GBSC 724 Metabolomics Class

Stephen Barnes

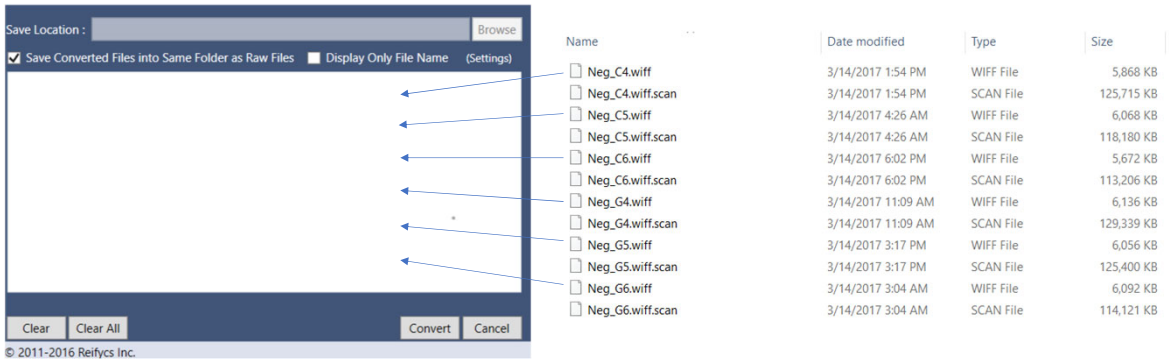
1

Setting up for MS-DIAL

- **Enter "MS-DIAL" into your browser and download MS-DIAL vs 4.12**
- **Scroll down to find the MSMS library files**
  - For this exercise, download the public negative ion library and place on the desktop
- **Click on ABF converter tab (top of the home page)**
  - Click download – this will open a box – enter your name, institution, country and email address – an email will be sent to you
  - Open the email and download ABD converter – unzip the file
  - Open the ABF converter folder and scroll down to find the program and double click to open it

2

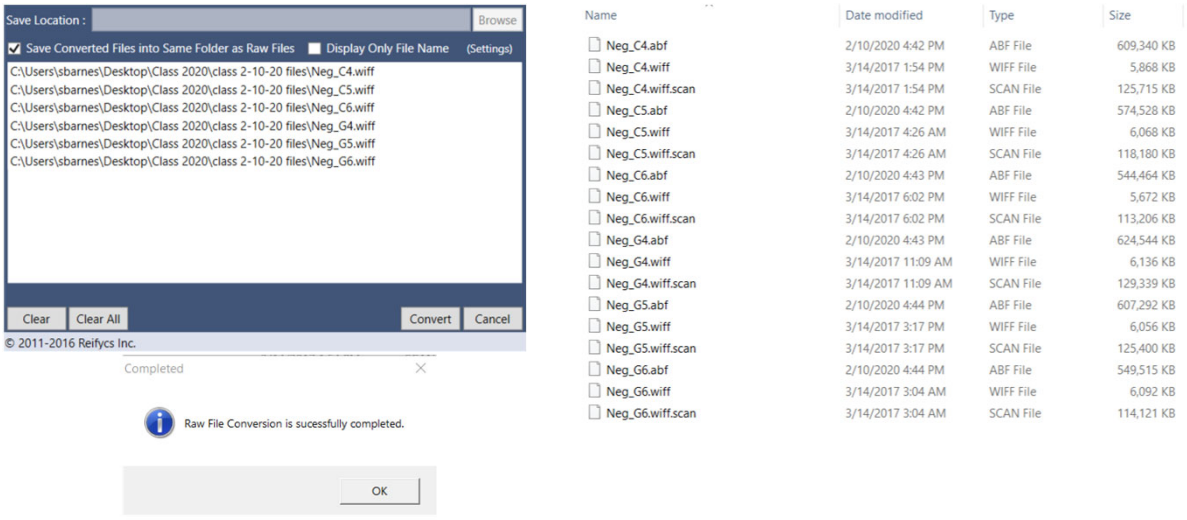
Getting prepared for conversion to .ABF files



Open conversion box

3

Creating .abf files

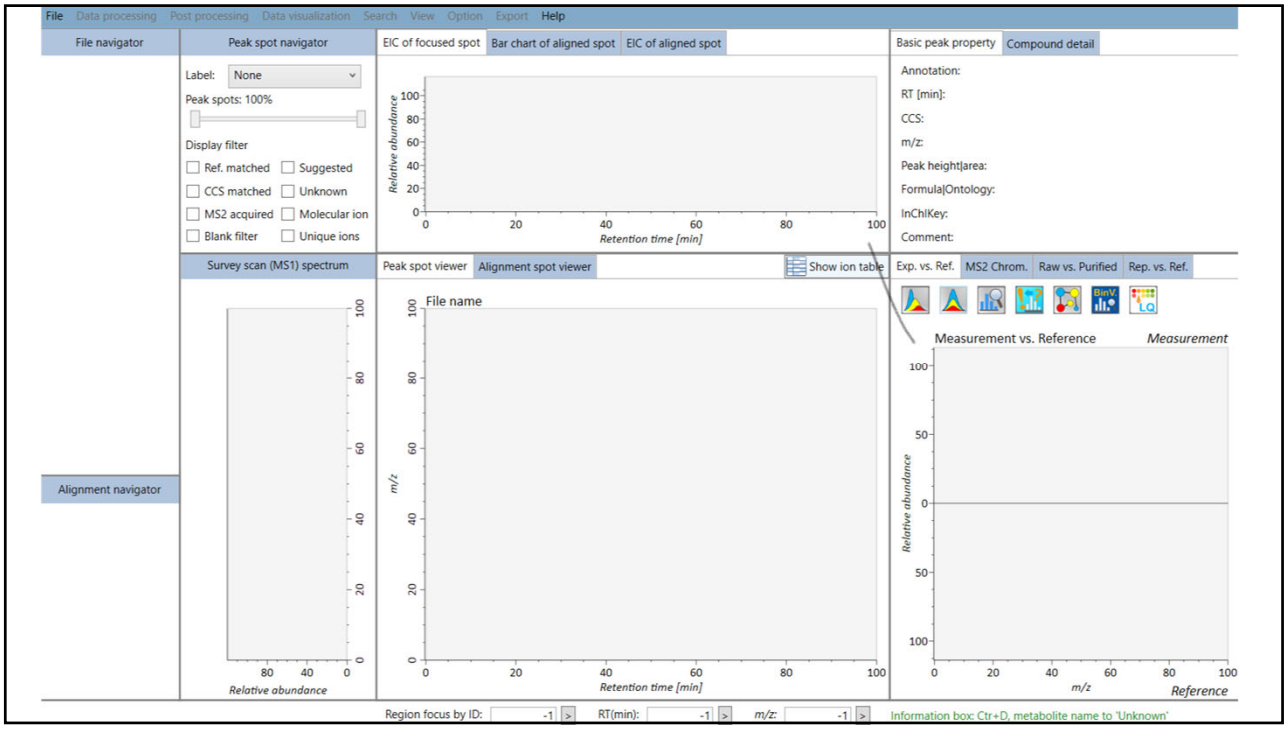


4

Loading MS-DIAL

Name	Date modified	Type	Size
MonaRestApi.dll	1/2/2020 12:28 AM	Application extens...	35 KB
MonaRestApi.dll.config	4/1/2019 11:03 PM	CONFIG File	2 KB
MSDIAL	1/7/2020 4:12 PM	Application	6,846 KB
MSDIAL.exe.config	9/20/2018 12:40 PM	CONFIG File	2 KB
MSDIAL	11/24/2019 4:38 PM	Configuration setti...	1 KB
MsdialCommon.dll	1/7/2020 4:12 PM	Application extens...	45 KB
MsdialConsoleApp	1/7/2020 4:12 PM	Application	132 KB
MsdialConsoleApp.exe.config	7/1/2016 12:39 PM	CONFIG File	1 KB
MsdialDataExporter.dll	5/30/2019 4:29 PM	Application extens...	41 KB
MsdialGcmsProcess.dll	1/7/2020 4:12 PM	Application extens...	165 KB

