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Unraveling Differential Lipids in Aging Tissues Using a Novel Data Analysis Workflow

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Introduction

Aging alters lipid metabolism across multiple tissues, driving metabolic inflexibility. While lipid remodeling in adipose and liver tissues is recognized, high-resolution approaches are required to characterize these dynamic shifts.

Here, nontargeted LC/Q-TOF lipidomics was performed on a multi-tissue sample set (WAT, BAT, liver) across three age cohorts (72 samples) using the 1290 Infinity III Bio LC coupled to the Revident LC/Q-TOF system.

To streamline discovery, we employed a novel workflow coupling MassHunter Explorer 2.0 (MH Explorer) for data extraction and chemometrics and MS-DIAL for complementary lipid annotation. This approach enabled the identification of 743 lipid species, using visualization tools in MH Explorer to quickly identify age-dependent changes.

These findings provide a comprehensive view of metabolic adaptation and underscore how integrated software solutions transform complex lipidomic datasets into actionable biological insights.

Figure 1. Agilent's omics standardized LC configuration used a 1290 Infinity III Bio LC (left) which is iron free to give best peak shape of metal-sensitive analytes. Coupled to it is a Revident LC/Q-TOF (right) which provides robust and reliable lipidomics analysis.

Experimental

Sample Preparation

Age-matched tissue samples (Figure 2) were collected from each animal (10 – 15 mg) and was subjected to lipid extraction using a modified methanol/MTBE/water protocol. EquiSPLASH internal standard mix in methanol was added prior to tissue pulverization using a BeadBug homogenizer. Samples were resuspended in 9/1 methanol/chloroform for LC-MS/MS analysis.

Experimental

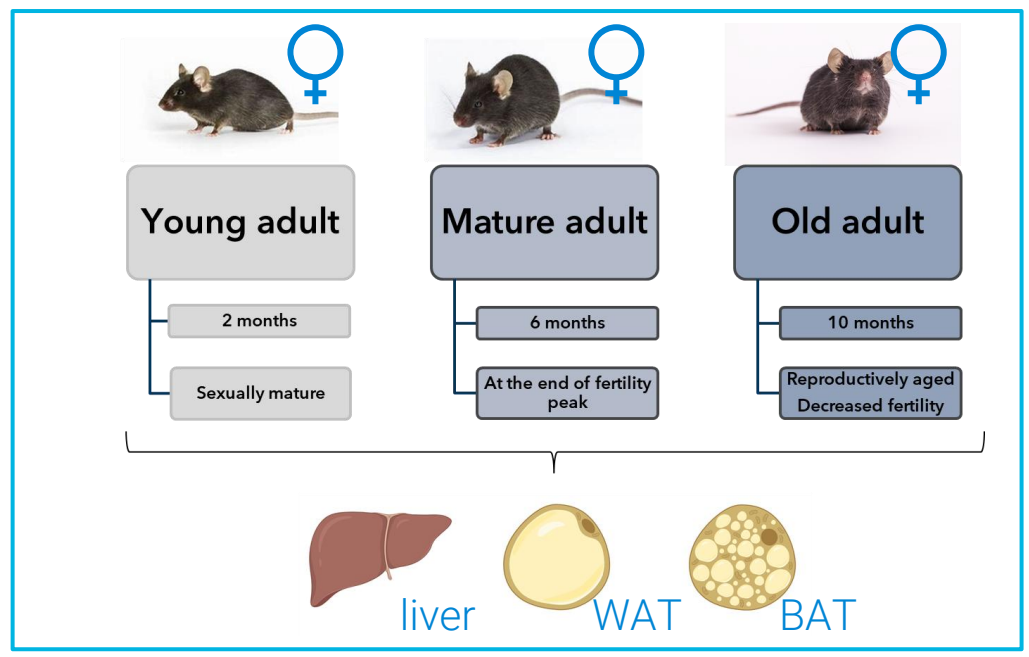

Figure 2. Study design for the aging lipidomics study.

Instrumentation

Lipid separation was performed using a 16-minute reversed-phase method on a ZORBAX Eclipse Plus C18 column with previously established chromatography[1]. Samples were analyzed on the Agilent Revident LC/Q-TOF, tuned in *m/z* 1700 stable (+/-) ion modes.

A pooled QC sample was used for both system suitability and for Iterative MS/MS acquisition (n = 6) per polarity for in-depth lipid identification coverage. MS1 acquisition was used for individual samples. The high resolution, isotopic fidelity and dynamic range of Revident enabled confident identification of 743 lipid species.

