Technology advancements

Pushing the limits of structural lipidomics: enhanced sensitivity and MS³-driven annotation on Orbitrap Tribrid Apex Small Molecule MS

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Abstract

Purpose: In this study, a Thermo Scientific™ Orbitrap™ Tribrid™ Apex Small Molecule Mass Spectrometer, is assessed for use in structural lipidomics using multiple fragmentation techniques including ultraviolet photodissociation (UVPD).

Methods: Preliminary analyses were done using Total Lipid Extract (TLE) from Bovine Liver (Avanti) on Orbitrap Tribrid Apex Small Molecule MS. This was followed up with analysis of Lipid Extract from the flower and parts of the plant *Agapanthus.*

Result: Resolution of isomers and annotation of unknown lipid species is shown.

Introduction

Advances in lipidomic analysis require mass spectrometry deliver high sensitivity, rapid acquisition, and efficient parallel use of multiple mass analyzers. Orbitrap Tribrid Apex Small Molecule MS was evaluated to meet these demands through improved sensitivity, faster polarity switching, and enhanced parallelization between the Orbitrap analyzer and linear ion trap analyzer. High-resolution MS enables effective resolution of isobaric lipid species, while alternative fragmentation strategies with rapid MS^n, supports confident lipid identification and structural annotation of lipid classes. The parallel operation of the Orbitrap analyzer and linear ion trap analyzer minimizes duty-cycle limitations while maximizing data density and throughput. Leveraging these instrument enhancements, robust lipidomics workflows were demonstrated through method evaluation with lipid reference materials and application to complex biological samples from plants.

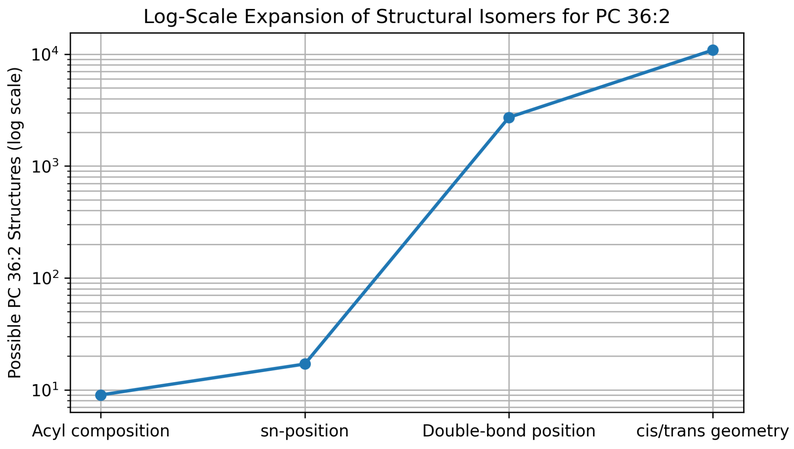


Figure 1. Structural diversity within a single lipid annotation. For example, the lipid with the sum composition of PC (36:2) comprises of thousands of isomeric species.

Structural diversity and isomers

Lipids exhibit extraordinary structural diversity, encompassing thousands of molecular species that vary in headgroups, acyl chain length, degree of unsaturation, stereochemistry, and chemical modifications. This diversity enables lipids to fulfill a wide range of biological functions, including energy storage, membrane architecture, cellular signaling, and molecular transport. The combinatorial possibilities arising from different lipid backbones, fatty acyl chains, and modifications generate a lipidome that is orders of magnitude more structurally complex than many other classes of biomolecules.

Materials and methods

Instrumentation and methods: A Thermo Scientific™ Accucore™ C30 HPLC Column (150 mm x 2.1 mm, 2.6 µm particle size) connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC System and a Orbitrap Tribrid Apex Small Molecule MS were used to acquire the data.

Sample: Lipids were extracted from 100 mg of various parts of the flower and parts of the plant Agapanthus using chloroform, methanol and water.

