SHIMADZU
Excellence in Science

Optional Kit For Oxygen Attachment Dissociation MS/MS OAD RADICAL SOURCE 1
High Performance Liquid Chromatograph Mass Spectrometer LCMS-9050

Application News

Identification of Double Bond Positions and Relative Acyl Chain Positions in Egg Yolk Phosphatidylcholines Using OAD-TOF System

Feng Ji, Masaki Yamada, Xiaodong Li
Shimadzu China Innovation Center

User Benefits

- Position-specific fragmentation with the OAD RADICAL SOURCE I allows the identification of double bond positions.
- Relative acyl chain position assigned using *sn*-1 signature ions produced by sequential CID and OAD is possible.
- Fragment ions are detected with high mass accuracy and structure characterization is annotated with high certainty.

■ Introduction

As a major class of lipids, phosphatidylcholines (PCs) often contain mixtures of structural isomers in terms of relative acyl chain positions on the glycerol backbone (*sn*-positions) and the locations of carbon-carbon double bonds (C=Cs) in unsaturated acyl chains. Studies have revealed these structural diversity has deep impacts on cell membrane permeability, proteins binding, enzyme selectivity, and plasticity of cancer cells. However, profiling PC isomers at the aforementioned level remains challenging as any relating variation structural isomers cannot be differentiated by traditional CID.

In this Application News, we present a novel workflow for confidently profiling PCs down to *sn*- and C=C location level. This proposed method can be simply incorporated into liquid chromatography coupled with OAD-TOF system* without sample derivatization or any instrument modification. The performance of the workflow is demonstrated by identification of 24 distinct PC molecular species from the egg yolk sample, including 8 identified down to C=C level only and 4 to both.

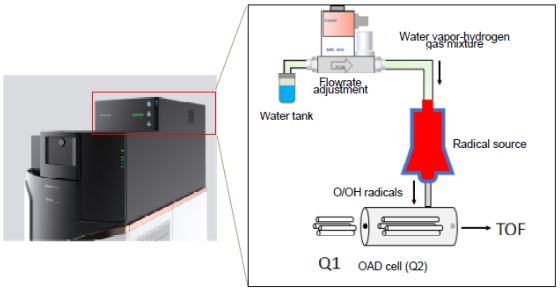

Fig. 1 Principle of OAD
* OAD-TOF system: LCMS-9050 equipped with OAD RADICAL SOURCE I

■ Oxygen Attachment Dissociation (OAD)

Shimadzu's OAD-TOF system integrates a newly designed fragmentation technology called oxygen attachment dissociation (OAD). The principle of this OAD equipment is shown in Fig.1. This technology effectively causes C=C-specific fragmentation and facilitates annotation of C=C positions in lipidomics[1], which has been its primary application focus to date.

However, *sn*-isomers cannot be differentiated by CID or OAD alone. To address this limitation, we employed a sequential CID and OAD strategy, termed OAciD. This approach generates unique, *sn*-position-specific fragment ions, enabling further identification. The scheme of the proposed workflow is shown in Fig.2. Furthermore, the workflow provides confident annotation of the native C=C positions as well. Therefore, the proposed workflow enables comprehensive identification of the *sn*-positions and native C=C locations in a single OAciD run simultaneously.

As demonstrated in mechanistic studies[2,3], C_α-H on fatty acyl chains participated in the phospho-head group from CID of $[M+Na]^+$ PC ions with a preference to removing the C_α-H on *sn*-2 over *sn*-1 chain, forming a five-membered 1,3-dioxolan ring in product ions (structure shown in Fig.2). By targeting the newborn C=C bond in sequential OAD, consequently, R2 is selectively cleaved, thus yielding an MS^3 fragment that contains only information from the *sn*-1 chain. Since PCs only contain two acyl chains, unambiguous assignment of the *sn*-2 chain is achieved as well. Notably, only in OAciD, diagnostic ions, capable of analyzing PCs at the *sn*-position level, are obtained.

