SHIMADZU

WP 520

Identifying Double-Bond-Positions of Phospholipids in Mouse Liver by Using Simultaneous Positive/Negative Ion Switching Analysis of LCMS-9050 and OAD-MS/MS

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1. Introduction

- Lipids, a class of biomolecules, play a significant role in the physiological system. Non-targeted lipidomics by High Resolution Accurate Mass Spectrometer (HRAM) is currently one of the major analytical workflows for the clinical research such as biomarker discovery as well as the basic study. However, it has been difficult to identify double-bond positions in lipids using conventional methods.
- A brand-new oxygen attachment dissociation (OAD) provides diagnostic fragment ions enabling the assignment of double-bond positions. Identifying the double-bond positions by OAD-MS/MS is expected to contribute for understanding of the lipid metabolism. In this study, it was focused on the fragmentation derived from phosphatidylcholine (PC) by using OAD-MS/MS and identified the chemical structures.

2. Methods

All experiments were performed using an ESI Q-TOF LCMS-9050 (Shimadzu, Kyoto, Japan). OH and O radicals generated by a compact microwave-driven radical source was introduced into the collision cell (Q2) through the quartz tube to obtain OAD-MS/MS spectrum [1][2]. Shimadzu Nexera™ LC system was used for the separation of the lipids in the extracts of mouse liver.

Extracts were subjected to analysis with LCMS-9050, which allows simultaneous positive/negative ion switching analysis. The positive ion mode was used to generate OAD-MS/MS based spectra to determine the positions of the double-bonds, and spectra based on collision induced dissociation (CID) was generated to confirm the lipid classes and fatty acids.

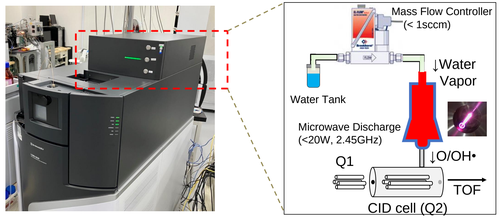


Fig. 1 Shimadzu LCMS-9050 and OAD system

2.2. Sample Preparation & Analytical Condition

The 160 mg of mouse-liver was homogenized using a multi-beads shocker with a metal cone at 1500 × g for 15 s, and 800 µL of MeOH was added to the homogenate. After the solvent was homogenized again under the same conditions, an appropriate amount of MeOH (30 mg tissue weight per 200 µL) was transferred to a 2 mL glass tube. After the solvent was removed up to 95% of the total volume of the sample, 200 µL of $CHCl_3$ was added to the solvent and vortexed for 10 s. After the solvent was incubated for 1 h on ice, 20 µL of ultra pure water was added, and the mixture was vortexed for 10 s. After 10 min incubation on ice, the solvent was centrifuged at 2000 × g for 10 min at 20 °C, and the supernatant was transferred to an LC-MS vial[2].

Table 1 Analytical Condition

HPLC condition	MS condition
System: Shimadzu Nexera X3	System: Shimadzu LCMS-9050
Flow rate: 0.3 mL/min	Mode: CID/OAD
Gradient Elution	Polarity: Pos/Neg switching
Mobile phase A: 20mM Ammonium formate	CE: 25V/ - 35V (pos/neg)
Mobile phase B ACN/IPA = 1/1 (v/v)	
Column: Shim-pack Scepter Claris C18-120 (1.9 µm, 2.1. mmI.D. x 100 mmL.)	

3. Results

Two peaks at m/z = 782.5695 $[M+H]^+$ from positive ion analysis mode and at 826.5604 $[M+HCOO]^-$ from negative ion analysis mode were detected from a mouse liver sample(Fig. 2) when Data Dependent Acquisition(DDA) with positive/negative ion switching analysis was performed. It was estimated as PC(36:4).

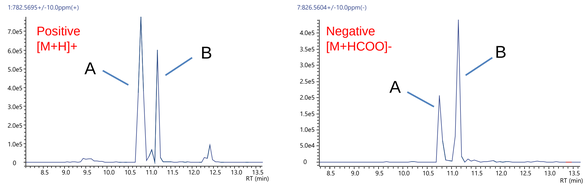


Fig. 2 Left) XIC Chromatogram at m/z = 782.5695(pos) and Right) at m/z = 826.5604(neg)

Carbon chain lengths and the number of double-bonds were estimated from CID-based negative ion analysis, and double-bond positions were identified with OAD-based positive ion analysis.

