Application Note: 367

Essential Lipidomics Experiments Using the LTQ Orbitrap Hybrid Mass Spectrometer

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Key Words

- LTQ Orbitrap™
- Accurate mass
- High resolution
- Structural elucidation

Overview

We tested the performance of a novel mass spectrometer, the Thermo Scientific LTQ Orbitrap, in a multitude of different applications in the field of lipidomics. The experiments presented here take advantage of the LTQ Orbitrap capabilities of multiple stage fragmentation, high resolution and mass accuracy to solve challenges of quantitating isomeric glycerophospholipids, resolving isobaric species, and elucidating fragmentation pathways and compositional assignment of various lipid species.

Introduction

Lipids are exceptionally diverse in their structural, chemical, and physical properties. A large number of lipids of various classes are present in the cell membranes. That said, the compositional differences between various membranes, membrane compartments, and microdomains are quantitative, rather than qualitative.

The LTQ Orbitrap hybrid mass spectrometer system, coupled to automated nanoflow chip-based electrospray ionization source, is a powerful and versatile tool for lipid analysis. We demonstrated its capabilities in three areas of interest to lipidomics experts:

1. *Quantitation of isomeric phosphatidylcholines:* It has been shown that MS^3 fragmentation of phosphatidylcholine (PC) peaks on an ion trap mass spectrometer enables quantitation of the relative amounts of the positional isomers.[1] This provided hitherto the most detailed and comprehensive characterization of the molecular composition of the pool of PC species in biological extracts. The LTQ Orbitrap performs higher orders of MS analysis with similar efficiency and significantly higher mass accuracy.
2. *Resolving isobaric species:* Lipid extracts are often so complex that even within a single lipid class there are multiple species with the same nominal mass. Sufficient resolution is typically not achievable using standard triple quadrupole, ion trap or quadrupole time-of-flight instrumentation.[2] On the other hand, the ability of the LTQ Orbitrap to provide high resolution/accurate mass information is of great benefit in these complex and peculiar cases.
3. *Compositional assignment and fragmentation pathways:* Accurately determined mass of a species limits considerably the number of suggestions for its elemental composition, and hence points towards its unique chemical structure. By enabling an accurate mass assignment of the observed fragments the LTQ Orbitrap helps with elucidating fragmentation mechanisms of various lipid classes.[3]

Materials and Methods

Synthetic lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL). Liquid Chromatography grade chloroform and methanol, and ammonium acetate were purchased from Fluka (Buchs SG, Switzerland). Standards were prepared in $CHCl_3$/MeOH 1:2 (v/v) containing 5 mM ammonium acetate. Prior to the analysis samples were vortexed thoroughly and centrifuged for five minutes at 14,000 rpm on a Thermo Scientific IEC Centra CL5R. For the experiments, 1 µM stock solutions of glycerophospholipids and ceramides (Cer) were used.

Mass spectrometric analysis was performed on the LTQ Orbitrap (Figure 1) equipped with the automated nanospray ion source NanoMate HD (Advion BioSciences Ltd.). Ionization voltage was set to 1.05 kV, gas pressure to 0.1 psi, and the source was controlled by *Chipsoft 6.3.2* software (Advion BioSciences Ltd.). The LTQ Orbitrap was operated in negative ion mode under various resolution settings controlled by Xcalibur™ software.

