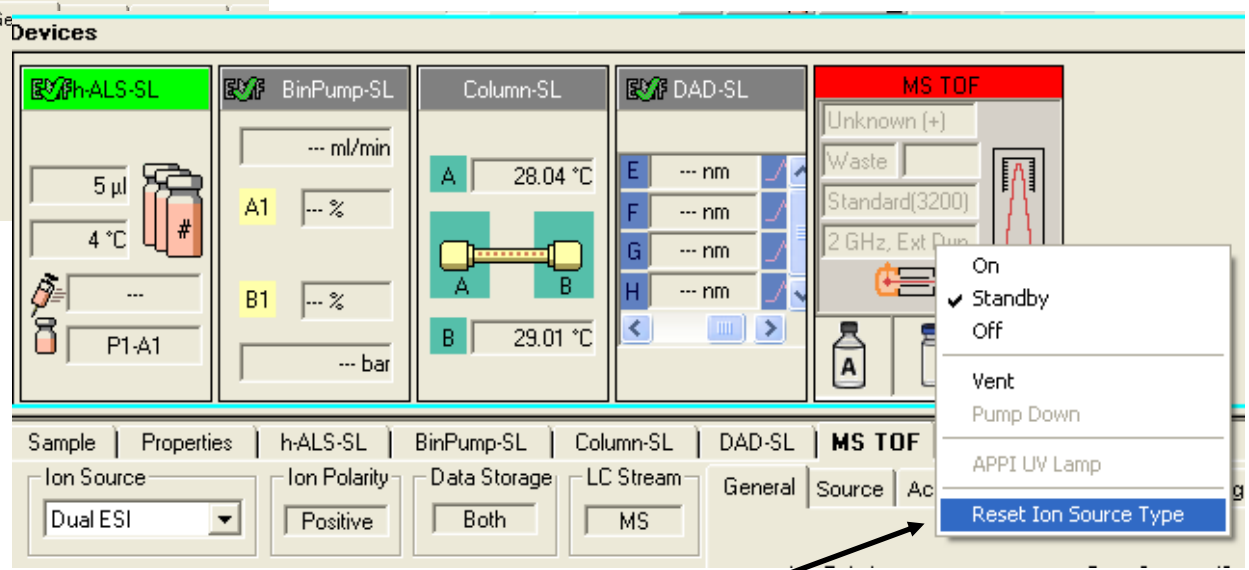




How To Reset Ion Source (No I-Button)

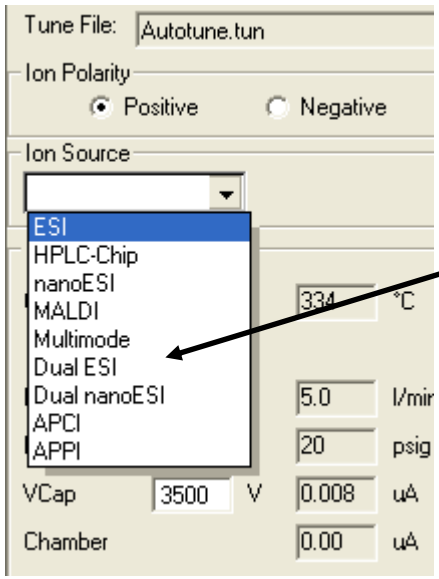


No I-Button on Ion Source
Unable to ID Ion Source Type



Right Click in MS TOF
Select Reset Ion Source Type

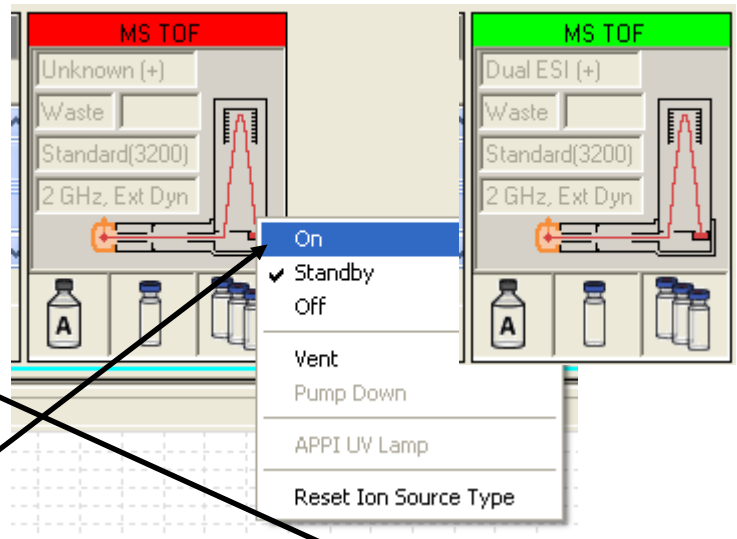
How To Reset Ion Source (No I-Button)

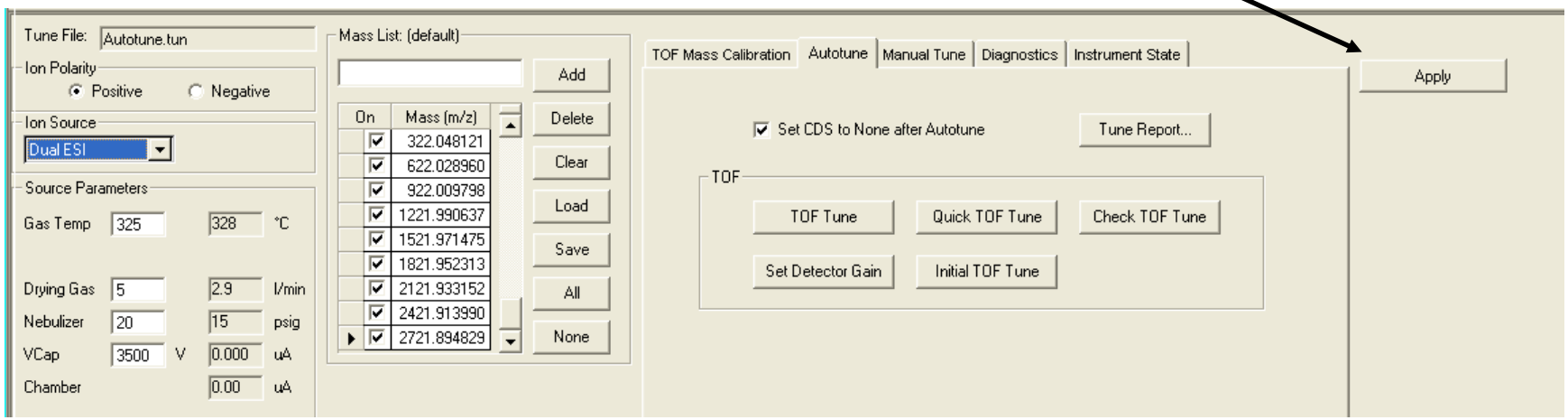


In MS TOF Tab
Select Ion Source
"Dual ESI"

Select Apply

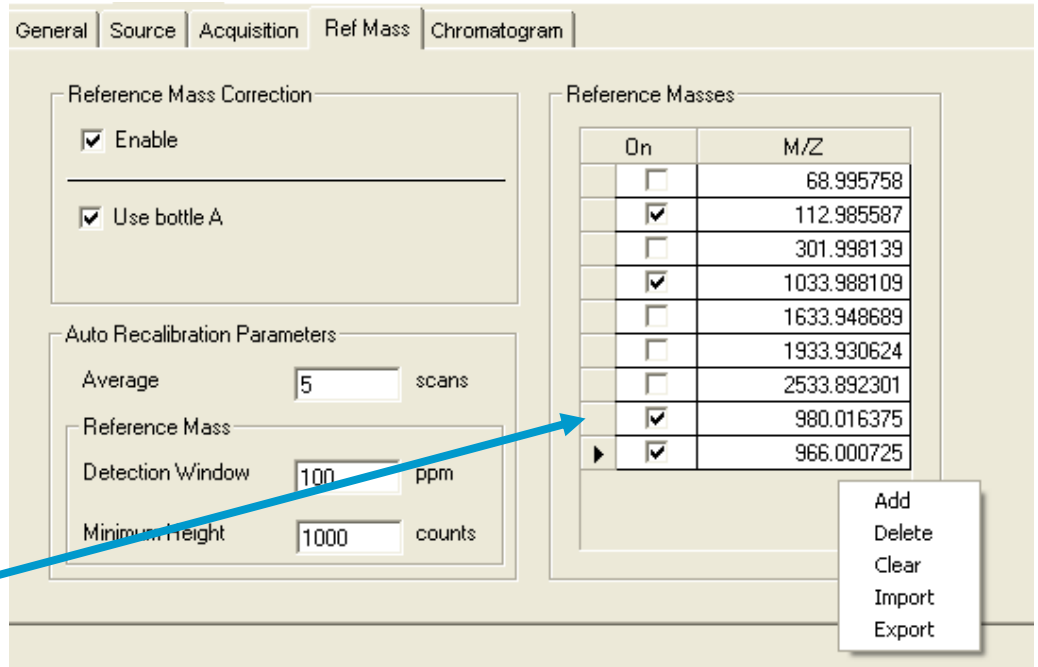
Right Click Select "On"





Addition of Negative Ion Reference Ions

- **From Acquisition**
- **MS TOF Tab**
- **General Tab**
  - Ion Polarity – Negative
- **Ref Mass Tab**
  - Right Click “Add”
  - Input Mass 966.000725
  - Input Mass 980.016375



Reference Solution can also be introduced using a Binary or Isocratic with a 100:1 splitter designed by Agilent. Flow rate of LC set at between 0.5-1.0 mL/min and gives very stable flow.

New Makeup Procedure for Reference Solution

2 mL of HP121 into 98 mL of 95/5 MeOH/Water

0.8 mL of HP921 into 99.2 mL of 95/5 MeOH/Water

Using a 500 mL volumetric flask, mix 25 mL of HP121 and 921 into 450 mL of 95/5 MeOH/Water with 0.2% Acetic Acid.

Better for Negative ion performance and also universal for use with MMI and APCI sources without changing reference solution.