Data Analysis

Iterative MS/MS data files were searched in Lipid Annotator and in MS-DIAL 5.6[2]. Prior to exporting as an SDF format, data was manually curated within the MS-DIAL software.

The Instrument type in the Property Settings of MS-DIAL was set to "LC-ESI-QTOF" for the MS/MS data be annotated using this instrument type. Once the SDF file has been created, it was subsequently imported to ChemVista Library Manager. ChemVista can further refine the database or be exported as a CDB file.

Positive and Negative ion data (Iterative MS/MS and MS1 data) were imported to MassHunter Explorer 2.0 as two separate projects, in which peak picking, statistical analysis and identification using databases created from MS-DIAL data and/or Lipid Annotator.

Additional databases can be used for non-targeted lipidomics such as the Plasma Lipid PCDL (5994-7627EN).

Seamless Incorporation of MS-DIAL, Lipid Annotator, ChemVista and MassHunter Explorer

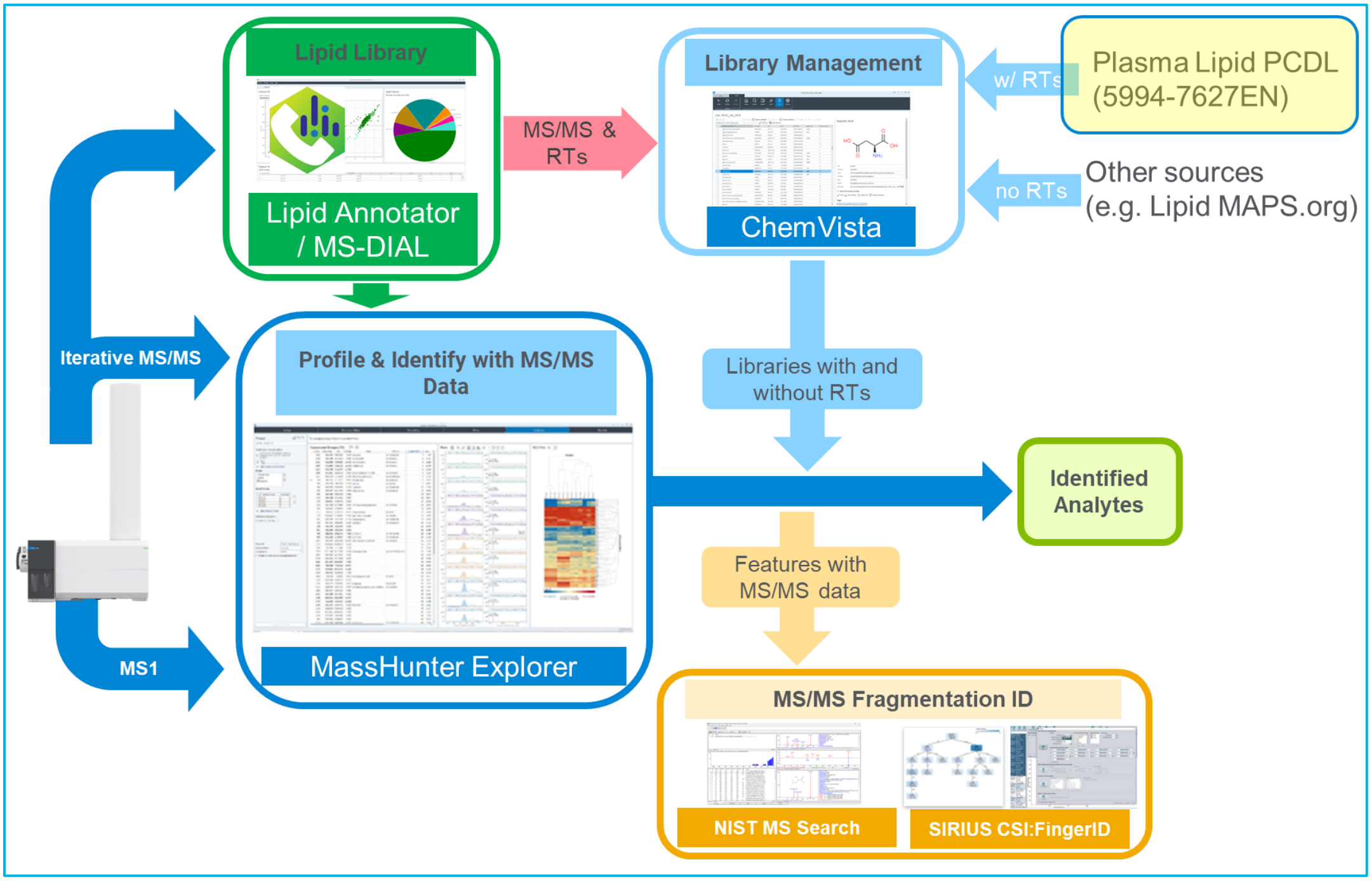

Figure 3. Lipidomics acquisition and data analysis workflow. MS1 data is collected for each individual sample and Iterative MS/MS is collected on pooled sample(s) to build a custom database to the study set. Iterative MS/MS data can be analyzed by Lipid Annotator or MS-DIAL and can be directly imported to Agilent ChemVista Library Manager. ChemVista can then export it as a .CDB file to be used with MassHunter Explorer 2.0.