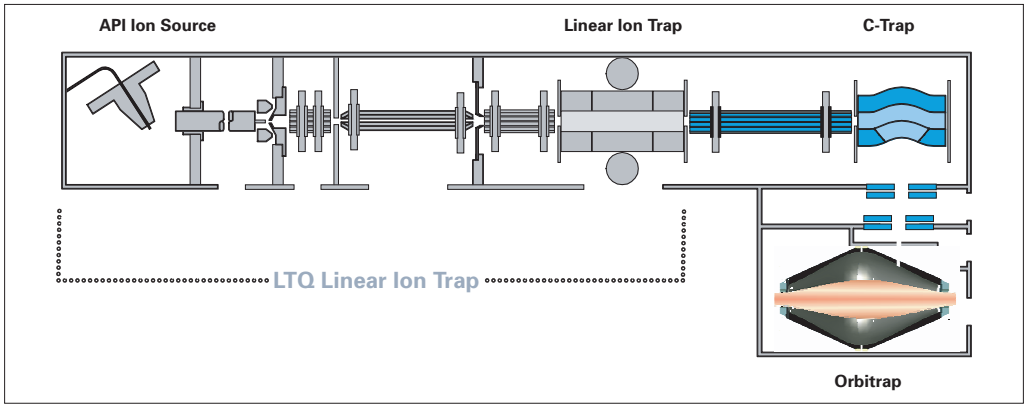

Figure 1: Diagram of the LTQ Orbitrap

Results and Discussion

The LTQ Orbitrap is a hybrid mass spectrometer consisting of two mass analyzers that can act in parallel or in series with each other. The linear ion trap provides the MS^n capability while the orbitrap determines *m/z* of precursor and fragment ions with high accuracy.

Quantitation of Isomeric PCs

Quadrupole time-of-flight analyzers that deliver reasonably accurate mass measurements have been used for phospholipid profiling.[4] However, it was the ability of ion traps to do multiple stage fragmentation (MS^3) that revealed the differential positional specificity of fragmentation pathways and its utility for relative quantitation of isobaric and isomeric PC species.[1] Patterns of PCs fragmentation pathways in the linear trap and conventional 3D trap are similar. After the loss of the methyl group from the choline part of the molecule in the first fragmentation event (MS/MS, Figure 2a), the MS^3 (Figure 2b) spectrum reveals that the neutral loss of fatty acid as ketene accounts for ~17% of the total intensity of fragment ions and occurs prevailingly by the cleavage of *sn-2* fatty acid. Acyl anion fragments are abundant in the MS^3 spectra (~83% of the total fragment intensity) and are mostly (although not exclusively) derived from *sn-2* fatty acid (~72% of intensity of acyl anion fragments; Figure 2). The highly selective and structurally informative MS^3 spectra were used for relative quantification of these isomeric PCs (Figure 3) without the need for an internal standard.

Resolving Isobaric Species

The lipid class of phosphatidylethanolamines (PE) comprises species containing fatty acids linked to glycerol backbone either by ester or ether bonds. It is not straightforward to distinguish those in mixtures.[2] Taking advantage of the high resolution and mass accuracy of the LTQ Orbitrap we were able to distinguish the isobaric PE ether species with 7 double bonds (which are common in several model organisms, such as *C. elegans*) from the PE ester species with two saturated fatty acid moieties having a mass difference of 0.0575 Da (Figure 4). In such a case, the resolving power of quadrupole time-of-flight instruments (ca. 10,000 FWHM) is insufficient to resolve the corresponding peaks if they were simultaneously present. As a minimum, a resolving power of 30,000 (FWHM) is required (Figure 5). It is worth pointing out that at this resolution of the LTQ Orbitrap the speed of data acquisition is still compatible with chromatographic separation, if required.

In MS^n experiments the high resolution of the LTQ Orbitrap allows you to distinguish between the acyl ions of FA 18:0 and the result of CO_2 loss from the acyl anion of FA 22:6 (Figure 6). This is very useful for identifying the individual fatty acyl residues, and confirming that the sample was indeed a mixture of two isobaric PE species. Note that, otherwise, the unequivocal assignment of species would have been impossible since these molecules are isobaric and the ether bond cleavage in PE O-16:1p/22:6 resulted in only very low intensity signals under low energy CID conditions.

