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

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Overview

Purpose: Analysis of a complex lipid extract with high resolution and high mass accuracy including fast polarity switching and fragmentation.

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

Results: Accurate mass data of different lipid species within the different lipid classes ionizing in different polarity modes could be obtained in one single LC-MS run.

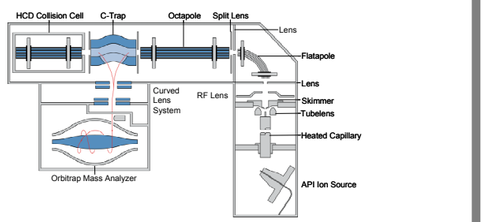
Introduction

The analysis of lipids is challenging to mass spectrometry due to its 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 structure. Information can be obtained regarding the molecular weight, the acid moieties and the residue attached to the phosphoric acid.
Some lipid classes like the phosphatidylinositols (PI) are best analyzed in negative ionization mode where most other lipid classes are best ionized in positive mode of operation. Nevertheless, both ionization modes deliver complementary information and therefore, to save time, it is beneficial to run the mass spectrometer in the alternated mode of operation. 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 high mass accuracy. In addition the instrument can perform CID experiments in the HCD collision cell delivering accurate fragment mass information which is of high value 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 um 150x2.1 mm Hypersil Silica Column (Thermo Fisher Scientific, USA) at a flow rate of 300 ul/min.
Solvents: A – $CHCl_3$ / MeOH / 80:20, 5 mM NH_4OAc, B – $CHCl_3$ / MeOH / H2O 60:34:6, 5 mM NH_4OAc.
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.
The mass spectrometer was operated with standard electrospray ionization performing 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.

FIGURE 1. Schematic layout of the instrument



Results

As can be seen from the total ion chromatograms generated in both ionization mode polarities (figures 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.
Therefore, resolution settings of up to 100,000 were used in both polarity modes to ensure the best possible mass spectrometric separation for these complex extracts. Mass accuracies in the low to sub ppm range were obtained without averaging data and were applied 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.

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

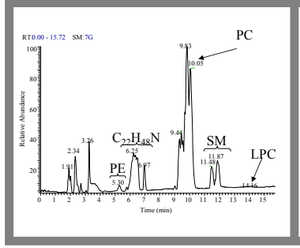
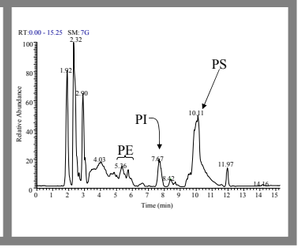


FIGURE 3. Base peak chromatogram of the lipid extract in negative ionization mode.



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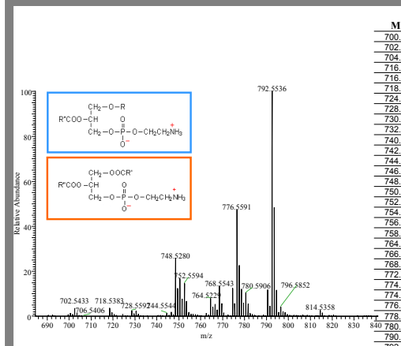
The high mass accuracy fragmentation data inherent to HCD offers the ability to generate pseudo parent ion mass chromatograms which are ideally suited to confirm the specific lipid classes. In combination with high resolution (key for separation of interferences) the applied narrow mass tolerance window (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.



Mass	Composition	Delta(ppm)
700.5277	C39 H75 O7 N1 P1	0.20
702.5432	C39 H77 O7 N1 P1	-0.01
704.5600	C39 H79 O7 N1 P1	1.43
716.5222	C39 H75 O8 N1 P1	-0.37
716.5586	C40 H79 O7 N1 P1	-0.38
718.5382	C39 H77 O8 N1 P1	0.02
724.5262	C41 H75 O7 N1 P1	-1.91
728.5588	C41 H79 O7 N1 P1	-0.12
730.5744	C41 H81 O7 N1 P1	-0.16
732.5540	C40 H79 O8 N1 P1	0.24
740.5220	C41 H75 O8 N1 P1	-0.60
742.5389	C41 H77 O8 N1 P1	1.09
744.5535	C41 H79 O8 N1 P1	-0.42
746.5693	C41 H81 O8 N1 P1	-0.21
748.5275	C43 H75 O7 N1 P1	-0.05
750.5429	C43 H77 O7 N1 P1	-0.49
752.5579	C43 H79 O7 N1 P1	-1.25
754.5751	C43 H81 O7 N1 P1	0.74
756.5898	C43 H83 O7 N1 P1	-0.51
758.6060	C43 H85 O7 N1 P1	0.18
764.5223	C43 H75 O8 N1 P1	-0.19
766.5385	C43 H77 O8 N1 P1	0.50
768.5533	C43 H79 O8 N1 P1	-0.65
772.5850	C43 H83 O8 N1 P1	-0.16
774.5426	C45 H77 O7 N1 P1	-0.79
774.6008	C43 H85 O8 N1 P1	0.13
776.5586	C45 H79 O7 N1 P1	-0.35
778.5750	C45 H81 O7 N1 P1	0.56
780.5898	C45 H83 O7 N1 P1	-0.41
790.5375	C45 H77 O8 N1 P1	-0.75
792.5534	C45 H79 O8 N1 P1	-0.47
796.5848	C45 H83 O8 N1 P1	-0.30