3.1. Detailed Processing of MS/MS spectra Based on CID

MS/MS spectra of PC(36:4) acquired with CID-based negative ion analysis were shown in Fig. 3.

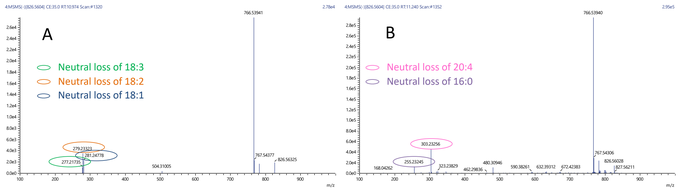
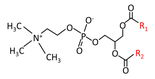


Fig. 3 MS/MS spectra of PC(36:4) acquired with CID-based negative ion analysis

Table 2 Combination list of fatty acids in PC(36:4)



PC(36:4)	R1	R2
A	18:2	18:2
	18:3	18:1
B	16:0	20:4

3.2. Detailed Processing of MS/MS spectra Based on OAD

MS/MS spectra of PC(36:4) acquired with OAD-based negative ion analysis were shown in Fig. 4 and 5.

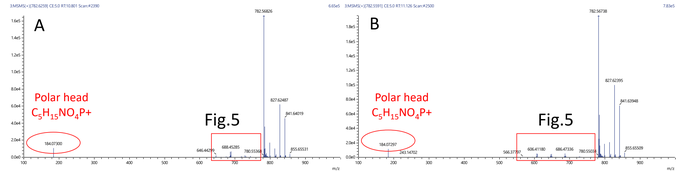


Fig. 4 MS/MS spectra of PC(36:4) acquired with OAD-based positive ion analysis

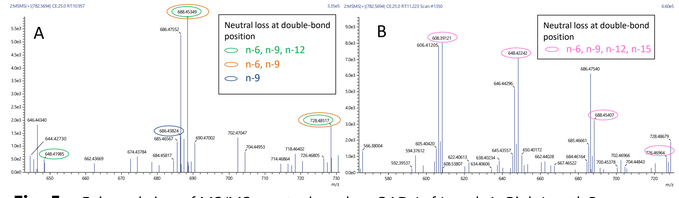
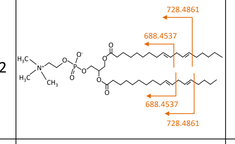
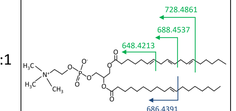
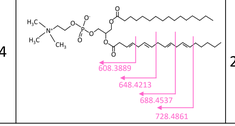


Fig. 5 Enlarged view of MS/MS spectra based on OAD. Left) peak A, Right) peak B

3.3. Identification of double bond positions

A combination of CID/OAD and positive/negative switching analysis by Q-TOF was used to identify lipids in mouse liver. The result was shown in Table 3. Three compounds were found from two peaks(A and B) detected at the same m/z.

Table 3 Structure identification result of PC(36:4)

PC(36:4)	R1	R2	Fragmentation	Double-bond position
A	18:2	18:2		18:2(n-6, n-9) Linoleic acid
	18:3	18:1		18:3(n-6, n-9, n-12) Gamma-Linolenic acid 18:1(n-9) Oleic acid
B	16:0	20:4		16:0 palmitic acid 20:4(n-6, n-9, n-12, n-15) Arachidonic Acid

4. Conclusion

- ✓ By using the combination of OAD and pos/neg switching analysis, the chemical structure of lipids in a mouse liver were identified.
- ✓ It was suggested that alpha-linolenic acid and gamma-linolenic acid are able to be distinguished by OAD.

5. Reference

[1] Takahashi.H et al. *Mass Spectrometry*. 2019, S0080.
[2] Uchino.H et al. *Commun Chem.* 5, 162 (2022)