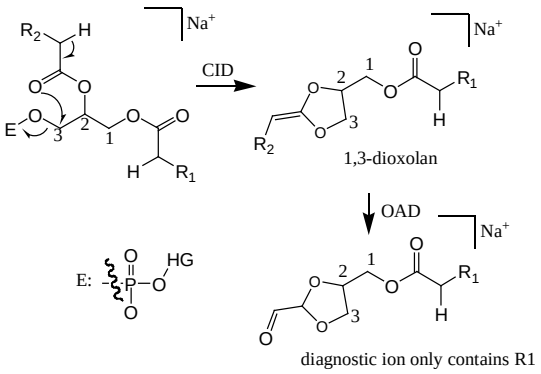

Fig. 2 Scheme of The Proposed Method

■ Analytical Conditions

Shimadzu's OAD-TOF system operates in three distinct modes: CID-only, OAD-only and OAciD, with rapid switching capability (<1 min). Notably, an OAciD spectrum is not a simple combination of the other two. Instead, product ions generated from CID undergo a relatively slower OAD dissociation process as new precursors, producing a highly hybrid and complex spectrum, rich in structural information. The analytical conditions are shown in Table 1.

Table 1 Analysis Conditions of OAD-TOF System

System	: Prominence™+OAD-TOF system
Column	: Reversed-phase column
Temperature	: 45 ˚C
Injection volume	: 3 µL
Mobile phases	: A) 20 mM Ammonium formate- Water B) Acetonitrile/2-propanol (1:1, v/v)
Mode	: Gradient elution
Flow rate	: 0.3 ml/min
Polarity	: Positive
DL temp	: 250 ˚C
Heat block temp	: 400 ˚C
Interface Voltage	: 4.0 kV
CE	: 30 V(OAciD)
TOF-MS	: *m/z* 150-1250
Measurement Time	: 38 min

■ Sample Preparation Conditions

Egg yolk lecithin, commonly used as pharmaceutical formulation, is obtained from local manufacturer. A 20 mg of lecithin was weighed and transferred into a 1.5 mL microtube, and then 1 mL of methanol/isopropanol (50/50, v/v) was added, and then ultrasonicated at 40˚C for 5 min. After centrifugation at room temperature (15,000,10 min), the supernatant was transferred into sample vial and subjected to LC-OAD-TOF system.

■ Identification of Candidate Lecithin PCs

Fig. 3 shows the TIC of the egg yolk lecithin sample obtained by OAciD. Data from the untargeted DDA-MS/MS analysis was processed by MS-DIAL (ver. 5.2.240424.3-net472) with the parameter settings shown in Table 3, and lipids were identified based on $[M+H]^+$ ion *m/z*. The candidate PCs in the egg yolk lecithin that were identified using MS-DIAL are shown in Table 2. CID and OAD analysis were performed to obtain detailed structural data, and the resulting MS/MS spectra were used for characterization. A total of 24 PCs were identified, with 12 of them annotated at C=C locations automatically. The MS-DIAL processing and PCs identified in egg yolk lecithin are shown in Fig. 4.

