Trapped Ion Mobility Spectrometry Enables Electron-Activated FT-ICR Tandem Mass Spectrometry of Lipid Isobars in Complex Matrices

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Overview

- Mass spectrometry analysis of lipids in complex mixtures can be convoluted due to high sample heterogeneity.
- Trapped ion mobility spectrometry (TIMS) was coupled with electron-based tandem mass spectrometry (MS^2), for characterization of phytoplankton lipids.
- Coupling TIMS separation to electron induced dissociation (EID) enabled quick separation of isobaric compounds and yielded unique fragments when compared to collision induced dissociation (CID).

Introduction

- Phytoplankton lipids have potential interest as jet fuel precursors.
- Phytoplankton lipid organic solvent extraction yields a mixture of lipids, pigments, and degradation products that contain many isobaric compounds.
- Liquid chromatography (LC) separation is slow and EID can provide unique fragments as compared to CID at typical parameters.

Methods

Lipids were extracted from marine coccolithophores, cultured under controlled conditions, with dichloromethane-methanol-water. The resulting samples were diluted to 10 ppm in a 90:10 mixture of acetonitrile:water with 1-10 mM ammonium acetate and analyzed with a prototype 9.4 T timsMRMS mass spectrometer (Bruker) in positive ion mode. MS^2 was performed by locking onto the desired scan number of each corresponding TIMS elution voltage peak and quadrupole isolating. For EID, ions were irradiated for 100 ms at a cathode bias voltage of -27 V, a lens voltage of 0 V, with a cathode heating current of 1.5 A.

Figure 1: Photo and instrument schematic of a prototype timsMRMS instrument coupled to a 9.4 T magnet.[1]

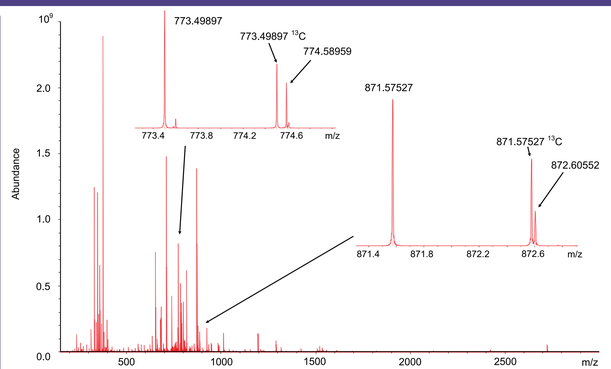

Figure 2: MS^1 of Adriatic Sea phytoplankton lipid extract. The inset spectra shows the existence of isobaric compounds in the MS^1 spectrum that cannot be isolated from each other via a traditional quadrupole.

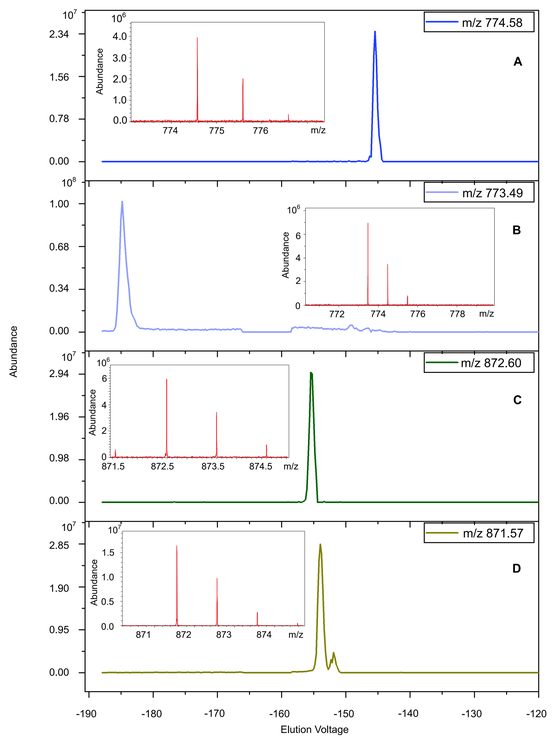

Figure 3: TIMS elution profiles of two pairs of isobaric compounds selected for MS^2 analysis. Compounds are separated based on their collision cross section (CCS). TIMS was conducted at ~2.59 mbar with a sweeping voltage ramp across a range of - 187.9 V to - 100.4 V.

