Poster Note 90117

Rapid Phospholipid Characterization Using a Novel Intelligent Workflow on a Tribrid Orbitrap Mass Spectrometer

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Overview

Purpose: Develop an intelligent and comprehensive LC/MS^n workflow which enables high throughput, high confidence lipid characterization and simultaneous quantitation by employing multiple lipid precursor ion dissociation techniques (HCD MS^2, CID MS^2 and CID MS^3) within a single LC/MS run on a new Tribrid Orbitrap mass spectrometer.

Methods: The new Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer connected with a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system is used for the method development. A Thermo Scientific™ Accucore™ C18 column is used for the lipid extract separation using a 28 min gradient. For instrument method set up, two different experiment designs are used based on the retention time range. During 0 min to 18 min, alternate positive data-dependent (dd) HCD MS^2 and negative dd HCD MS^2 data are acquired. A further positive CID MS^2 data is collected if a diagnostic phosphatidylcholine (PC) fragment ion (*m/z* 184.0733) is detected from a positive HCD MS^2 scan. During 18 min to 28 min, positive dd MS^2 data is collected and further CID MS^3 data are collected for top three HCD fragment ions if fatty acid neutral losses are detected from a HCD MS^2 scan.

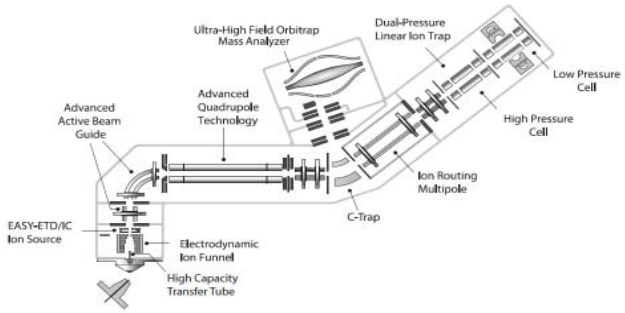
Results: The high-throughput, intelligent instrument method was used for a lipid profiling experiment of bovine heart lipid extracts. The number of identified molecular lipids were compared with those observed from other approaches including positive dd HCD MS^2, alternate positive dd HCD MS^2 and negative dd HCD MS^2, separate positive HCD MS^2 and negative dd HCD MS^2 analyses. The new intelligent workflow identified the most molecular lipids among all four different approaches by using comprehensive fragment ion information from the HCD MS^2 and product-dependent CID MS^2, MS^3.

Introduction

High energy HCD MS^2 is widely used for the characterization of lipid extracts. Both positive and negative HCD MS/MS data are often required to fully characterize individual molecular species, such as PCs. It takes extra instrument time to collect both positive and negative MS/MS data and the ionization efficiency in the negative ion mode is generally lower compared to the positive ion mode. In addition, CID MS^3 data are often needed for identifying the triglyceride (TG) molecular species if several TG isomers co-elute.

The Orbitrap Fusion MS architecture is based on a mass resolving quadrupole, a collision cell, an Orbitrap mass analyzer, and a linear ion trap mass analyzer (Q-OT-qIT) (Figure 1). This revolutionary architecture enables multiple dissociation techniques, including HCD and CID, to be performed at any fragmentation stage, followed by analysis in either the linear ion trap or Orbitrap mass analyzer. Taking advantage of this unique architecture, we developed a novel workflow on the new Orbitrap Fusion Lumos mass spectrometer which uses HCD MS^2 for characterization of most lipid classes and complementary CID MS^2 or CID MS^3 to enhance and improve the coverage for characterization of PC and TG molecular lipids. Here, we report that PC and TG molecular species can be fully characterized together with other lipids within a single intelligent LC/MS^n run using this new approach.

FIGURE 1. Instrument layout of Orbitrap Fusion Lumos Tribrid Mass Spectrometer.



Methods

Sample Preparation

Bovine heart lipid extract (2.5 mg/ml) was purchased from Avanti. The stock sample was diluted to the concentration of 500 ng/µL using a solvent of IPA/MeOH (1/1).

HPLC Conditions

A Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system performed separations using the gradient conditions shown in Table 1. Mobile phase A was 60:40 Acetonitrile / Water and mobile phase B was 90:10 IPA / Acetonitrile; both A and B contained 10mM ammonium formate and 0.1% formic acid. The column was an Accucore C18 (2.1 x 150mm, 2.6µm) operated at 45°C, flow rate of 260 µL/min and the injection volume was 2 µL.

MS Conditions

An Orbitrap Fusion Lumos MS was used for general HCD MS/MS lipid profiling experiments (pos, alternate pos/neg, separate pos and neg) and the high throughput intelligent lipid profiling experiments.