5



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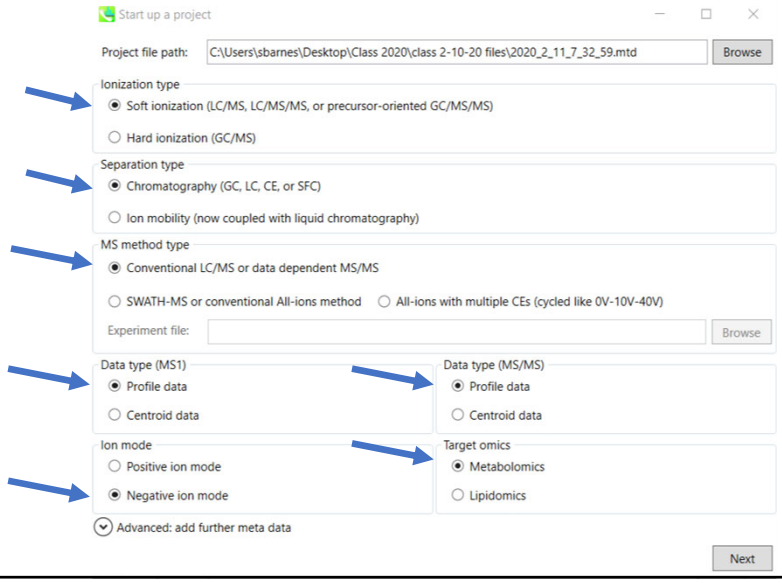
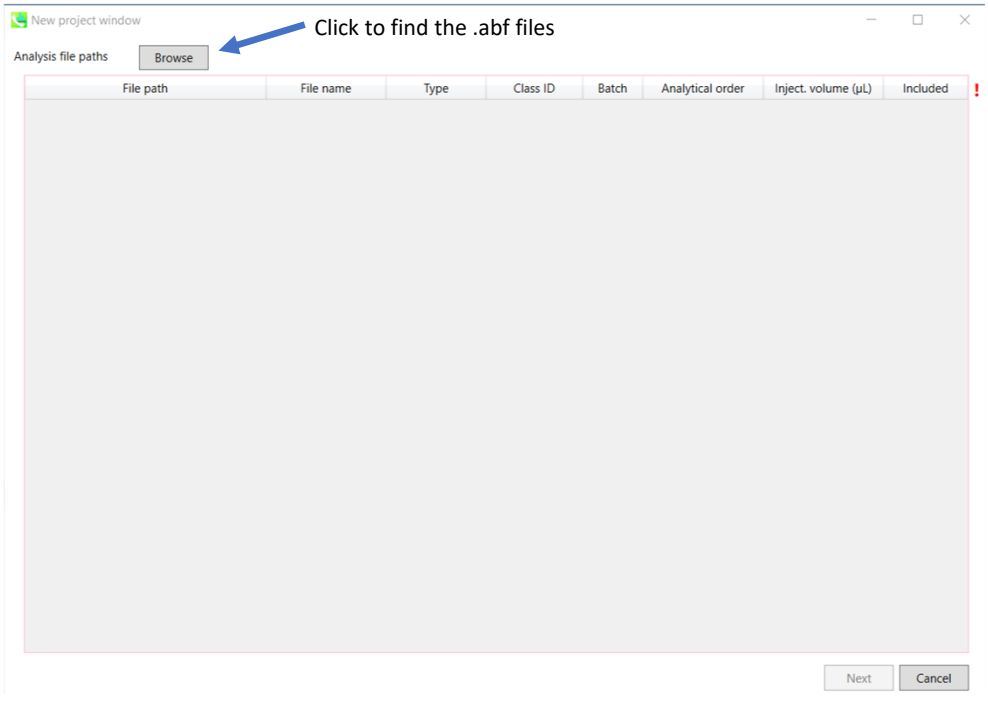
Setting the initial parameters

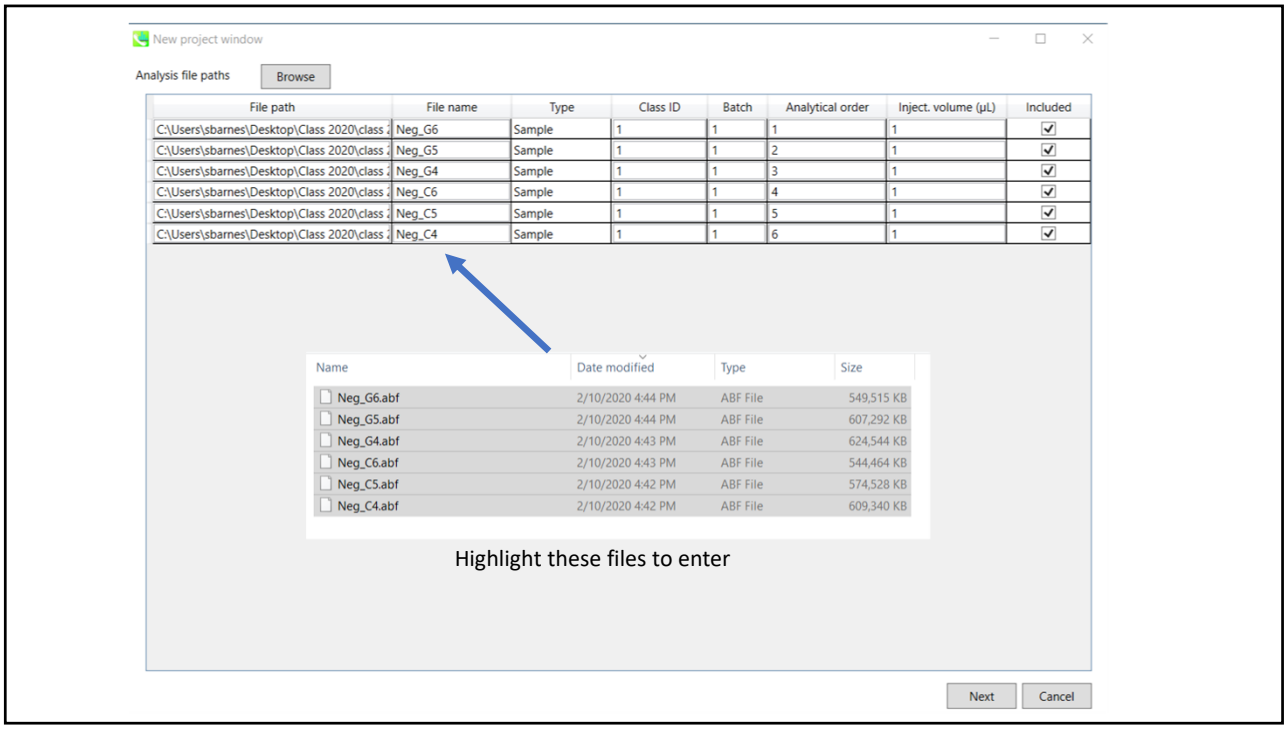


Select the path to the .abf files

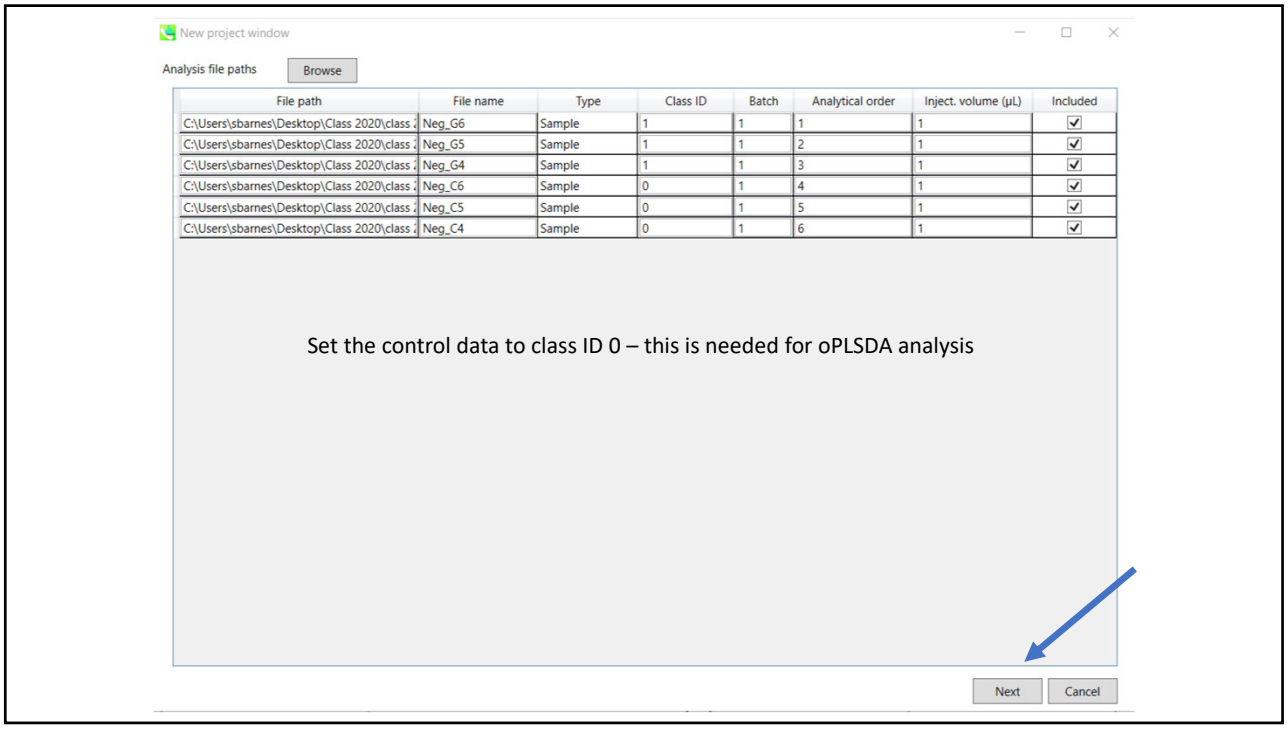
7



8


New project window
Analysis file paths
Browse
File path
File name
Type
Class ID
Batch
Analytical order
Inject. volume (µL)
Included
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G6
Sample
1
1
1
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G5
Sample
1
1
2
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G4
Sample
1
1
3
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C6
Sample
1
1
4
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C5
Sample
1
1
5
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C4
Sample
1
1
6
1
Name
Date modified
Type
Size
Neg_G6.abf
2/10/2020 4:44 PM
ABF File
549,515 KB
Neg_G5.abf
2/10/2020 4:44 PM
ABF File
607,292 KB
Neg_G4.abf
2/10/2020 4:43 PM
ABF File
624,544 KB
Neg_C6.abf
2/10/2020 4:43 PM
ABF File
544,464 KB
Neg_C5.abf
2/10/2020 4:42 PM
ABF File
574,528 KB
Neg_C4.abf
2/10/2020 4:42 PM
ABF File
609,340 KB
Highlight these files to enter
Next
Cancel