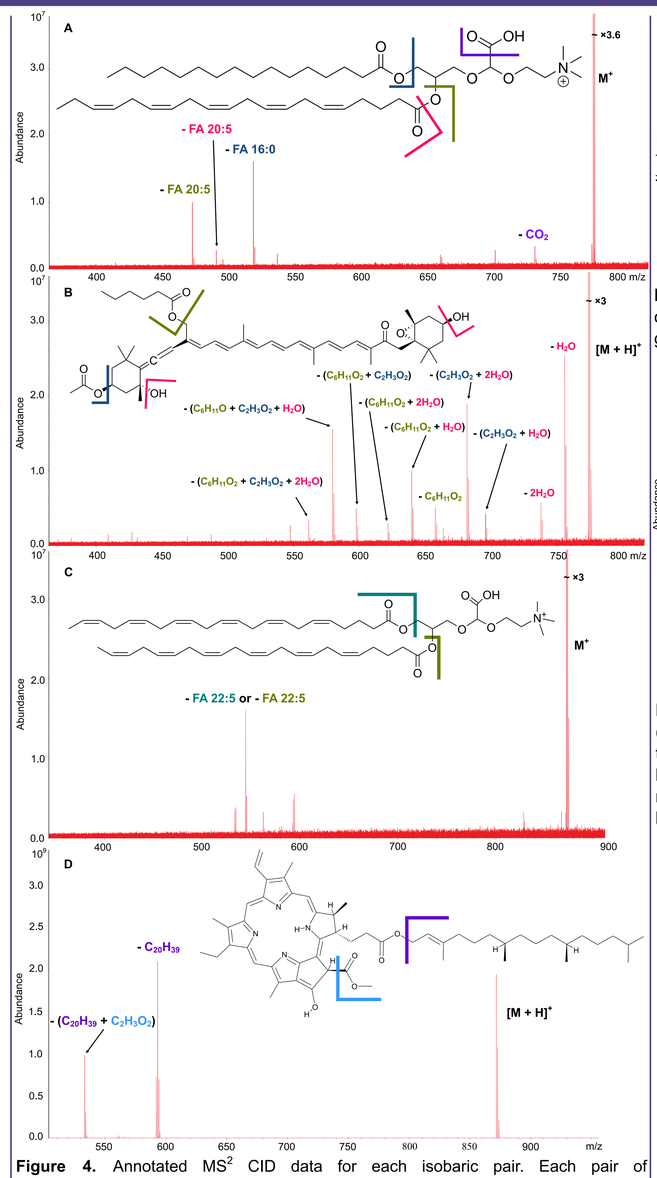

Figure 4. Annotated MS^2 CID data for each isobaric pair. Each pair of compounds contains a lipid and a pigment. Compound A was identified as 1,2-diacylglyceryl-3-*O*-carboxy-(hydroxymethyl)-choline (DGCC) 20:5/16:0. Compound B was identified as 19'-hexanoyloxyfucoxanthin, Compound C was identified as DGCC 22:5, Compound D was identified as the chlorophyl Pheophytin a.

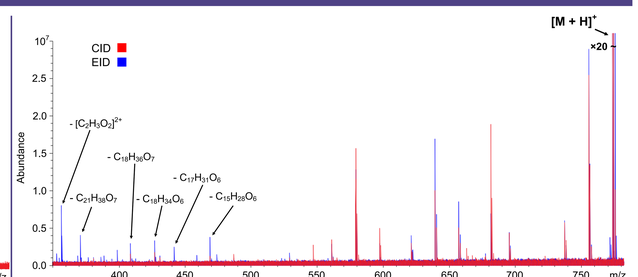

Figure 5. EID (blue trace) of 19'-hexanoyloxyfucoxanthin (m/z 773.49) overlaid with CID data (red trace). Multiple unique fragments are generated via EID that are not observed in the CID data.

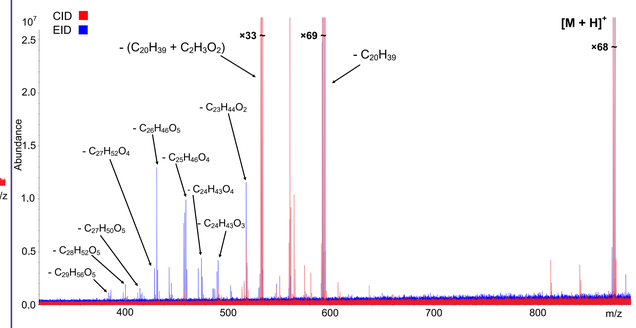

Figure 6. EID (blue trace) of Pheophytin a (m/z 871.57) overlaid with CID data (red trace). Several unique fragments were generated by EID. The m/z range of the fragments, from m/z 375 to m/z 510, indicate that they result from multiple bond cleavages within the highly conjugated chlorin ring head. These fragments are not observed in CID, likely because the bonds are not vibrationally labile enough to be broken with collision energy alone.

Conclusions

Overall, TIMS enables separation of isobaric lipid and pigment species in complex phytoplankton samples. Coupling TIMS with EID enables greater structural identification of each isobaric species compared with CID.

References

1: Wootton, C. A.; Maillard, J.; Theisen, A.; Brabeck, G. F.; Schat, C. L.; Rüger, C. P.; Afonso, C.; Giusti, P. A Gated TIMS FTICR MS Instrument to Decipher Isomeric Content of Complex Organic Mixtures. *Anal. Chem.* **2024**, *96* (28), 11343–11352.

Acknowledgments

Alina Theisen is thanked for insightful discussion. This work was performed at the Ion Cyclotron Resonance User Facility at the National High Magnetic Field Laboratory at Florida State University, supported by the National Science Foundation Divisions of Materials Research and Chemistry (DMR-2128556), and the State of Florida The research project was supported by National Science Foundation grant CHE2528759 to K. H. COI: *C.A.W. is an employee of Bruker Daltonics GmbH & Co., which is a supplier of commercial TIMS-FT-ICR instruments.*

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