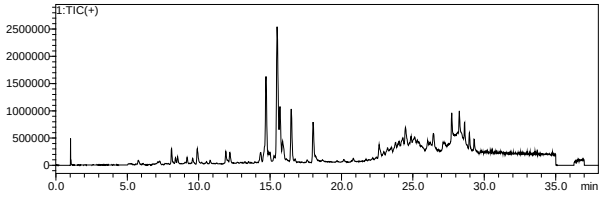

Fig. 3 TIC of Egg Yolk Lecithin Sample Obtained by OAciD

Table 2 List of PCs Candidates in Egg Yolk Lecithin Obtained with MS-DIAL

MS1 (*m/z*)	Adduct	Predicted structure
622.4386	$[M+H]^+$	PC 12:0_12:0
730.5324	$[M+H]^+$	PC 16:1_16:1
756.5472	$[M+H]^+$	PC 10:0_24:3
782.5613	$[M+H]^+$	PC 18:2_18:2
806.5620	$[M+H]^+$	PC 12:0_26:6(8,11,14,17,20,23)
732.5474	$[M+H]^+$	PC 16:0_16:1(9)
808.5782	$[M+H]^+$	PC 10:0_28:5
782.5626	$[M+H]^+$	PC 16:0_20:4(5,8,11,14)
804.5462	$[M+H]^+$	PC 8:1_30:6
758.5638	[M+H]+	PC 16:0_18:2(9,12)
808.5779	$[M+H]^+$	PC 10:0_28:5
784.5788	$[M+H]^+$	PC 18:1(9)_18:2(9,12)
808.5784	$[M+H]^+$	PC 16:0_22:5(4,7,10,13,16)
834.5944	$[M+H]^+$	PC 12:0_28:6(10,13,16,19,22,25)
756.5439	$[M+H]^+$	PC 10:0_24:3
734.5624	$[M+H]^+$	PC 16:0_16:0
760.5797	$[M+H]^+$	PC 16:0_18:1(9)
810.5938	$[M+H]^+$	PC 18:0_20:4(5,8,11,14)
786.5941	$[M+H]^+$	PC 18:1(9)_18:1(9)
812.6099	$[M+H]^+$	PC 10:0_28:3
836.6092	$[M+H]^+$	PC 12:0_28:5(10,13,16,19,22)
812.6091	$[M+H]^+$	PC 10:0_28:3
838.6220	$[M+H]^+$	PC 20:2_20:2
788.6102	$[M+H]^+$	PC 18:0_18:1(9)

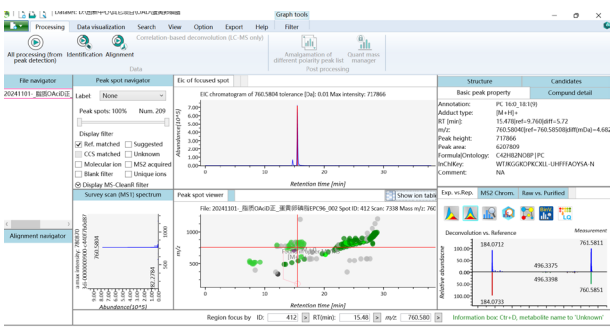

Fig. 4 MS-DIAL Processing and PCs Identified in Egg Yolk Lecithin

Table 3 Parameters of MS-DIAL Software

Measurement parameters	
Ionization type	: Soft ionization
Separation type	: Chromatography
Collision type	: OAD
Data type (MS1)	: Centroid data
Data type (MS/MS)	: Centroid data
Ion mode	: Positive ion mode
Target omics	: Lipidomics
Data collection	
MS1 tolerance	: 0.01 Da
MS2 tolerance	: 0.025 Da
Peak detection	
Peak detection parameters	
Minimum peak height	: 100 amplitude
Mass slice width	: 0.1 Da
Spectrum deconvolution	
Deconvolution parameters	
Sigma window value	: 0.5
MS/MS abundance cut off	: 0 amplitude
Adduct ion	: $[M+H]^+$, $[M+Na]^+$ (positive ion mode)

■ Identification of PC 16:0/18:1(n-9) at *sn*-Position

PC 16:0_18:1(9) was selected from Table 2 to illustrate the process of determining *sn*-positions and native C=C locations. Fig. 5 shows the MS/MS spectra of egg yolk lecithin sample acquired in CID^- (A), CID^+ (B), OAD^+ (C), and $OAciD^+$ (D) modes. Although OAciD alone is sufficient to achieve this level of structural characterization, spectra from the other three dissociation modes are also provided for comparison.

Interpretation of the spectrum A confirms the presence of C16:0 and C18:1 acyl chains. Since negative MS^2 CID of PC typically produces more abundant fragment ions from *sn*-2, it would probably be PC(16:0/18:1). But we are not sure, because uncertainty from instrument conditions.

In spectrum B, *sn*-2 is favorably lost producing more abundant ion than *sn*-1. The intensity of *m/z* 496 (loss of C18:1) is higher than *m/z* 522 (loss of C16:0), suggesting acyl chain C18:1 is probably *sn*-2. Still, we are not sure. Notably, *m/z* 577 is $[M+H]^+$ form of five-membered 1,3-dioxolan ring, which shows a considerable intensity, although fails to produce *sn*-specific fragment ions further.

Clearly shown in spectrum C, a neutral loss of 96.1298 Da corresponds to C=C location information. based on the established rules for OAD-specific neutral losses, we identify the acyl chain as C18:1(n=9).

