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Lipid Insight Unblocked: Combining Nontargeted LC/MS Chemometrics With Automated Lipid Annotation

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Introduction

As adoption of non-targeted lipidomics expands, software tools must provide high confidence, high coverage annotations in a user-friendly framework.

MassHunter Explorer has a workflow-driven interface that enables MS and MS/MS feature extraction, statistical analysis, and MS/MS-based identification tools, while LipidMatch offers complementary deep lipid annotation and the ability for the user to review these annotations for confident lipid assignment.

We demonstrate a workflow that exchanges information between these software packages conveniently and show in-depth coverage with >800 lipid annotations from a porcine brain lipid sample.

These results highlight the importance of chemometrics, streamlined curation, and integrated visualization tools to support robust lipidomics interpretation.

Experimental

Sample Preparation

A simple experiment was conducted as a proof of principle for this workflow. A commercially available porcine brain lipid extract was spiked with Avanti Splash II Lipidomix standard. Three replicates were heated at 99 °C for 2 hours, and three replicates were left untreated (control) (Fig 1). The samples were dried under N_2 and resuspended in 1:1:0.1 butanol:methanol:10mM ammonium acetate.

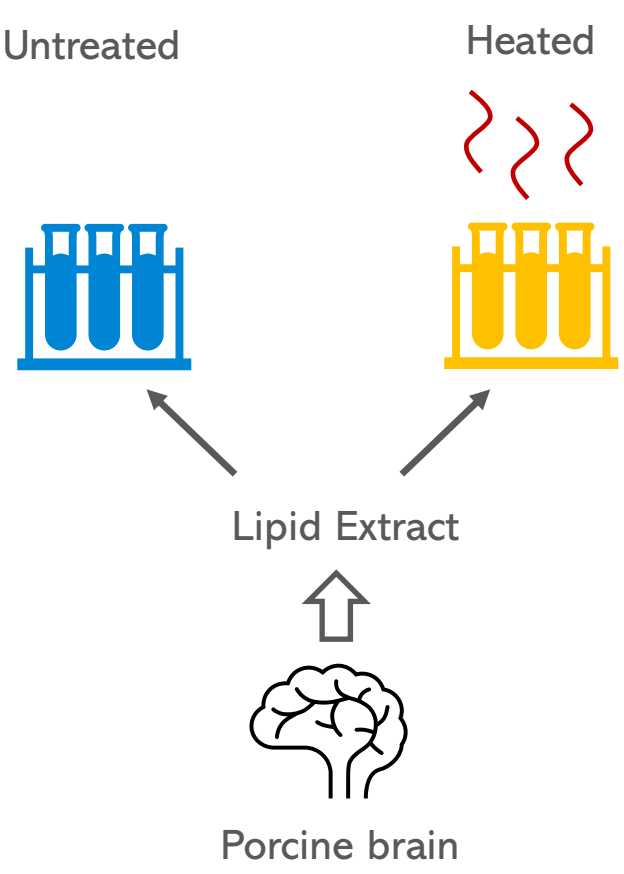


Figure 1. Sample study design

Experimental

Method

A previously optimized 20-minute reverse-phase chromatography method [1] was applied with two modifications: an Agilent Altura ZORBAX Eclipse Plus C18 column was used and 10mM ammonium formate was replaced with 10mM ammonium acetate. Lipid extracts were analyzed in + and - ion modes with MS1-only and Auto MS/MS (Iterative) methods (Fig 2).

Figure 2. Agilent's standardized Omics LC configuration used a 1290 Infinity III Bio LC coupled to a Revident LC/Q-TOF that provides robust and reliable lipidomics analysis.

Software Workflow

Data analysis was performed with a combination of MassHunter Explorer 2.0, LipidMatch Suite 5.73 (Innovative Omics), and Mass Profiler Professional (MPP) 15.1 software. File format exchange is depicted in Fig 3.