9


New project window
Analysis file paths
Browse
File path
File name
Type
Class ID
Batch
Analytical order
Inject. volume (µL)
Included
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G6
Sample
1
1
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1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G5
Sample
1
1
2
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_G4
Sample
1
1
3
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C6
Sample
0
1
4
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C5
Sample
0
1
5
1
C:\Users\sbarnes\Desktop\Class 2020\class 2
Neg_C4
Sample
0
1
6
1
Set the control data to class ID 0 – this is needed for oPLSDA analysis
Next
Cancel

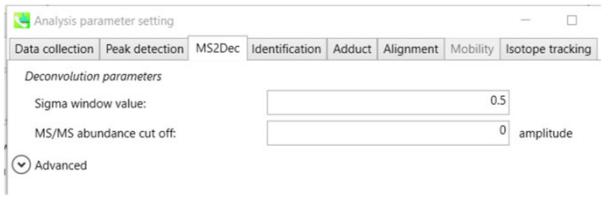
10

Setting the parameters under the tabs

Do not click the finish tab in the righthand corner until later

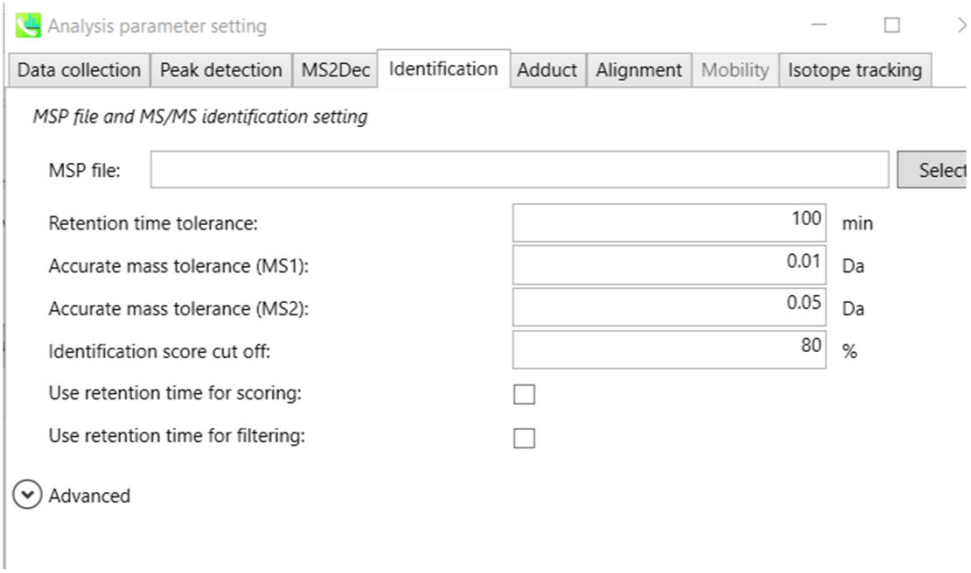
11

No changes to these tabs



12

Need to locate the data library



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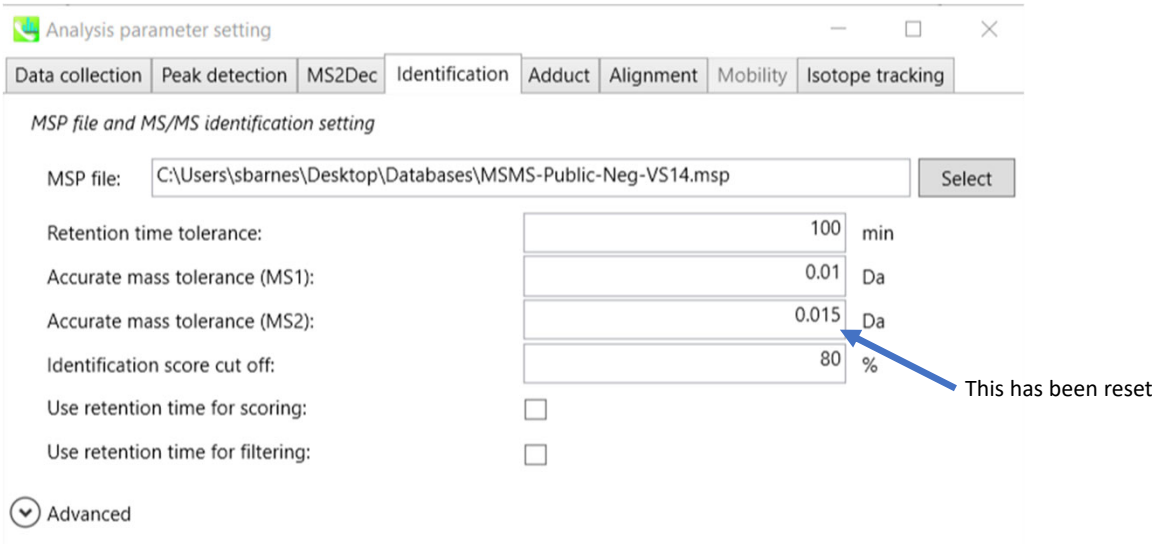
The database has been added



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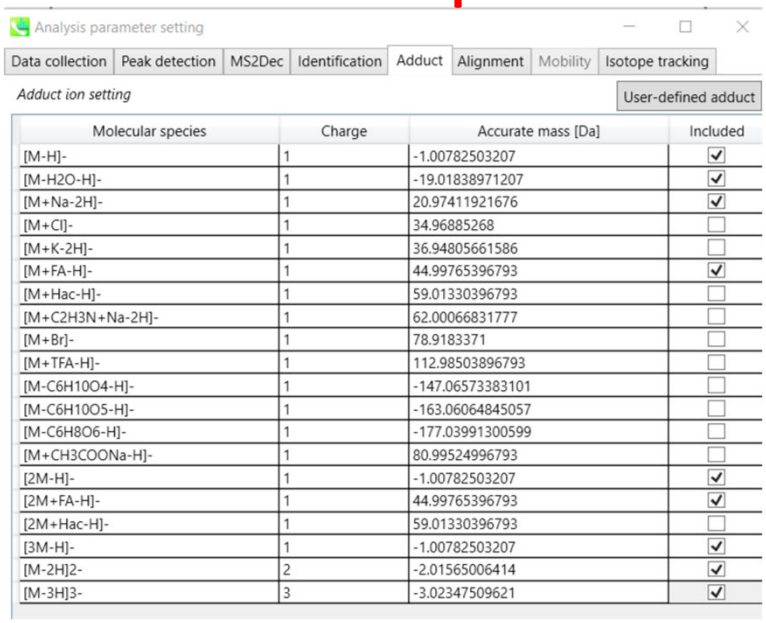
Check the boxes for possible adducts



Molecular species	Charge	Accurate mass [Da]	Included
[M-H]-	1	-1.00782503207	☑
[M-H2O-H]-	1	-19.01838971207	☑
[M+Na-2H]-	1	20.97411921676	☑
[M+Cl]-	1	34.96885268	☐
[M+K-2H]-	1	36.94805661586	☐
[M+FA-H]-	1	44.99765396793	☑
[M+Hac-H]-	1	59.01330396793	☐
[M+C2H3N+Na-2H]-	1	62.00066831777	☐
[M+Br]-	1	78.9183371	☐
[M+TFA-H]-	1	112.98503896793	☐
[M-C6H10O4-H]-	1	-147.06573383101	☐
[M-C6H10O5-H]-	1	-163.06064845057	☐
[M-C6H8O6-H]-	1	-177.03991300599	☐
[M+CH3COONa-H]-	1	80.99524996793	☐
[2M-H]-	1	-1.00782503207	☑
[2M+FA-H]-	1	44.99765396793	☑
[2M+Hac-H]-	1	59.01330396793	☐
[3M-H]-	1	-1.00782503207	☑
[M-2H]2-	2	-2.01565006414	☑
[M-3H]3-	3	-3.02347509621	☑

