thermo scientific

Ultraviolet photodissociation (UVPD) mass spectrometry for structural characterization of lipids in biological matrices on chromatographic time scales

Rahul Deshpande[1], Bashar Amer[1], Susan Bird[1], and Thomas Moehring[2]
[1]Thermo Fisher Scientific, San Jose, USA, [2]Thermo Fisher Scientific, Bremen, Germany

ABSTRACT

Purpose
Establish a workflow for structural characterization of lipids using multiple fragmentation techniques including Ultraviolet photodissociation (UVPD).

Methods
Experiments were conducted to characterize lipid structure for resolution of isomers based on sn-position, chain length and double bond positions.

Results
Lipid standard were used for optimization of parameters for characterization of isomers using high resolution chromatographic separation and multiple fragmentation techniques. Resolution of isomers and annotation of unknown lipid species were shown in the bovine liver lipid extract.

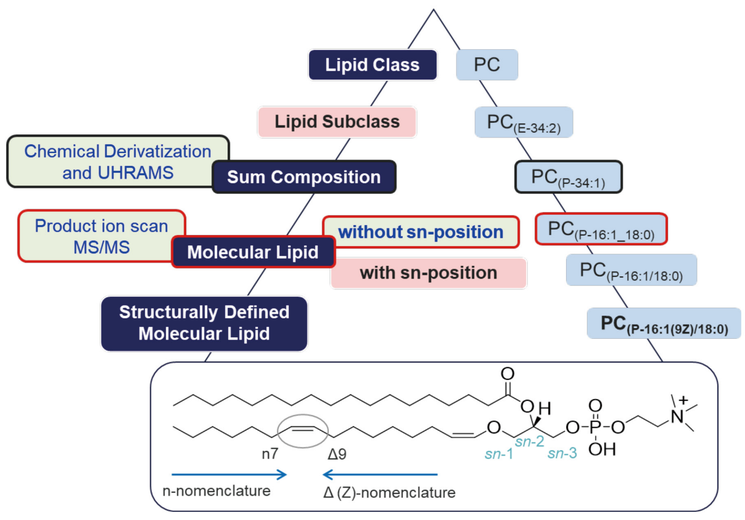
INTRODUCTION
Lipidomics is the comprehensive analysis of molecular lipid species. Separation of lipids using liquid chromatography followed by analysis by mass spectrometry has become the method of choice for lipidomic studies. Lipid structures are extremely diverse, in principle rendering over 100,000 unique species by theoretical estimation. The exact number of lipid molecules is even larger due to the presence of structural isomers. The identification of lipids based on their classification is shown in figure 1. Complete structural characterization of complex lipids, such as glycerophospholipids, by mass spectrometry continues to present a major challenge. An ideal analytical tool should be able to characterize the structure of lipids in a discovery platform as well as being feasible for large scale studies. Here we present preliminary work for structural characterization of lipids in a biological matrix on a chromatographic time scale.

FRAGMENTATION

Collision-induced dissociation (CID):
CID or CAD refers to the process whereby the ions kinetic energy has been increased prior to colliding with neutral molecules converting kinetic energy to internal energy that can induce fragmentation. Cannot detect low MW fragments (1/3 Rule)

Higher-energy collisional dissociation (HCD):
HCD is a CID technique associated with Thermo Scientific Orbitrap instruments. Voltage offsets between components increase the kinetic energy of the precursor ion and dissociated in the ion routing multipole cell from collisions with nitrogen molecules. Can detect low mw ions.

Ultraviolet photodissociation (UVPD) -213 nm:
UVPD differs from CID and HCD in that it utilizes photons generated from a laser source to increase the internal energy of a selected precursor ion until there is sufficient internal energy present to overcome the barrier to dissociation. It generates complimentary fragments to HCD/CID.



MATERIALS AND METHODS

Chemicals and Standards
Lipid standards and bovine liver lipid extract were purchased from Avanti Lipids

LC Methods
Column: **Thermo Scientific™ Accucore™ C30**, 2.6 µm, 150 x 2.1 mm (RP)
Column Temperature: 45 ˚C
Autosampler Tray Temperature: 15 ˚C
Injection Volume: 2 µL
Flow rate: 0.26 ml / min
Run Time: 45 Minutes

Mass Spectrometer
Thermo Scientific™ Orbitrap IQ-X™ Tribrid Mass Spectrometer was used.

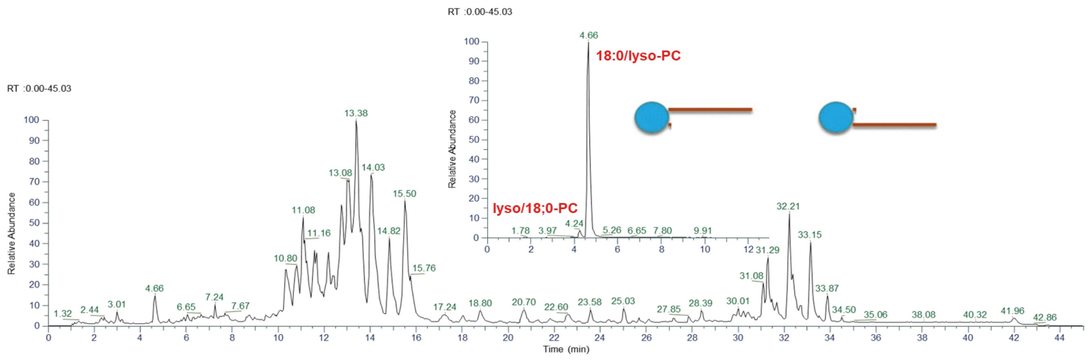
MS Conditions
MS^1 at 240K resolution (FWHM @ m/z 200) and data-dependent MS^n experiments were performed.