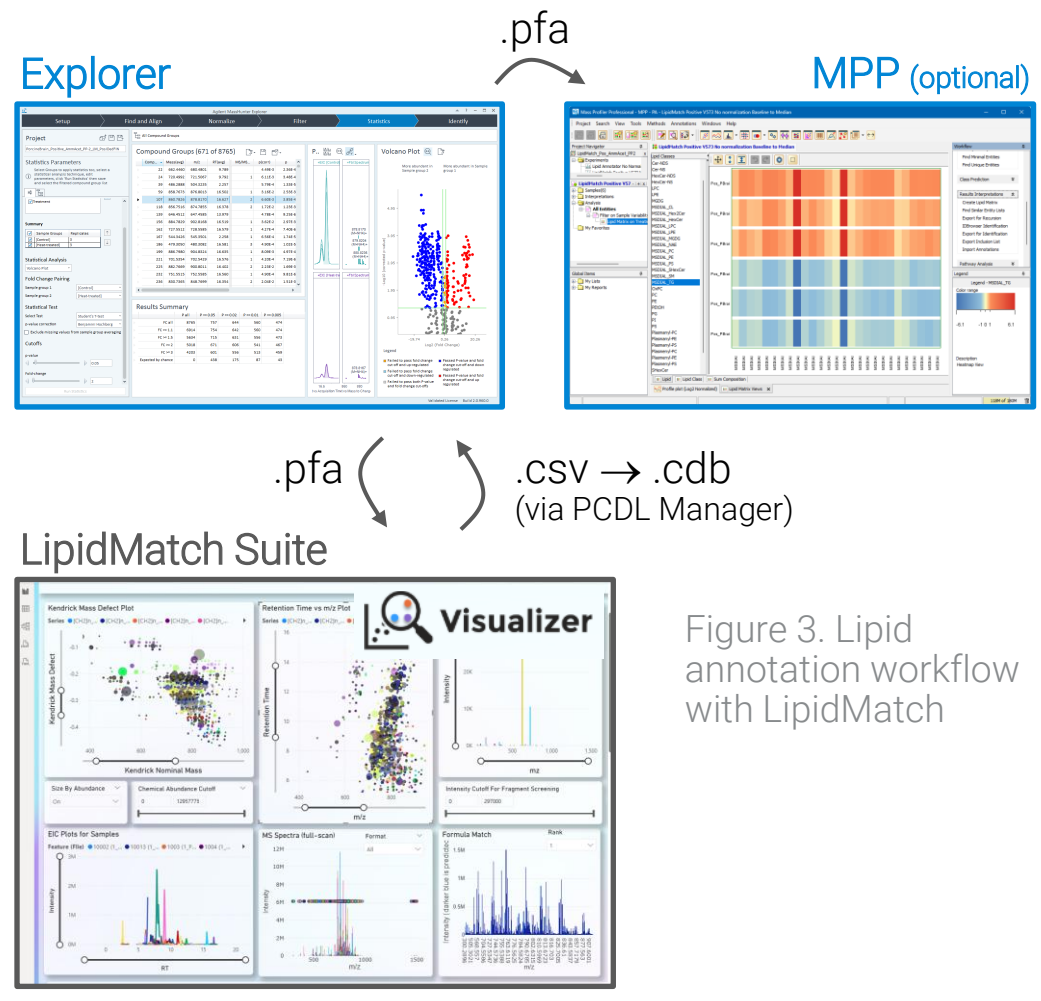


Figure 3. Lipid annotation workflow with LipidMatch

Results and Discussion

MassHunter Explorer- Compound (Feature) Extraction and Chemometrics

MS-only and iterative MS/MS data files were acquired from control and heat-treated porcine brain lipid extracts and were processed in Explorer, where feature extraction, MS/MS extraction, normalization, filtering, and statistical analysis were performed (Fig 4).

All the extracted compound information and associated MS/MS in a project can be exported as a single .pfa file for easy import into other applications.

