Compound Discoverer Software

Reducing false annotations in lipidomics using Compound Discoverer 3.4 software

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Abstract

Purpose: Confident annotation is essential for translating untargeted lipidomics data into meaningful biological information. Thermo Scientific™ Compound Discoverer™ 3.4 software with Thermo Scientific™ LipidSearch™ software was evaluated for its ability to reduce false annotations by utilizing some of its key features including the detection of adducts and in-source or unintentional fragmentation.

Methods: Lipid Standards and bovine liver total lipid extracts were used for evaluation. Separation was carried out either using analytical flow or nano flow UHPLC coupled. Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer or Thermo Scientific™ Orbitrap™ Excedion™ Pro hybrid mass spectrometer were used for detection.

Results: Adducts and unintentional fragments combined form more than 50% of all the features detected irrespective of the chromatographic flow rate or the mass spectrometer used. The Orbitrap Excedion Pro MS with its new front end for ion transmission can significantly reduce the insource or unintentional fragmentation.

Introduction

False annotations and unintended or in-source MS1 fragmentation are critical challenges in lipidomics, affecting data accuracy and interpretation. False annotations arise when lipids are misidentified due to spectral similarities, database limitations, or incorrect adduct assignments. Unintentional MS1 fragmentation occurs when lipids break down within the ion source or the ion path of the mass spectrometer, generating fragment ions that may be mistaken for distinct lipid species. This complicates lipid identification and quantification, increasing false discovery rates. Here we show the utility of Thermo Scientific Compound Discoverer 3.4 software with LipidSearch software for grouping of adducts and unintentional MS1 fragments to avoid false annotations and for confident annotation of lipids.

Materials and methods

Sample Preparation

Avanti Research™ SPLASH™ LIPIDMIX™ (mixture of internal standard) and liver total lipid extract were purchased from Avanti Lipids. 1:100 dilution of the liver extract was used as the stock concentration of matrix.

Instrument Method

Lipid standards and bovine liver lipid extracts were purchased from Avanti Research™ . Separation was carried out on a Thermo Scientific™ Accucore™ C30 HPLC column connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Extracted Lipids were also analyzed using nanoLC on a Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC column connected to a Thermo Scientific™ Vanquish™ Neo UHPLC system. The run time for analytical flow was 30 Minutes and for the nanoflow was 15 minutes. Data was acquired on Orbitrap Astral MS and Orbitrap Excedion Pro MS. 20X amount was loaded on the analytical flow compared to the nanoflow.

Compound Discoverer 3.4 Software

- Includes LipidSearch software node for simplified lipid identification
- QC for small molecule experiments using internal standards
- Automatically detects and groups MS1 fragments
- Connects to Thermo Scientific™ mzCloud™ mass spectral library 2.0 for improved compound identification

LipidSearch software in Compound Discoverer software

- LipidSearch software provides automated identification of lipids with a comprehensive database, containing 1.5 million lipid ions and their predicted fragment ions
- LipidSearch software node can be added to any untargeted data processing workflow
- New workflow template: *Lipidomics\Untargeted Lipidomics using LipidSearch software*

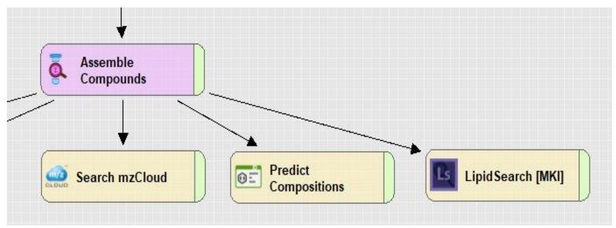

Figure 1. LipidSearch software available as a workflow node in Compound Discoverer software with a new template for untargeted lipidomics.