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

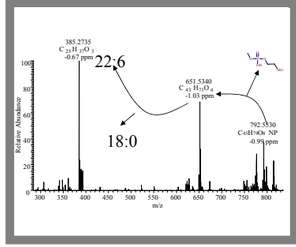
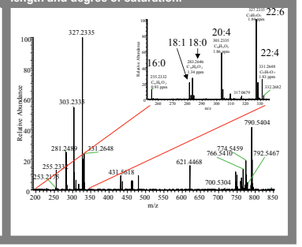
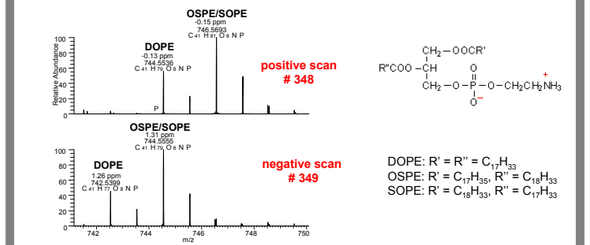


FIGURE 6. Negative HCD MS/MS spectrum of the PEs at RT 5.3 minutes. Inset shows the fatty acid region with assignment of the fatty acids chain length and degree of saturation.



Detailed structural analysis of the phosphatidylethanolamines (PE)
Within the different lipid classes it is difficult to separate by LC the individual species. 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 did find a number of PEs with one ether function in the molecule (figure 4, blue masses). In any case, as can be seen from the list of PEs in Figure 4, all compounds could be identified with a mass error of less than 2 ppm. Similar results are obtained from the full scan data taken in negative ionization mode (showing [M-H]-, table not shown) and ensures that all detected species within the lipid class.
All HCD spectra show the same good mass accuracy as the full scan spectra (figure 5 and 6). Mass deviations of less than 2 ppm are routinely obtained using external 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.

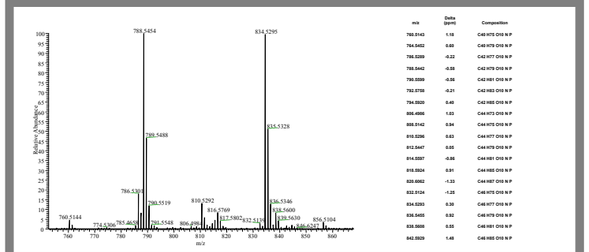


CH_2-OOCR'
$R''COO-CH$
$CH_2-O-P-O-CH_2CH_2NH_3$
O^-

DOPE: R' = R'' = $C_{17}H_{33}$
OSPE: R' = $C_{17}H_{35}$, R'' = $C_{18}H_{33}$
SOPE: R' = $C_{18}H_{33}$, R'' = $C_{17}H_{33}$

In order to obtain all the accurate mass information of lipids ionizing in the positive as well as in the 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 well below 3 ppm for all ions.

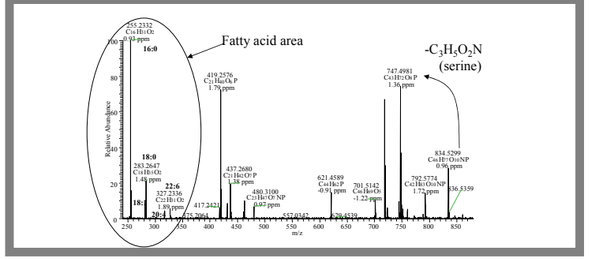
FIGURE 8. Negative full scan spectrum of the lipid extract recorded during the elution of the PSs together with an accurate mass list of all detected PS species.



m/z	Delta (ppm)	Composition
760.5143	1.18	C40 H75 O10 N P
764.5452	0.60	C40 H79 O10 N P
786.5289	-0.22	C42 H77 O10 N P
788.5442	-0.58	C42 H79 O10 N P
790.5599	-0.58	C42 H81 O10 N P
792.5755	-0.21	C42 H83 O10 N P
794.5920	0.40	C42 H85 O10 N P
806.4996	1.03	C44 H73 O10 N P
808.5142	0.54	C44 H75 O10 N P
810.5296	0.63	C44 H77 O10 N P
812.5447	0.65	C44 H79 O10 N P
814.5597	-0.06	C44 H81 O10 N P
816.5924	0.91	C44 H83 O10 N P
820.6062	-1.33	C44 H87 O10 N P
832.5124	-1.25	C46 H75 O10 N P
834.5283	0.36	C46 H77 O10 N P
836.5440	0.02	C46 H79 O10 N P
838.5606	0.55	C46 H81 O10 N P
842.5929	1.48	C46 H85 O10 N P

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 phosphatityl-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 (figure8). 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 and MS/MS scans in both polarity modes at very high resolution ensuring accurate masse measurements independent of the polarity allows fast, precise and unambiguous identification of the lipid classes as well as the individual lipid species. Operation of the instrument in the alternated mode of operation saves time without sacrificing analytical performance.

Acknowledgements

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