Orbitrap Tribrid Apex Small Molecule MS

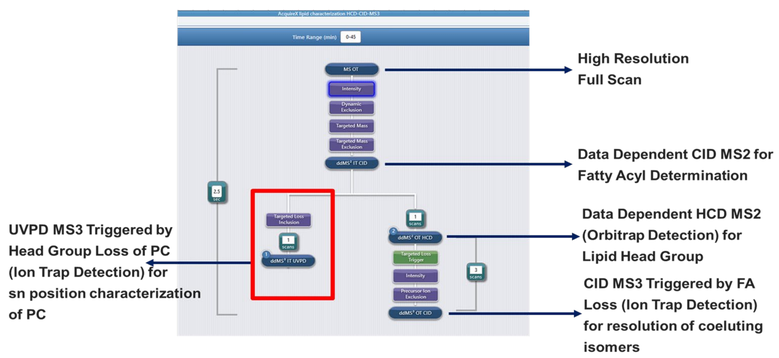


Figure 2. Class based MS/MS, UVPD and MS^3 triggers built into untargeted method templates.

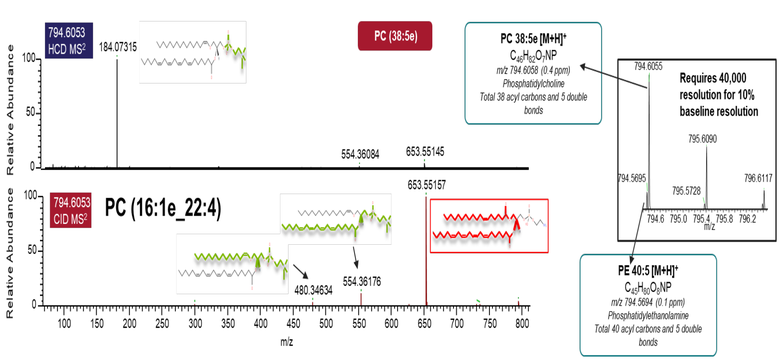


Figure 3. Importance of high resolution along with alternate fragmentation for confident annotation. The HCD spectra of PC gives the head group while the CID spectra is able to provide the acyl chain information.

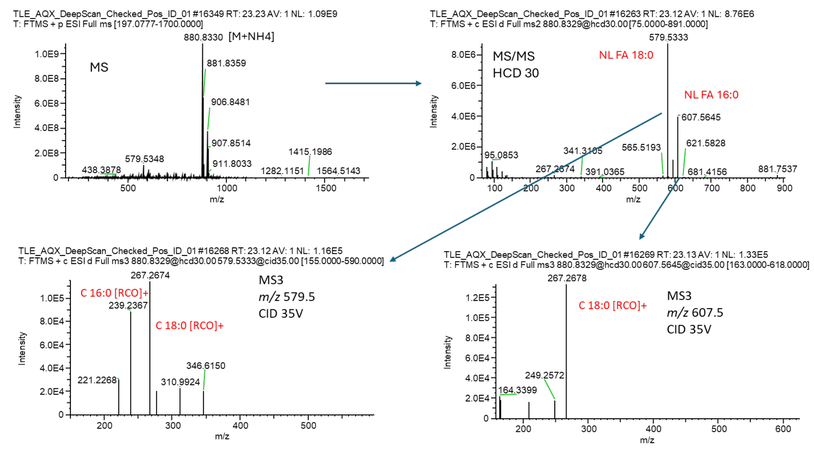


Figure 4. Complete characterization of TG (16:0_18:0_18:0) found in TLE utilizing MS^3 based fragmentation.