Compositional Assignment and Fragmentation Pathways

The accurate determination of molecular and fragment ion masses can assist greatly in assigning the elemental composition of an unknown compound, and consequently help with chemical structure determination. The mass accuracy of the fragment ion Cer 18:1;2/24:1;0 measured on the LTQ Orbitrap was all within 3 ppm (Figure 7). That enabled an unambiguous assignment of chemical structures for the observed fragment ions, and helped outline the fragmentation pathways of the ceramide molecule.

Conclusions

The LTQ Orbitrap is a novel mass spectrometer capable of delivering high resolution and high mass accuracy.

- The MS^n capability proved essential for identifying positional isomers of PCs in mixtures.
- It also allowed for the relative quantitation of isomeric PCs in a mixture without internal standards.
- Resolving power of at least 30,000 is needed in the full scan mode to resolve isobaric species of glycerophospholipids.

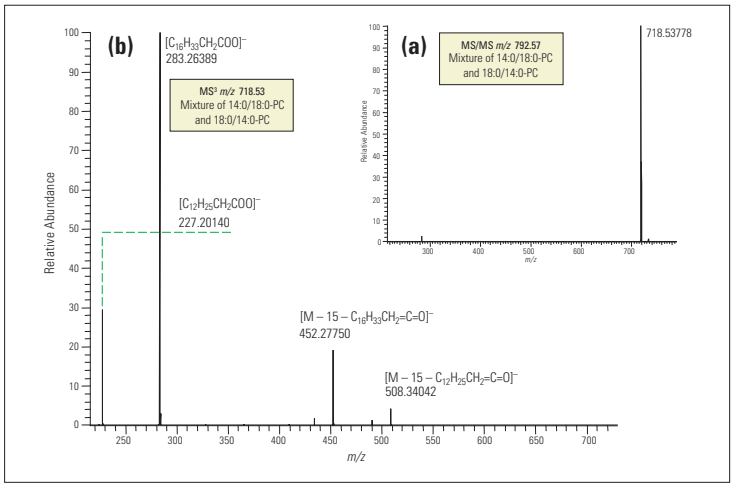

Figure 2: Relative quantitation of two positional isomers, 14:0/18:0-PC and 18:0/14:0-PC, in a mixture.
a: MS/MS spectrum of *m/z* 792.57 (acetate adduct). Abundant fragment at *m/z* 718.54 corresponds to demethylated PC $[M–15]^-$.
b: MS^3 spectrum of *m/z* 718.54. The $[M-15]^-$ ions undergo futher fragmentation by neutral loss of ketenes giving $[M–15–C_{12}H_{25}CH_2{=}C{=}O]^-$ (*m/z* 508.34) and $[M–15–C_{16}H_{33}CH_2{=}C{=}O]^-$ (*m/z* 452.28), and by yielding acyl anions of stearic (*m/z* 283.26; C18:0) and myristic (*m/z* 227.20; C14:0) acids directly.

- The presence of isobaric species was also confirmed in MS/MS fragmentation spectrum acquired at high resolution.
- The mass accuracy of the measurement in MS and MS^n mode was always better than 3.5 ppm for either parent or fragment ions applying external calibration.
- The time scale for acquiring such high quality data is compatible with on-line chromatographic separation.

