

Analysis of whole lipid extracts using on-line high resolution LC-MS

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Overview

Purpose: Analysis of a complex lipid extract using high resolution accurate mass LC-MS with fast polarity switching and HCD fragmentation.

Methods: On-line LC-MS using a stand-alone Orbitrap mass spectrometer.

Results: Accurate mass data of different lipid species within various different lipid classes can be obtained in both positive and negative ionization modes in a single LC-MS experiment.

Introduction

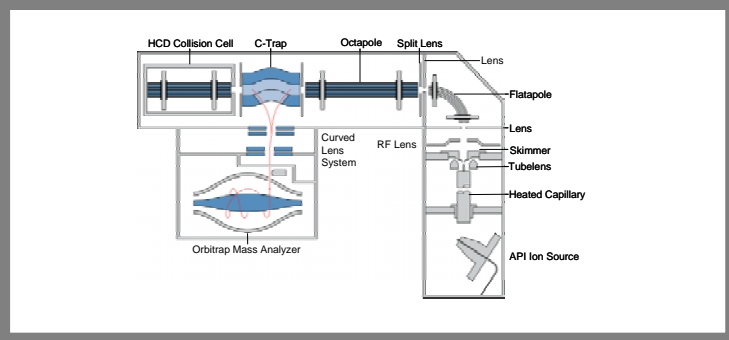
The analysis of lipids presents an analytical challenge for mass spectrometry due to the complexity and variety of lipid classes. Electrospray ionization both in positive and in negative ionization mode has been shown to be a useful method for structural studies of lipids and especially for phospholipids because of their zwitterionic composition. Information can be obtained regarding the molecular weight, the acid moieties and the residue attached to the phosphoric acid.

Some lipid classes such as phosphatidylcholines (PC) are best analyzed in negative ionization mode whereas most other lipid classes are best ionized in positive mode. Nevertheless, both ionization modes deliver complementary information and therefore, in order to save time, it is beneficial to run the mass spectrometer in alternating positive / negative ionization mode. The non hybrid benchtop Orbitrap™ mass spectrometer is capable of providing a full cycle (one positive and one negative high resolution full scan) in less than one second while maintaining precise mass accuracy. In addition the instrument can perform CID experiments in the HCD collision cell delivering accurate fragment mass information for structural analysis.

Methods

All on-line LC-MS Experiments were performed on an Exactive™ mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an Accela™ HPLC system (Thermo Fisher Scientific, San Jose, CA) using a 5 µm 150x2.1 mm Hypersil Silica Column (Thermo Fisher Scientific, USA) at a flow rate of 300 µL/min. Solvents: A – CHCl₃ / MeOH / 80:20, 5 mM NH₄OAc, B – CHCl₃ / MeOH / H₂O 60:34:6, 5 mM NH₄OAc. Gradient: 0–2 min 20% B, 2–10 min 20% B – 100% B, 10–18 min 100% B, 18–19 min 100% B – 20% B, 19–27 min 20% B.

FIGURE 1. Schematic layout of the Exactive instrument



The mass spectrometer was operated with standard electrospray ionization mode with alternating full scan and CID fragment ion scans carried out in the HCD collision cell (see Figure 1). Resolution settings of 100,000 were applied in both polarity modes. For the fast polarity switching experiments a resolution setting of 25,000 was used to achieve a full cycle (one positive full scan and one negative full scan) in less than one second.

Results

As can be seen from the total ion chromatograms generated in both ionization mode polarities (Figure 2 and 3), the chromatographic conditions employed allow good separation of the different lipid classes but not complete separation of the different lipid species within one lipid class.

FIGURE 2. Base peak chromatogram of the lipid extract in positive ionization mode.

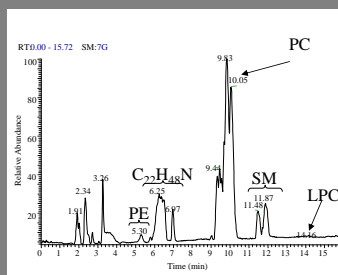
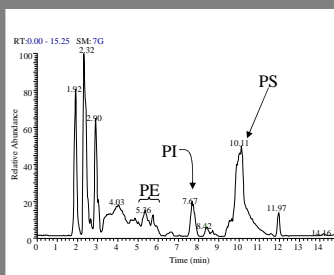


FIGURE 3. Base peak chromatogram of the lipid extract in negative ionization mode.



