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Application News

Oxygen Attachment Dissociation for the Structural Characterisation of Lipids Involved in Pancreatic Cancer

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User Benefits

- Oxygen Attachment Dissociation (OAD) is a novel radical-induced dissociation technology that uses charge-neutral hydroxyl radicals (OH•) and oxygen atoms (O) used to annotate the positions of double bonds (C=C) in complex lipids.
- Simultaneous acquisition of OAD-MS/MS and CID-MS/MS in positive or negative ESI mode enables lipid identification to the structural level.
- OAD-MS/MS increases reporting confidence in lipid identification.

■ Introduction

Clinical lipidomics research has resulted in a significant advance in the understanding of the dysregulation of lipid metabolism and the association with the development and progression of cardiometabolic and neurological diseases, endocrine system diseases, inflammatory disorders, and several forms of cancer. Mass spectrometry-based technologies have been applied to characterising the lipidome, however, the structural diversity of lipids is complex as a result of different combinations of the molecular backbone, polar head groups, acyl chains, C=C positional isomers, sn-positions, and stereocentres.

Oxygen Attachment Dissociation OAD-MS/MS based mass fragmentation, also referred to as radical-induced dissociation, results in C = C-specific fragmentation and annotation of C=C positional isomers. As OAD-MS/MS and CID-MS/MS spectra are acquired at the same time (in either positive or negative ion mode) it results in the identification of lipids at the structural level (Fig. 1).

In this study, the novel radical induced MS/MS technique of OAD-MS/MS was applied to provide double bond specific dissociation in lipids previously identified as potential biomarkers in pancreatic ductal adenocarcinoma (PDAC) through untargeted metabolomics using similar analytical conditions but with collision induced dissociation MS/MS.

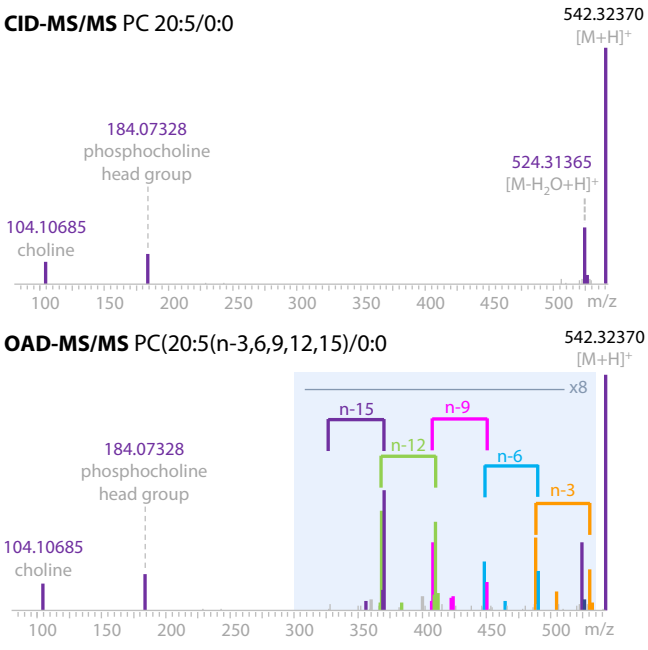

Fig. 1 CID-MS/MS identified a lipid that was differentially expressed in a PDAC sample as PC 20:5/0:0. OAD-MS/MS enabled the C=C positional assignments in lipids resulting in the lipid identification as PC(20:5(n-3,6,9,12,15)/0:0).

■ Materials and Methods

Human serum extracts were obtained from PDAC patients and healthy controls. The research project was approved by the Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) Access Committee. Phenotypic pooled quality control samples were analysed by OAD-MS/MS to structurally characterise lipids found to be significantly diminished in PDAC patient serum profiles compared to healthy control profiles in an untargeted metabolomics analysis (30 healthy controls and 30 PDAC). The analysis was performed using the analytical conditions described in Table 1.

LabSolutions Insight Explore™ and MS-DIAL were used to process the data. OAD-DDA-MS/MS spectra acquired in positive and negative ESI mode for each lipid were used 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.