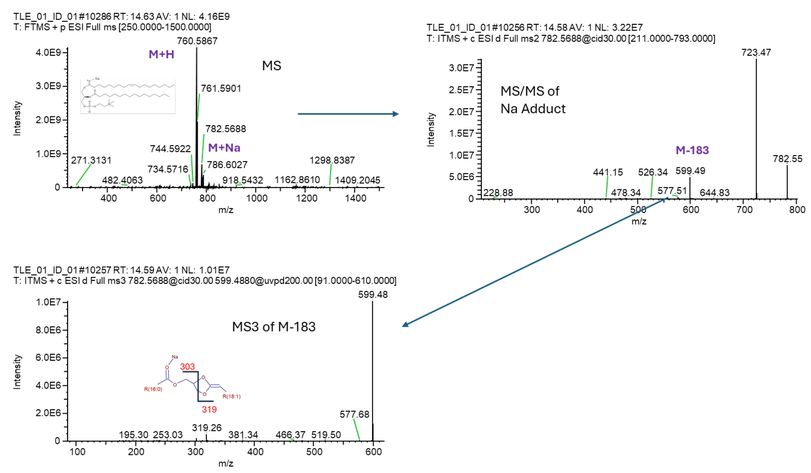


Figure 5. UVPD based MS^3 fragmentation for sn-position determination of PC. The diagnostic ions of 303/319 determine the sn-position of the lipid PC (16:0/18:1). Only one isomer is found in the TLE.

Plant and flower lipid extracts

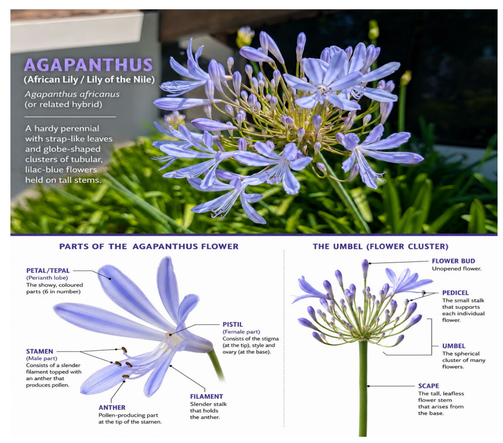
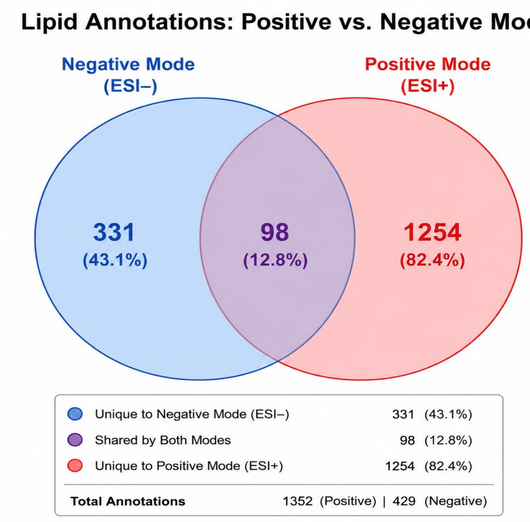


Figure 6. Lipids from the flower, leaf, stem and bud of the Agapanthus plant were extracted and the lipidome was assessed using the optimized workflow. 100 mg of different parts of the plant were used in the study.



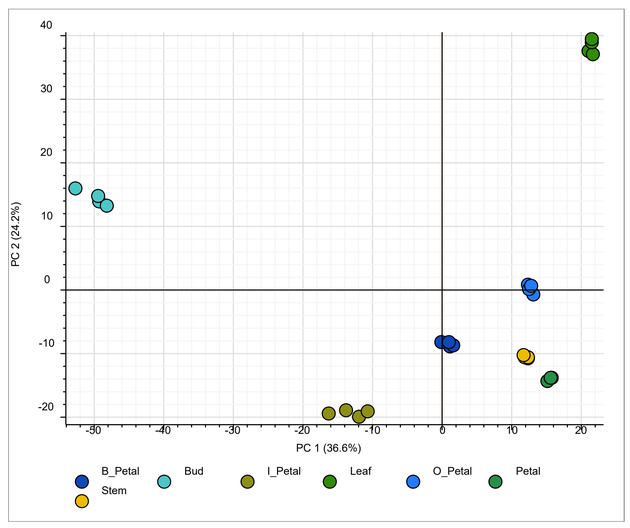


Figure 7. Thermo Scientific™ Compound Discoverer™ 3.5 Software was used for the data processing of the lipid extracts. Around 1700 high grade annotated lipids are detected in the extracts which show clear separation on the PCS plot.

Conclusions

We have successfully demonstrated the utility of the Orbitrap Tribrid Apex Small Molecule MS for structural lipidomics, using various fragmentation strategies on an LC timescale. We applied this to study lipid extracts from a plant and annotated around 1700 high grade lipids.

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