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Application News

Rapid Structural Elucidation of Lipids Including C=C Positions Using Dual-Polarity CID/OAD in a Single LC-MS Run

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User Benefits

- Combining positive/negative polarity switching and simultaneous CID/OAD analysis acquires all the information required for detailed structural analysis of lipids in a single LC-MS analysis run.
- Carbon-carbon double bond positions in co-eluted lipid structural isomers are also determined.

■ Introduction

Lipids are essential for life and serve a variety of physiological functions, such as cell wall components, signal transmitters, energy storage, and epithelial barrier formation. Disruption of lipid homeostasis is implicated in many diseases, and non-targeted lipidomics is a powerful multi-omics research technique for elucidating disease pathologies and identifying specific biomarkers that show lipid changes in the body. Carbon-carbon double bond (C=C) position lipid isomers have a significant impact on enzyme selectivity, the physical properties of biomembranes, and other biological functions. However, these isomers are not easily distinguishable by collision-induced dissociation (CID) analysis. Analytical techniques capable of distinguishing double bond position isomers are extremely important in understanding the mechanisms of lipid homeostasis in different tissues and how these mechanisms change with various diseases.

■ Oxygen Attachment Dissociation Technology

Identifying the position of double bonds in lipids is a new and increasingly popular approach in lipidomics for the study of biological effects. Shimadzu's OAD-TOF system* integrates a new proprietary fragmentation technology called oxygen attachment dissociation (OAD).[1)] This technology was developed by Shimadzu to identify the positions of double bonds in compounds by generating double bond position-specific fragment ions with no derivatization or other pretreatment. The principle of this OAD method is shown in Fig. 1.

This Application News describes a detailed analysis of the chemical structure of phosphatidylcholines (PCs) detected in mouse liver extract, including the position of double bonds.

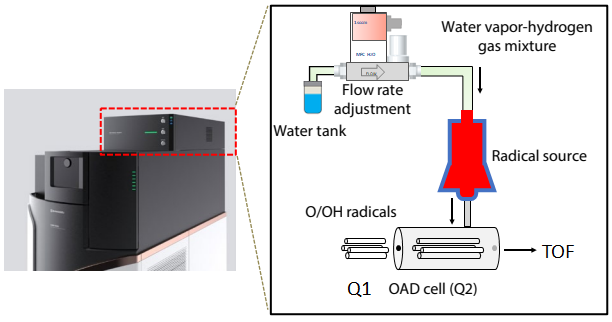


Fig. 1 Principle of OAD

* OAD-TOF system: LCMS-9050 equipped with OAD RADICAL SOURCE I

■ Preparation of Mouse Liver Extract

The samples used in this Application News were provided by Makoto Arita of RIKEN and were prepared *according* to the following method, as described by Uchino *et al.*[1)]

Approximately 160 mg of mouse liver was homogenized for 15 seconds in a multi-beads shocker with metal cones at 1500 × *g*. Next, 800 µL of MeOH was added to the homogenate, and the mixture was homogenized again under the same conditions. A sample of the resulting tissue suspension was transferred to a 2 mL glass tube and made up to 400 µL with MeOH (resulting in a mixture containing 30 mg of tissue per 200 µL). Next, 200 µL of $CHCl_3$ was added, and the mixture agitated with a vortex mixer for 10 seconds. After incubating the mixture on ice for 1 hour, 20 µL of ultrapure water was added, and the mixture was agitated with a vortex mixer again for 10 seconds. The mixture was then incubated on ice for 10 minutes, centrifuged at 2000 × *g* and 20 °C for 10 minutes, and the supernatant was transferred to an LCMS vial.

■ Analytical Conditions

Analysis was performed using a Nexera™ X3 and an OAD-TOF system comprising an LCMS-9050 that was equipped with an OAD RADICAL SOURCE I (Fig. 2).

The rapid positive/negative polarity switching feature of the LCMS-9050 was used to detect CID- and OAD-derived fragment ions in positive ion mode and CID-derived fragment ions in negative ion mode and to also perform a detailed structural analysis of PCs based on a single LC-MS analysis run.