Table 1 Analytical conditions

UHPLC:	Nexera X2
Column	C18 (2.1x100mm 1.7 µm); column maintained at 50 ˚C
Flow Rate	0.4 mL/min
Mobile Phase	A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid
Rinse (R0/R1/R2)	IPA/MeOH/MeCN/H_2O (1:1:1:1) + 1% formic acid
Gradient	2% (0-1min); 40% B (2 min); 90% B (25 min, curvi-linear gradient); 100% B (27.5-31 min); 2% B (31.01-35 min)
Injection	1 µL (ESI+) / 2 µL (ESI-); autosampler maintained at 4 ˚C
Mass Spectrometer:	LCMS-9050 with OAD Radical Source I
Interface	300 ˚C, 4.0 kV (ESI+) / −3.0 kV (ESI−)
Heat block, DL	400 ˚C, 250 ˚C
Gas flow rates	Nebulizing gas 3 L/min; Heating gas 10 L/min Drying gas 15 L/min
OAD Radical Source	Atomic oxygen (O) and hydroxyl radicals (OH•) are generated in a radical source. Collision gas pressure 17 kPa
MS	TOF-MS (*m/z* 60-1250)
MS/MS	5 DDA-MS/MS (ESI+) / 3 DDA-MS/MS (ESI -); *m/z* 40-1250; 1 second cycle time; collision energy spread 6-30 V; Precursor isolation 0.8 Da (ESI+) / 3 Da (ESI−)

■ Results

OAD MS/MS is an innovative technology, providing additional fragmentation to CID in positive and negative ion mode on the LCMS-9050 QTOF Mass Spectrometer. Figure 2 shows a schematic of the OAD Radical Source I which can be attached to the LCMS-9050 QTOF.

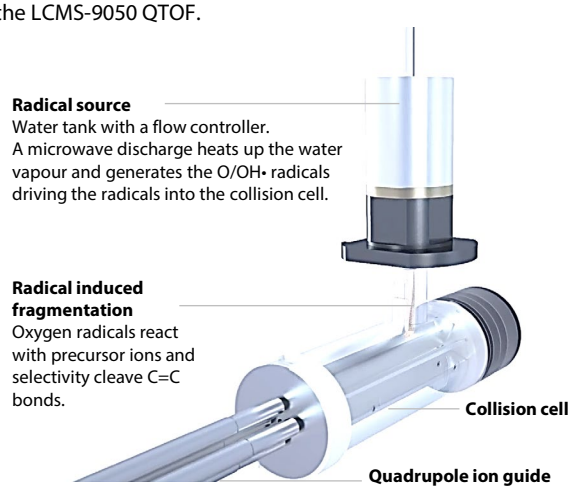

Fig. 2 Schematic of the OAD Radical Source I . Configuration supports OAD, CID or OAD/CID modes, controlled using LabSolutions™ data acquisition software.

In this study, potential biomarkers of PDAC identified in a previous metabolic profiling study have been analysed in greater depth using OAD-MS/MS to enable C=C position assignments. This involved identification of OAD-MS/MS characteristic neutral loss fragments for each of the candidates as described for lipids[1]. The relative intensity of the fragments can be influenced by lipid subclass, chain type and adduct, although the limit of annotation is defined as the detection of the main 'fragment pair'.

An example of OAD-MS/MS spectral interpretation is shown in Fig. 3 for LPC 20:5 sn-1, which was one of the lipids that was significantly diminished in the PDAC patient serum compared to healthy controls. OAD-MS/MS fragments are coloured to denote the double bond position they correspond to, and the main fragment pairs are labelled by detected m/z. OAD-MS/MS analysis of LPC 20:5 sn-1 enabled the identification of a carbon-carbon double bonds in positions 3, 6, 9, 12, and 15 from the methyl (omega) end (or positions 5, 8, 11, 14, and 17 from the carboxylic end corresponding to the delta nomenclature of lipid structural characterisation).

Consistent with previously published LC-MS/MS literature, phospholipids containing linoleic acid were significantly reduced in PDAC compared to healthy controls. Lipids included PC(18:2/0:0), PE(18:2/0:0), PC(18:1_18:2), PC(18:2_18:2) and PC(18:2_20:4). OAD-MS/MS revealed that all contained linoleic acid, with double bonds in the n-6 and n-9 positions corresponding to omega-6 linoleic acid (18:2(n-6,9)). Figure 4 highlights the spectral interpretation of OAD-MS/MS indicating the double bond positions in these lipids.

