Trapped Ion Mobility Spectrometry (TIMS) for lipid quantification in combination with HILIC separation

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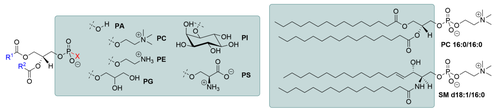
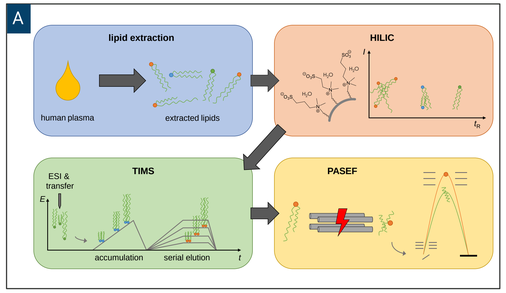
Introduction

The sum of all lipids in an organism, also called lipidome, is characterized by a variety of important functions at cellular level such as stabilizing the cell membrane and the formation of mediators. Therefore, a change in lipid composition and concentration often correlates with various neurodegenerative and cardiovascular diseases such as Alzheimer disease and atherosclerosis. An important role is assumed by polar phospholipids as a major component of the cell membrane. Due to the high progress of lipidomics research in the last decade the number of biomedical applications with lipids as biomarkers has increased significantly. Nevertheless, quantification of lipids remains analytically challenging due to the large number of lipids and their structural diversity.



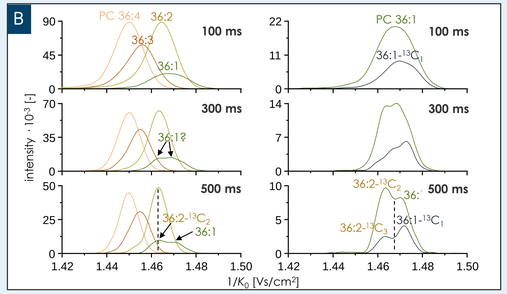
In this work, a method for lipid quantification of polar phospholipids in human plasma was developed using trapped ion mobility spectrometry (TIMS) after chromatographic lipid class separation (A).



Methods

- Sample preparation, acquisition in Münster (Germany)
- Sample: Human blood plasma (Sigma Aldrich) + SPLASH® LIPIDOMIX® (Avanti Polar Lipids)
- LC separation: HILIC column, 18 min gradient
- MS instrument: timsTOF fleX (Bruker)
- Data acquisition:
  - ESI negative
  - PASEF mode, 500 ms ramp time
- Processing in Münster: MetaboScape 2022

Results

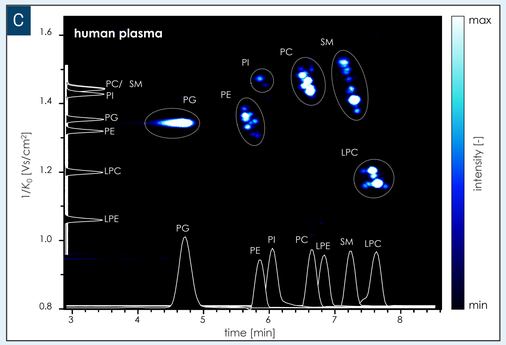


B . Type-II overlap

- Type-II overlap[1] caused from lipids with increasing double bond number due to the natural isotopic distribution of carbon isotopes.
- At high ramp times, and thus higher IMS resolution, isobaric and isomeric interferences can be identified and separated via mobility.

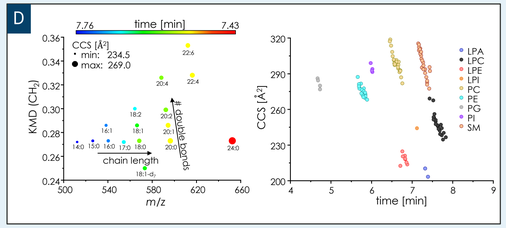
C. Separation

- Lipid class separation was achieved by hydrophilic interaction liquid chromatography (HILIC).
- The additional use of the orthogonal, postionization separation TIMS hyphenated to mass spectrometry (MS) allowed an unambiguous assignment of lipids based on mobility and retention time.



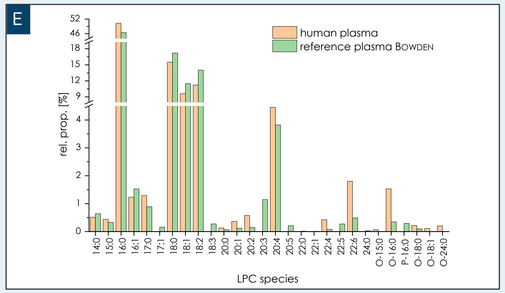
D. Annotation

- A variety of phospholipids were detected in human plasma using the MetaboScape identification software based on accurate mass, specific fragmentation, natural isotope distribution and collisional cross section (CCS) values.
- The lipid annotation could be confirmed by the lipid class-specific retention time windows and by trends in 4D Kendrick mass plots.



E. Quantitation

- For quantitative profiling of phospholipids in human plasma, an isotopically labeled internal standard (IS) per lipid class with external calibration was used. IS coeluted with the corresponding phospholipid class applying HILIC separation conditions.
- Mobilogram signals integrated as areas were used for quantification.



- For isobaric interferences, the first isotopic peak (M+1) of a lipid was used for quantification..
- The bar graph depicts the comparison of the relative proportions of lysoPC species in the investigated human plasma sample and in the extensively characterized reference plasma by BOWDEN[2] .

References

[1] M. Höring, C. S. Ejsing, S. Krautbauer, V. M. Ertl, R. Burkhardt, G. Liebisch, Journal of lipid research 2021, 62, 100050.

[2] J. A. Bowden, A. Heckert, C. Z. Ulmer, C. M. Jones, J. P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A. M. Armando, J. M. Asara et al., Journal of lipid research 2017, 58, 2275.

Conclusion

- The additional use of the orthogonal, postionization separation TIMS hyphenated to mass spectrometry (MS) allowed an unambiguous assignment of lipids based on mobility and retention time.
- Clean mobilogram signals integrated as areas were used for quantification.
- Isobaric and isomeric interferences can be identified and separated via mobility. This is especially true for type-II overlaps[1] caused from lipids with increasing double bond number due to the natural isotopic distribution of carbon isotopes.
- The lipid annotation by MetaboScape could be confirmed by the lipid class-specific retention time windows and by trends in 4D Kendrick mass plots.

4D-Lipidomics