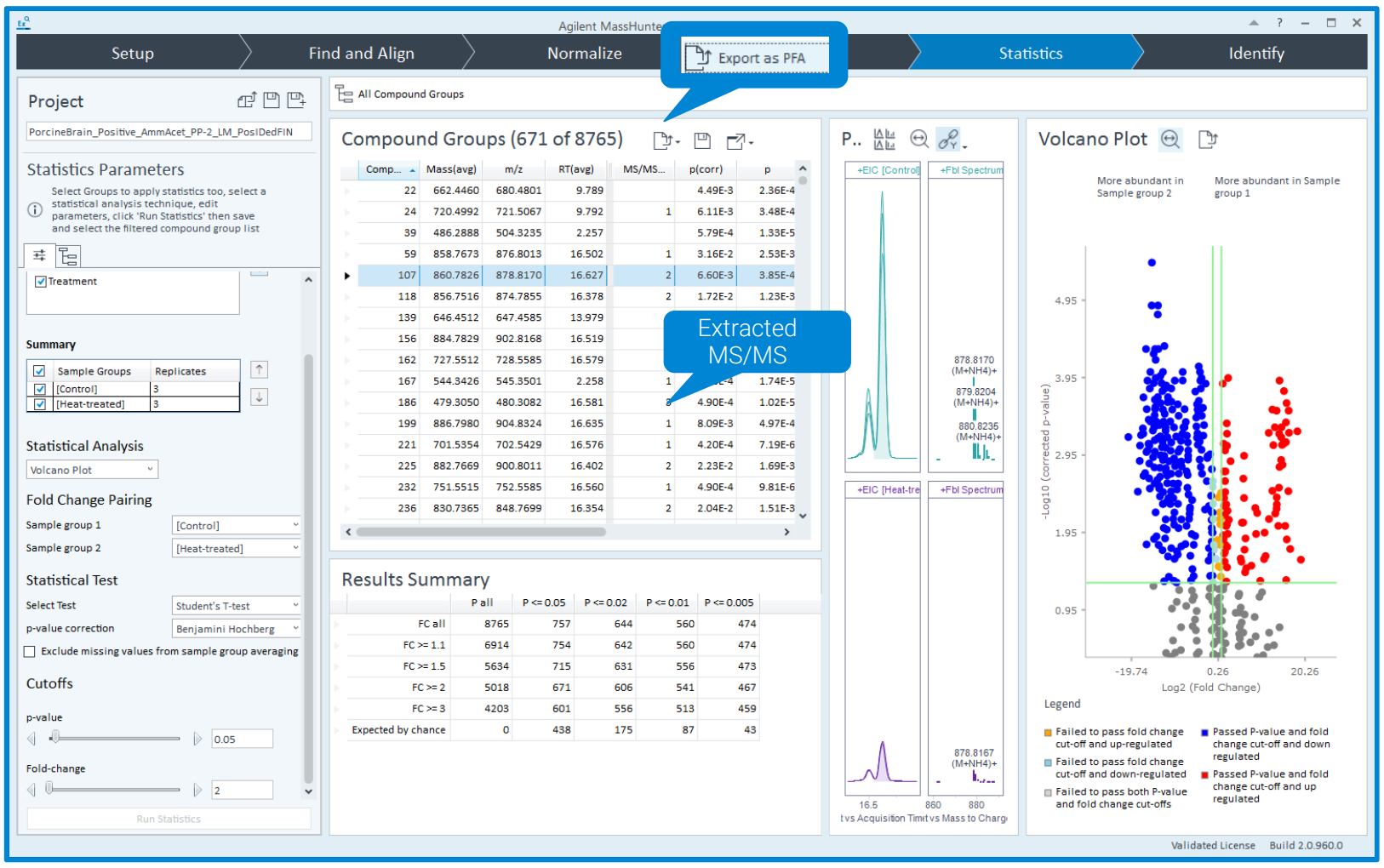


Figure 4. Explorer UI displaying results from the Statistics procedure. A volcano plot was used to find compounds that change in abundance with heat. The "Export to PFA" option is highlighted.

LipidMatch Software for Automated Lipid Annotation

Explorer .pfa files from + and – ion mode projects were directly imported into the LipidMatch Flow interface, a software enabling deep annotation based on >500,000 lipids with fragmentation in libraries and a <5% false-positive annotation rate.[2] 827 and 256 high-scoring lipids (score=1+3) were annotated from + and – ion mode data, respectively.

Resulting lipid annotations were then reviewed with LipidMatch Visualizer (Fig 5). The lipid class phosphatidylserine (PS), known to be reduced with heat treatment, was inspected further with a Kendrick mass defect plot.

Reviewing homologous series patterns revealed further lipids as confirmed by carbon:double bond content even without MS/MS. In this manner, annotation evidence could be reviewed, filtered, and curated.