Integrating MS-DIAL MS/MS Results into MassHunter Explorer 2.0

1. MS-DIAL Setup and Export: Set the instrument type to "LC-ESI-QTOF" in Project Properties and export curated MS/MS spectra data as an SDF file (Figure 4).
2. Import to ChemVista: Load the SDF file into ChemVista Library Manager. This also automatically transfers curated lipid data which includes retention times and other chemometric data (precursor mass, formula, InChiKey, SMILES).
3. Export or Refine: The database is ready for immediate export to MH Explorer, with the option for additional curation within ChemVista if required.
4. Integrate with Explorer: In the Identify Tab of MH Explorer, add the MS-DIAL database to finalize the workflow. Additional databases from Lipid Annotator and the Plasma Lipid PCDL can also be added.

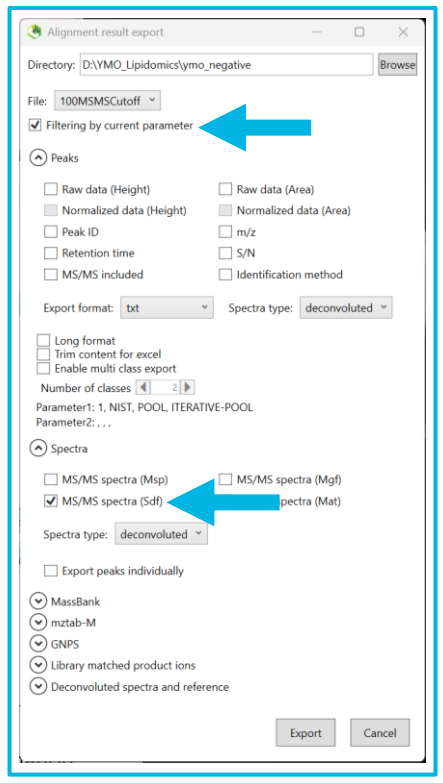

Figure 4. Exporting from MS-DIAL. After curating the results in the software, export the alignment results by checking the highlighted boxes in the Alignment Result Window. This will generate an SDF file that contains MS/MS data of the filtered results, complete with retention time, precursor mass, ion type, chemical formula and structure.