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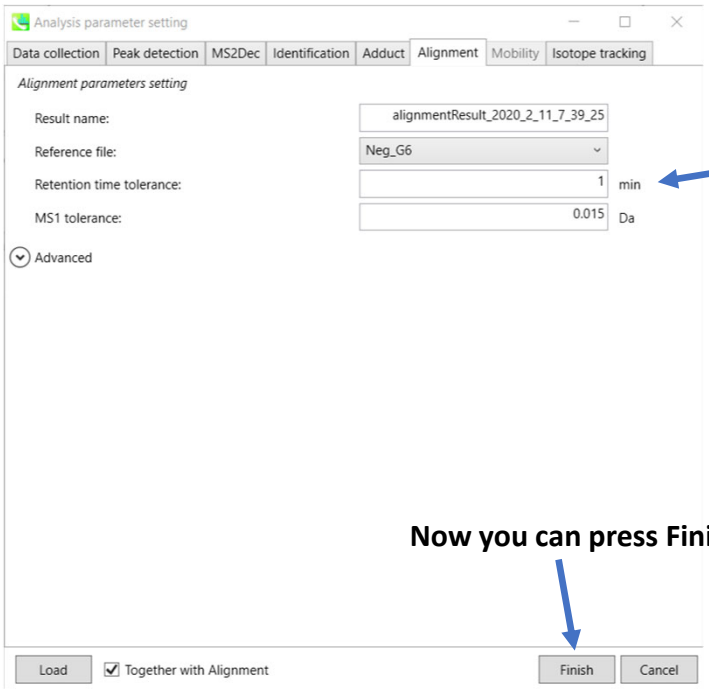

This time has been reset

Now you can press Finish

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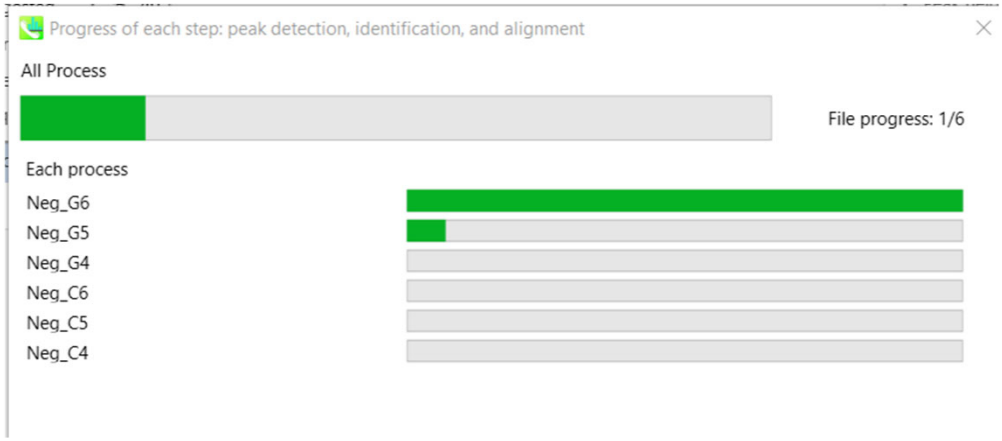
Showing the progress of the analysis



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When the green bar is fully over to the right, secondary programs will run. Finally, the MS-DIAL analysis panes will appear.

Double click on the alignment result in the left side bar

All the detected ions (known and unknown) are displayed

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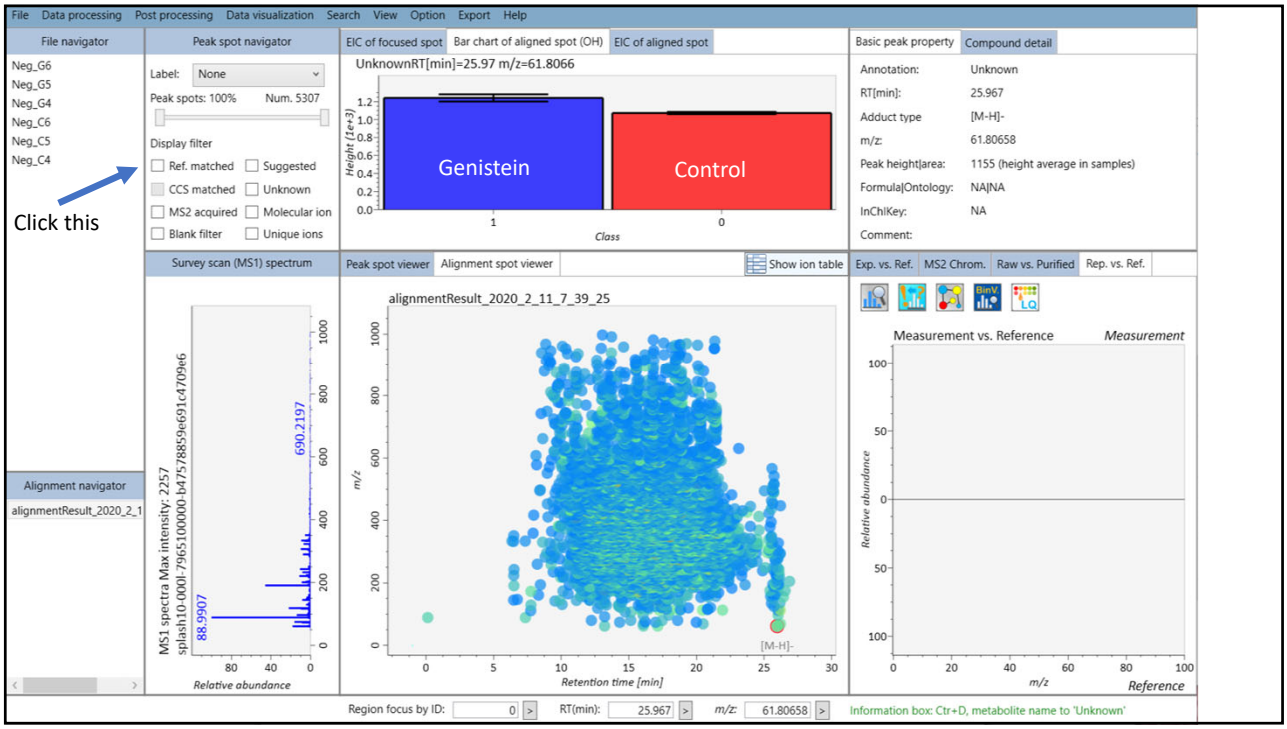
File Data processing Post processing Data visualization Search View Option Export Help
File navigator
Neg_G6
Neg_G5
Neg_G4
Neg_C6
Neg_C5
Neg_C4
Click this
Peak spot navigator
Label: None
Peak spots: 100% Num. 5307
Display filter
Ref. matched Suggested
CCS matched Unknown
MS2 acquired Molecular ion
Blank filter Unique ions
EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot
UnknownRT[min]=25.97 m/z=61.8066
Genistein
Control
Class
Basic peak property Compound detail
Annotation: Unknown
RT[min]: 25.967
Adduct type [M-H]-
m/z: 61.80658
Peak height|area: 1155 (height average in samples)
Formula|Ontology: NA|NA
InChIKey: NA
Comment:
Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2257
splash10-0001-7965100000-b47578859e691c4709e6
690.2197
88.9907
Relative abundance
Peak spot viewer Alignment spot viewer Show ion table
alignmentResult_2020_2_11_7_39_25
[M-H]-
Retention time [min]
Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.
Measurement vs. Reference
Measurement
Reference
Alignment navigator
alignmentResult_2020_2_1
Region focus by ID: 0 RT(min): 25.967 m/z: 61.80658
Information box: Ctr+D, metabolite name to 'Unknown'