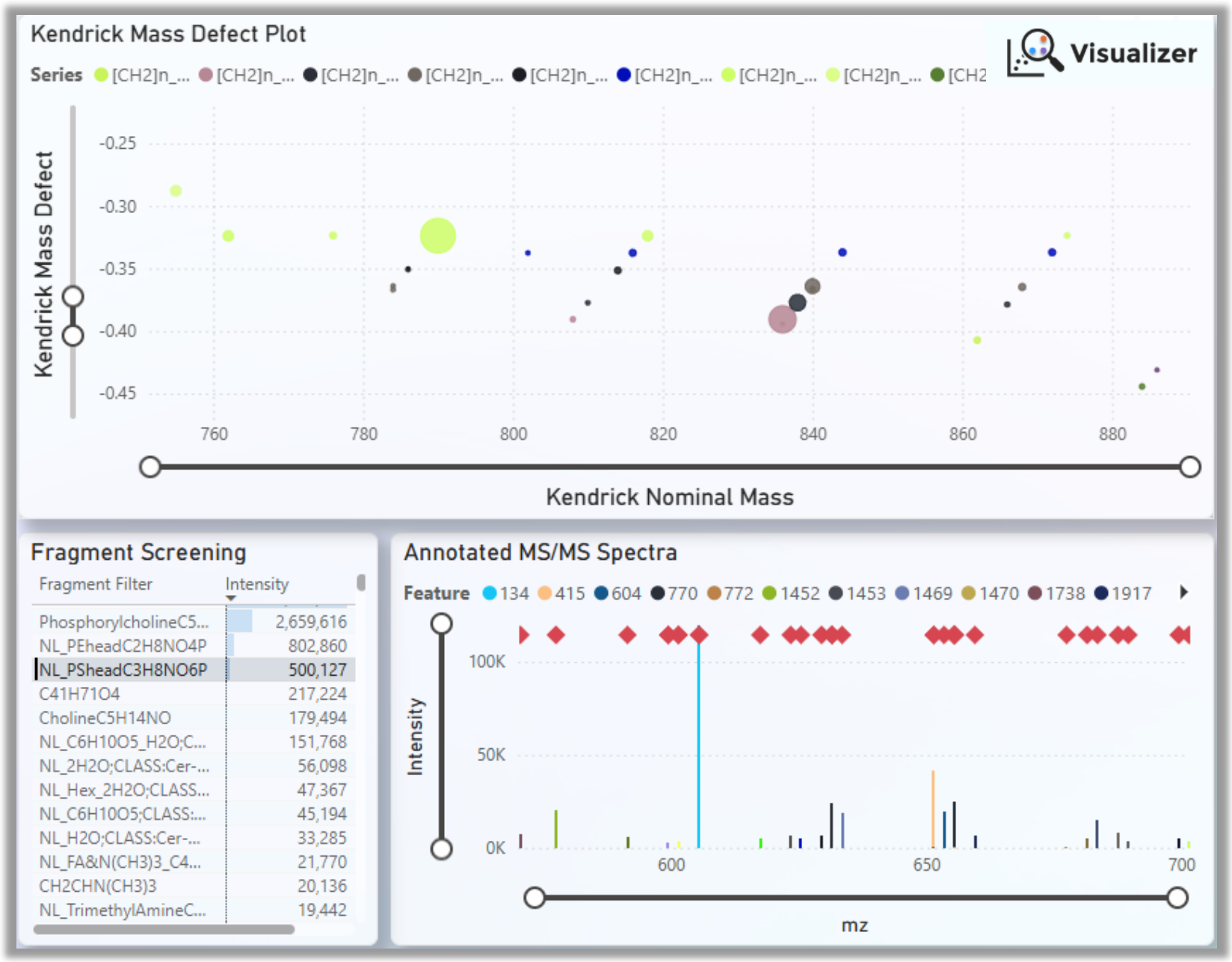


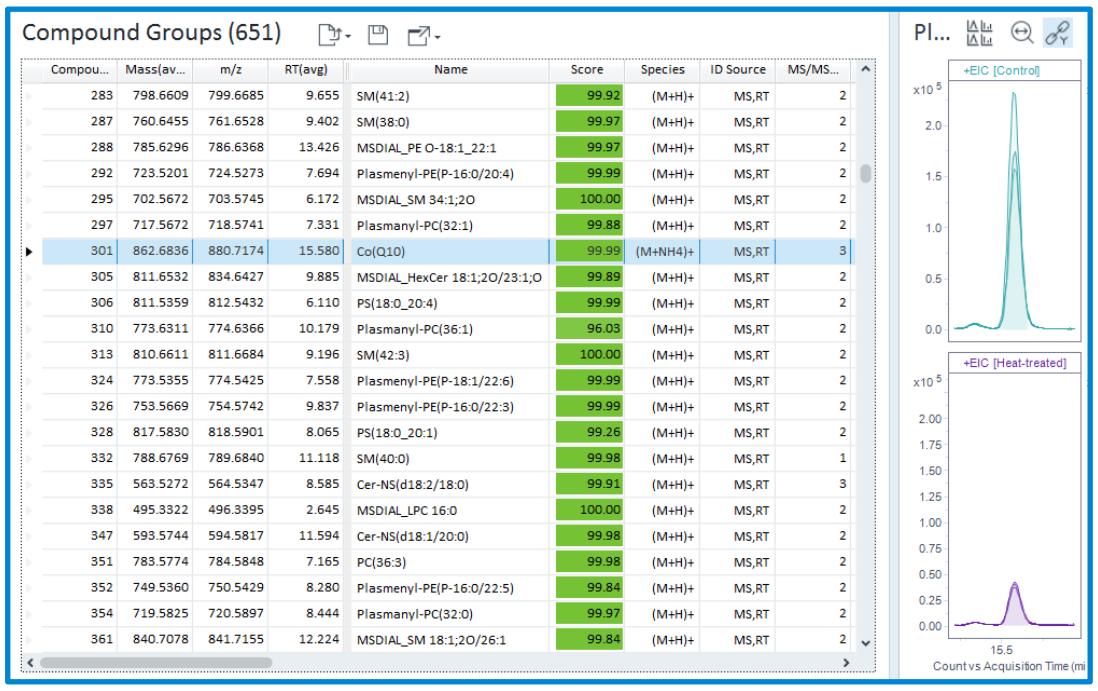
Figure 5. LipidMatch Visualizer zoomed view of the Kendrick Mass Defect Plot. The neutral loss (NL) corresponding to the PS headgroup was used to filter features indicating PS lipids.

Results and Discussion

