Oxygen Attachment Dissociation MS/MS for the structural ID of double-bond positions in different lipid classes associated with alcohol toxicity

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Overview

- Oxygen Attachment Dissociation (OAD) MS/MS is a novel method for fragmentation, providing additional fragmentation to CID in ESI+ and ESI- mode on the LCMS-9050.
- Carbon-carbon double bond position-specific fragmentation by OAD enables lipid identification to the structural level.
- OAD MS/MS has been applied in the analysis of mouse tissues exposed to chronic ethanol toxicity to enhance the identification of lipids implicated in the mechanism of toxicity from a previous study using CID-MS/MS.

1. Introduction

Structural classification of lipids requires the identification of carbon number as well as the number and position of double bonds. Identifying carbon-carbon double bond position(s) presents a major challenge in unsaturated lipid characterisation, however it is vital to understand a biological mechanism, since minor structural differences between positional isomers can alter the biochemical function of a lipid.

In a previous study into the toxicity of ethanol in a mouse model, marked differences were found in the lipidome of the liver, pancreas, and gut. In this study, Oxygen Attachment Dissociation (OAD) MS/MS has been applied to enhance the identification of these lipids.

2. Materials and Methods

2.1 LC-MS/MS method

Tissue extracts (liver, pancreas, and gut) were obtained from C57BL/6 mice that were subjected to chronic exposure to ethanol and un-dosed controls. The study was conducted in accordance with EU and National ethical guidelines and approved by the Aristotle University of Thessaloniki.

- **Reverse phase LC separation**
  - Acquity C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min, cycle time 35 minutes.
  - Binary gradient; water + 0.1% formic acid, and acetonitrile + 0.1% formic acid.
- **LC-MS/MS Mass Spectrometry detection with simultaneous CID and OAD**
  - Untargeted metabolite profiling method used a TOF MS mass scan followed by DDA-MS/MS mass scans in ESI+ and ESI- mode.
  - TOF MS mass scan m/z 60-1250; 100 msecs.
  - DDA-MS/MS m/z 40-1250; five scans of 200 msecs for ESI+ mode, three scans of 300msec for ESI-; collision gas pressure 17kPa; collision energy spread 6-30V.
  - External mass calibration for positive and negative mode ESI.
- **Data processing and lipid identification**
  - LabSolutions Insight™ (Shimadzu Corporation) and MS-DIAL were used to process data. (https://systemsomicslab.github.io/compms/msdial/main.html).
  - DDA spectra acquired in ESI+ and ESI- for each lipid were analyzed to assign C=C double bond positions and matched to the OAD database in MS-DIAL.
  - Lipids were identified according to the omega and delta nomenclature, counting carbons from the methyl end or the carboxylic end of the fatty acyl, respectively.

3. Results

OAD MS/MS enabled the structural characterization of lipid isomers indistinguishable by CID-MS/MS. Figure 1 highlights two isomers of PC(22:5/0:0) detected in pancreas tissue.

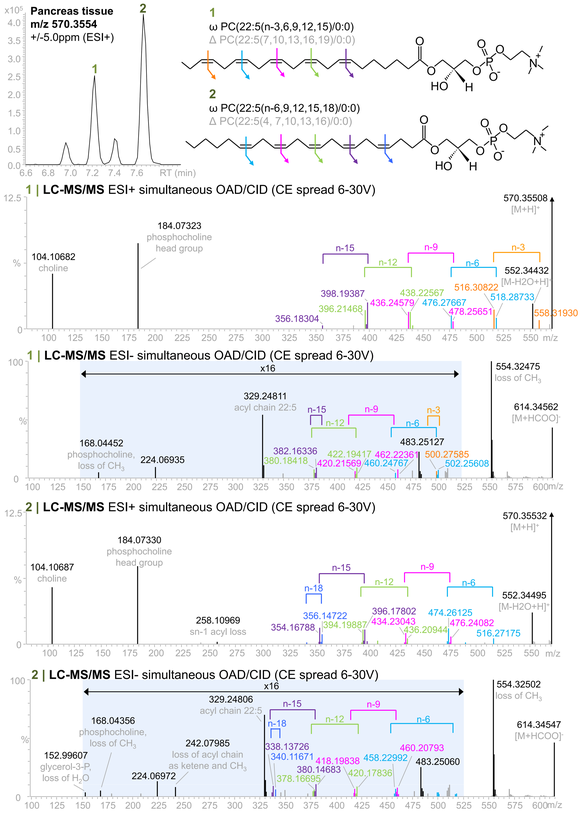

Figure 1. Structural characterization of PC(22:5/0:0) positional isomers using OAD MS/MS in ESI+ and ESI- mode. CID specific fragments (black) cannot distinguish the isomers. OAD specific fragments highlighted in different colors to show the ions corresponding to each C=C double bond in each isomer. Omega and delta nomenclature given for each ID.