Therefore, resolution settings of up to 100,000 were used in both polarity modes to ensure optimum mass separation of these complex extracts. Mass accuracies in the low to sub ppm range were obtained without averaging data and were used to identify and confirm the different lipids in each scan. Due to the complexity of the extracts, with many isobaric compounds amongst the different lipid classes, it is essential to obtain fragmentation information in addition to the full scan accurate mass data. This allows confirmation of the identity of the different lipid classes and furthermore to determine the fatty acid pattern within these lipid classes.

The precise mass accuracy obtained from HCD fragmentation data provides the ability to generate pseudo parent ion mass chromatograms which are ideally suited to confirm specific lipid classes. In combination with high resolution (key for separation of interferences) the applied narrow mass tolerance window (as a result of stable accurate masses) ensures correct identification of the lipid classes even for very complex samples. Examples for the benefits of these instrumental features are shown using complex natural lipid extracts.

FIGURE 4. Positive full scan mass spectrum of the PE section taken at RT 5.3 min and list of identified PEs obtained from the positive full scan experiment.

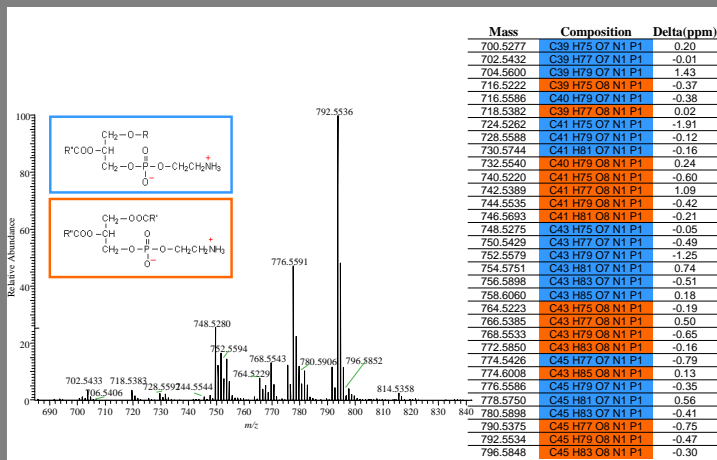


FIGURE 5. Positive HCD MS/MS spectrum of the PEs at RT 5.3 minutes

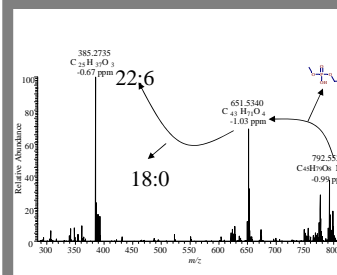
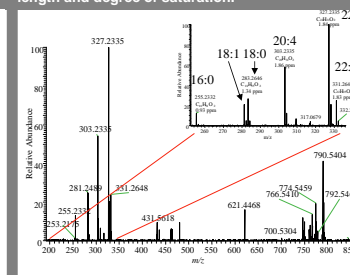


FIGURE 6. Negative HCD MS/MS spectrum of the PEs at RT 5.3 minutes. Inset shows the fatty acid region with assignment of the fatty acids chain length and degree of saturation.

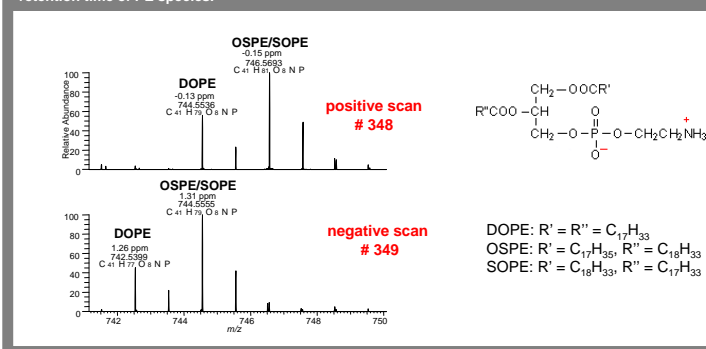


Detailed structural analysis of the phosphatidylethanolamines (PE)

Within the different lipid classes it is difficult to separate individual species by LC. The lack of chromatographic separation is compensated for by high resolution in the mass spectrometer with the full scan and HCD fragmentation scans. We used a resolution setting of 100,000 for the alternating full scan / HCD scan experiment and performed separate runs in each polarity mode.