Fig. 2 Nexera™ X3 and an OAD-TOF system

Table 1 OAD-TOF System Analytical Conditions

System	: Nexera X3 + OAD-TOF System
HPLC Conditions	
Flow rate	: 0.3 mL/min
Column	: Shim-pack Scepter™ Claris (100 mm x 2.1 mm I.D., 1.9 µm)*
Mobile Phase A	: 20 mM Ammonium formate
Mobile Phase B	: ACN/IPA = 1/1 (v/v)
Column Oven Temp.	: 40 °C
MS Conditions	
Mode	: CID/OAD
Polarity	: Positive/Negative switching
Collision Energy (CE)	: 25 V (Positive), 35 V (Negative)
DL Temp.	: 250 °C
Heat Block Temp.	: 50 °C
Interface Voltage	: 4 kV (Positive), -3 kV (Negative)
MS Scan Range	: *m/z* 100-1000
Measurement Time	: 20 min

* P/N: 227-31210-02

■ XICs of Lipids Subjected to Structural Elucidation

The mouse liver extract was analyzed using data-dependent acquisition (DDA) with rapid positive/negative polarity switching, with the OAD mode enabled. As a demonstration, we focused on the precursor ion of phosphatidylcholine (PC) 36:4, detected at *m/z* 782.5695 $[M+H]^+$ in positive ion mode and at *m/z* 826.5604 $[M+HCOO]^-$ in negative ion mode to identify the lipid structure. Fig. 3 shows the extracted ion chromatograms (XICs) for the targeted precursor ion, acquired in both polarities from a single LC-MS run.

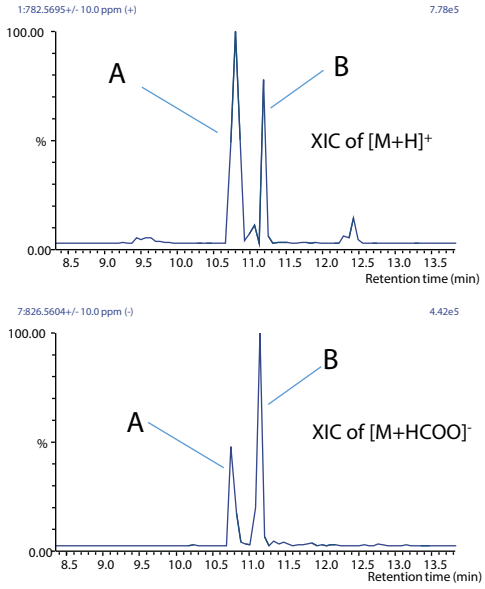


Fig. 3 MS Chromatograms of PC 36:4
m/z = 782.5695 $[M+H]^+$ (Top) and *m/z* = 826.5604 $[M+HCOO]^-$ (Bottom)

■ Negative-OAD Spectra for Fatty Acyl Chain Composition Analysis

Fig. 4 shows the OAD spectra corresponding to peaks A and B in Fig. 3, acquired in negative ion mode with a collision energy (CE) of 35 V. In negative ion mode, fragment ions derived from fatty acyl chains—commonly observed in conventional CID—were detected. These ions enabled identification of fatty acyl chain lengths and number of C=Cs. Peak A was found to consist of three fatty acyl chains: C18:3, C18:2, and C18:1, while peak B consisted of two fatty acyl chains: C20:4 and C16:0. These results indicate that even when OAD mode is enabled, CID-derived fragment ions appear at higher CE.