The isomer specifically identified as PC(22:5(n-6,9,12,15,18)/0:0) in Figure 1 was found to be significantly reduced by ethanol toxicity in the pancreas, while there was no significant difference reported for the isoform PC(22:5(n-3,6,9,12,15)/0:0). Following the process described in Figure 1, other lipids involved in ethanol toxicity were identified to the structural level including PC(20:5(n-3,6,9,12,15)/0:0) and PC(16:0_22:5(n-6,9,12,15,18)) in the liver, as well as PC (O-18:1(n-9)/0:0), PC(14:0_18:2(n-6,9)) and PC(18:2(n-6,9)/18:2(n-6,9)) in the gut. Examples of OAD spectral annotation are shown in Figure 2. The high specificity of OAD helped to simplify the process of structural elucidation by generating spectra that are easy to interpret.

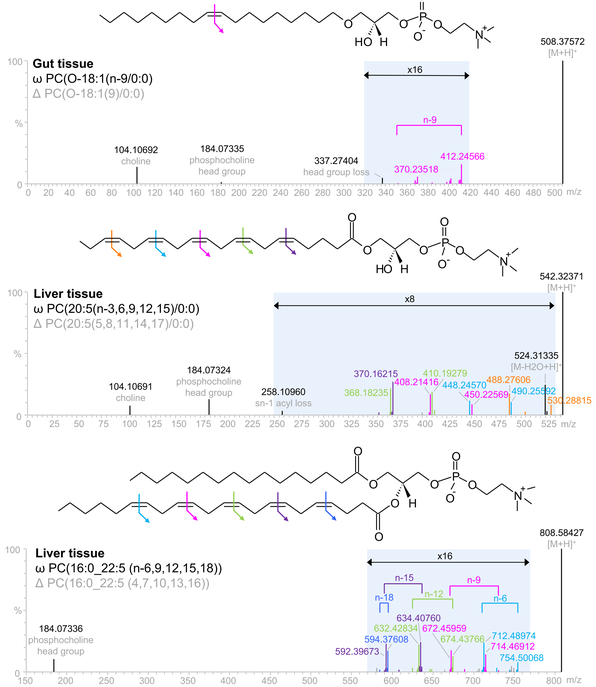

Figure 2. Structural characterization of PC(O-18:1/0:0), PC(20:5/0:0) and PC(16:0_22:5) using DDA-MS/MS with simultaneous OAD and CID MS/MS in ESI+. CID specific fragments are highlighted in black and OAD specific fragments are highlighted in different colors to show the ions corresponding to each C=C double bond in the structure. The omega and delta nomenclature are given for each identification.

3.1 Automated data processing and lipid identification

Data from the untargeted DDA-MS/MS analysis of gut, liver and pancreas tissue extracts were processed in MS-DIAL which includes the OAD database for lipid identification to the structural level. Figure 3 highlights the identification of PC 18:2(9,12)_18:2(9,12), also known as PC(18:2(n-6,9)/18:2(n-6,9)), in the gut.

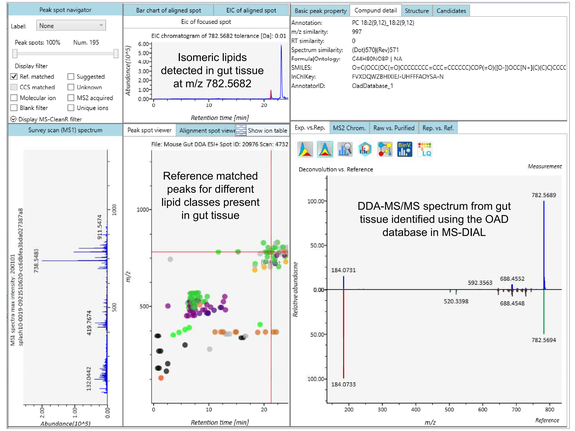

Figure 3. Data processing and lipid identification using the OAD database in MS-DIAL.

4. Conclusions

- OAD-MS/MS is a novel fragmentation method providing complementary structural information to CID in ESI+ and ESI- mode. It is less hazardous than other methods and provides higher efficiency fragmentation, especially for singly charged positive and negative ion precursors.
- OAD-MS/MS enables C=C positional assignments in lipids and has been applied to structurally characterize lipids found to be potential markers of ethanol toxicity in a previous study using high resolution metabolomics analysis by LC-CID-MS/MS.
- Isomers that could previously only be distinguished by retention time as the CID-MS/MS spectra were similar have now been identified as unique structures using OAD-MS/MS.
- The enhanced level of structural identification provided by OAD-MS/MS allows the potential to improve our understanding of the biological roles of lipids.
- MS-DIAL version 5 supports the automated processing and identification of lipids in OAD-MS/MS data acquired using the LCMS-9050.

The authors declare no competing financial interest.