An Orbitrap Fusion MS was used for general HCD MS/MS lipid profiling experiments (pos only).

Table 2 shows the MS setup for the general HCD MS/MS experiments on both Orbitrap Fusion and Orbitrap Fusion Lumos MS.

Figure 2 shows the flow chart of MS setup for the high throughput intelligent lipid profiling experiment with the Orbitrap Fusion Lumos MS.

Data Processing

Thermo Scientific™ LipidSearch™ 4.1 software was used for all data processing.

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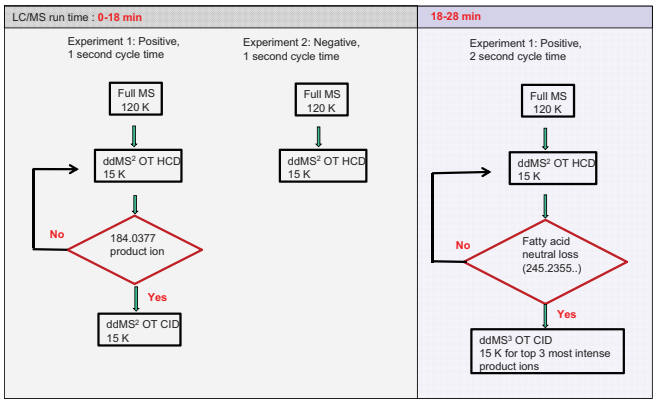
TABLE 1. HPLC Gradient.

Time	% A	% B
-3	70	30
0	70	30
2	57	43
2.1	45	55
12	35	65
18	15	85
20	0	100
25	0	100
25.1	60	40
28	60	40

TABLE 2. MS Setup for HCD MS^2 Lipid Profiling.

HESI Source	Orbitrap Fusion, Orbitrap Fusion Lumos
Sheath gas 40	Pos ion (250 - 1200 amu) Neg ion (200 - 1200 amu)
Aux gas 3	MS resolution R=120K (FWHM at m/z 200)
Spray volt. 3.5 kV	Top speed dd-MS^2 1s cycle at 15K; 2.4 cycle at 30K
RF-Lens 50	MS^2 Isolation width 1 Da
Cap. temp. 320°C	Stepped NCE – Pos. 27 ± 3 Stepped NCE – Neg. 30 ± 10
Heater temp. 350°C	AGC target 4E+5 MS, 50 ms max. 5E+4 MS2, 80 ms max for 2.4s cycle 35ms for 1s cycle

FIGURE 2. Flow Chart of High Throughput, Intelligent Lipid Profiling Workflow.



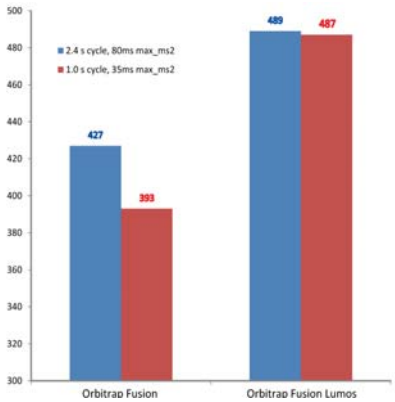
Results

Increased lipid ID coverage and sensitivity with Orbitrap Fusion Lumos MS.

By employing the new ion source and segmented quadrupole, the Orbitrap Fusion Lumos mass spectrometer allows more ions into the mass spectrometer relative to the Orbitrap Fusion mass spectrometer. Figure 3 shows more lipid species were identified from 1µg bovine heart lipid extracts with the Orbitrap Fusion Lumos MS Platform.

Due to the brighter ion source, there were no losses of lipid identifications when using shorter ion injection times with the Orbitrap Fusion Lumos MS platform (Figure 3) which allows the general dd HCD MS^2 experiment to be performed using a 1 second experiment cycle time with sufficient sensitivity and frequency for lipid profiling experiments.

FIGURE 3. Comparison of Lipid IDs with the Orbitrap Fusion MS and Orbitrap Fusion Lumos MS Instruments with 1 Second and 2.4 Second Cycle Times.



The brighter source and segmented quadrupole implemented on the Orbitrap Fusion Lumos MS allow ions to enter the MS at a higher rate, yielding good quality MS/MS data even with very short ion injection time (35 ms).

Shorter cycle times allow a higher frequency of full scans for profiling. Only Orbitrap Fusion Lumos MS has the sensitivity to maintain the identification rate when injection times are decreased to support the desired full scan rate.