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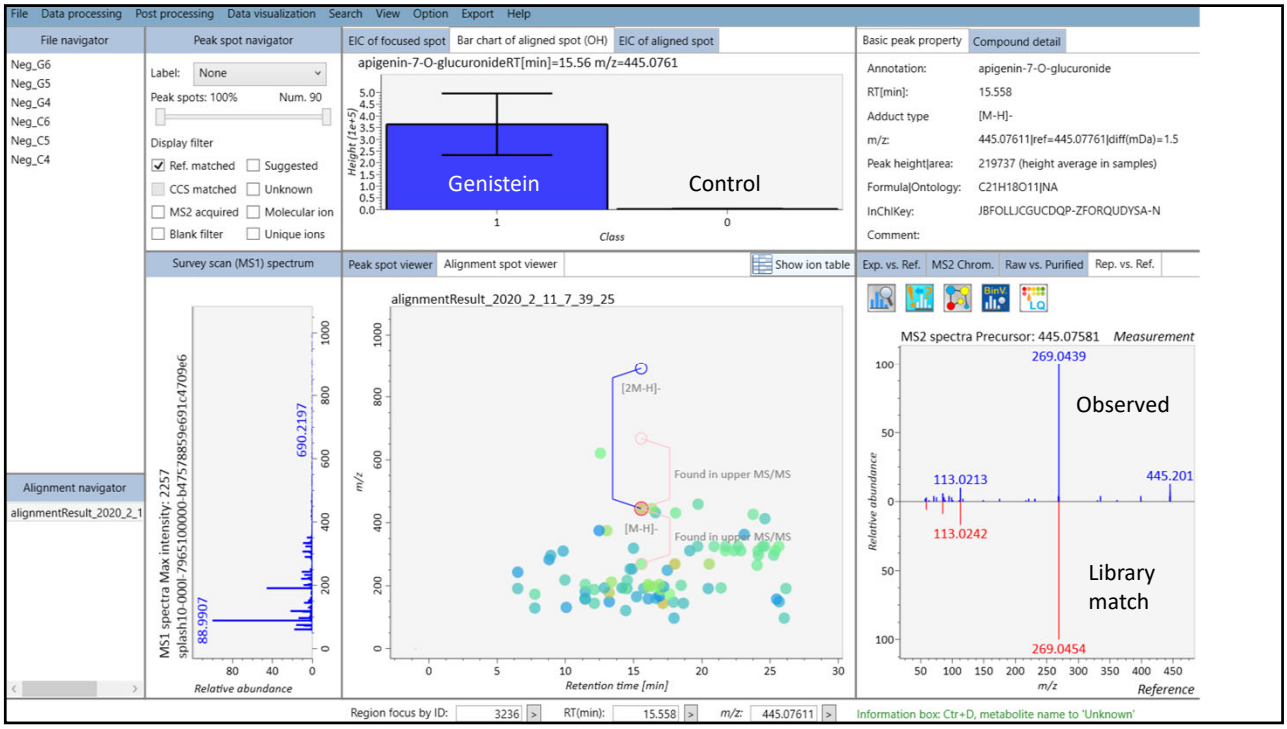
File Data processing Post processing Data visualization Search View Option Export Help
File navigator
Neg_G6
Neg_G5
Neg_G4
Neg_C6
Neg_C5
Neg_C4
Peak spot navigator
Label: None
Peak spots: 100% Num. 90
Display filter
Ref. matched Suggested
CCS matched Unknown
MS2 acquired Molecular ion
Blank filter Unique ions
EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot
apigenin-7-O-glucuronideRT[min]=15.56 m/z=445.0761
Genistein
Control
Class
Basic peak property Compound detail
Annotation: apigenin-7-O-glucuronide
RT[min]: 15.558
Adduct type [M-H]-
m/z: 445.07611|ref=445.07761|diff(mDa)=1.5
Peak height|area: 219737 (height average in samples)
Formula|Ontology: C21H18O11|NA
InChIKey: JBFOLLJCGUCDQP-ZFORQUDYSA-N
Comment:
Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2257
splash10-0001-7965100000-b47578859e691c4709e6
690.2197
88.9907
Relative abundance
Peak spot viewer Alignment spot viewer Show ion table
alignmentResult_2020_2_11_7_39_25
[2M-H]-
Found in upper MS/MS
[M-H]-
Found in upper MS/MS
Retention time [min]
Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.
MS2 spectra Precursor: 445.07581
Measurement
269.0439
Observed
113.0213
445.201
113.0242
Library match
269.0454
Reference
Alignment navigator
alignmentResult_2020_2_1
Region focus by ID: 3236 RT(min): 15.558 m/z: 445.07611
Information box: Ctr+D, metabolite name to 'Unknown'

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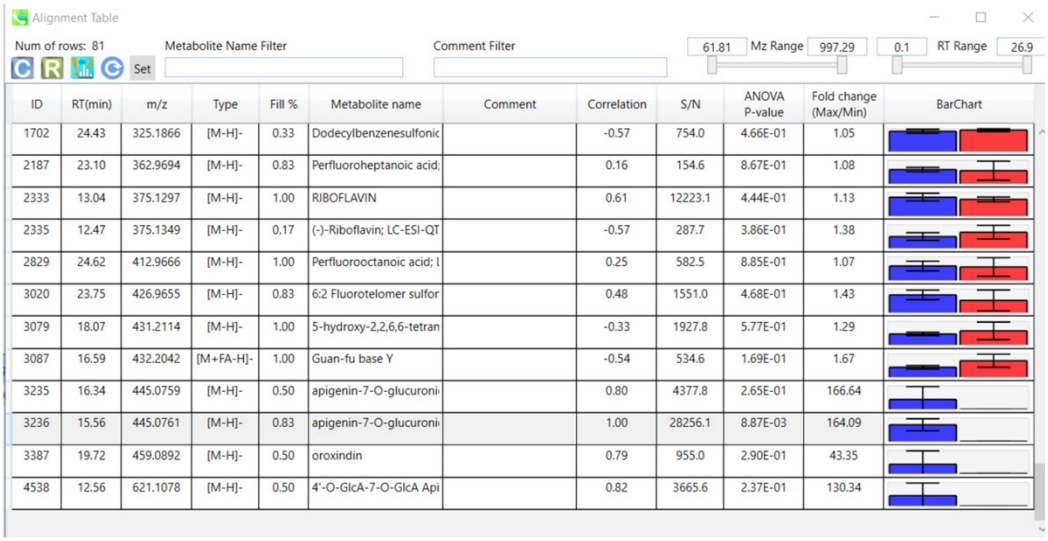
Click on ”Ion table” to show the “identified” ions



ID	RT(min)	m/z	Type	Fill %	Metabolite name	Comment	Correlation	S/N	ANOVA P-value	Fold change (Max/Min)	BarChart
1702	24.43	325.1866	[M-H]-	0.33	Dodecylbenzenesulfonic		-0.57	754.0	4.66E-01	1.05	
2187	23.10	362.9694	[M-H]-	0.83	Perfluoroheptanoic acid;		0.16	154.6	8.67E-01	1.08	
2333	13.04	375.1297	[M-H]-	1.00	RIBOFLAVIN		0.61	12223.1	4.44E-01	1.13	
2335	12.47	375.1349	[M-H]-	0.17	(-)-Riboflavin; LC-ESI-QT		-0.57	287.7	3.86E-01	1.38	
2829	24.62	412.9666	[M-H]-	1.00	Perfluorooctanoic acid; L		0.25	582.5	8.85E-01	1.07	
3020	23.75	426.9655	[M-H]-	0.83	6:2 Fluorotelomer sulfor		0.48	1551.0	4.68E-01	1.43	
3079	18.07	431.2114	[M-H]-	1.00	5-hydroxy-2,2,6,6-tetran		-0.33	1927.8	5.77E-01	1.29	
3087	16.59	432.2042	[M+FA-H]-	1.00	Guan-fu base Y		-0.54	534.6	1.69E-01	1.67	
3235	16.34	445.0759	[M-H]-	0.50	apigenin-7-O-glucuroni		0.80	4377.8	2.65E-01	166.64	
3236	15.56	445.0761	[M-H]-	0.83	apigenin-7-O-glucuroni		1.00	28256.1	8.87E-03	164.09	
3387	19.72	459.0892	[M-H]-	0.50	oroxindin		0.79	955.0	2.90E-01	43.35	
4538	12.56	621.1078	[M-H]-	0.50	4'-O-GlcA-7-O-GlcA Api		0.82	3665.6	2.37E-01	130.34	

All the ions in the table can be highlighted, copied and transferred to Excel

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	A	B	C	D	E	F	G	H	I	J	K
1	ID	RT	Mass	Ion	Fractio			Correl	S/N	p-value	FC
2	20	26.03	96.96125	[M-H]-	1	Phosphoric acid		-0.46775	70.35927	0.870505	1.046085
3	21	17.95	96.96135	[M-H]-	0.333333	Phosphoric acid		-0.22581	11.09236	0.5714	1.266831
4	44	14.42	121.0303	[M-H]-	1	2-Hydroxybenzaldehyde		-0.02084	230.3153	0.392505	1.58231
5	56	7.76	129.0212	[M-H]-	1	CITRACONIC ACID		0.207015	89.03239	0.344172	1.530682
6	57	8.26	129.0212	[M-H]-	0.333333	CITRACONIC ACID		0.435113	34.15426	0.218116	1.594494
7	63	10.07	131.0361	[M-H]-	0.333333	Glutaric acid; LC-ESI-QTOF; MS2; CE		0.611154	81.5596	0.644436	1.588785
8	84	12.12	144.0458	[M-H]-	1	4-Hydroxyquinoline		0.968062	268.3839	0.609065	1.246376
9	85	17.14	144.0478	[M-H]-	1	4-Hydroxyquinoline		0.470719	13043.28	0.380413	1.298935
10	86	18.64	144.048	[M-H]-	0.5	4-Hydroxyquinoline		0.285642	118.1601	0.333711	1.610268
11	95	17.92	148.0242	[M-H]-	1	Benzyl Isothiocyanate		0.087637	150.4269	0.67476	1.374674
12	96	13.21	149.0088	[M-H]-	0.166667	L-(+)-tartaric acid; LC-ESI-QTOF; MS2; CE		-0.42978	19.05287	0.4837	1.8518
13	97	25.66	149.0106	[M-H]-	0.333333	(R,R)-TARTARIC ACID		-0.43203	52.50727	0.54429	1.590465