LipidSearch: Lipid Identification

- Database contains >1.7 million lipid ions and predicted fragment ions
- Fragmentation patterns are utilized to grade identification quality and provide all possible annotations
- Lipid adduct ions and MS^2 and MS^3 fingerprints are also included

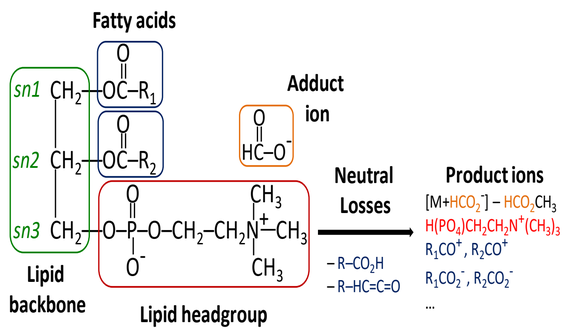

Figure 2. Predicted fragmentation of a typical lipid included in the LipidSearch software database

Category	Definition
C0	Headgroup specific ion
C01	Class specific product ion
C1	Substituent product ion
C2	Other non-specific ions

Condition	Definition
H1	C0 and C01 (or more than 2 C0) (or C01 for all substituents) are assigned
H2	One of C0 or C01 is assigned
S1	C1 and C01 from all substituents are assigned
S2	More than one of C1 or C01 from substituents are assigned
Other	C2 – headgroup and substituents not defined

Grade	Rule
A	H1 *and* S1
B	(H2 *and* S1) *or* (H1 *and* S2)
C	H1 *or* S2
D	Other

Note:
Grade A assigned based on product ions for one class specific, **C01**, one headgroup specific, **C0**, and all substituent ions, **C1**
Grade B assigned based on the lack of **H1** or **S1** conditions
Grade C assigned based on the lack of **H** or **S** conditions
Grade D assigned based on lack of headgroup/substituent ions

Figure 3. Rule based gradation of lipid annotation in LipidSearch software. The grade of a lipid is determined by comparing its fragmentation spectra with the predicted fragmentation. Higher grade implies more confident annotation.

Calc. *m/z*	Ion	Category	PC 16:0_20:4	PC 20:1_16:3	PS 16:0_23:3	PS 23:0_16:3
826.56036	$C_{45}H_{81}NO_{10}P^-$		M+HCOO⁻	M+HCOO⁻	M-H⁻	M-H⁻
766.53923	M-CH_3^-	C0	☑	☑	☒	☒
480.30957	LPC(16:0)-CH_3^-	C01	☑	☒	☒	☒
303.23295	20:4-H⁻	C1	☑	☒	☒	☒
259.24312	20:4-H-CO_2^-	C1	☑	☒	☒	☒
255.23295	16:0-H⁻	C1	☑	☒	☑	☒
224.06934	$C_3H_{11}NO_6P^-$	C2	☑	☑	☒	☒
205.19617	16:3-H-CO_2^-	C1		☑		☑
168.04312	(P-CHO)-CH_2-H	C0	☑	☑	☒	☒
		Rank	1	2	3	4
		ID score	1.88	0.86	0.31	0.01
		Grade	H1 and S1 → A	H2 → C	D	D

Figure 4. The gradation of isomeric lipid species based on the fragmentation spectra for confident annotation.

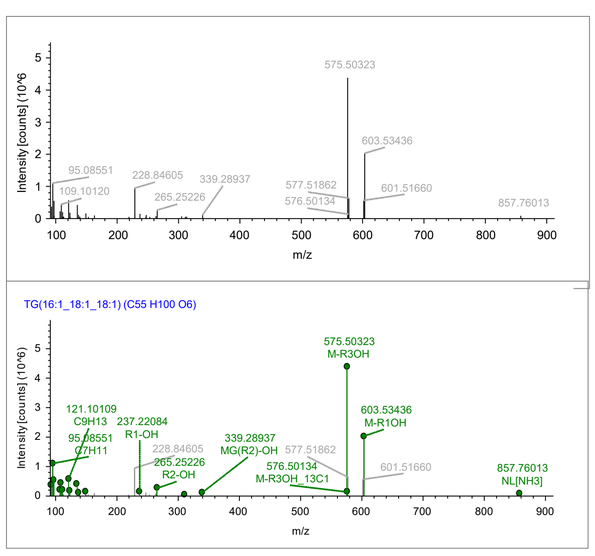

Figure 5. Annotation of fragments in the MS2 spectra by LipidSearch software to help improve annotation confidence. The above spectra is for TG(16:1_18:1_18:1)

Adduct Ions

Adduct ions are formed by the interaction of a precursor ion with one or more atoms or molecules to form an ion containing all the constituent atoms for the precursor ion as well as the additional atoms from the associated atoms or molecules. Common lipid adducts include +Na, +K, $-H_20$ etc. depending on the lipid class.