Determining Saturation Level of TOF

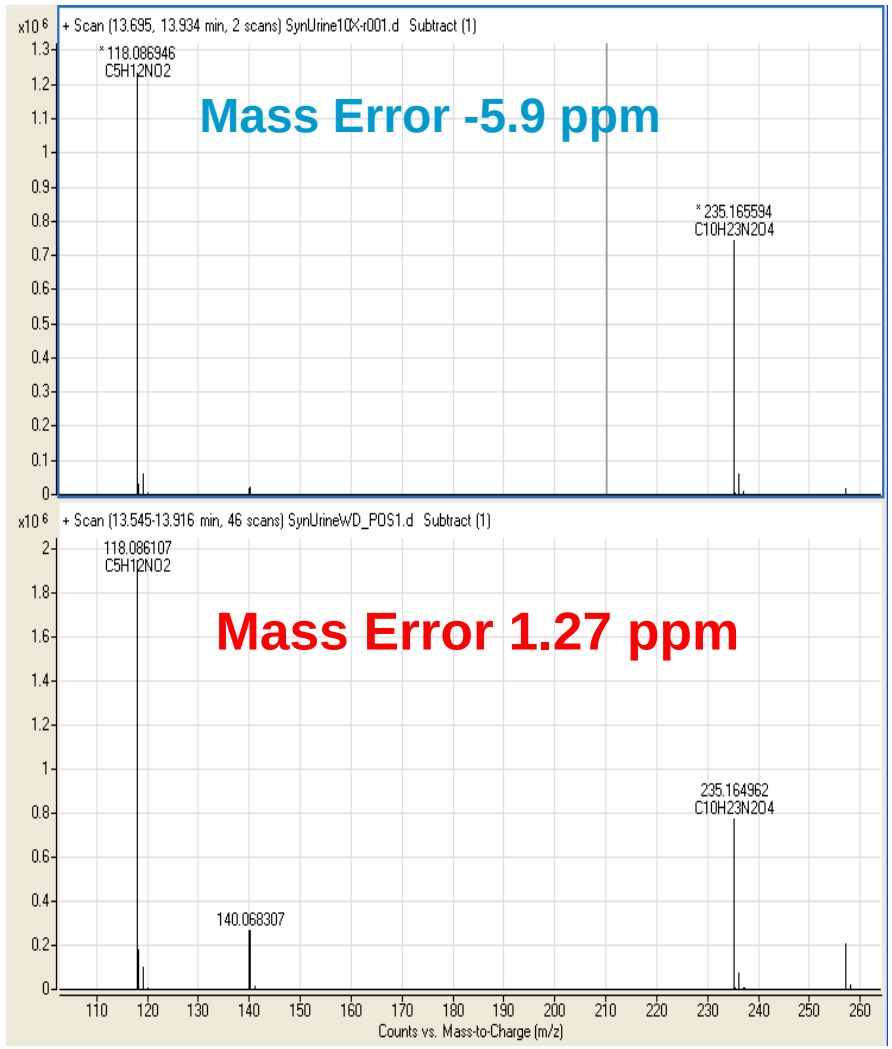
High Resolution 4GHz Mode

1 Spectrum/s 10,000 transients*255
= 2.6 Million Counts

Extended Dynamic Range 2GHz Mode

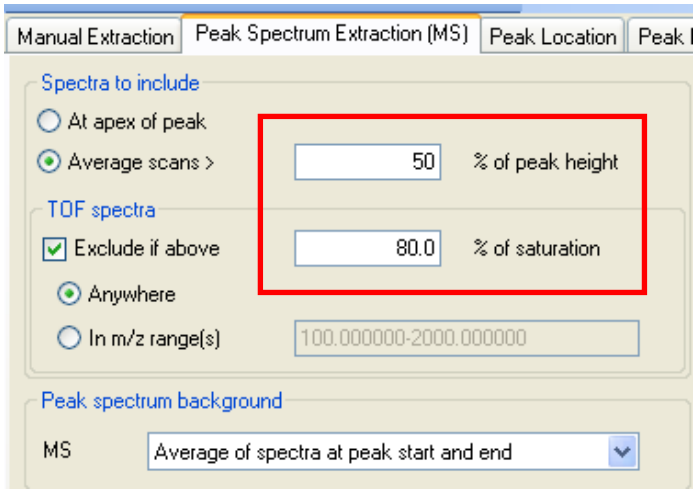
Ratio Low to High Gain Preamplifier is 12

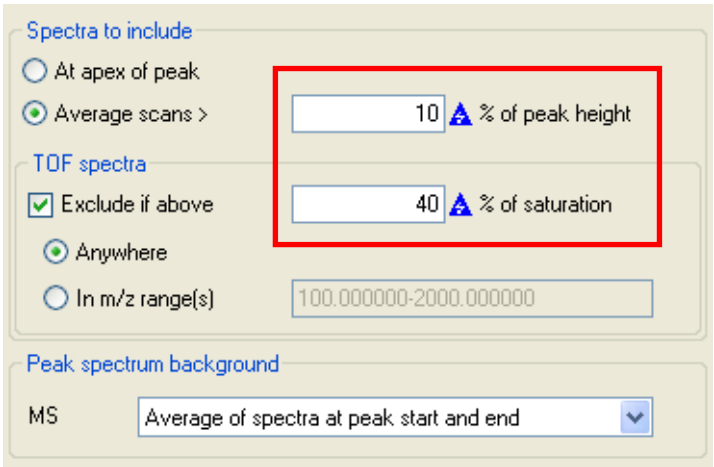
1 Spectrum/s 10,000 transients*255*12
= 30 Million Counts

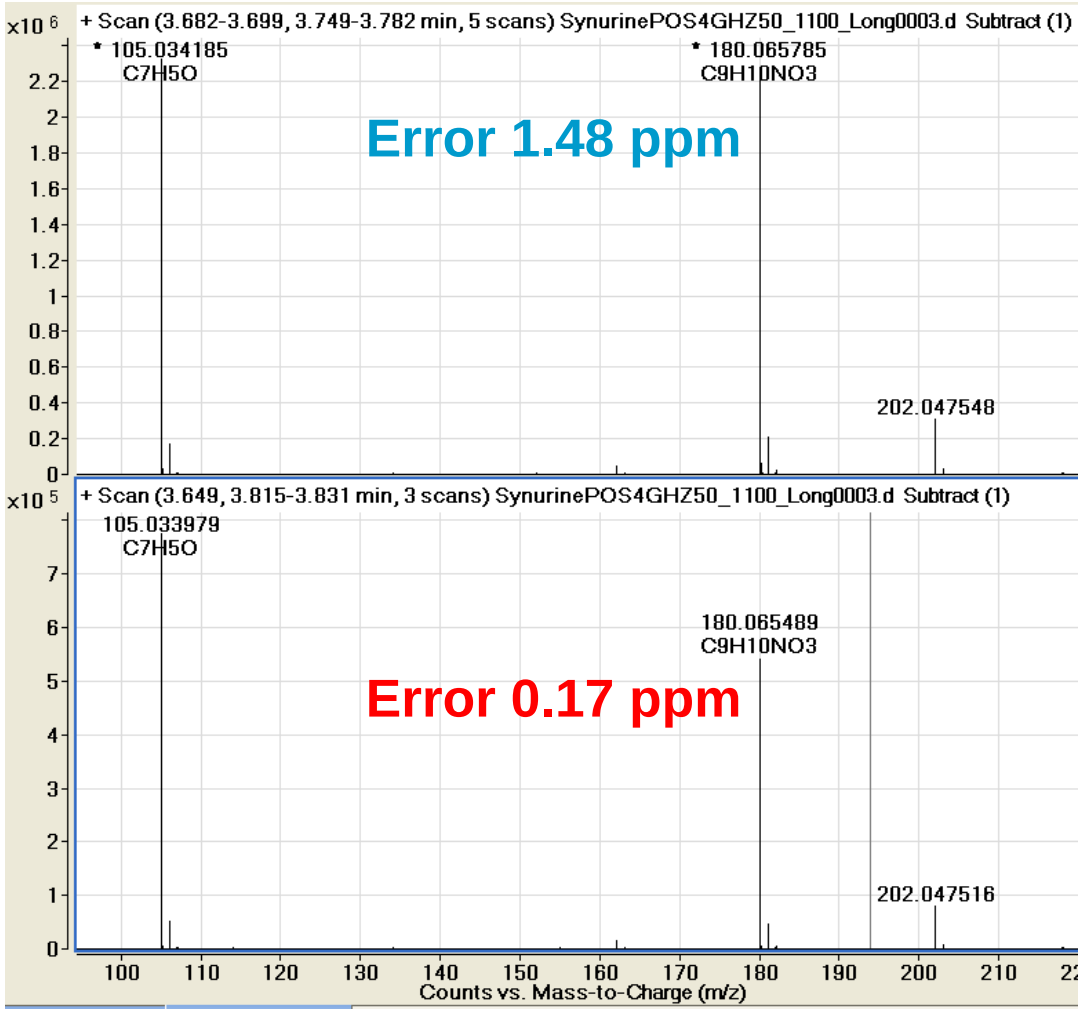


Saturation Correction in MassHunter QUAL

Spectrum: Extract (MS)







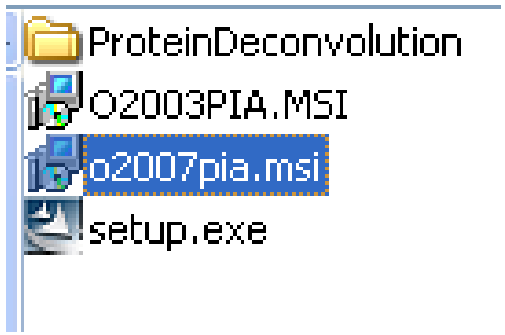
We Listen..
Your Submitted Questions Answered Here…

Question - Answers

1. Does MassHunter B.01.03 Qual and B.01.04 Quant Support Newer Versions of Excel (from 2003)?

   The New Version of MassHunter Software now Supports both Microsoft Excel 2003 and 2007.

   If upgrading either MassHunter or Excel Software make sure to apply the o2007pia.msi file after installation.



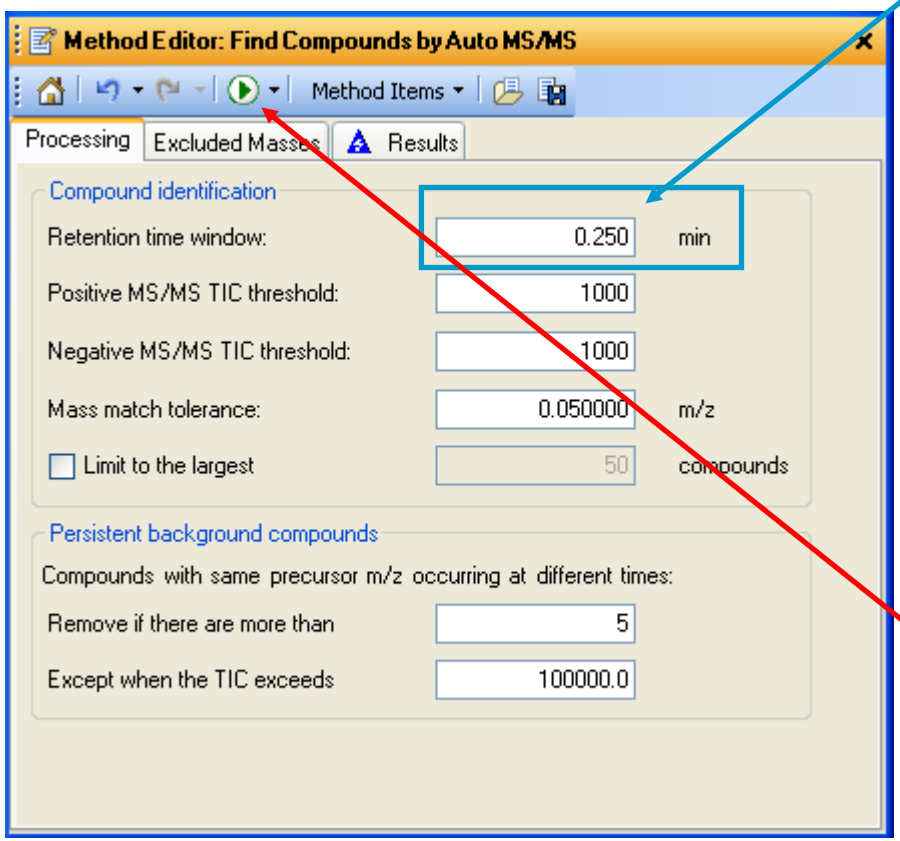
MassHunter Qual B.01.03
MS/MS Data Analysis Questions