Data Processing
Thermo Scientific™ LipidSearch™ 5 was used for data analysis.

RESULTS

REGIOISOMERS (SN-POSITION)
Lysophosphatidylcholines (lysoPCs) are products or metabolites of phosphatidylcholines (PCs), which are structural components of animal cell membranes. LysoPCs occur in two forms, with the fatty acyl groups at positions 1 (sn-1) or 2 (sn-2) on the glycerol backbone. These two forms can be resolved with high resolution chromatography.

Figure 2 Separation of isomeric lysoPC's in a biological matrix using high resolution liquid chromatography using C30 column.



Phosphatidylcholines (PCs) contain two fatty acyl chains which can result in two regioisomers. These can be resolved using UVPD for MS^3 fragmentation of specific neutral loss fragments.

Figure 3 Mass Spectrometric Template on IQ-X Utilizing Complimentary Multiple Fragmentation Strategies for determination and resolution of structural isomers.

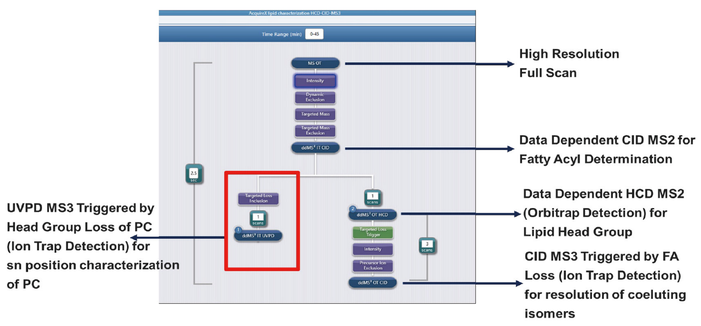
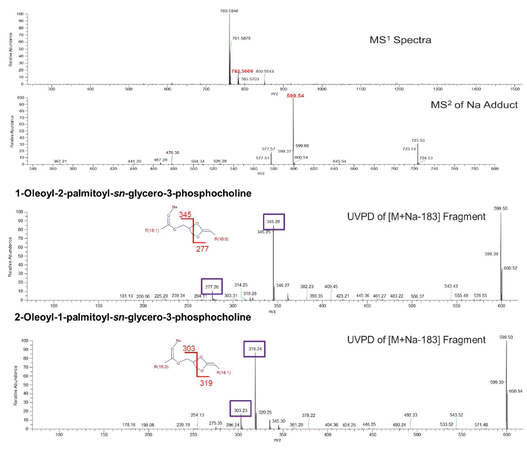
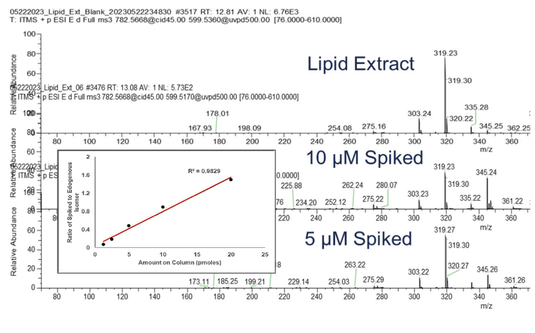


Figure 4 UVPD based MS^3 fragmentation for sn-position determination of PC. Lipid standards PC(16:0/18:1) and PC(18:1/16:0) were used for determination of diagnostic fragments for differentiation in biological sample.



QUANTITATION
Bovine Liver Lipid Extract was spiked with 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine at different ratio. Relative quantification of each isomer was calculated by ratio of diagnostic fragments.

Figure 6 Relative Quantification of regioisomers in biological matrix. Ratio of the diagnostic fragments 345.24 and 319.24 were used for the quantification.



Double Bond
Lipid Standards 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine {PC(18:1(9Z)/18:1(9Z))} and 2-dipetroselinoyl-sn-glycero-3-phosphocholine {PC(18:1(Δ6)/18:1(Δ6))} which are PC isomers differing in the position of the double bond were baseline separated by the high-resolution chromatographic method using C30 column. However, none of these two PC's were present in the bovine liver lipid extract with an isomer at different retention time.

Figure 8 Isomers of PC differing in their double bond position were separated by the high-resolution chromatographic method using C30 column.