The intelligent and comprehensive HPLC/MS^n workflow for increased throughput and higher confidence identification of molecular lipid species on the Orbitrap Fusion Lumos MS.

The increased sensitivity and multiple fragmentation capabilities of Orbitrap Fusion Lumos MS enabled the development of a unique, intelligent, and comprehensive LC/MS^n workflow for lipid profiling (Figure 2).

For relatively early-eluting lipids, including PCs, alternate positive and negative high resolution dd HCD MS/MS (15 K resolution) acquisitions were performed using Top Speed mode. An additional high resolution CID MS/MS acquisition was performed upon detection of a diagnostic fragment ion of the PC class (*m/z* 184.0733) from the positive HCD MS/MS run. The Top Speed cycle time was 1 second per polarity.

For later-eluting lipids, such as TG, positive HCD MS/MS (15 K resolution) acquisitions were performed using Top Speed mode. Additional CID high resolution MS3 acquisitions were performed on the three most intense fragment ions when a fatty acid neutral loss (Table 3) was detected in the positive HCD MS/MS spectrum. The frequency of full scans was 2 second.

Figure 4 shows the extracted base peak chromatograms of the bovine heart sample from both positive and negative ion modes using the intelligent workflow. High full MS scan frequency across each lipid peak enabled precise quantification (Figure 4, insert). Figure 5 shows the CID MS/MS triggered from the PC diagnostic fragment ion, providing comprehensive fatty acid side chain information that is required for determining the PC molecular species confidently.

TABLE 3. Fatty Acid Neutral Loss Inclusion List Used for Triggering CID MS^3.

FA	Formula	NL (FA + NH3)	FA	Formula	NL (FA + NH3)
(12:1)	C12 H22 O2	215.1885	(20:3)	C20 H34 O2	323.2824
(14:0)	C14 H28 O2	245.2355	(20:4)	C20 H32 O2	321.2668
(14:1)	C14 H26 O2	243.2198	(20:5)	C20 H30 O2	319.2511
(16:0)	C16 H32 O2	273.2668	(21:0)	C21 H42 O2	343.3450
(16:1)	C16 H30 O2	271.2511	(22:0)	C22 H44 O2	357.3607
(16:2)	C16 H28 O2	269.2355	(22:1)	C22 H42 O2	355.3450
(17:0)	C17 H34 O2	287.2824	(22:2)	C22 H40 O2	353.3294
(18:0)	C18 H36 O2	301.2981	(22:3)	C22 H38 O2	351.3137
(18:1)	C18 H34 O2	299.2824	(22:4)	C22 H36 O2	349.2981
(18:2)	C18 H32 O2	297.2668	(22:5)	C22 H34 O2	347.2824
(18:3)	C18 H30 O2	295.2511	(22:6)	C22 H32 O2	345.2668
(19:0)	C19 H38 O2	315.3137	(23:0)	C23 H46 O2	371.3763
(20:0)	C20 H40 O2	329.3294	(24:0)	C24 H48 O2	385.3920
(20:1)	C20 H38 O2	327.3137	(24:1)	C24 H46 O2	383.3763
(20:2)	C20 H36 O2	325.2981	(26:0)	C26 H52 O2	413.4233

FIGURE 4. Base Peak Chromatograms of Positive and Negative Ion Modes of Bovine Heart Lipid Extracts in a single run using the novel Intelligent Workflow.

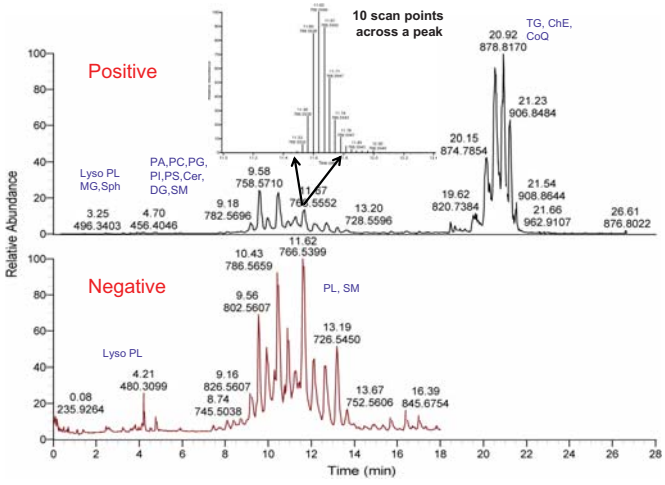
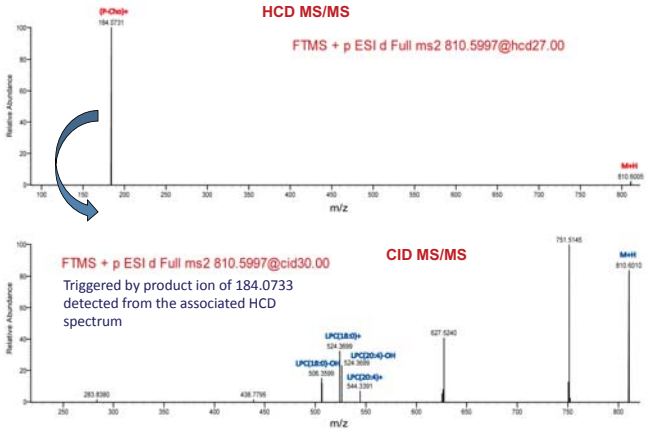