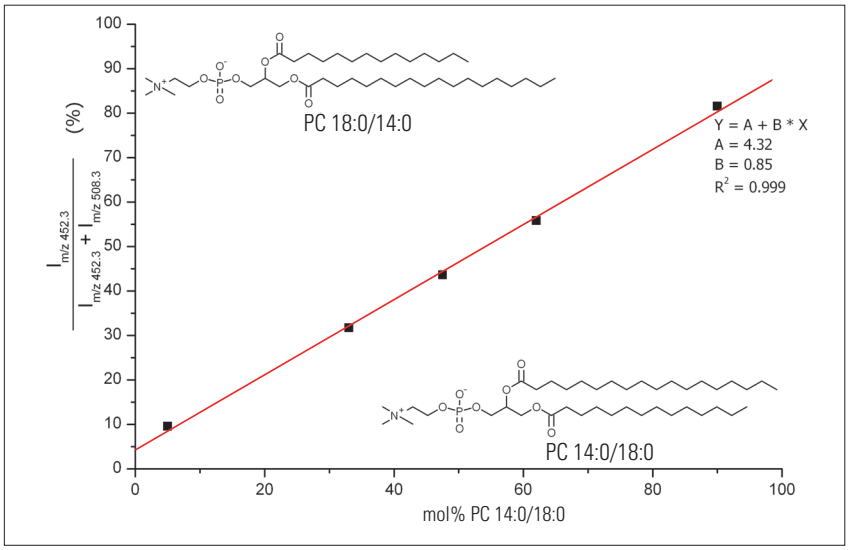

Figure 3: LTQ Orbitrap MS^3 response as a function of mol % PC 14:0-18:0 isomers

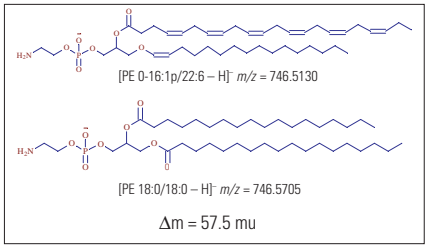

Figure 4: Structures and mass difference between isobaric phosphatidylethanolamine species PE O-16:1p/22:6 and PE 18:0/18:0

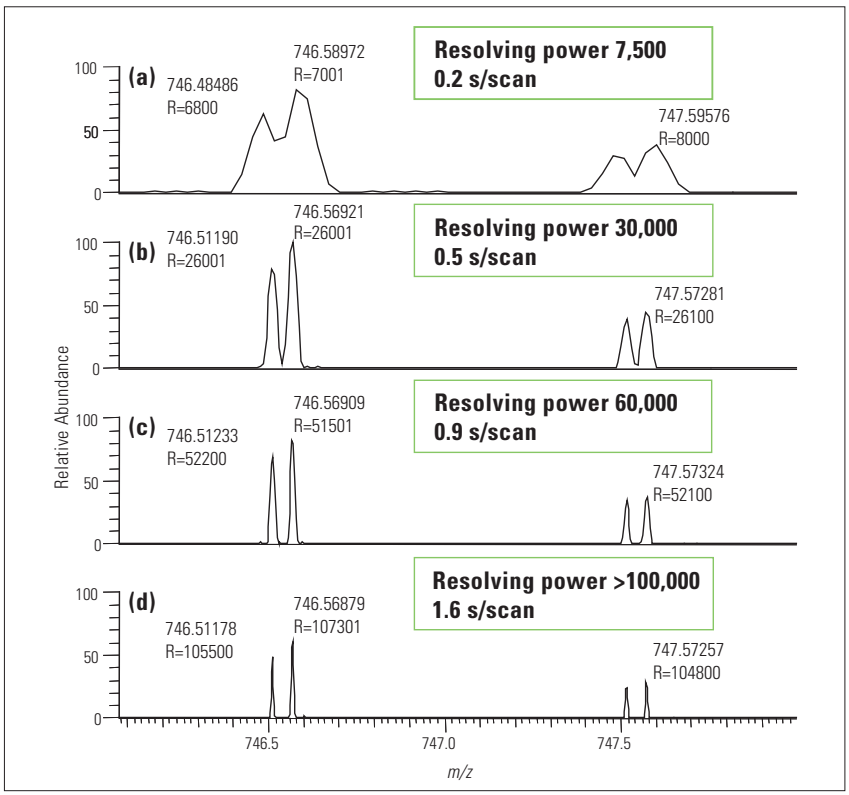

Figure 5: LTQ Orbitrap analysis of isobaric species PE 18:0/18:0 and PE O-16:1p/22:6. The time needed for performing one scan at given resolution settings is highlighted in boxes