Compound Review in Explorer and LipidMatch

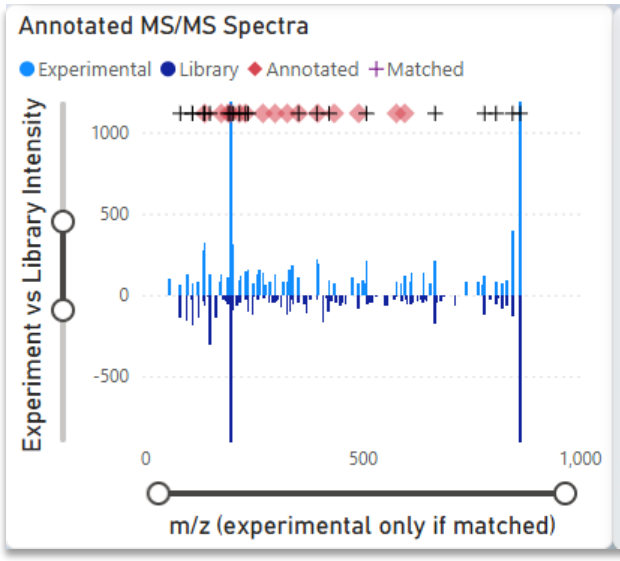
LipidMatch annotations were mapped to the MassHunter Explorer project to enable easier review of compound behavior in statistical tests and visualization of abundance changes across sample groups (Fig 6).