Clean up the columns to make it easier to appreciate the data

1	ID	RT	Mass	Ion	Fractic			Corre	S/N	p-valu	FC
2	20	26.03	96.9613	[M-H]-	1.00	Phosphoric acid		-0.4678	70.36	0.87050	1.05
3	21	17.95	96.9614	[M-H]-	0.33	Phosphoric acid		-0.2258	11.09	0.57140	1.27
4	44	14.42	121.0303	[M-H]-	1.00	2-Hydroxybenzaldehyde		-0.0208	230.32	0.39251	1.58
5	56	7.76	129.0212	[M-H]-	1.00	CITRACONIC ACID		0.2070	89.03	0.34417	1.53
6	57	8.26	129.0212	[M-H]-	0.33	CITRACONIC ACID		0.4351	34.15	0.21812	1.59
7	63	10.07	131.0361	[M-H]-	0.33	Glutaric acid; LC-ESI-QTOF; MS2; CE		0.6112	81.56	0.64444	1.59
8	84	12.12	144.0458	[M-H]-	1.00	4-Hydroxyquinoline		0.9681	268.38	0.60907	1.25
9	85	17.14	144.0478	[M-H]-	1.00	4-Hydroxyquinoline		0.4707	13043.28	0.38041	1.30
10	86	18.64	144.0480	[M-H]-	0.50	4-Hydroxyquinoline		0.2856	118.16	0.33371	1.61
11	95	17.92	148.0242	[M-H]-	1.00	Benzyl Isothiocyanate		0.0876	150.43	0.67476	1.37
12	96	13.21	149.0088	[M-H]-	0.17	L-(+)-tartaric acid; LC-ESI-QTOF; MS2; CE		-0.4298	19.05	0.48370	1.85
13	97	25.66	149.0106	[M-H]-	0.33	(R,R)-TARTARIC ACID		-0.4320	52.51	0.54429	1.59

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Reorganizing the data according to RT

	RT	Mass	Ion	Fraction			Correl	S/N	p-value	FC
258	6.51	191.0212	[M-H]-	0.67	CITRATE		0.0265	59.66	0.51275	1.29
677	6.51	243.0623	[M-H]-	0.83	Pseudouridine		0.6598	57.52	0.34761	1.37
678	7.37	243.0630	[M-H]-	0.50	Pseudouridine		0.4058	48.79	0.38523	1.53
259	7.37	191.0213	[M-H]-	0.17	Citric acid		-0.0381	25.39	0.63893	1.23
56	7.76	129.0212	[M-H]-	1.00	CITRACONIC ACID		0.2070	89.03	0.34417	1.53
167	7.76	173.0114	[M-H]-	1.00	cis-Aconitate		0.2025	231.84	0.37510	1.51
57	8.26	129.0212	[M-H]-	0.33	CITRACONIC ACID		0.4351	34.15	0.21812	1.59
1151	8.81	283.0680	[M-H]-	0.50	Xanthosine; LC-ESI-QTOF; MS2; CE		0.4278	74.18	0.49296	1.53
1356	8.93	296.1021	[M-H]-	1.00	N2-Methylguanosine		0.5310	330.68	0.49288	1.36
1565	9.83	310.1140	[M-H]-	1.00	N2,N2-Dimethylguanosine		0.2243	264.50	0.51888	1.36
460	9.96	218.1030	[M-H]-	0.83	D-PANTOTHENIC ACID		-0.5419	603.07	0.49295	1.91
63	10.07	131.0361	[M-H]-	0.33	Glutaric acid; LC-ESI-QTOF; MS2; CE		0.6112	81.56	0.64444	1.59
114	11.44	157.0404	[M-H]-	0.33	ALLANTOIN		0.5857	23.50	0.41526	1.42

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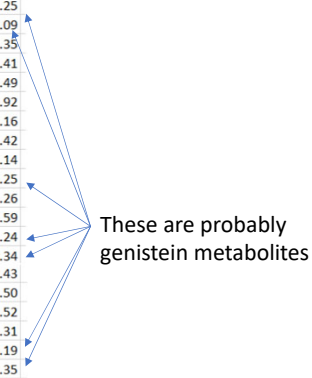
Organizing the data according to p-value

RT	Mass	Ion	Fraction			Correl	S/N	p-value	FC
15.57	269.0468	[M-H]-	0.50	Emodin		-0.5117	2344.65	0.00537	81.25
15.56	445.0761	[M-H]-	0.83	apigenin-7-O-glucuronide		-0.5247	28256.12	0.00887	164.09
16.89	167.1055	[M-H]-	0.83	Chrysanthemic Acid		0.3701	115.90	0.01392	1.35
16.07	204.0672	[M-H]-	1.00	Indolelactic acid		0.1934	5353.15	0.02399	1.41
16.07	158.0597	[M-H]-	1.00	Indole-3-acetaldehyde; LC-ESI-QTOF; MS2; CE		0.1690	110.97	0.09263	1.49
17.62	174.0565	[M-H]-	1.00	Indoleacetic acid; LC-ESI-QTOF; MS2; CE		0.4052	2283.56	0.11465	1.92
21.80	311.1691	[M-H]-	1.00	Triptophenolide		-0.7362	2340.87	0.14646	1.16
14.14	193.0354	[M-H]-	0.67	Glucuronate		0.8812	134.13	0.16489	1.42
22.60	311.1691	[M-H]-	1.00	Triptophenolide		-0.9200	1109.05	0.16520	1.14
15.21	165.0575	[M-H]-	0.33	3-(3-Hydroxyphenyl)propionic acid		0.0486	235.63	0.17098	12.25
19.69	325.1794	[M-H]-	0.17	Dodecylbenzenesulfonic acid		-0.5846	427.25	0.17189	1.26
8.26	129.0212	[M-H]-	0.33	CITRACONIC ACID		0.4351	34.15	0.21812	1.59
18.02	269.0439	[M-H]-	1.00	Apigenin; LC-ESI-QTOF; MS2; CE		-0.4217	7616.71	0.22022	11.24
12.56	621.1078	[M-H]-	0.50	4'-O-GlcA-7-O-GlcA Apigenin (NMR)		-0.4362	3665.59	0.23690	130.34
16.89	204.0664	[M-H]-	1.00	N-Cinnamoylglycine		0.4430	2884.94	0.26166	2.43
21.40	193.0399	[M-H]-	1.00	D-(+)-Galacturonic acid		0.7855	412.51	0.26947	1.50
18.02	432.2045	[M+FA-H]-	0.33	Guan-fu base Y		0.7312	615.06	0.26950	1.52
16.95	187.0982	[M-H]-	1.00	Azelaic acid (Not validated); PlaSMA ID-221		0.5178	3418.23	0.27544	1.31
20.53	269.0465	[M-H]-	0.83	Aloe-emodin		-0.4072	2886.64	0.28086	38.19
19.72	459.0892	[M-H]-	0.50	oroxindin		-0.4125	955.03	0.28982	43.35
18.64	144.048	[M-H]-	0.50	4-Hydroxyquinoline		0.2856	118.16	0.33371	1.61
25.48	157.039	[M-H]-	0.50	ALLANTOIN		0.5537	20.31	0.34204	1.63
7.76	129.0212	[M-H]-	1.00	CITRACONIC ACID		0.2070	89.03	0.34417	1.53
25.69	325.1855	[M-H]-	1.00	Dodecylbenzenesulfonic acid		0.9645	3118.95	0.34721	1.31
6.51	243.0623	[M-H]-	0.83	Pseudouridine		0.6598	57.52	0.34761	1.37
11.45	181.0501	[M-H]-	0.83	DL-3-(4-Hydroxyphenyl)lactic acid; LC-ESI-QTOF; MS2; CE		-0.1964	85.72	0.35649	1.50
24.04	265.1473	[M-H]-	1.00	C12-AS (TENTATIVE)		-0.4008	1845.40	0.35770	2.09
14.54	191.0211	[M-H]-	0.33	CITRATE		0.5951	40.59	0.37175	1.74



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More information about the experiment

- Although you might think that if you take mice on controlled diet with and without genistein, the variation in observed metabolites would be smaller
- In the experiment described in the PLoS One paper, what we had done is to collect feces from patients with advanced breast cancer who were undergoing chemotherapy
- The fecal organisms were introduced into germ-free mice to establish a close-to-humanized microbiome in each mouse
- **Therefore, the mice had individualized, humanized microbiomes and therefore were not uniform**

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Multivariate statistics

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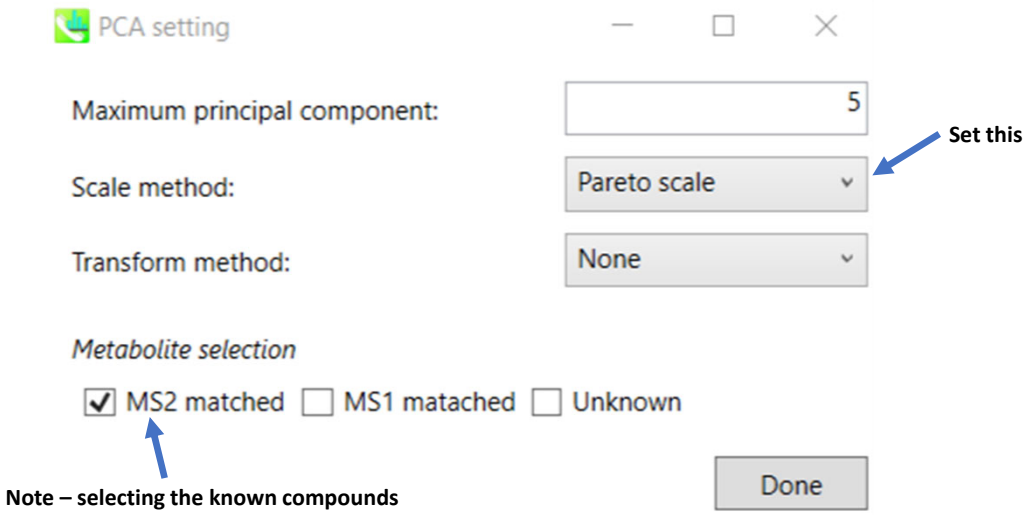
Set up for PCA analysis