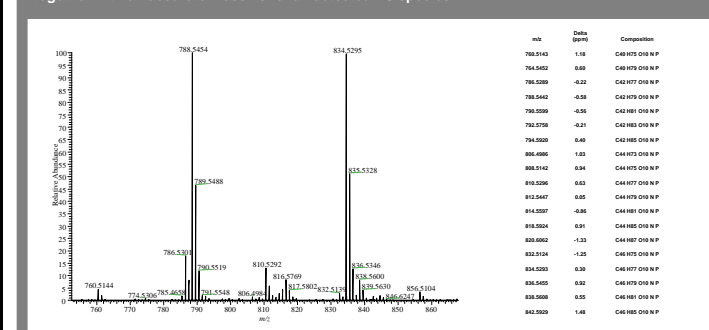
As can be seen from the mass spectrum of the PE section of the lipid extract, a number of different PEs co-elute (Figure 4). Using the accurate masses for the determination of the elemental composition and subsequently for the identification of the different PE species, we found a number of PEs with one ether function in the molecule (Figure 4, blue masses). As can be seen from the list of PEs in Figure 4, all compounds were identified with a mass error of less than 2 ppm. Similar results were obtained from the full scan data taken in negative ionization mode (showing [M-H]⁻, table not shown) and ensured that all detected species within the lipid class. All HCD spectra showed similar mass accuracy as the full scan spectra (Figure 5 and 6). Mass deviations of less than 2 ppm were routinely obtained using external mass calibration. The fragmentation pathway can be easily confirmed with the use of the accurate masses of the fragments allowing the determination of the elemental composition of the fragments in both polarity modes.

FIGURE 7. Example of alternating positive and negative scan during fast polarity switching at the retention time of PE species.



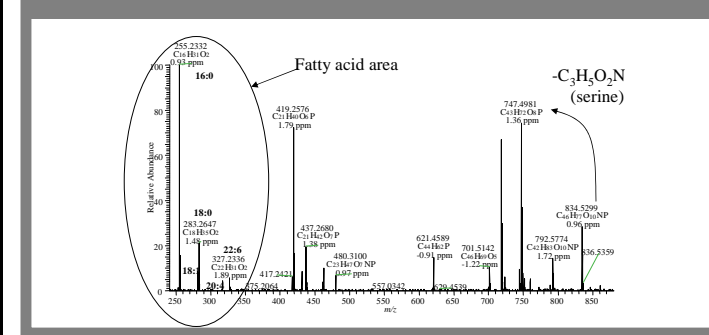
In order to obtain all the accurate mass information for lipids in positive and negative ion mode, the instrument was operated in the fast polarity switching mode. Two consecutive scans show the [M+H]⁺ and [M-H]⁻ ion respectively for the different fatty acid containing phosphatidylethanolamines (Figure 7). One full cycle (one positive and one negative scan) was acquired in less than one second at 25,000 resolution. Mass accuracy was better than 3 ppm for all ions.

FIGURE 8. Negative full scan spectrum of the lipid extract recorded during the elution of the PSs together with an accurate mass list of all detected PS species.



As an example for a different lipid class the negative full scan spectrum (Figure 8) and the HCD spectrum (Figure 9) are shown for the phosphatidyl-serines (PS). Similar to the other lipid classes, the full scan data for the PSs are used to determine the accurate molecular weights and subsequently their elemental compositions (Figure 8). In the HCD scan significant fragments are seen for the loss of the serine group and for the fatty acid residues. This allows the determination of the lipid group and the distribution of the fatty acids in the PS molecules (Figure 9).

FIGURE 9. Negative HCD MS/MS spectrum of the lipid extract recorded during the elution of the PSs.



Conclusions

The Exactive is an ideal instrument for the analysis of complex lipid extracts. The ability to perform full scans MS and MS/MS scans in both polarity modes at very high resolution ensures accurate mass measurements independent of the polarity. This provides fast, precise and unambiguous identification of the lipid classes as well as the individual lipid species. Operation of the instrument in the alternating positive / negative mode saves time without sacrificing analytical performance.

Acknowledgements

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