Results and Discussion

Multivariate Lipidomics Analysis with MassHunter Explorer 2.0

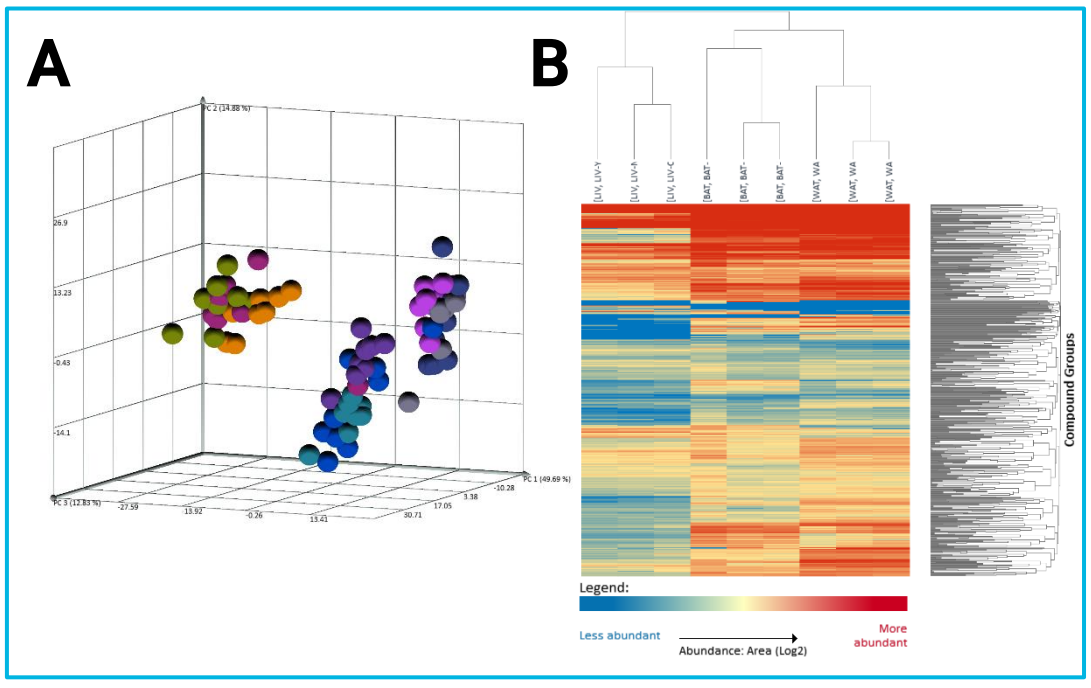

Figure 5. Visualize data as you filter with publication-quality figures. A: 3D Principal Component Analysis (PCA) plot of multi-tissue sample set of liver, BAT, and WAT. B: Hierarchical clustering heatmap of 645 lipids identified by MS/MS and retention time in positive ion mode.

- Following sample normalization by tissue weight, a PCA plot verified tissue-specific clustering (Figure 5A). Furthermore, after filtering for features identified by MS/MS and RT, a heatmap was produced which validates the tissue specific separation and also lipid class-specific alterations (Figure 5B).
- Volcano plots (Figure 6) enable rapid identification of statistically significant features across experimental cohorts.
- Integration with MS-DIAL results supports high-confidence identification of additional lipid species. As shown, a TG-estolide was found elevated in the OLD liver cohort, supported by a robust MS/MS spectral match (Figure 7).

Conclusions

- **Complementary and Flexible:** MassHunter Explorer 2.0 easily integrates with current MS-DIAL analysis pipelines to enable an end-to-end workflow for non-targeted lipidomics.
- **Comprehensive:** ChemVista manages custom MS/MS libraries from MS-DIAL, Lipid Annotator and other databases sources to use for Identification in MH Explorer 2.0.
- **Confident:** Statistically significant results can be obtained quickly and efficiently using powerful feature finding algorithms and statistical tools. Publication-quality visualizations can be created in the same workflow.

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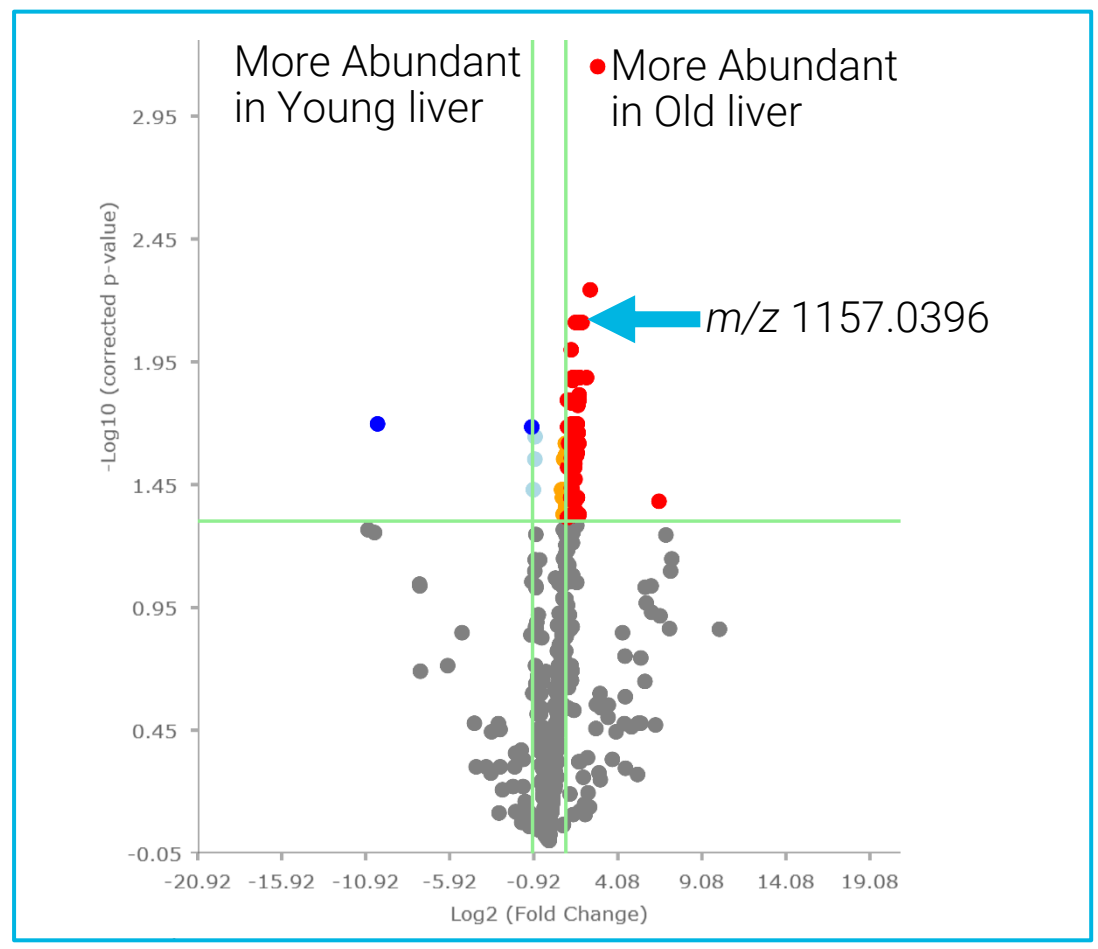