1) How to generate empirical formulas from MSMS spectra
2) How to use find compounds by AutoMSMS
3) How to use find compounds by Targeted MSMS
4) How to get empirical formula of peaks from MSMS spectra without using find compound MSMS feature
5) How to generate simple Compound and Analysis Reports

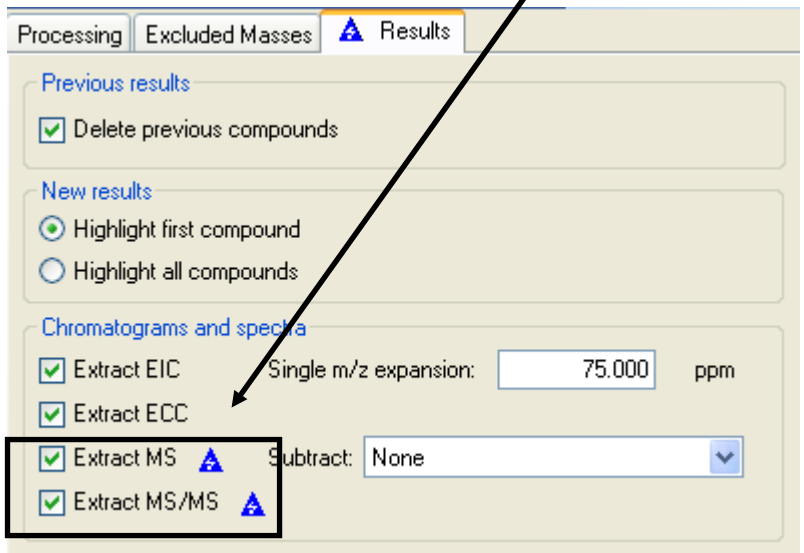
How to Generate Empirical Formula Information from Auto MS/MS Data Files? (Part 1)

Set Parameters for Find Compounds by AutoMSMS

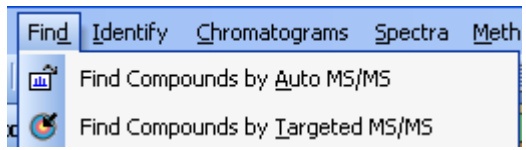
Set Retention Time to Match Chromatographic Peak Width



Check Extract MS and MS/MS



Execute Command from Menu

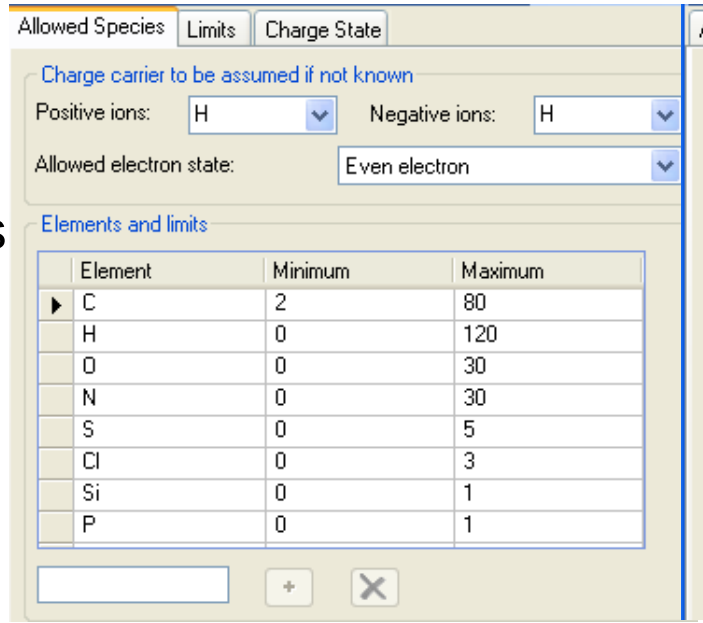


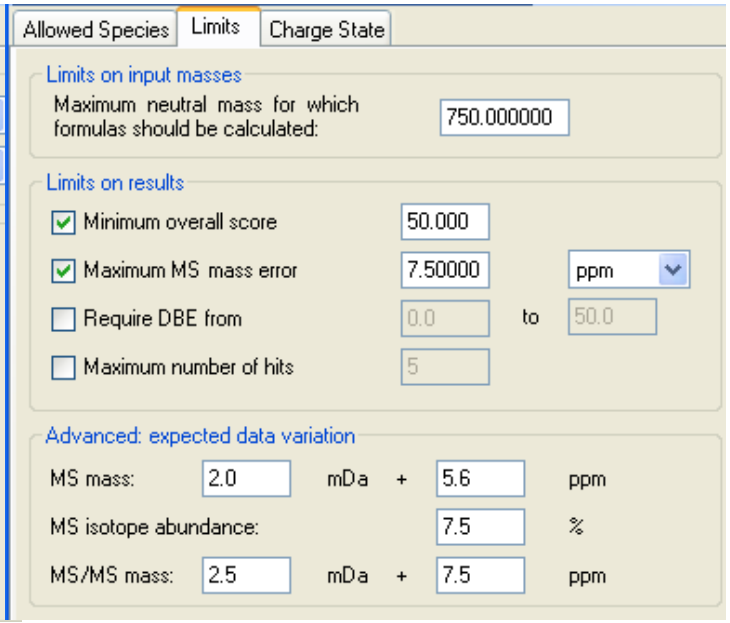
How to Generate Empirical Formula Information from Auto MS/MS Data Files? (Part 2)

Compound List

Name	Show/Hide	File	RT	Height	Mass	MFG Formula	MFG Diff (ppm)
Compound 1	☑	Synurine10xautom	0.418	6566			
Compound 2	☑	Synurine10xautom	0.495	99922			
Compound 3	☑	Synurine10xautom	0.504	8428			
Compound 4	☑	Synurine10xautom	0.505	138591			
Compound 5	☑	Synurine10xautom	0.514	39616			
Compound 6	☑	Synurine10xautom	0.538	25018			
Compound 7	☑	Synurine10xautom	0.544	7138			
Compound 8	☑	Synurine10xautom	0.567	11664			
Compound 9	☑	Synurine10xautom	0.575	8059			

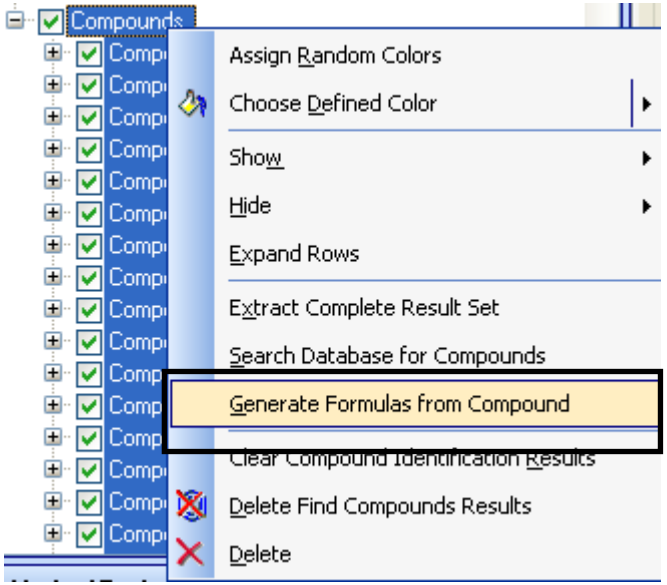
Generate Formulas Set Elements and Limits



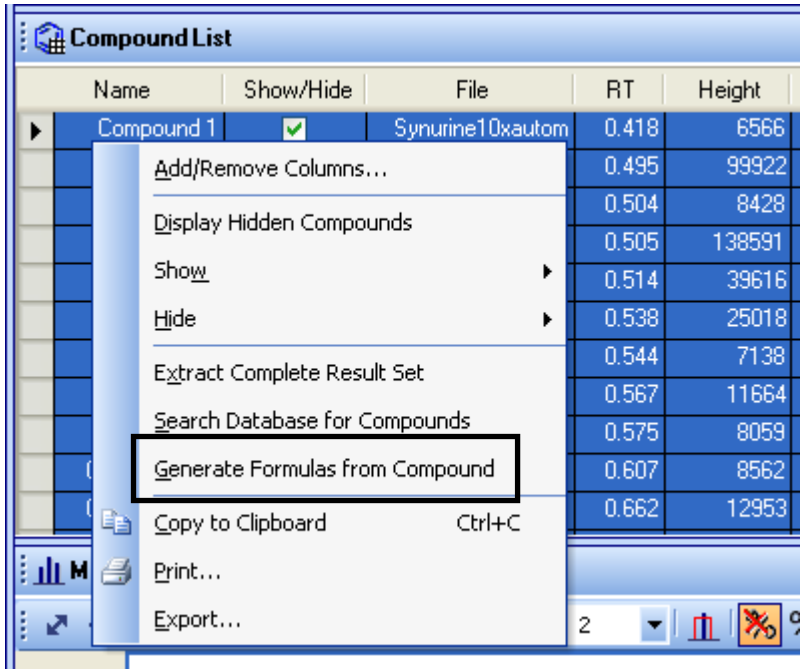


How to Generate Formulas from Compounds

From Method Explorer



From Compound Table



How to Generate Empirical Formula Information from Auto MS/MS Data Files? (Part 3)

Name	Show/Hide	File	RT	Height	Mass	MFG Formula	MFG Diff (ppm)
Compound 1	☑	Synurine10xautom	0.418	6566	146.105447	C6H14N2O2	0.55
Compound 2	☑	Synurine10xautom	0.495	99922		<none>	
Compound 3	☑	Synurine10xautom	0.504	8428	119.058125	C4H9NO3	1
Compound 4	☑	Synurine10xautom	0.505	138591	117.079213	C5H11NO2	-2
Compound 5	☑	Synurine10xautom	0.514	39616	161.105043	C7H15NO3	0.93
Compound 6	☑	Synurine10xautom	0.538	25018	131.069372	C4H9N3O2	0.8
Compound 7	☑	Synurine10xautom	0.544	7138		<none>	
Compound 8	☑	Synurine10xautom	0.567	11664	115.063353	C5H9NO2	-0.21
Compound 9	☑	Synurine10xautom	0.575	8059	279.095487	C10H17NO8	-0.25

MS Formula Results Table

Coverage **MS/MS**

m/z	Ion	Formula	Abundance
377.146449	(M+H)+	C17H21N4O6	343544.9

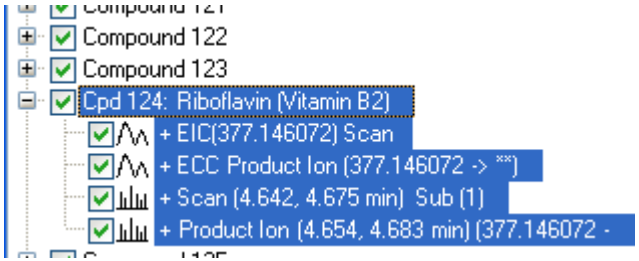
Best	Formula (M)	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Coverage	MS Score	MS/MS Score	DBE
☑	C17H20N4O6	100		376.139172	376.138284	-2.36	100	92.32	100	10

Isotope	Abund%	Calc Abund%	m/z	Calc m/z	Difference (ppm)
1	100	100	377.146449	377.145561	-2.35
2	17.25	20.32	378.149377	378.148506	-2.3
3	2.11	3.19	379.151912	379.1508	-2.93