In-source or Unintentional Fragmentation

In-source or unintentional fragmentation refers to the breakdown of ions into smaller fragments within the instrument's ion source or ion path. This phenomenon occurs before the ions are analyzed in the mass analyzer leading to complex MS1 spectra.

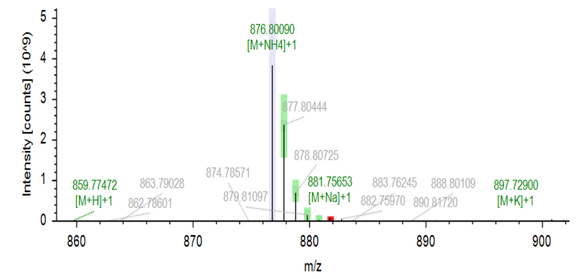

Figure 6. Compound Discoverer software is able to detect all the adduct forms and group them together. The above spectra shows TG(16:1_18:1_18:1) with the isotopes and adducts detected by Compound Discoverer 3.4 software.

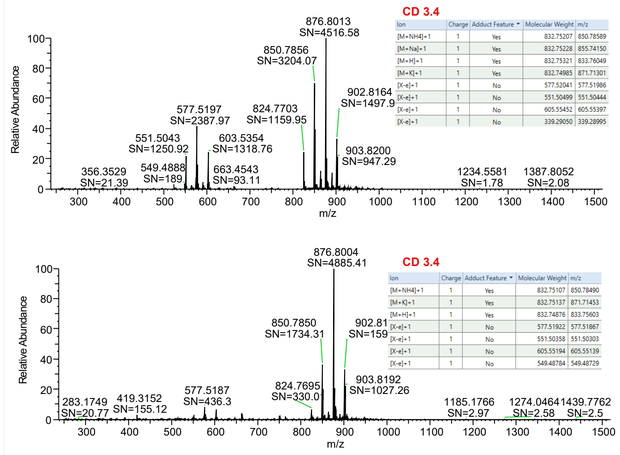

Figure 7. The legacy full scan in Orbitrap Excedion Pro MS shows lower unintentional fragmentation compared to Orbitrap Astral mass spectrometer MS1 scan. The new version of Compound Discoverer 3.4 software can map these unintentional fragments and other adducts to reduce false annotation. The spectra of TG(16:0_16:0_18:1) is shown above.

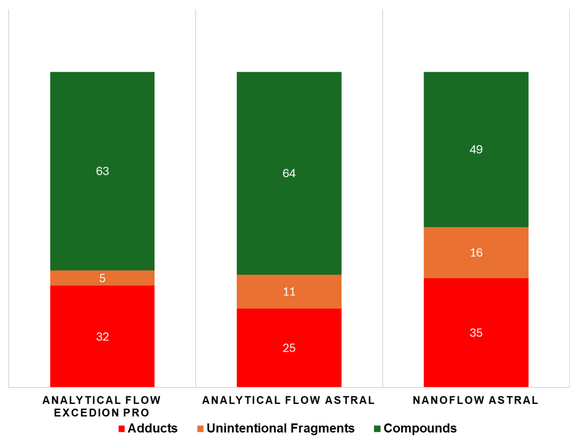

Figure 8. Distribution of adducts, MS1 fragments and compounds as percentage of total features found by Compound Discoverer 3.4 software.

Conclusion

Compound Discoverer 3.4 software with LipidSearch software aids in confident annotation of lipids. Data analysis shows only 60% of all features detected in a chromatographic run are actual compounds with the rest of the features being adducts and MS1 fragments.

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