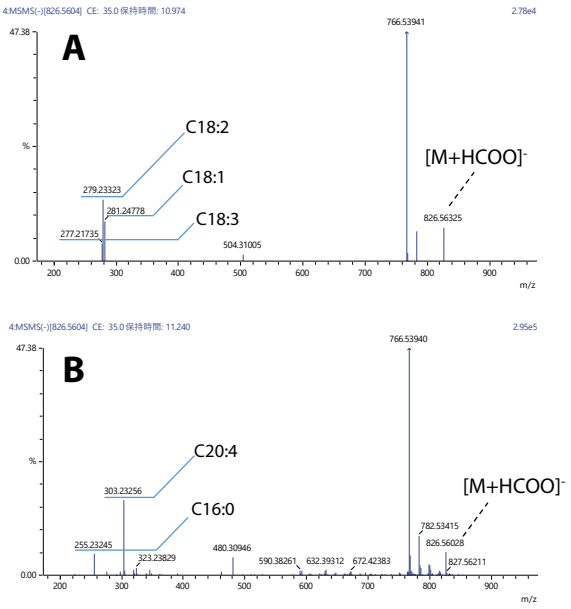


Fig. 4 MS/MS Spectra of PC 36:4 Peaks Detected in Negative Ion Mode (CE = 35 V). Top: Peak A, Bottom: Peak B.

■ Positive-OAD Spectra for Lipid Class Analysis

Fig. 5 shows the OAD spectra corresponding to peaks A and B in Fig. 3, acquired in positive ion mode with CE = 25 V. As shown in Fig. 5, OAD-derived neutral losses (NLs) related to C=C positions—typically not observed in CID—were detected. These NLs provide information about C=C positions within fatty acyl chains. In addition, the fragment ion at *m/z* 184.0731, which is derived from the polar head group of PC and is commonly observed in conventional CID, was also confirmed. These results indicate that both peaks A and B are PCs. Even when the OAD mode is enabled, optimizing the CE allows simultaneous detection of both CID- and OAD-derived fragment ions, enabling more detailed structural information to be obtained in a single LC-MS run.

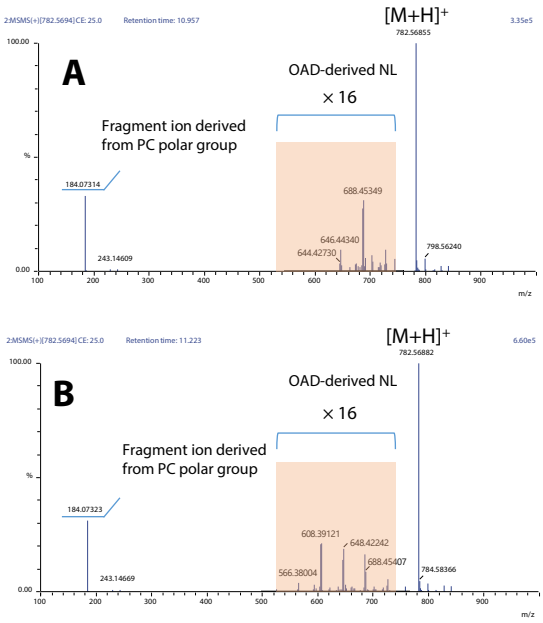
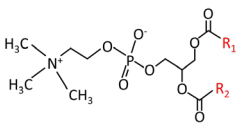


Fig. 5 MS/MS Spectra Obtained in Positive Ion Mode (CE = 25 V).
Top: Peak A, Bottom: Peak B

■ Estimation of *sn*-Positions

MS/MS analysis in negative ion mode typically provides more intense fragment ions derived from fatty acyl chains at the *sn*-2 position than at *sn*-1 [2]. Based on this, the MS/MS spectra in Fig. 4 were analyzed, and the *sn*-positions of the fatty acyl chains in peaks A and B were assigned as listed in Table 2. Phospholipids often have saturated chains at *sn*-1 and unsaturated ones at *sn*-2, consistent with these results.

Table 2 Fatty Acyl Chains Combinations in Peaks A and B



PC:36:4	R1 (*sn*-1)	R2 (*sn*-2)
A	C18:2	C18:2
	C18:3	C18:1
B	C16:0	C20:4

■ Identification of C=C Positions via OAD-Derived Neutral Losses

When lipids undergo ion fragmentation via OAD, NLs associated with C=C positions are observed, as shown in Fig. 5 and Fig. 6. This allows for the identification of the C=C positions within the structure. Furthermore, when polyunsaturated fatty acids are present, NLs corresponding to each C=C position are also produced, enabling the identification of all C=C positions. Table 3 summarizes the C=C-related NLs obtained through OAD.