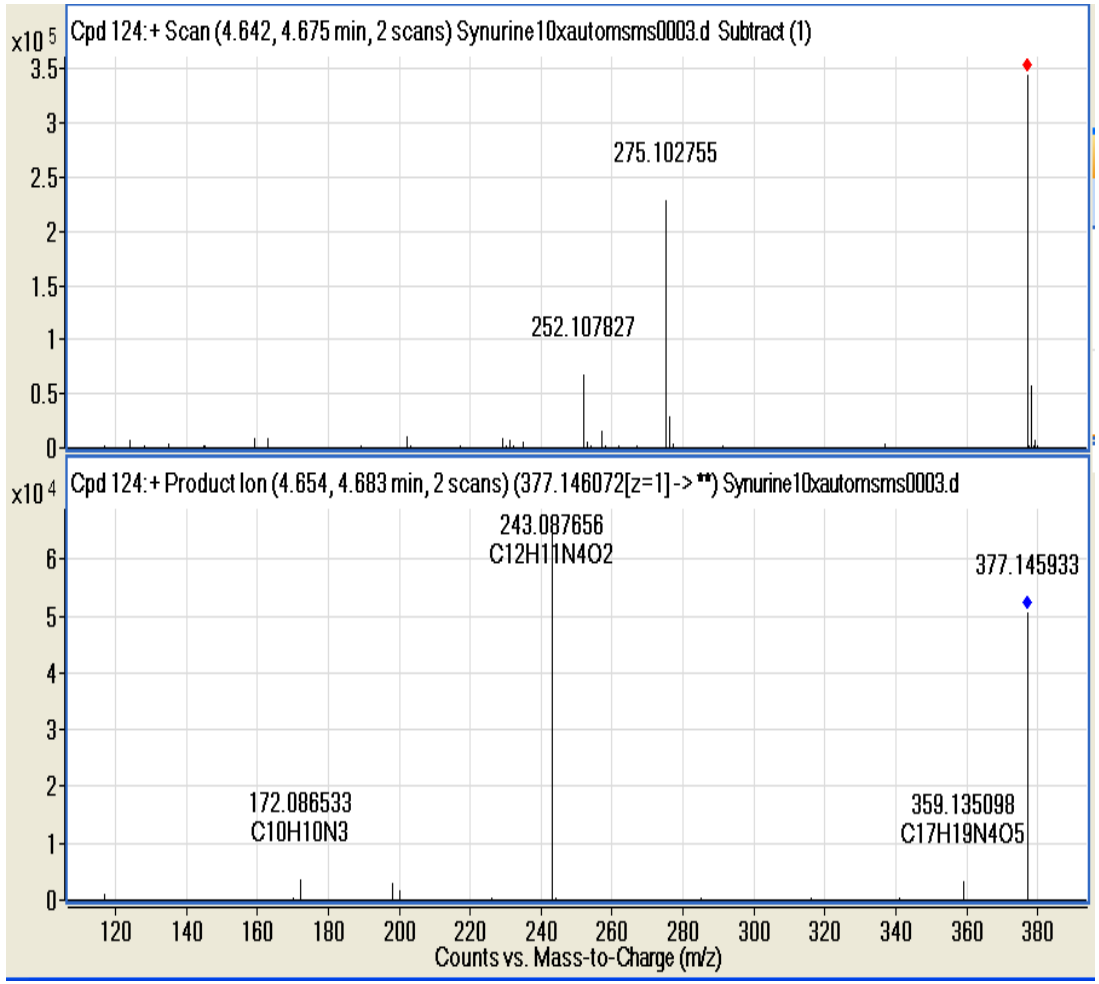
Best	Formula (M)	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Coverage	MS Score	MS/MS Score	DBE
☐	C15H25N2O7P	97.06		376.139172	376.139938	2.03	100	100	89.6	5
☐	C12H17N12OP	95.23		376.139172	376.13859	-1.55	100	90.5	97.15	11
☐	C18H16N8O2	87.93		376.139172	376.139622	1.19	100	81.18	99.99	15
☐	C16H21N6O3P	82.04		376.139172	376.141275	5.59	100	75.87	99.82	10

Empirical Formulas Auto MS/MS Data Files(Part 4)

Highlight Compound from Navigator Window to Display both MS and MS/MS Spectra



Select MSMS Formula Details from Menu to View Empirical Formulas for MSMS Peaks



MSMS Formula Details Table:
Vitamin B2 Example

MS/MS Formula Details: Cpd 124: Riboflavin (Vitamin B2) C17H20N4O6

m/z	Calc m/z	Formula	Abund%	Abund	Difference (ppm)	Loss Mass	Loss Formula
359.135098	359.134996	C17H19N4O5	4.56	3317	-0.28	18.010565	H2O
243.087656	243.087652	C12H11N4O2	90.48	65796	-0.01	134.057909	C5H10O4
243.087656	243.086315	C11H15O6	90.48	65796	-5.52	134.059246	C6H6N4
172.086533	172.086924	C10H10N3	4.96	3609	2.27	205.058637	C7H11NO6

Mass Loss and Elemental Composition is Based on Elemental Composition Determined for Precursor Ion (M+H)+ or (M-H)-.

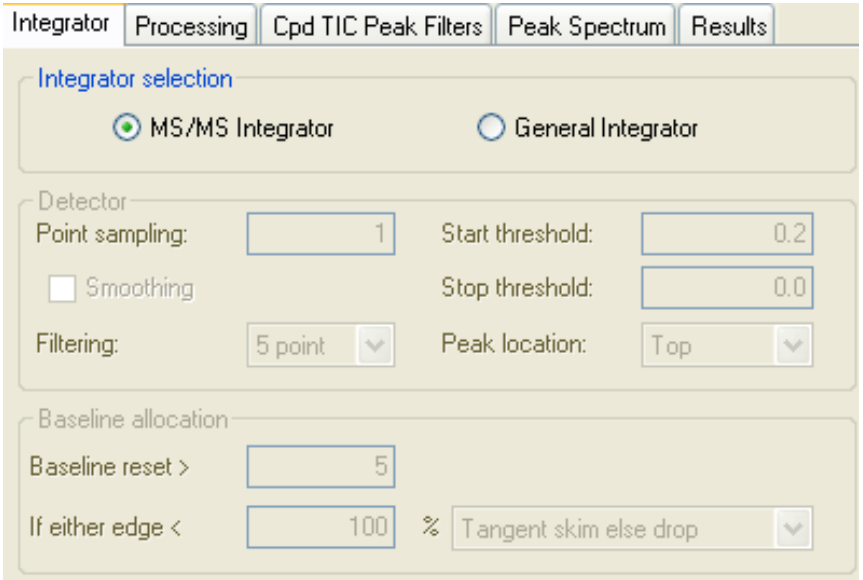
Loss Mass and Loss Formula show Composition of Neutral Losses.

Note: Abundance Values < 100 counts may give higher mass errors

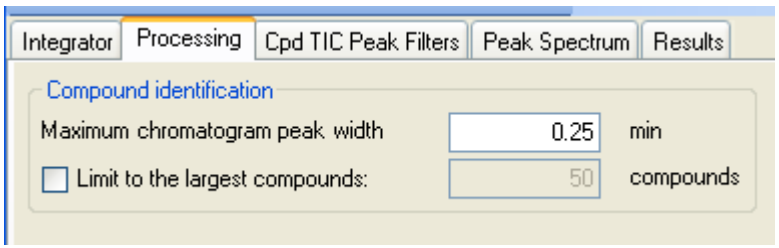
Colored Peaks Indicate the Presence of Multiple Compositions/Mass

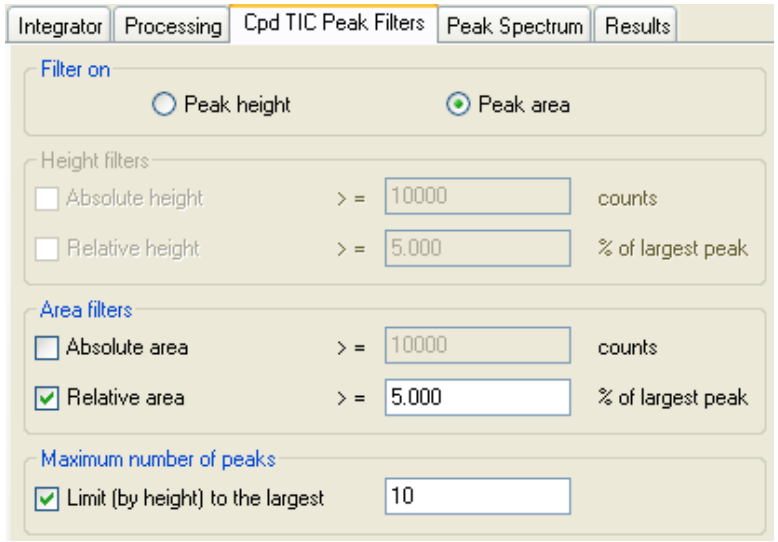
How to Generate Empirical Formula Information from Targeted MS/MS Data Files? (Part 1)