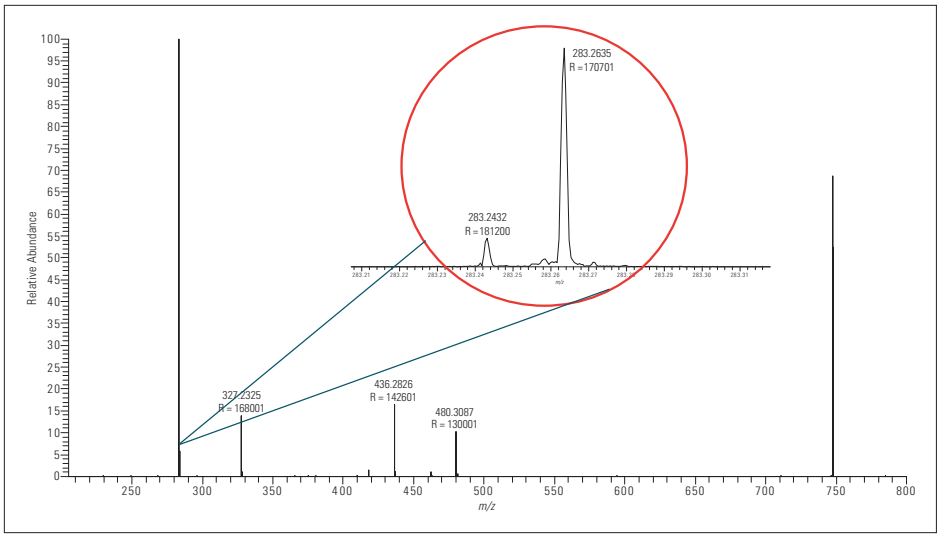

Figure 6: MS/MS spectrum of a mixture of PE 18:0/18:0 and PE O-16:1p/22:6 (*m/z* 746.57) acquired at 100,000 resolution settings. Inset: Zooming onto the region *m/z* 283 reveals fragments that confirm the presence of two isobaric species

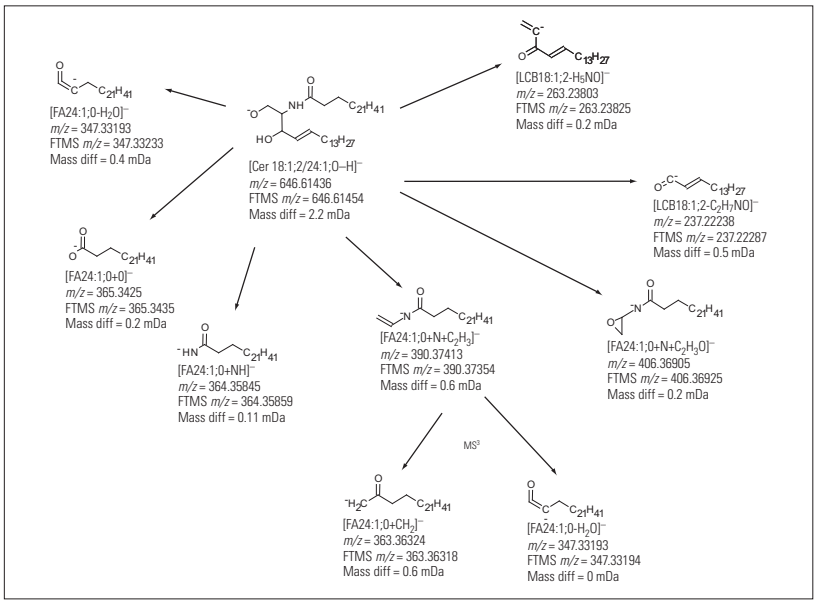

Figure 7: Fragmentation pathways suggested for Cer 18:1;2/24:1;0. The notation stands for a ceramide molecule whose long chain base (LCB) contains 18 carbon atoms, one double bond and two hydroxyl groups, whereas its N-linked fatty acid moiety contains 24 carbon atoms and one double bond. Note the close agreement between theoretical and measured masses of all detected fragments.

References

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