FIGURE 5. Comprehensive Positive Fragment Ion Information from the HCD and product dependent CID MS² Identified the PC species as PC (18:0_20:4).



To evaluate the improvement in throughput for lipid molecular species identification using the novel intelligent workflow, the same bovine heart sample was analyzed using three standard HCD MS/MS approaches (Positive only, Alternate Positive/Negative in a single run, Positive and Negative in separate runs). All raw data were processed using the Lipid Search 4.1 software. LipidSearch software was able to automatically recognize and use the comprehensive fragment ion information from the HCD MS^2, CID MS^2 and CID MS^3, from the novel intelligent workflow, for characterization of individual lipid species (Figure 6). Figure 7 shows the comparison results of lipid identifications from the 4 different approaches. As expected, the intelligent workflow identified and characterized the most lipid species in a single LC/MS run compared with the two alternative single run approaches. When comparing the lipid identification results with the separate positive and negative LC/MS runs, the new workflow was still able to characterize a comparable number of lipid molecular species in a single run, yielding 2 times higher throughput and also higher confidence especially for TG characterization by using comprehensive CID MS^2 and CID MS^3 fragment ion information.

FIGURE 6. Lipid Search Software Automatically Uses Comprehensive Fragment Ion formation from Multiple Fragmentation Techniques for Molecular Lipid IDs.

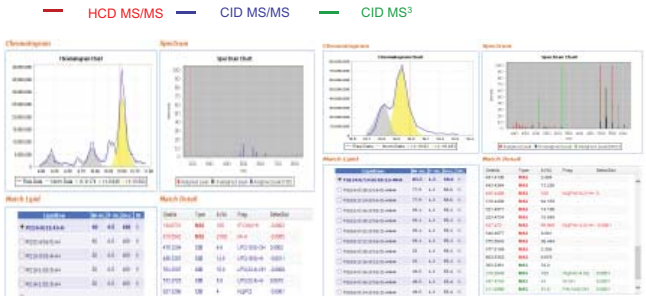
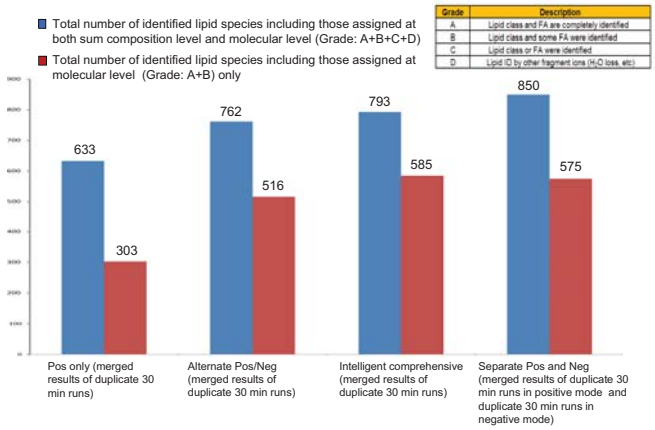


FIGURE 7. Comparison of Identified Lipid Species Across Four Different Approaches on the Orbitrap Fusion Lumos MS.



Conclusion

- Due to the increased sensitivity, the new Orbitrap Fusion Lumos MS allows use of lower ion injection times to collect MS2 data, yielding faster cycle times without significantly losing sensitivity for lipid identification and quantification.
- The new intelligent comprehensive LC/MS^n workflow with the Orbitrap Fusion Lumos MS enables confident molecular lipid ID using a single run.
- The new workflow provided the highest number of lipid identifications compared to positive ion only and alternating positive/negative ion runs.
- The comprehensive workflow provided a comparable number of molecular lipid identifications compared to merged search results from separate positive and negative LC/MS/MS workflows, yielding two times higher throughput.
- The unique combination of HCD MS2 and CID MS3 data enables higher confidence for identifying TG molecular isomers.

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