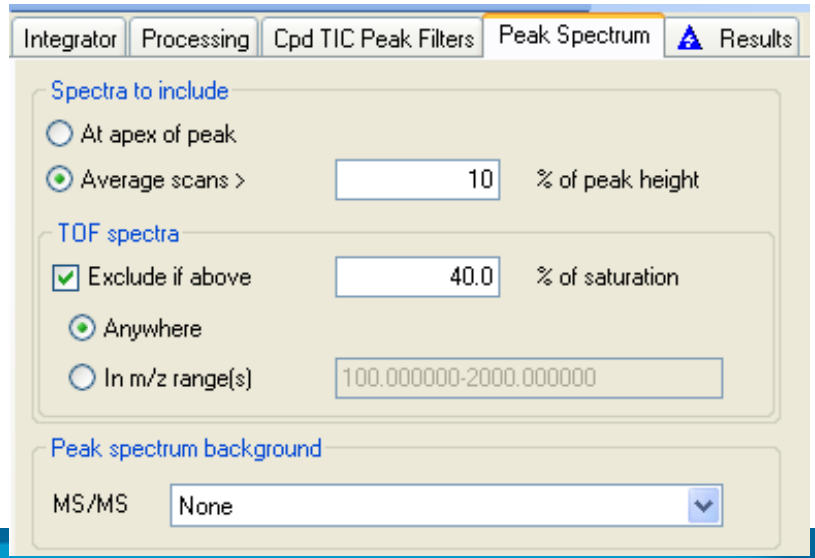
Integration Parameters



Chromatographic Peak Width

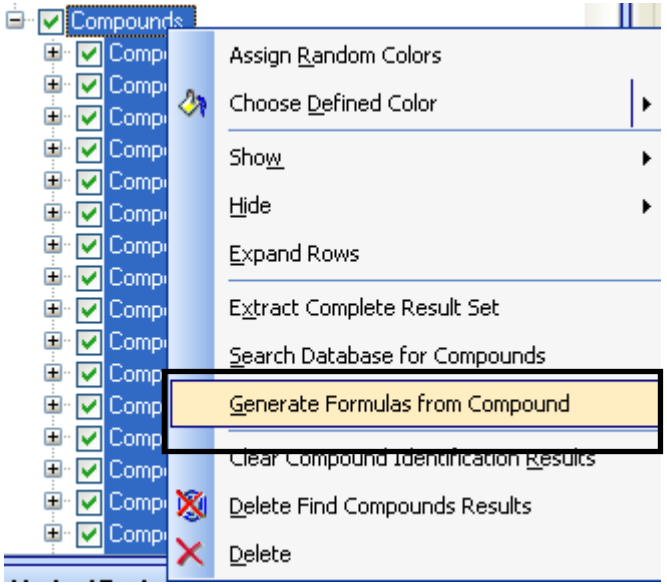




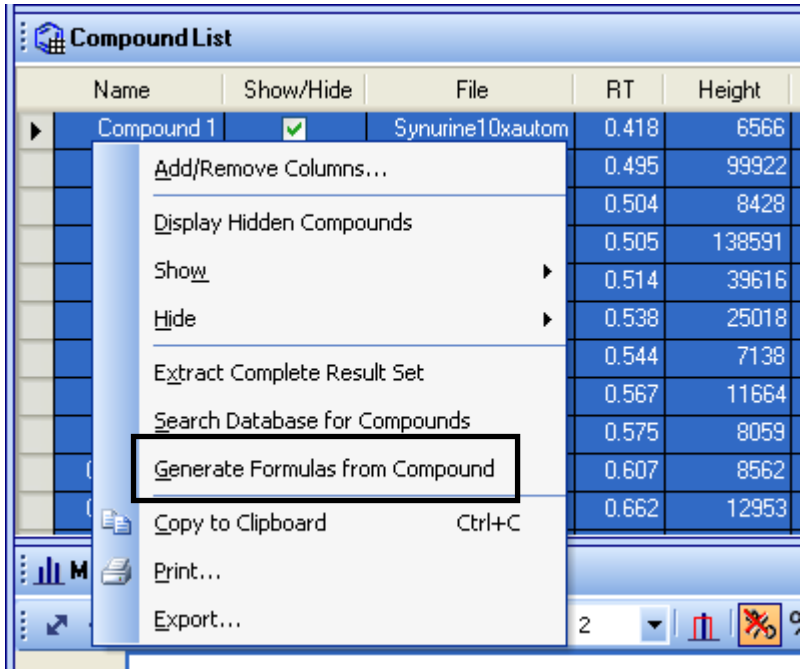


How to Generate Formulas from Compounds

From Method Explorer

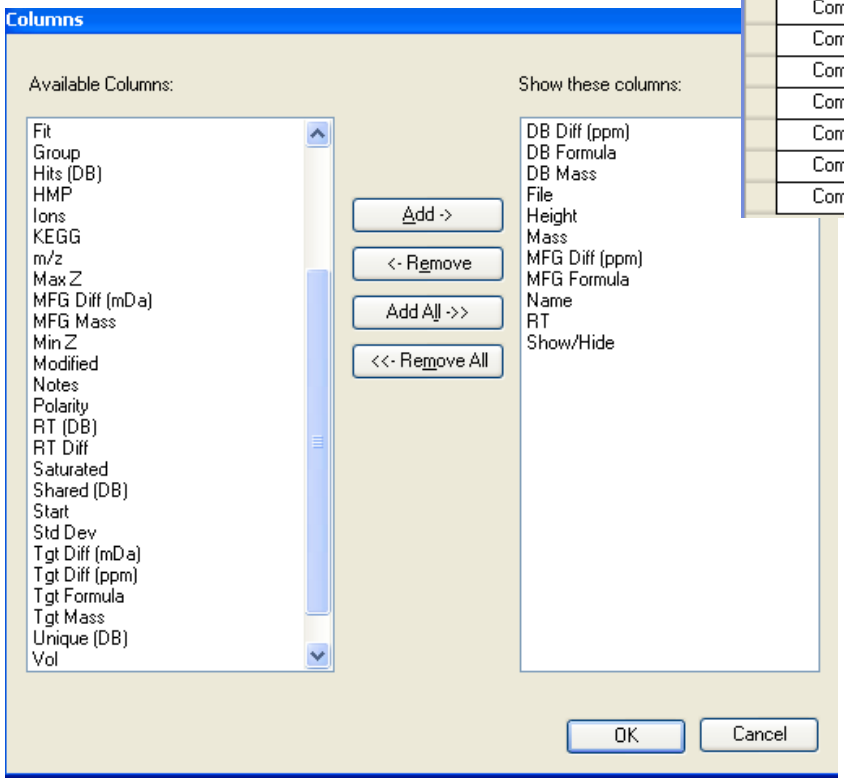


From Compound Table



How to Generate Empirical Formula Information from Targeted MS/MS Data Files? (Part 2)

Compound Table Addition of Columns



Compound List

Name	Show/Hide	File	RT	Height	Mass	MFG Formula	MFG Diff (ppm)
Compound 12	☑	DstdTargeted1.d	8.389	34029	304.239169	C20H32O2	3.49
Compound 10	☑	DstdTargeted1.d	6.881	31363	306.219793	C19H30O3	-0.97
Compound 8	☑	DstdTargeted1.d	6.766	122855	328.203239	C21H28O3	1.85
Compound 2	☑	DstdTargeted1.d	4.378	401617	403.226098	C25H29N3O2	-0.3
Compound 1	☑	DstdTargeted1.d	3.719	275963	403.226274	C25H29N3O2	-0.74
Compound 3	☑	DstdTargeted1.d	4.428	1539143	435.286335	C27H37N3O2	5.15
Compound 5	☑	DstdTargeted1.d	5.664	1325404	435.289034	C27H37N3O2	-1.05
Compound 6	☑	DstdTargeted1.d	5.797	218196	445.269159	C22H39NO8	-3.57
Compound 4	☑	DstdTargeted1.d	5.479	294005	445.272811	C28H35N3O2	0.26

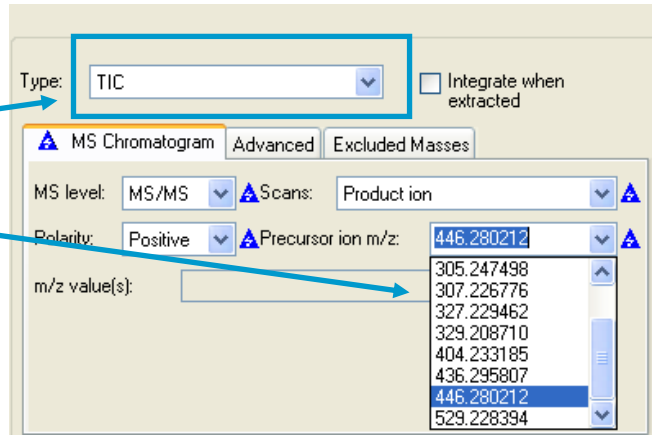
How to Generate Empirical Formula Information from Targeted MS/MS Data Files? (Part 3)

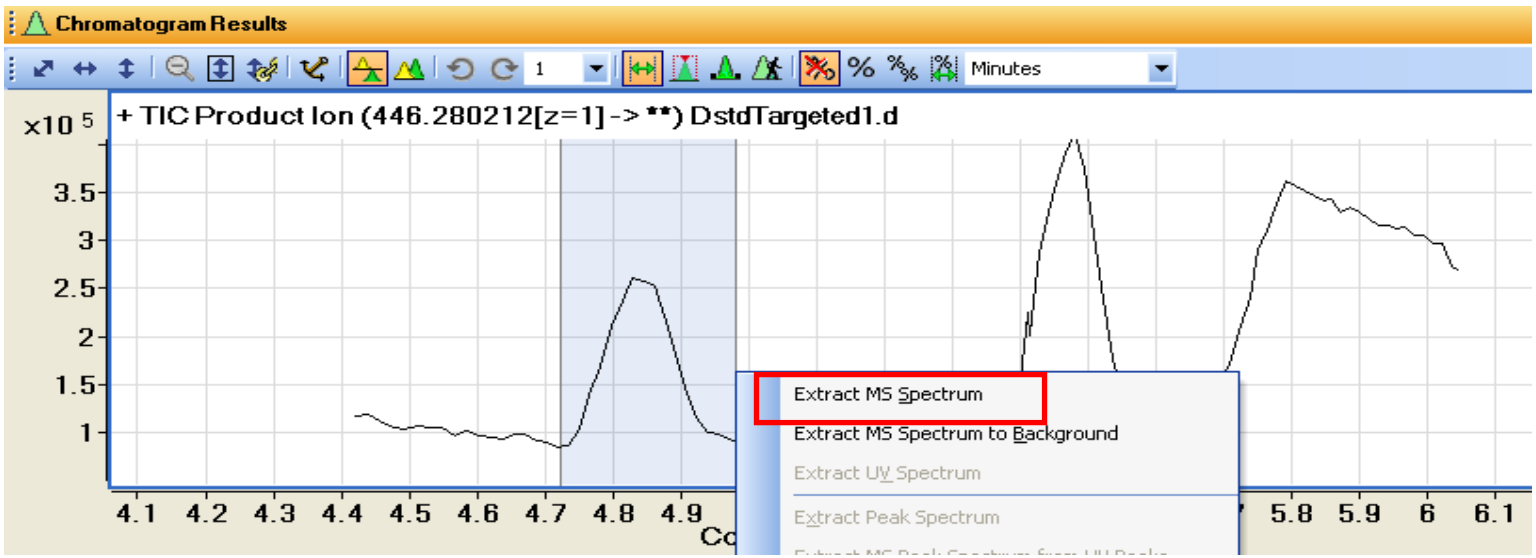
MS/MS Formula Details: Compound 10 C19H3003

m/z	Formula	Abund%	Abund	Difference (ppm)	Loss Mass	Loss Formula
307.189525		2.28	112			
289.216192	C19H29O2	30.08	1478	0.05	18.010565	H2O
271.204698	C19H27O	15.88	780	3.48	36.021129	H4O2
253.193137		5.76	283			
233.154435	C15H21O2	1.46	72	-3.55	74.073165	C4H10O
229.194411	C17H25	7.36	362	2.91	78.031694	C2H6O3
213.16398	C16H21	2.33	115	-0.95	94.062994	C3H10O3
193.122526	C12H17O2	1.45	71	-1.14	114.104465	C7H14O
181.124194	C11H17O2	1.38	68	-10.42	126.104465	C8H14O
179.105897	C11H15O2	1.26	62	4.24	128.120115	C8H16O
175.1473	C13H19	2.7	133	4.72	132.078644	C6H12O3
161.135052	C12H17	1.27	63	-15.98	146.094294	C7H14O3
159.117185	C12H15	1.95	96	-2.25	148.109944	C7H16O3
153.090096	C9H13O2	1.41	69	5.95	154.135765	C10H18O
149.129874	C11H17	3.52	173	17.46	158.094294	C8H14O3
147.116877	C11H15	2	98	-0.34	160.109944	C8H16O3
145.098907	C11H13	1.26	62	15.64	162.125594	C8H18O3
139.074598	C8H11O2	1.37	67	5.45	168.151415	C11H20O
135.11775	C10H15	3.27	161	-6.83	172.109944	C9H16O3
133.099182	C10H13	1.95	96	14.99	174.125594	C9H18O3
123.117351	C9H15	1.37	67	-4.26	184.109944	C10H16O3
121.100613	C9H13	6.83	335	4.66	186.125594	C10H18O3
119.085301	C9H11	1.86	91	1.9	188.141245	C10H20O3