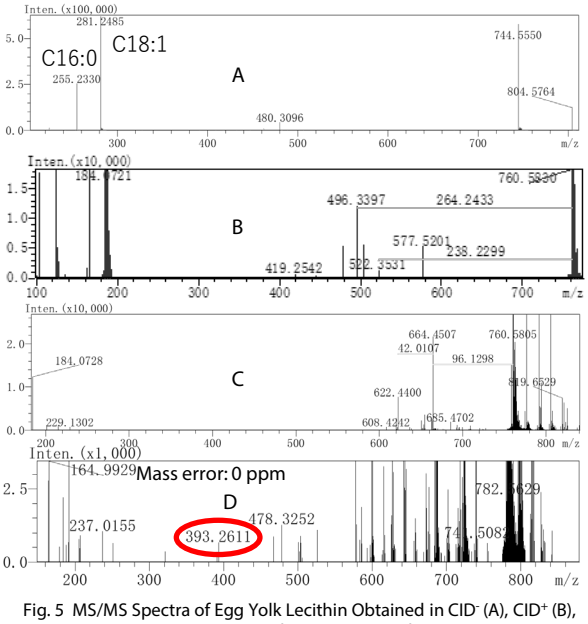

Fig. 5 MS/MS Spectra of Egg Yolk Lecithin Obtained in CID^- (A), CID^+ (B), OAD^+ (C), and $OAciD^+$ (D) Mode

In spectrum D, the signature *sn*-1 fragment ion at *m/z* 393 (circled in red, mass error 0 ppm) definitively identifies C16:0 at the *sn*-1 position, confirming the full identification as PC 16:0/18:1(n-9). Notably, information from headgroup, acyl chain and C=C position can also be read out form $[M+H]^+$ MS/MS spectrum under OAciD, enabling comprehensive identification of the *sn*-positions and native C=C locations in a single OAciD run simultaneously.

■ Diagnosis Ions for Common Acyl Chains at *sn*-1 Position

sn-1 acyl chain remains in fragment generated via OAciD, serving as a diagnosis ion for *sn*-isomers. Since PCs contain only two acyl chains, *sn*-2 could be deduced from the acyl composition as shown in Table 2. Table 4 lists diagnosis ions for common acyl chains located at *sn*-1 position.

Table 4 Diagnosis Ion *m/z* of *sn*-1 Generated by OAciD

No.	Original fatty acid	Carbon: DB	Diagnosis ion *m/z*
1	Lauric acid	C12:0	337.1985
2	Myristic acid	C14:0	365.2298
3	Palmitoleic acid	C16:1	391.2455
4	Palmitic acid	C16:0	393.2611
5	Linolenic acid	C18:3	415.2455
6	Linoleic acid	C18:2	417.2611
7	Oleic acid	C18:1	419.2768
8	Stearic acid	C18:0	421.2924
9	EPA	C20:5	439.2455
10	Arachidonic acid	C20:4	441.2611
11	Eicosenoic acid	C20:1	447.3081
12	Arachidic acid	C20:0	449.3237
13	DHA	C22:6	465.2611
14	DPA	C22:5	467.2768
15	Behenic acid	C22:0	477.3550
16	Nervonic acid	C24:1	503.3707

■ Identification of Other Major PCs in Egg Yolk Lecithin at *sn*-1 Position

As shown in Table 2, egg yolk lecithin contains a variety of PCs, we pinpoint *sn*-position for other three major PCs using the established method. Fig. 6 shows the *sn*-1 diagnosis ions detected in each PC. Fig. 7 shows presumed structures for each PC.

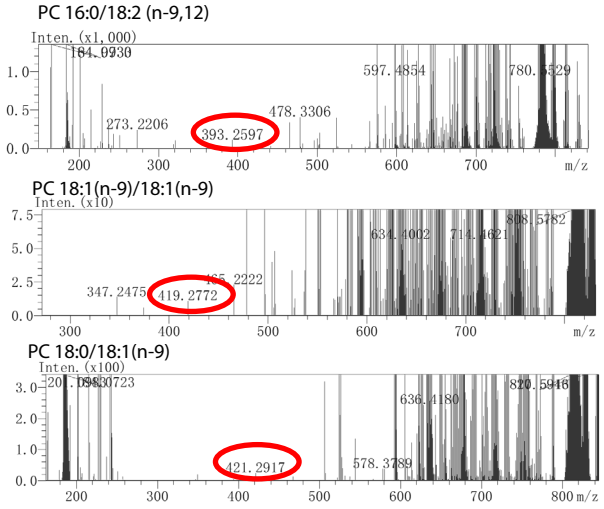

Fig. 6 *sn*-1 Diagnosis Ions Detected in (Top) PC 16:0/18:2 (n-9,12), (Middle) PC 18:1 (n-9)/18:1 (n-9) and (Bottom) PC 18:0/18:1 (n-9)