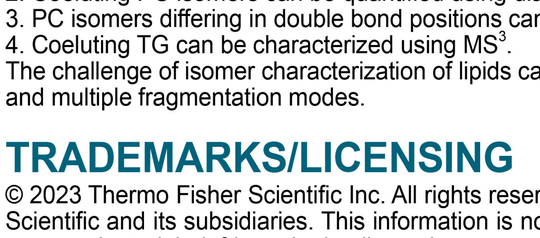
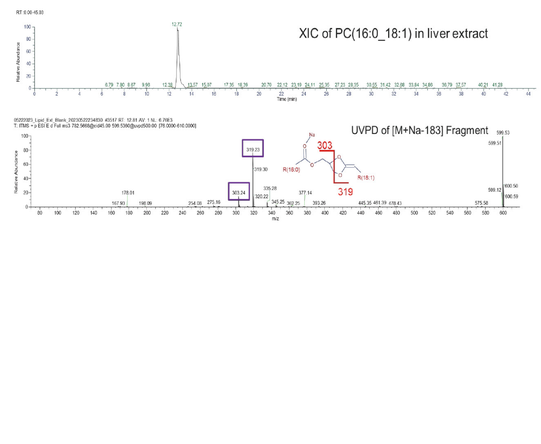
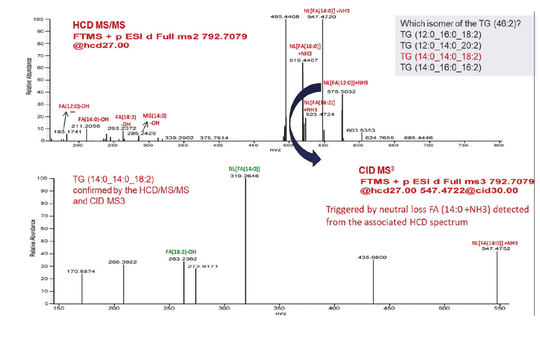


Figure 5 Bovine liver lipid extract contains only one isomer of PC(34:1), 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine.



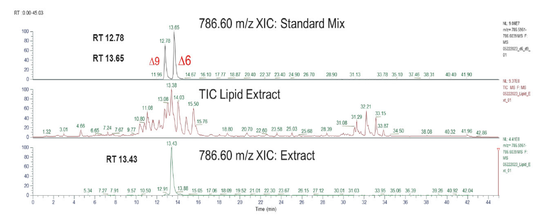
Chain Length
Co-eluting Triacylglycerol (TG) isomers of different fatty acyl chain lengths cannot be distinguished by MS/MS alone. MS^3 using CID of specific MS^2 neutral loss fragments helps in determination of the various coeluting TG species.

Figure 7 Coeluting isomers in bovine liver lipid extract can be differentiated using HCD MS/MS followed by CID MS^3.



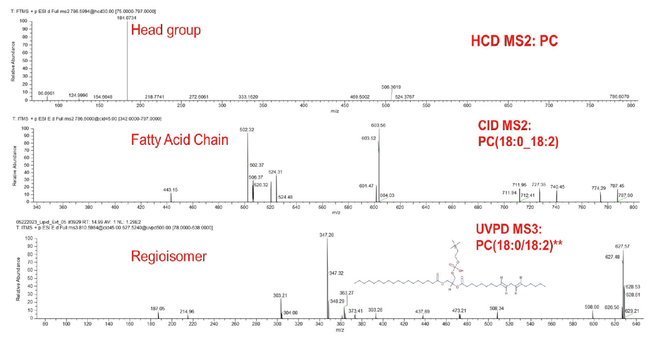
Structural Characterization of Unknown Lipid
The above chromatographical method using the MS template with multiple fragmentation was used to characterize an unknown lipid with m/z of 786.599 which showed up as an isomer to the lipid standards PC(18:1/18:1)

Figure 9 Isomers of PC differing in their double bond position were separated by the high-resolution chromatographic method using C30 column. An unknown isomer was found to be present in the liver extract.



The HCD MS^2 fragmentation of the unknown lipid gives the head group of the lipid which is identified as a PC lipid. The CID MS^2 fragmentation of the unknown PC gives the two fatty acyl chains associated and is identified as PC(18:2_18:0). The MS^3 UVPD spectra of the neutral loss fragment of the sodium adduct of the lipid is able to give the sn positions of the PC. The lipid is thus identified to be PC(18:0/18:2).

Figure 10 Structural characterization of an unknown lipid using complimentary fragmentation strategies. In a single chromatographic run, the unknown lipid undergoes HCD MS/MS and CID MS/MS. The sodium adduct of the lipid undergoes CID MS/MS and UVPD MS^3. These multiple fragmentation along with the retention time help to characterize the lipid.



OUTLOOK
More optimization needs to be done for double bond characterization. Templates for other lipid classes using multiple fragmentation have to be built for structural characterization.

CONCLUSIONS
A workflow for structural characterization of lipid class has been presented
1. Regioisomers can be separated either by using LC (lysoPC) or when coeluting by using UVPD fragmentation (PC).
2. Coeluting PC isomers can be quantified using diagnostic fragments.
3. PC isomers differing in double bond positions can be separated using LC
4. Coeluting TG can be characterized using MS^3.

The challenge of isomer characterization of lipids can be addressed using high resolution chromatography and multiple fragmentation modes.

TRADEMARKS/LICENSING

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

ThermoFisher SCIENTIFIC