How to Generate Empirical Formula Information from Extracted MS/MS Spectra

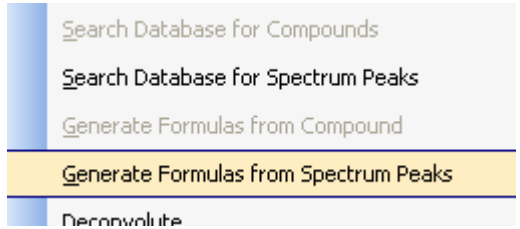
Generate TIC, BPC, or EIC for Specific Precursor Ion MS/MS Spectra

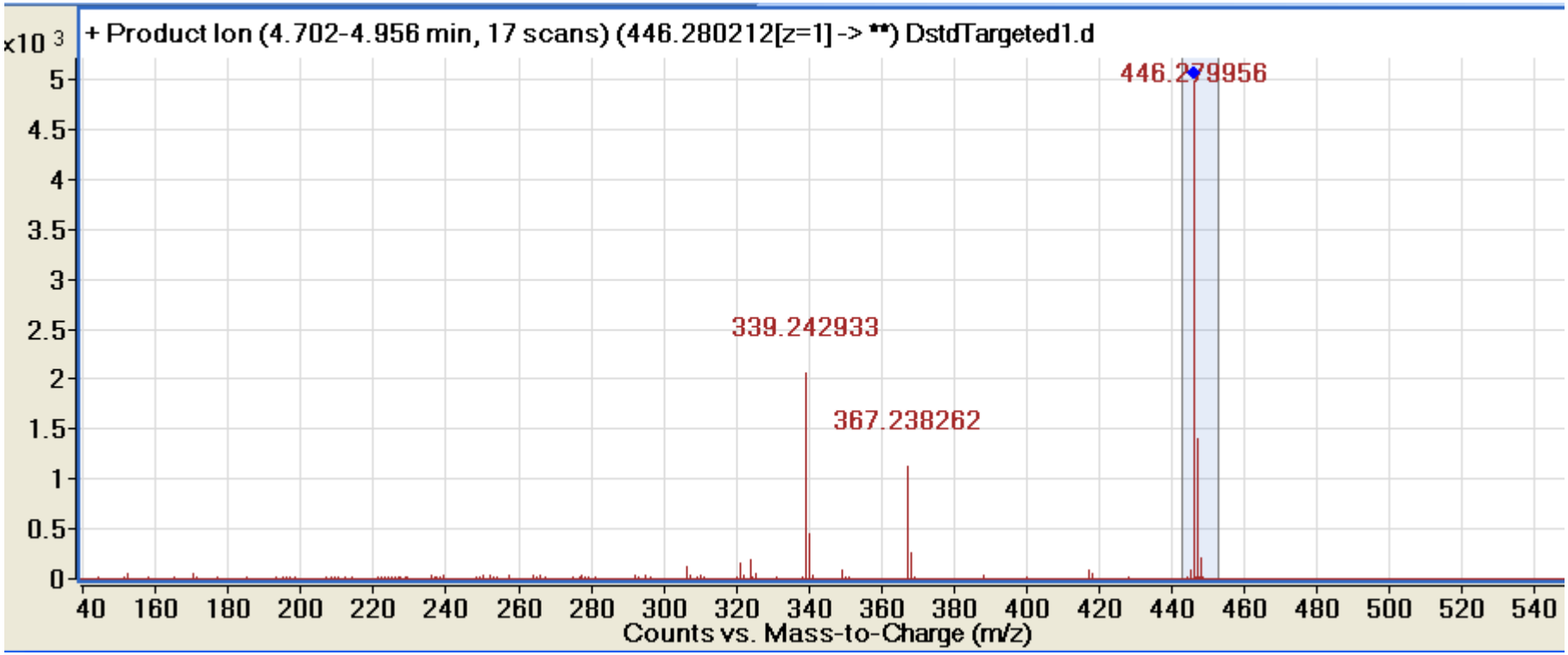




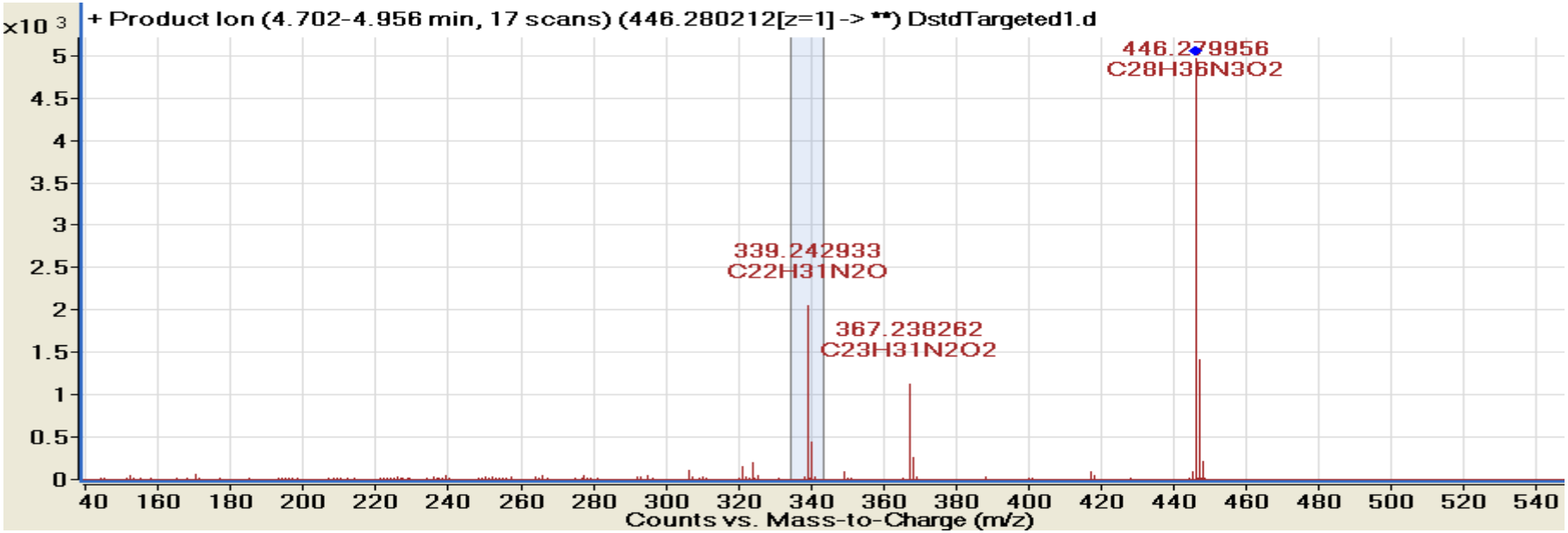
Generate Empirical Formula Information from Extracted MS/MS Spectra

Highlight Each Peak, then Right Click and Select Generate Formulas from Spectrum Peaks





Generate Empirical Formula Information from Extracted MS/MS Spectra – Formula for each peak



m/z	Ion	Formula	Abundance
339.242933	M*+	C22H31N2O	2051.2

Best	Formula (M)	Ion Formula	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm)	Difference (mDa)	DBE
☑	C22H31N2O	C22H31N2O	100		339.243481	339.243639	0.46	0.46	0.16	8.5

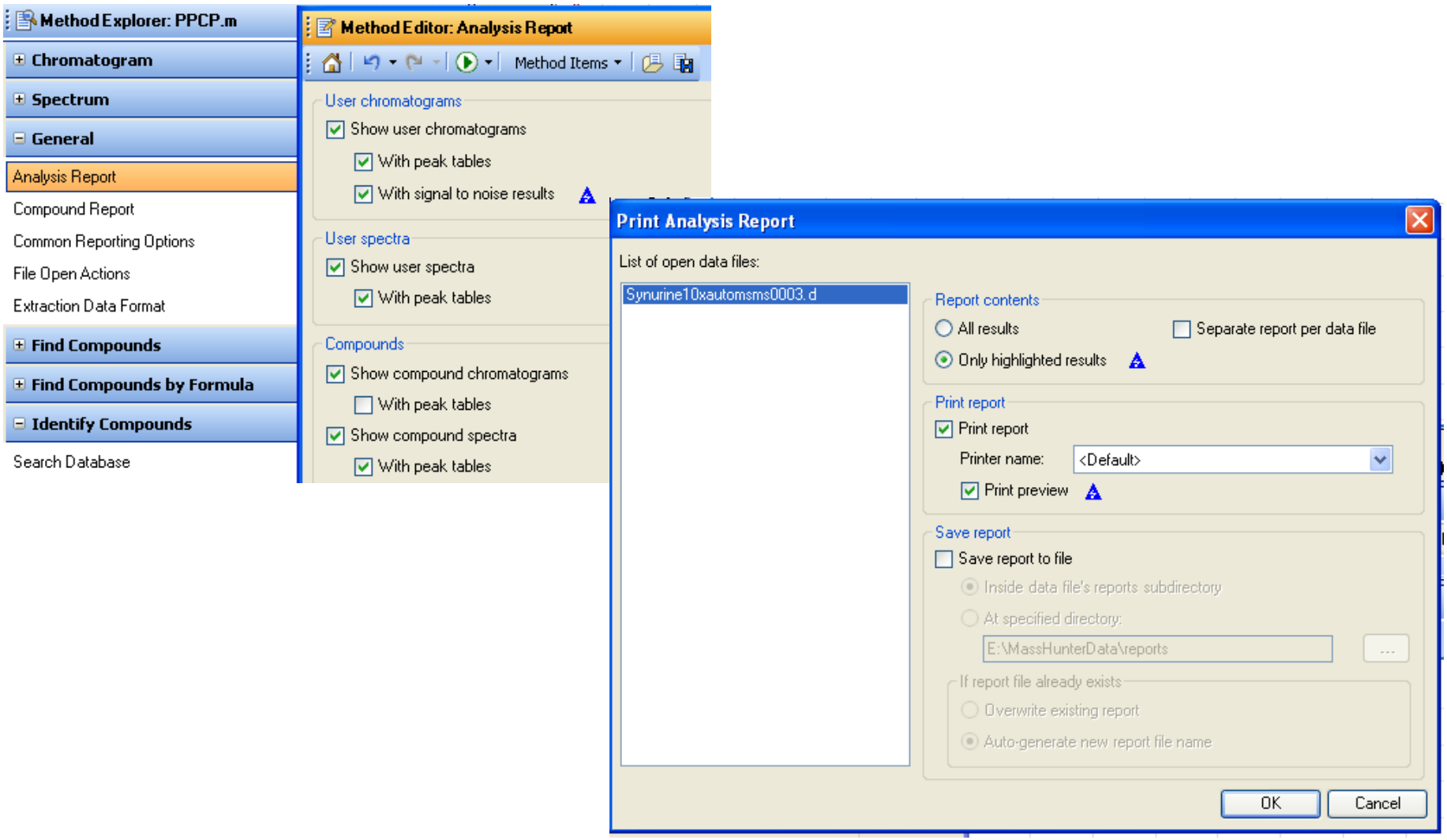
m/z	Ion	Formula	Abundance
367.238262	M*+	C23H31N2O2	1126.8

Best	Formula (M)	Ion Formula	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm)	Difference (mDa)	DBE
☑	C23H31N2O2	C23H31N2O2	100		367.238811	367.238553	-0.7	0.7	-0.26	9.5

m/z	Ion	Formula	Abundance
446.279956	M*+	C28H36N3O2	4967.8

Best	Formula (M)	Ion Formula	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm)	Difference (mDa)	DBE
☑	C28H36N3O2	C28H36N3O2	100		446.280505	446.280752	0.56	0.56	0.25	12.5