A



Compou...	Mass(av...	m/z	RT(avg)	Name	Score	Species	ID Source	MS/MS...
283	798.6609	799.6685	9.655	SM(41:2)	99.92	(M+H)+	MS,RT	2
287	760.6455	761.6528	9.402	SM(38:0)	99.97	(M+H)+	MS,RT	2
288	785.6296	786.6368	13.426	MSDIAL_PE O-18:1_22:1	99.97	(M+H)+	MS,RT	2
292	723.5201	724.5273	7.694	Plasmenyl-PE(P-16:0/20:4)	99.99	(M+H)+	MS,RT	2
295	702.5672	703.5745	6.172	MSDIAL_SM 34:1;2O	100.00	(M+H)+	MS,RT	2
297	717.5672	718.5741	7.331	Plasmanyl-PC(32:1)	99.88	(M+H)+	MS,RT	2
301	862.6836	880.7174	15.580	Co(Q10)	99.99	(M+NH4)+	MS,RT	3
305	811.6532	834.6427	9.885	MSDIAL_HexCer 18:1;2O/23:1;O	99.89	(M+H)+	MS,RT	2
306	811.5359	812.5432	6.110	PS(18:0_20:4)	99.99	(M+H)+	MS,RT	2
310	773.6311	774.6366	10.179	Plasmanyl-PC(36:1)	96.03	(M+H)+	MS,RT	2
313	810.6611	811.6684	9.196	SM(42:3)	100.00	(M+H)+	MS,RT	2
324	773.5355	774.5425	7.558	Plasmenyl-PE(P-18:1/22:6)	99.99	(M+H)+	MS,RT	2
326	753.5669	754.5742	9.837	Plasmenyl-PE(P-16:0/22:3)	99.99	(M+H)+	MS,RT	2
328	817.5830	818.5901	8.065	PS(18:0_20:1)	99.26	(M+H)+	MS,RT	2
332	788.6769	789.6840	11.118	SM(40:0)	99.98	(M+H)+	MS,RT	1
335	563.5272	564.5347	8.585	Cer-NS(d18:2/18:0)	99.91	(M+H)+	MS,RT	3
338	495.3322	496.3395	2.645	MSDIAL_LPC 16:0	100.00	(M+H)+	MS,RT	2
347	593.5744	594.5817	11.594	Cer-NS(d18:1/20:0)	99.98	(M+H)+	MS,RT	2
351	783.5774	784.5848	7.165	PC(36:3)	99.98	(M+H)+	MS,RT	2
352	749.5360	750.5429	8.280	Plasmenyl-PE(P-16:0/22:5)	99.84	(M+H)+	MS,RT	2
354	719.5825	720.5897	8.444	Plasmanyl-PC(32:0)	99.97	(M+H)+	MS,RT	2
361	840.7078	841.7155	12.224	MSDIAL_SM 18:1;2O/26:1	99.84	(M+H)+	MS,RT	2

B



Spectral Match Table

Frag	mz	Intensity	mz lib	int lib
	863.691	3,429	863.69	3429
C10H13O4	197.081	3,017	197.08	3229
	149.132	125	149.13	305
	109.103	72	109.10	192
	109.100	76	109.10	192
	667.621	213	667.62	174
			409.38	173
			95.09	155
			123.12	139
			163.15	138
	81.072	66	81.07	136
	845.680	393	845.68	130
			198.08	127
			327.30	125
	781.618	120	781.61	120
			247.24	120

Spectral Match Feature Table

Feature	m/z	RT	Ranked Library Matches	Identifiers	ppm Error	Cosine	Reverse Cosine	Entropy Score	Formula Pred	Frags Matched	Perc Coverage
1022	863.6910	15.58	Coenzyme Q10...	MSBNK-...	0.26 | 0.26 |...	0.9703 |...	0.9834 |...	0.6397 ...	C59H91O4	20 | 24 |...	0.6954 | 0.6148 |...

Figure 6. Example of compound review and inspection of annotation results. (A) In Explorer, the selected compound group 301 annotated as lipid Coenzyme Q10 was found to be markedly reduced with heat treatment. To enable this annotation mapping a curated list was exported from the LM Visualizer tool and a compound database (.cdb) was created. This database was then leveraged in Explorer to support compound matching based on accurate mass with a tight retention time tolerance, resulting in 651 compounds in the positive-ion project with lipid annotations. (B) Inspection of LipidMatch results showed that Coenzyme Q10 was identified via spectral library matching, in contrast to most other lipids that were annotated via in silico library matching. LipidMatch supports the identification of lipids with both in silico approaches and classical spectral library matching..

Advanced Visualizations with MPP

MPP software can import Explorer results (.pfa) to enable advanced chemometrics and lipid visualizations (Fig 7.)