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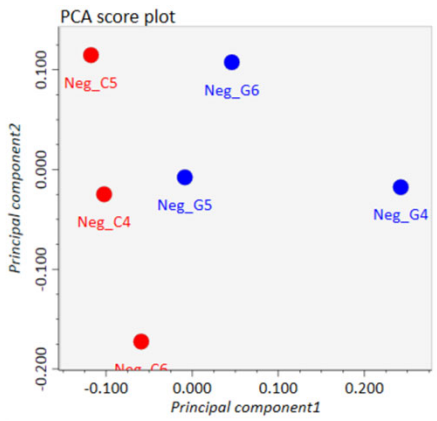
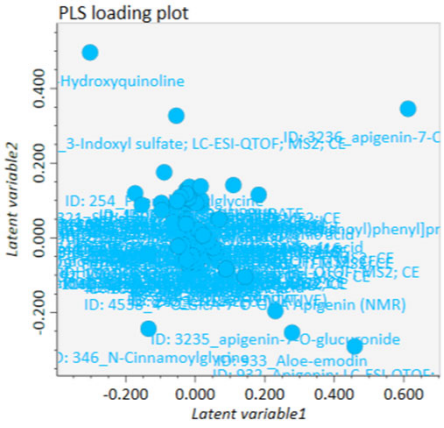
Results for PCA





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Plot of the components of the PCA

Red: positive correlation Blue: negative correlation

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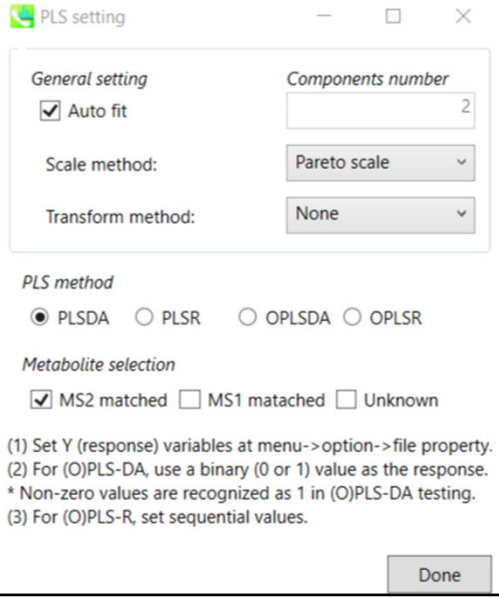
Set up for PLSDA analysis



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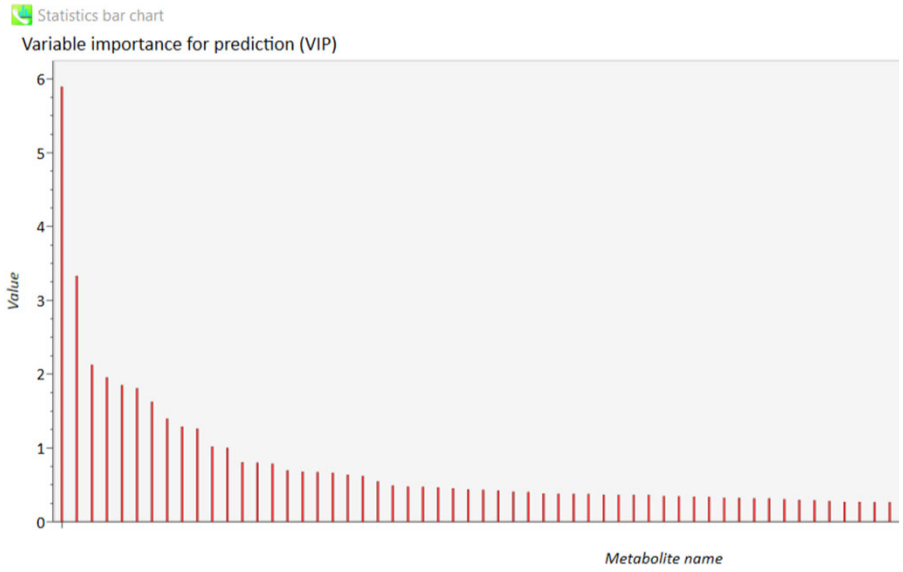
PLSDA VIP plot



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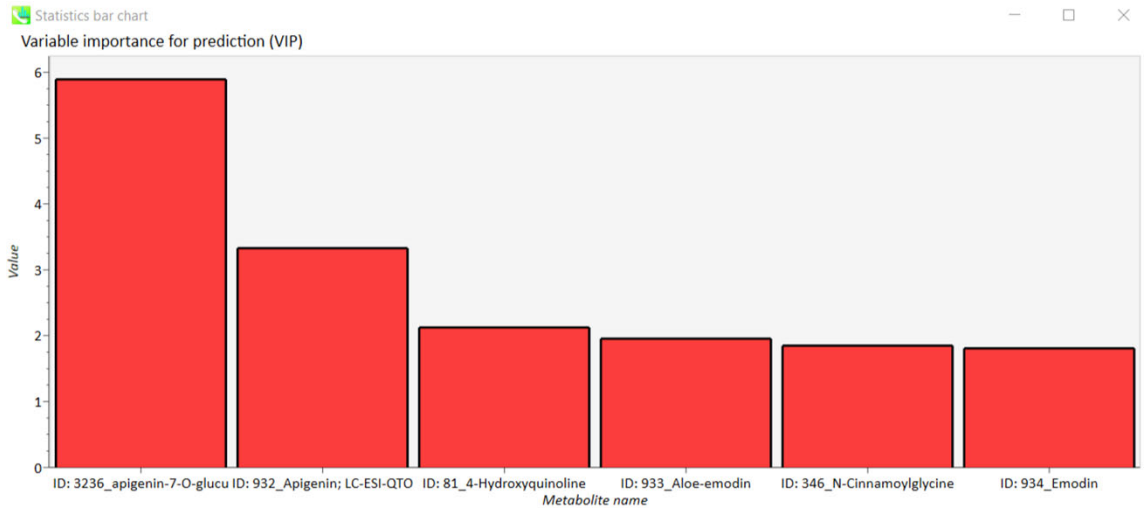
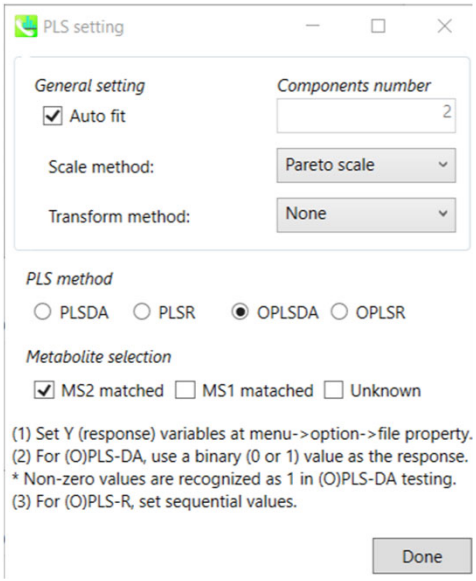
Expanded PLSDA VIP showing the top metabolites



32



Setting up the oPLSDA analysis

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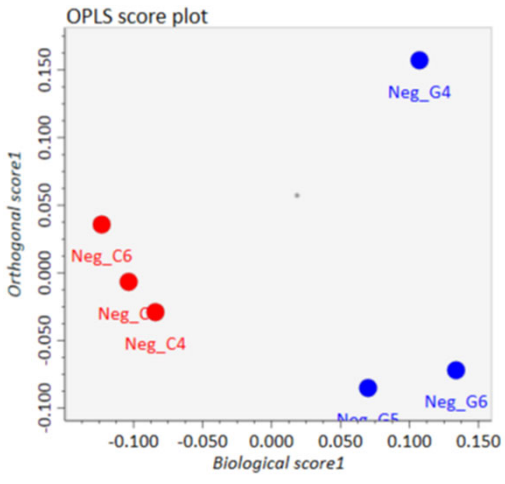
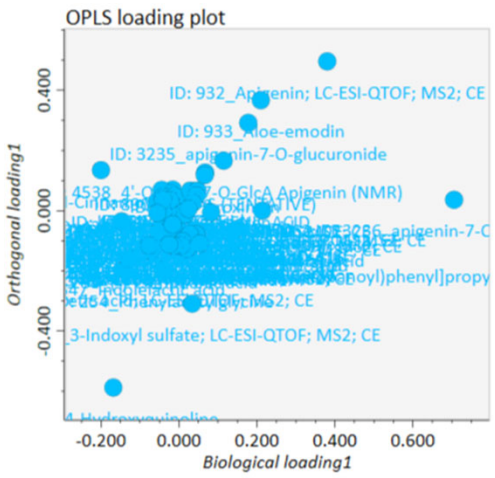
oPLSDA score and loading plots