How to Generate Analysis Reports



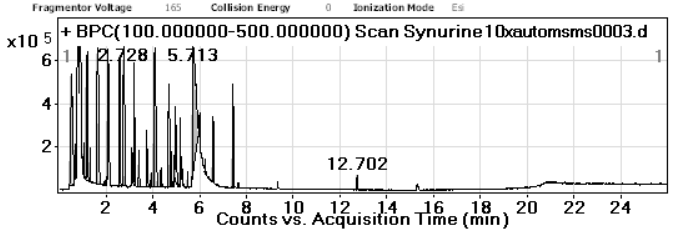
Example Analysis Report
Chromatograms, MS Spectra, Compounds

Qualitative Analysis Report

Data Filename	Synurine10xautomsms0003.d	**Sample Name**	Urine
Sample Type	Sample	**Position**	P1-F8
Instrument Name	Instrument 1	**User Name**	Training
Acq Method		**IRM Calibration Status**	Success
DA Method	PPCP.m	**Comment**	

User Chromatograms

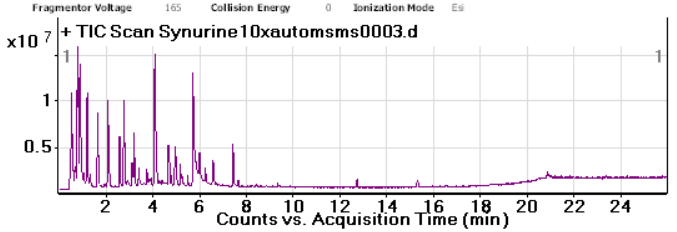
Fragmentor Voltage 165 Collision Energy 0 Ionization Mode Esi



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	0.402	0.479	0.589	515216	2856537	52.69
4	1.101	1.150	1.218	592696	1878044	34.64
6	1.563	1.602	1.751	627983	1738993	32.07
7	2.010	2.050	2.105	593569	1744319	32.17
8	2.505	2.560	2.617	596118	1006717	18.57
9	2.692	2.728	2.790	638155	1111172	20.49
11	3.132	3.172	3.243	553792	825141	15.22
15	3.990	4.041	4.187	641507	2666713	49.19
23	5.638	5.713	5.939	618349	5421754	100
26	7.346	7.389	7.453	476935	735713	13.57

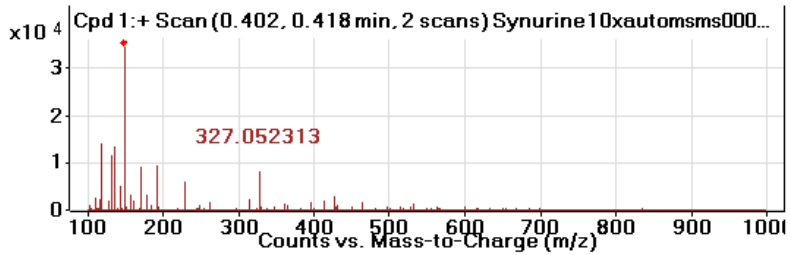
Fragmentor Voltage 165 Collision Energy 0 Ionization Mode Esi



User Spectra

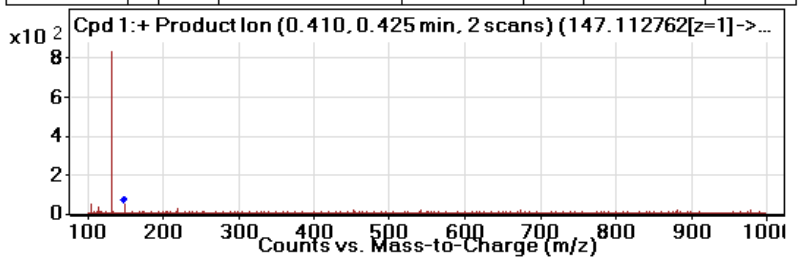
Fragmentor Voltage	Collision Energy	Ionization Mode
165	0	Esi

Qualitative Analysis Report



Peak List

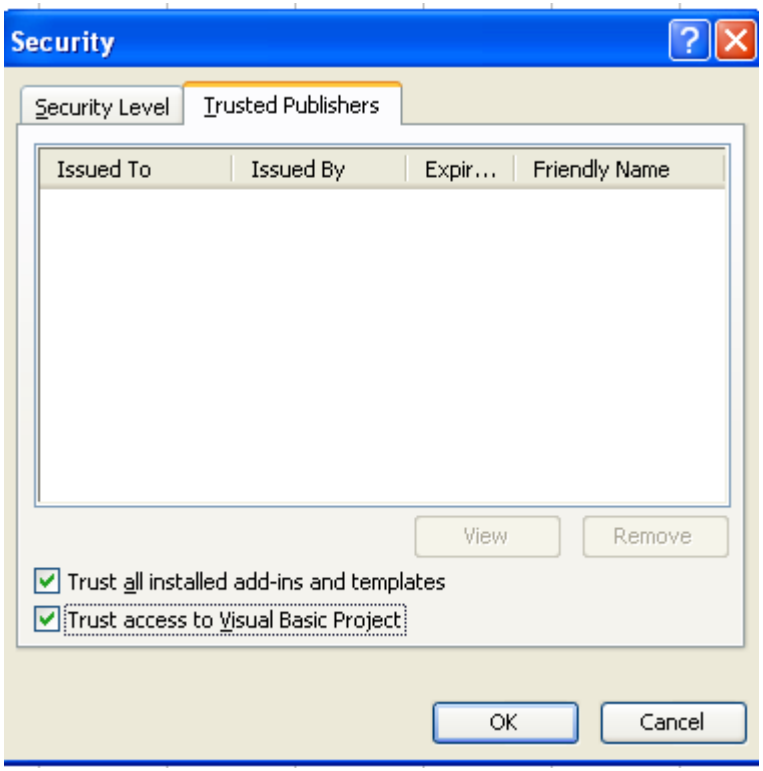
m/z	z	Abund.	Name	Formula	Ion	Score (DB)	Hits (DB)
116.070886	1	14272					
130.086043	1	11480					
133.097294	1	13721					
141.958133	1	5268					
147.112724	1	35930	Lysine	C6H15N2O2	(M+H)+	97.24	2
148.115074	1	2017		C6H15N2O2	(M+H)+		
169.094241	1	9178					
191.076238	1	9417					
226.951616	1	5988					
327.052313	1	8254					

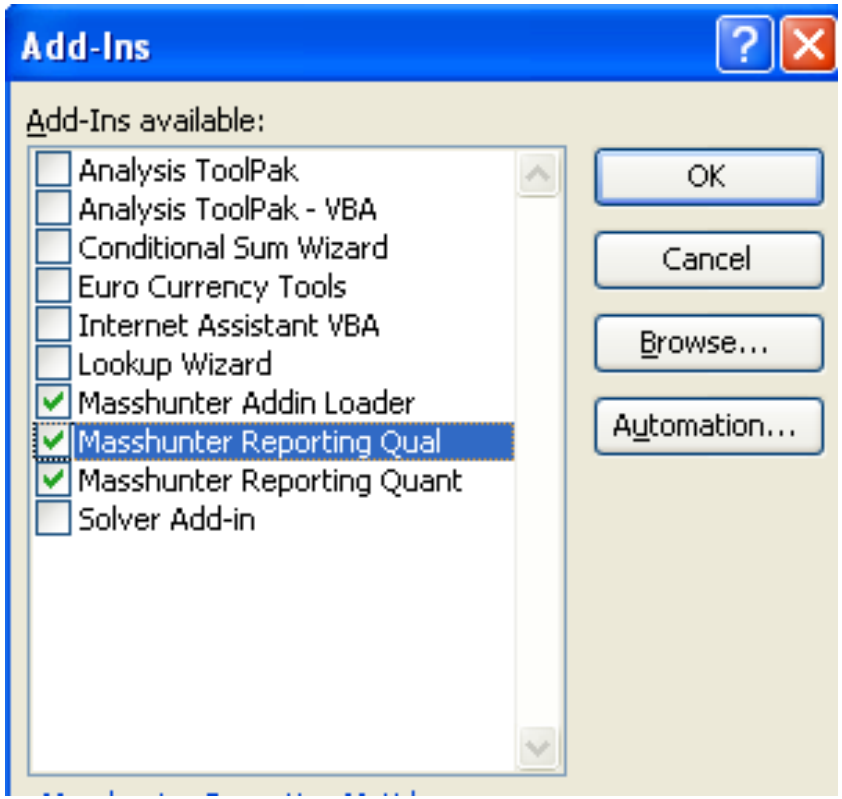


Peak List

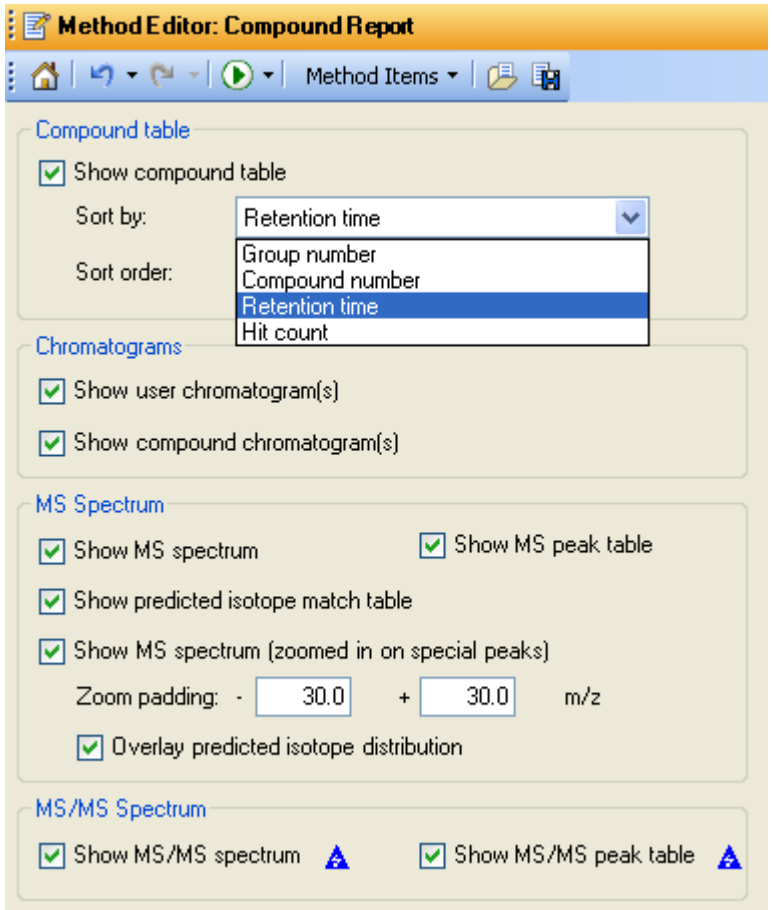
m/z	Abund.	Formula
102.091125	51	C5H12NO
130.085641	836	C6H12NO2
130.108475	51	
147.110984	43	

