Application of TIMS-MRMS to the study of lipids in human blood plasma

Viola Jeck, Alina Theisen, Christopher Wootton, Matthew Lewis
Bruker Daltonics GmbH and Co. KG, Fahrenheitstraße 4, 28359 Bremen, Germany

Introduction

TIMS-MRMS is a novel hyphenated technology which combines orthogonal trapped ion mobility spectrometry (TIMS) with ultra-high resolution magnetic resonance mass spectrometry (MRMS)

- Combination was applied to the analysis of lipids to determine the combined effect of TIMS and MRMS technologies on the selectivity of MS1-based quantification, tackling a major persistent challenge in the lipidomics field.
- Analytes were separated on the basis of their size/shape and mass, respectively (assuming singular charge state).
- Lipid reference standards and NIST-SRM-1950 reference plasma were used to demonstrate resolution of challenging separation scenarios including isomeric/isobaric and type-2 overlap.

Method

Experiments were conducted on a **prototype TIMS-MRMS** platform with **ExD capability** and a **7T magnet**.

- Samples were ionised using ESI and acquired in 2ω detection mode with a **resolving power** of 800k (at 400m/z).
- Gated **TIMS separation** was performed at 2.7 mbar, the voltage range was optimised for each sample, e.g. -183V to -15V, with a ramp time of 500ms split into 139 evenly sized bins for FT-ICR MS analysis.
- For **MS/MS**, species of interest were isolated in the quadrupole and subjected to ExD fragmentation in the ICR cell. Data was analysed using DataAnalysis.

TIMS

TIMS is used to provide prior separations of isomeric and isobaric lipids, providing additional benefits to selectivity that are analogous to those achieved through chromatographic separation, and quantitative mobility measurements for characterization of lipid species.

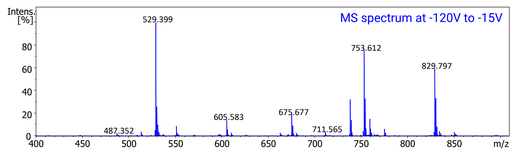


Fig. 1 Mass spectrum showing Avanti EquiSPLASH acquired with direct infusion in positive ESI ionisation mode.

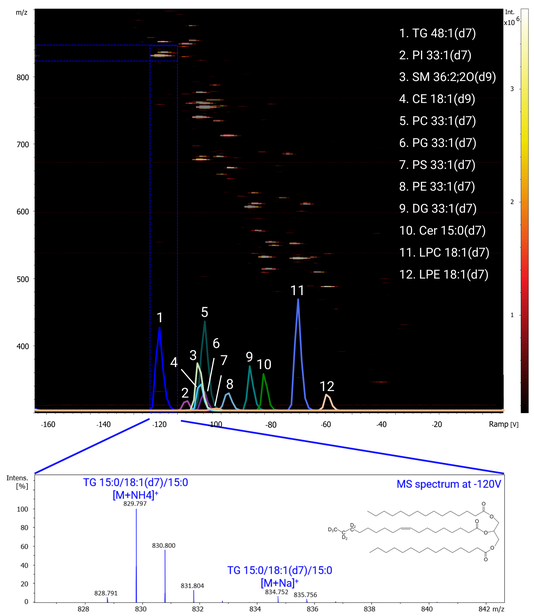


Fig. 2 Heatmap of Avanti EquiSPLASH (above) and exemplified mobility filtered mass spectrum of TG 15:0/18:1(d7)/15:0 (below).

MRMS

Here we demonstrate the ability of MRMS to resolve type-2 overlap challenges (M1+2 vs M2 isotope) which otherwise undermine confidence in selective MS1 measurement in lipidomics studies.

The phenomenon of **peak coalescence** has been described as a limiting characteristic of some FTMS instrumentation.[1] Overcoming peak coalescence is presented using MRMS. The high magnetic fields (i.e. 7-18 Tesla) make the system much more robust to peak coalescence.

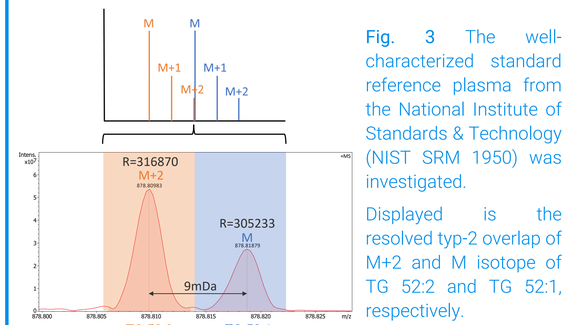


Fig. 3 The well-characterized standard reference plasma from the National Institute of Standards & Technology (NIST SRM 1950) was investigated.

Displayed is the resolved typ-2 overlap of M+2 and M isotope of TG 52:2 and TG 52:1, respectively.

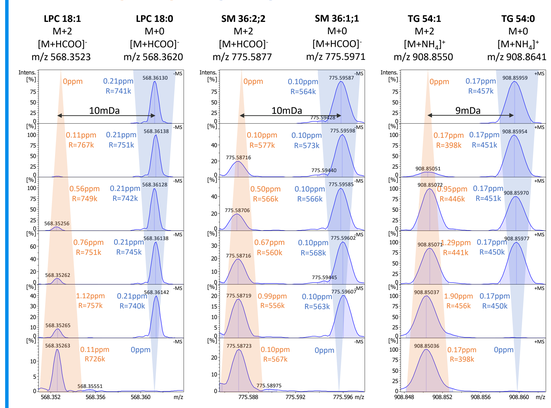
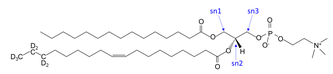


Fig. 4 Mass spectra showing the resolved typ-2 overlap of selected lipid species. Different concentration levels were measured to present the stability of the peak shape and m/z with increasing M+2 and decreasing M. (Spectra were not corrected for m/z offset.)

ExD Fragmentation

Gated TIMS and ExD analysis allows isolation of individual lipid species and unique fragmentation pathways for higher depth lipid characterization.

Fig. 5 Nomenclature of sn positions exemplified by PC 15:0/18:1(d7).



Electron-induced dissociation (EID) with MRMS has been described as analytical technique for structure characterization of lipids.[2] According to the literature we conducted EID fragmentation that enabled the generation of diagnostic fragments for the assignment of sn positions (e.g., in TG 48:1(d7) and PC 33:1(d7)). The sn position assignment and deep structure elucidation of lipids in combination with TIMS will be further pursued in future research.

References

[1] Höring M, Ejsing CS, Krautbauer S, Ertl VM, Burkhardt R, Liebisch G. J Lipid Res. 2021;62:100050. doi: 10.1016/j.jlr.2021.100050.

[2] Jones JW, Thompson CJ, Carter CL, Kane MA. J Mass Spectrom. 2015 Dec;50(12):1327-39. doi: 10.1002/jms.3698.

Conflict of interest

All contributing authors for this work are currently employed by Bruker Daltonics GmbH and Co. KG., which is a supplier of commercial FT-ICR MS/MRMS and TIMS instruments.

Conclusion

- **Novel combination of TIMS and MRMS**, applied to the analysis of common challenges in lipid analysis in complex samples.
- **TIMS** provides separations of isomeric and isobaric lipids as well as complex matrices, **providing additional benefits to selectivity** for characterization of lipid species.
- We demonstrate the ability of **MRMS to resolve type-2 overlap** challenges (M1+2 vs M2 isotope).
- Gated **TIMS and ExD** analysis allows isolation of individual lipid species and unique fragmentation pathways for higher depth lipid characterization.

Lipidomics by TIMS-MRMS