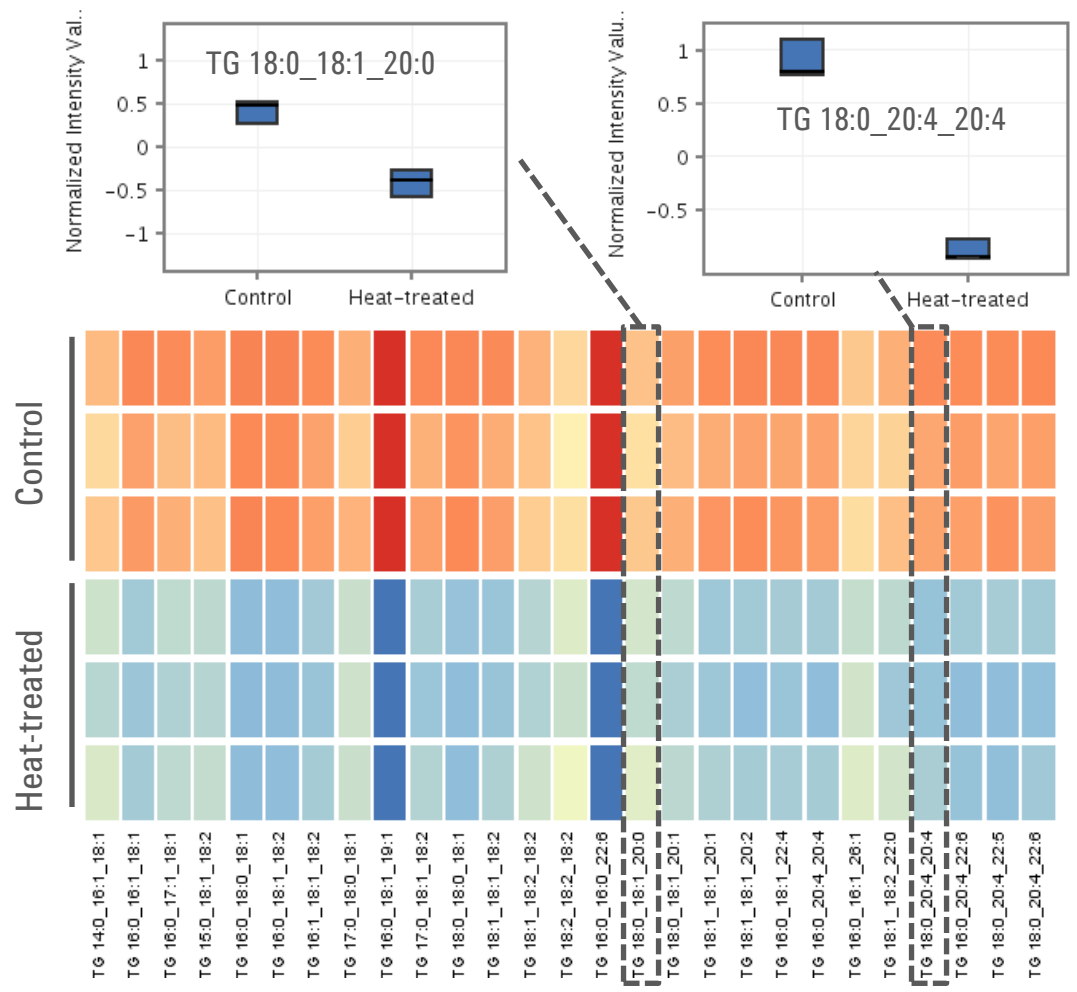


Figure 7. MPP lipid matrix plot of triacylglycerol (TG) compound abundances across control and heat-treated samples. Most TG lipids were reduced with heat. TGs with polyunsaturated fatty acids (PUFAs) were even more prone to degradation. The box plots highlight two TG lipids with different degrees of saturation.

Conclusions

Lipidomics workflow expands annotation through chemometrics, user curation, and visualization tools.

- Key enablement is .pfa file import to LipidMatch
- LipidMatch can be used to rapidly annotate lipids in an automatic fashion and confirm annotations and determine unknowns using an interactive visualizer
- Offers an alternative annotation approach, either independently or in conjunction with Agilent Lipid Annotator software.

References

[1] Hyunh, K et al. LC/MS dMRM Method Refinement Expands Targeted Lipidomics Studies from Plasma to Cells and Tissues. Agilent Application Note 5994-8365EN, 2026.

[2]Koelmel, JP et al. LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. BMC Bioinformatics. 2017 Jul 10;18(1):331. doi: 10.1186/s12859-017-1744-3.

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