Figure 6. Volcano plot of lipid alteration liver in old and young mice and highlighting a TG-estolide, 2-fold difference and p-value cutoff of 0.05.

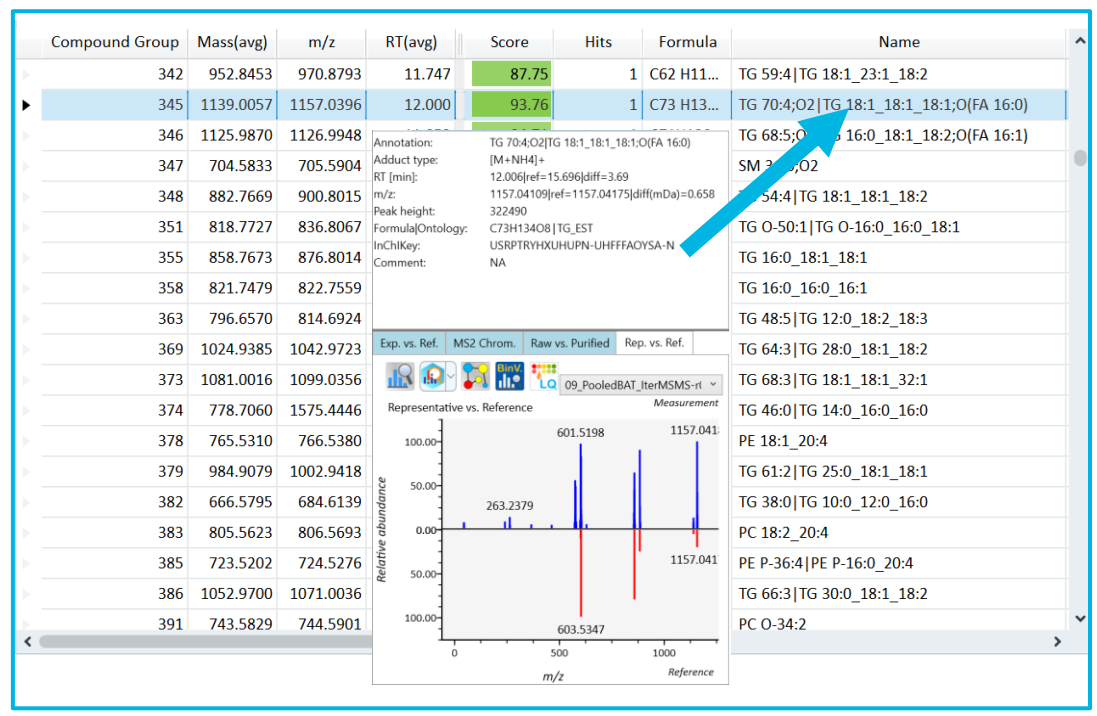

Figure 7. Highlighting TG 18:1_18:1_18:1;O(FA 16:0) as a high confident ID in MH Explorer and the corresponding annotation in MS-DIAL.

References

1. Huynh et al., A Comprehensive, Curated, High-Throughput Method for the Detailed Analysis of the Plasma Lipidome. Agilent Application Note 5994-3447EN, 2021.
2. Takeda et al. Nature Communications, 2024

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