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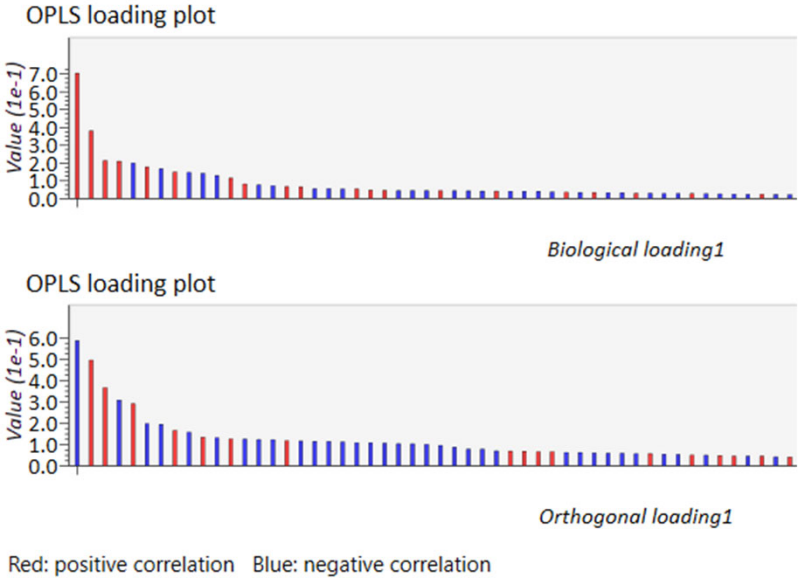
oPLSDA component plots



Red: positive correlation Blue: negative correlation

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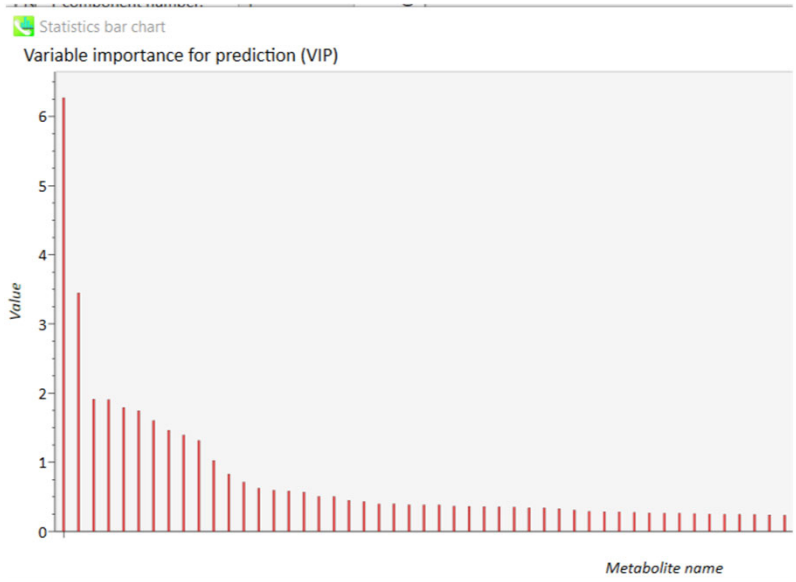
oPLSDA VIP components



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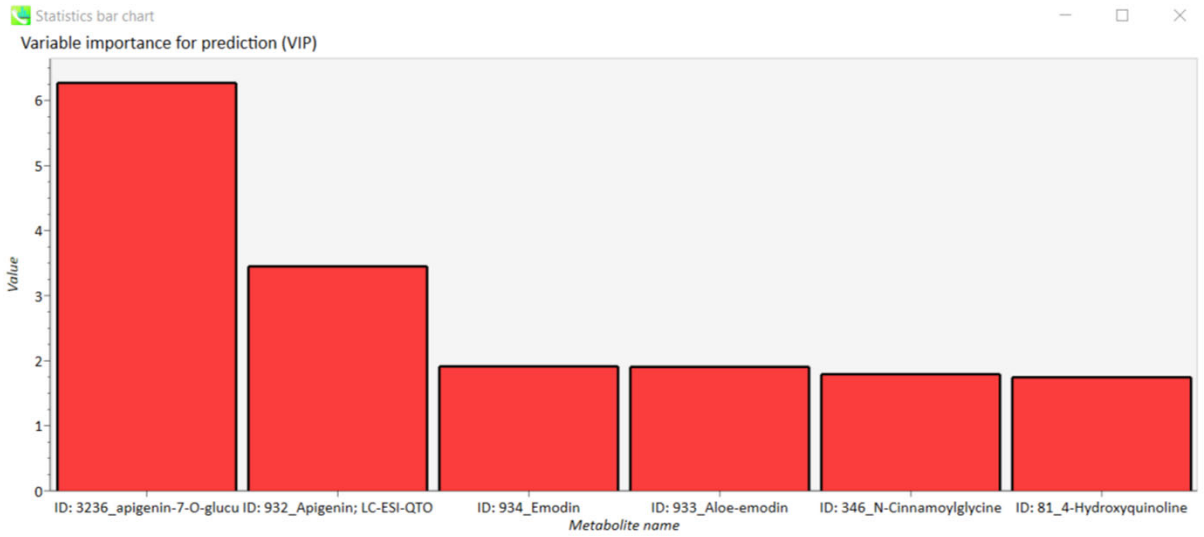
Expanded oPLSDA VIP items



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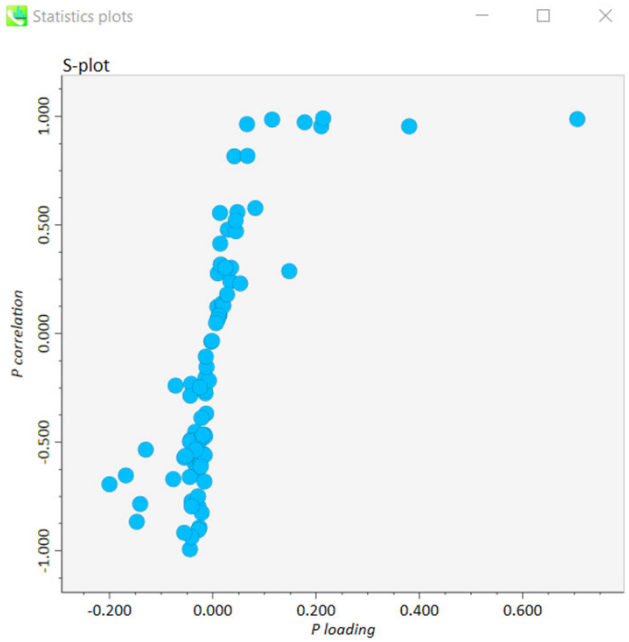

S-plot from oPLSDA

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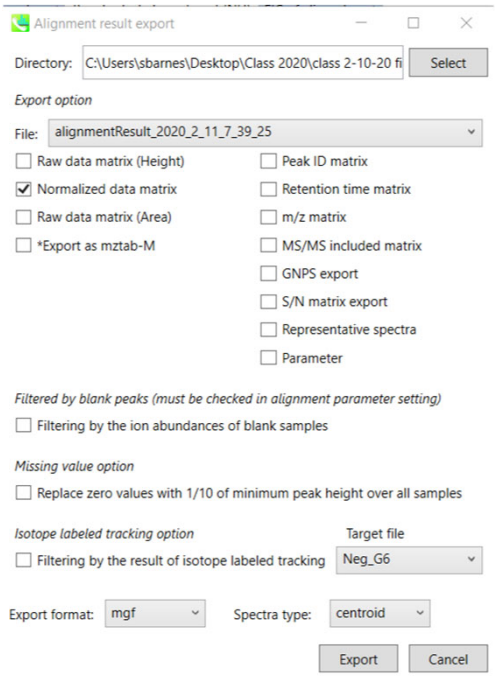

This outputs information about normalized areas of the metabolite peaks in each sample

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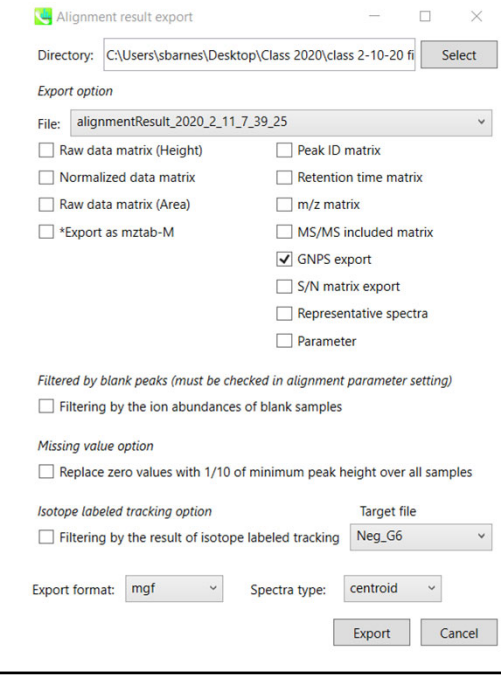

This output file allows us to look at adduct ions that are related to the known metabolite ions

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