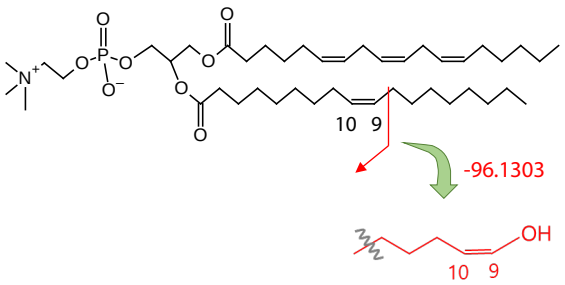


Fig. 6 Example NL Obtained by OAD

Table 3 Predicted NLs Obtained by OAD

First Double Bond	n-	NL [Da]
n-3	3	-12.0364
	6	-52.0687
	9	-92.1012
	12	-132.1335
n-6	6	-54.0833
	9	-94.1157
	12	-134.1481
	15	-174.1805
n-9	9	-96.1303
	12	-136.1626
	15	-176.1951
	18	-216.2274

■ Extracted-Ion Chromatograms of C=C Positional Isomers

Fig. 7 shows the XICs of the OAD-derived NLs listed in Table 3, corresponding to peaks A and B. As shown in Table 2, two types of PCs co-elute at peak A, but the C=C positions of each were successfully identified, as summarized in Table 4 (A). On the other hand, the NL at 12.0364, which corresponds to a C=C position at n-3, was not observed in either peak A or B.

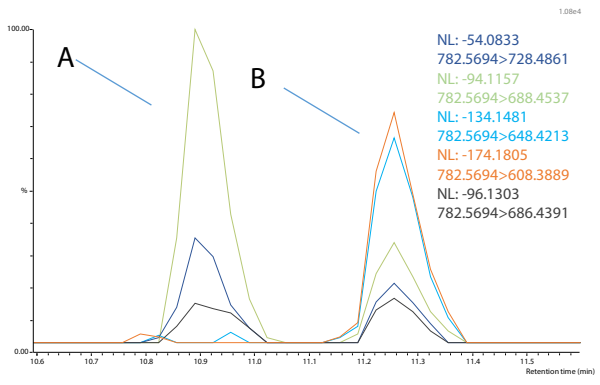
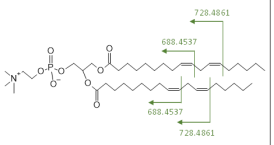
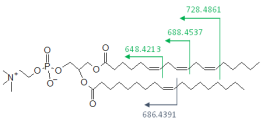
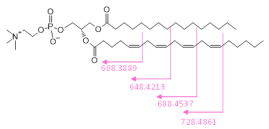


Fig. 7 MS/MS Chromatograms Obtained by OAD Analysis of PC 36:4

■ Detailed Structural Analysis of PC 36:4

Based on the results of Table 2 and Fig. 7, the compound identified as PC 36:4 was found to be a mixture of three structural isomers with the same exact mass: PC 18:2/18:2, PC 18:3/18:1, and PC 16:0/20:4, as listed in Table 4.

Table 4 Estimated Structure of PC 36:4

PC 36:4	R1	R2	Fragmentation	C=C 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

■ Conclusion

By combining rapid positive/negative polarity switching and simultaneous CID/OAD analysis using the OAD-TOF system, complementary fragment ions from CID and OAD were acquired in both positive and negative ion modes within a single LC-MS run. This enabled comprehensive structural analysis of lipids in mouse liver extract. Information such as polar head groups, fatty acyl chains, and the number of C=Cs was obtained from CID-derived ions, while C=C position information was derived from OAD. As a result, detailed structural elucidation of PCs was achieved in a single analysis.

<Acknowledgements>
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<References>

1) H. Uchino, H. Tsugawa, Takahashi, M. Arita, Computational mass spectrometry accelerates C=C position-resolved untargeted lipidomics using oxygen attachment dissociation, *Communications Chemi*stry, **5**, 162 (2022)
2) K. Ekroos, *et al.*, Charting molecular composition of phosphatidylcholines by fatty acid scanning and ion trap MS^3 fragmentation, *Journal of Lipid Research*, 44, 2181 (2003)

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