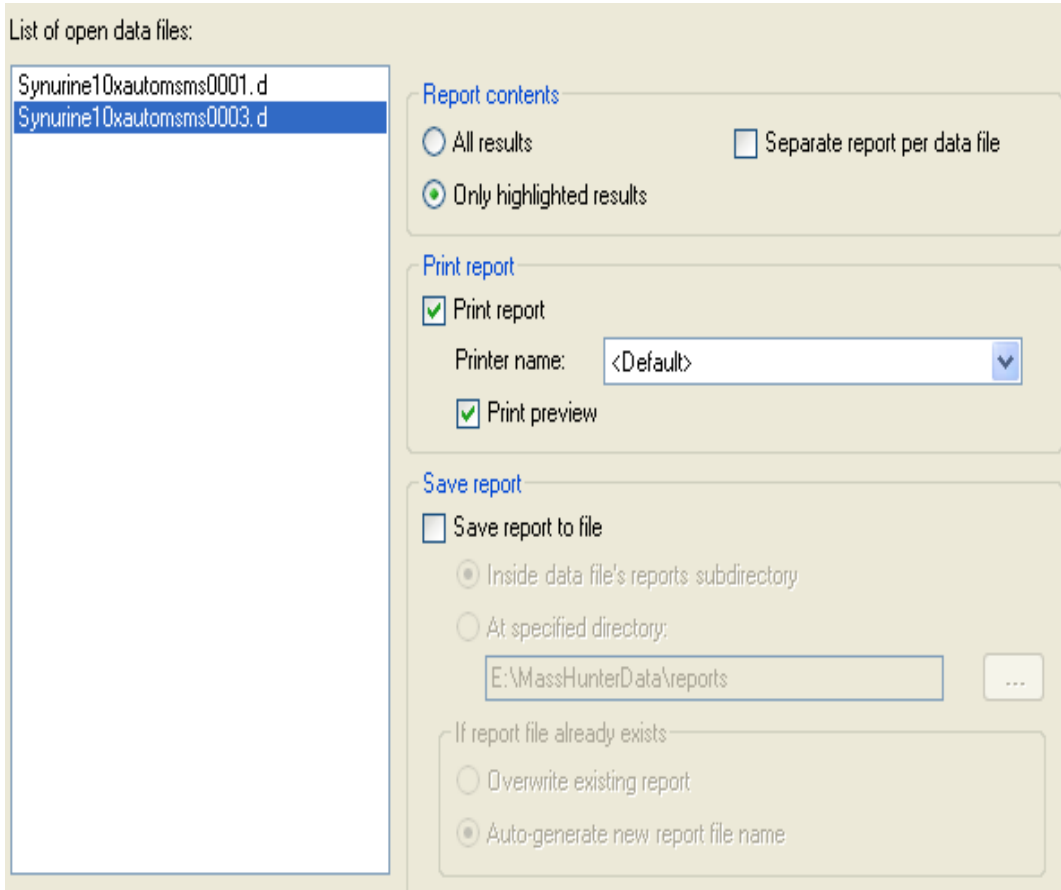
Microsoft Excel Error





How to Generate Compound Reports



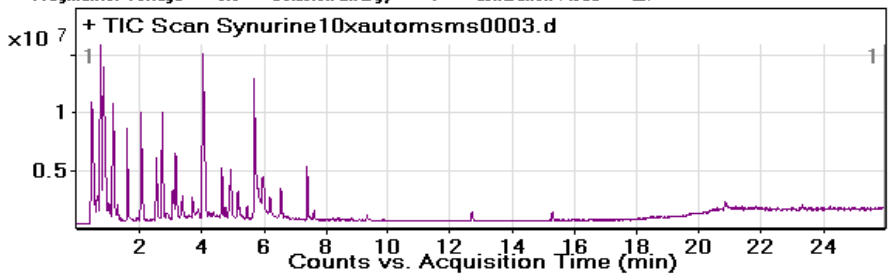


Qualitative Compound Report

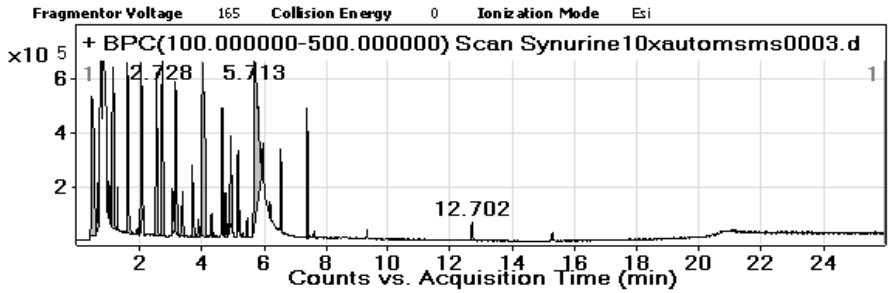
Data Filename	Synurine10xautomsms0003.d	**Sample Name**	Urine
Sample Type	Sample	**Position**	P1-F8
Instrument Name	Instrument 1	**User Name**	Training
Acq Method		**IRM Calibration Status**	Success
DA Method	PPCP.m	**Comment**	

User Chromatograms

Fragmentor Voltage 165 **Collision Energy** 0 **Ionization Mode** Esi



Fragmentor Voltage 165 **Collision Energy** 0 **Ionization Mode** Esi



Compound Table

Name	RT	Mass	Abund.	MFG Formula	MFG Diff (ppm)	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 1: Lysine	0.4	146.105447		C6H14N2O2	0.55	C6H14N2O2	0.55	2
Cpd 3: Homoserine	0.5	119.058125	78720	C4H9NO3	1	C4H9NO3	1	4

Example Compound Report
Chromatograms, MS Spectra, Compounds

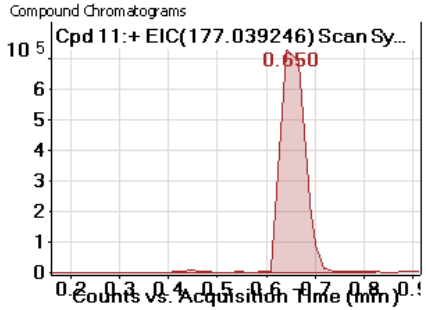
Compound Table

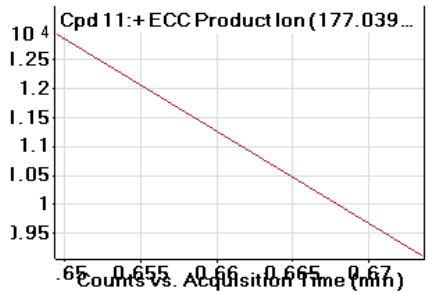
Name	RT	Mass	MFG Formula	MFG Diff (ppm)	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 11: Ascorbic acid (Vitamin C)	0.7	176.031927	C6H8O6	0.91	C6H8O6	0.91	2

Compounds

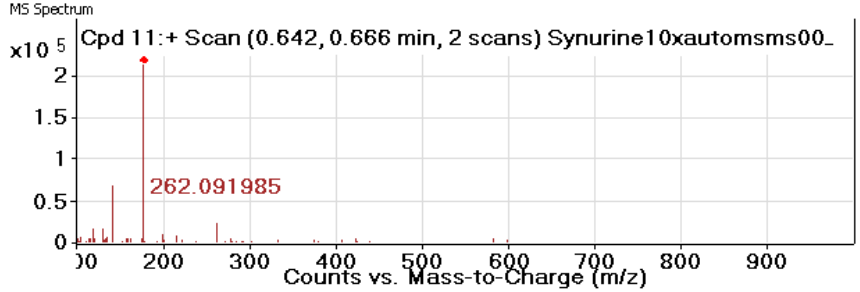
Name	m/z	RT	Algorithm	Mass
Cpd 11: Ascorbic acid (Vitamin C)	177.039204	0.662	Auto MS/MS	176.031927

Compound Chromatograms





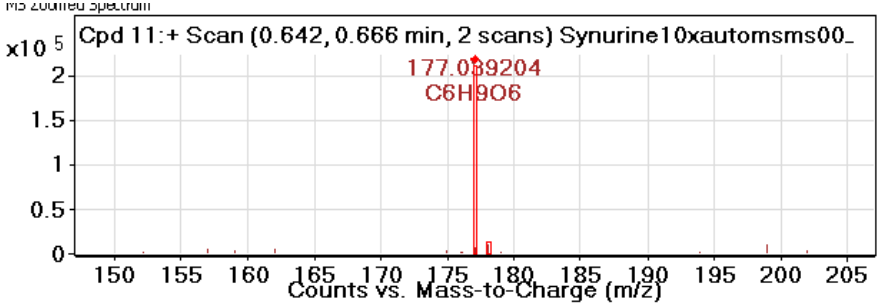
MS Spectrum



MS Spectrum Peak List

m/z	z	Abund.	Formula	Ion	Diff(ppm)
118.086409	1	16531			
129.018046	1	16234			
141.018168	1	68795			
177.039204	1	214526	C6H9O6	(M+H)+	0.91

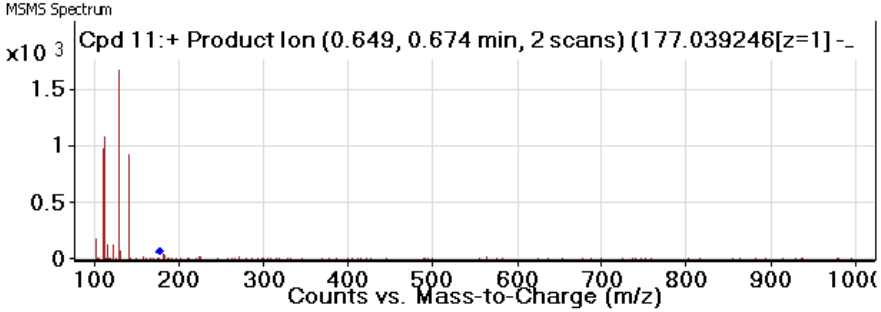
MS Zoomed Spectrum



Predicted Isotope match table

Isotope	Abund%	Calc Abund%	m/z	Calc m/z	Diff (ppm)
1	100	100	177.039204	177.039364	0.91
2	5.03	6.82	178.042423	178.042792	2.08

MSMS Spectrum



MSMS Spectrum Peak List

m/z	Abund.	Formula	Diff (ppm)
101.02363	190	C4H5O3	-3.07
111.008103	1000	C5H3O3	-3.9
113.023211	1111	C5H5O3	0.97
113.070715	144		
115.039679	128	C5H7O3	-6.16
123.006015	137		
129.018046	1678	C5H5O4	1.46
141.017402	929	C6H5O4	5.9

--- End Of Report ---

Mass Hunter Quant B.01.04

MassHunter Quantitative Analysis B.01.04

Provides enhancements for the QQQ, plus support for TOF/Q-TOF including dual mode data and use of MFE to Find Compounds in Samples

- **Support of Excel 2007**
- **Overlay all MRM chromatograms from one sample**
- **Support compound grouping**
- **Honor manual integrations on reprocessing of batch**
- **Add report templates which report “not detected”**
- **Significantly faster processing and browsing**
- **Support TOF/QTOF**
  - better quant method creation from full scan MS and MS/MS data
  - TOF/Q-TOF shows applicable mass precision, scan types, menu choices
  - support dual mode data (profile AND centroid)

Future Webex (Feb and March)

- **Overview of New MassHunter Profiler Software**
- **Overview of Metlin Personal Database Software**
  - Applications for Metabolomics, Metabolite ID
  - Applications for Small Molecule Analysis
- **MassHunter B.01.04 Quantitation MS and MS/MS Modes**

Questions and Answers