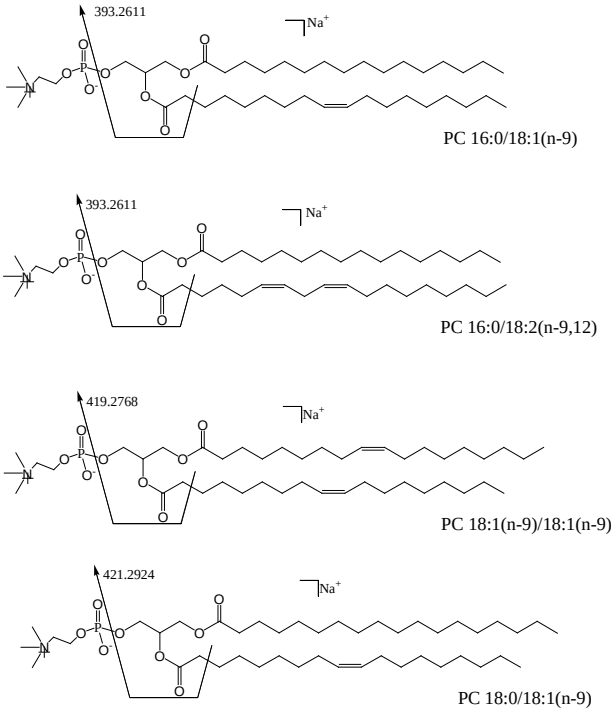

Fig. 7 Presumed Structures for 4 Major PCs in Egg Yolk Lecithin

■ Conclusion

√ For the first time, the *sn*-positions and C=C locations are confidently pinpointed for major PCs in egg yolk lecithin samples with both diagnosis ions in a single OAciD analysis, significantly enhancing the structural identification capacity of the OAD-TOF system.

√ OAciD could subject CID product ions for sequential OAD dissociation, fully compatible with LC separation, producing a highly hybrid and complex spectrum, rich in structural information.

√ The excellent mass accuracy of the LCMS-9050 allows verification of specific structural information with MS-DIAL software, which is automatic, handy and convenient.

√ Further optimization of the proposed workflow involves enhancing sensitivity for low-abundance PCs through the addition of sodium acetate to mobile phase and integrating *sn* diagnosis ion information into OAD database in MS-DIAL for an all-in-one automated annotation.

<References>

1) H. Uchino, H. Tsugawa, H. Takahashi, M. Arita, Computational mass spectrometry accelerates C = C position-resolved untargeted lipidomics using oxygen attachment dissociation, *Communications Chemistry*, 5, 162 (2022).
2) W. P. Zhang, R. J. Jian, J. Zhao, Y. K. Liu, Y. Xia, Deep-lipidotyping by mass spectrometry: recent technical advances and applications, *Journal of Lipid Research*, 63, 7 (2022).
3) D. L. Marshall, H. T. Pham, M. Bhujel, J. S. R. Chin, J. Y. Yew, K. Mori, T. W. Mitchell, S. J. Blanksby, *Analytical Chemistry, 88*, 2685 (2016).

<Related Applications>

1. Identification of Double Bond Positions in Butter Triacylglycerols Using DPiMS QT Kit and OAD-TOF system, Application News No. 01-00675

Prominence is a trademark of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

03-LCMSMS-1088-EN First Edition: Feb. 2026

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/
Shimadzu (China) Co., Ltd
www.shimadzu.com.cn

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
See http://www.shimadzu.com/about/trademarks/index.html for details.
Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.
The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

Copyright © 2026 Shimadzu Corporation and/or its affiliates. All rights reserved.

Please fill out the survey

Related Products Some products may be updated to newer models.

LCMS-9050

Quadrupole Time-of-Flight Liquid Chromatograph Mas···

OAD-TOF system

Quadrupole Time-of-Flight Mass Spectrometer system···

Related Solutions

Food and Beverages

Food Metabolomics

Price Inquiry

Product Inquiry

Technical Service / Support Inquiry

Other Inquiry