Following this process, other unsaturated lipids revealed as potential biomarkers of PDAC were structurally characterised (Table 2).

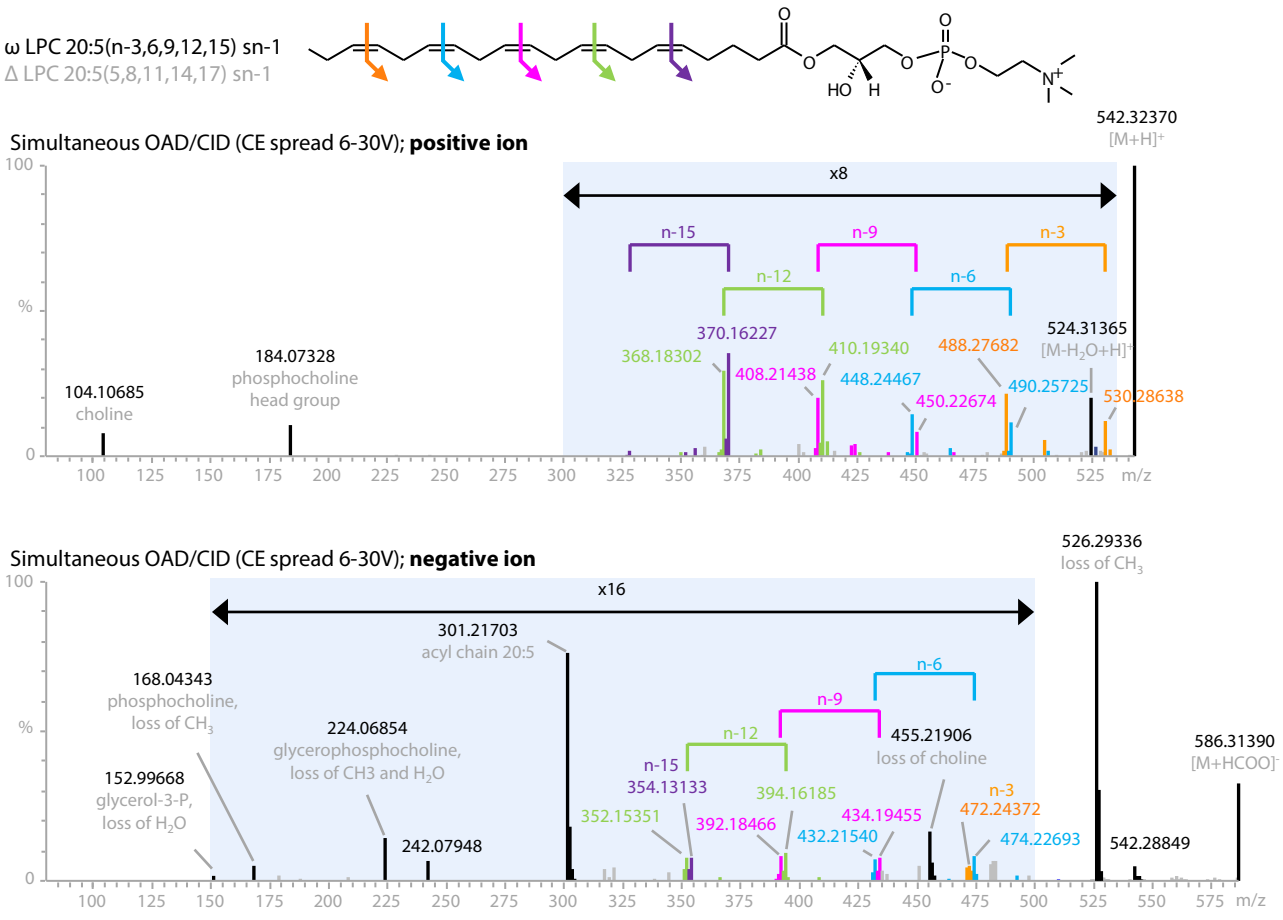

Fig. 3 Structural characterisation of LPC 20:5sn-1 using OAD and CID MS/MS. DDA-MS/MS acquired with simultaneous OAD and CID in positive and negative ion mode on the precursor for this lipid. CID specific fragments are highlighted in black and OAD specific fragments are highlighted in different colours to show the ions corresponding to each C=C double bond in the structure. The double bond positions are given in both the omega and delta nomenclature.

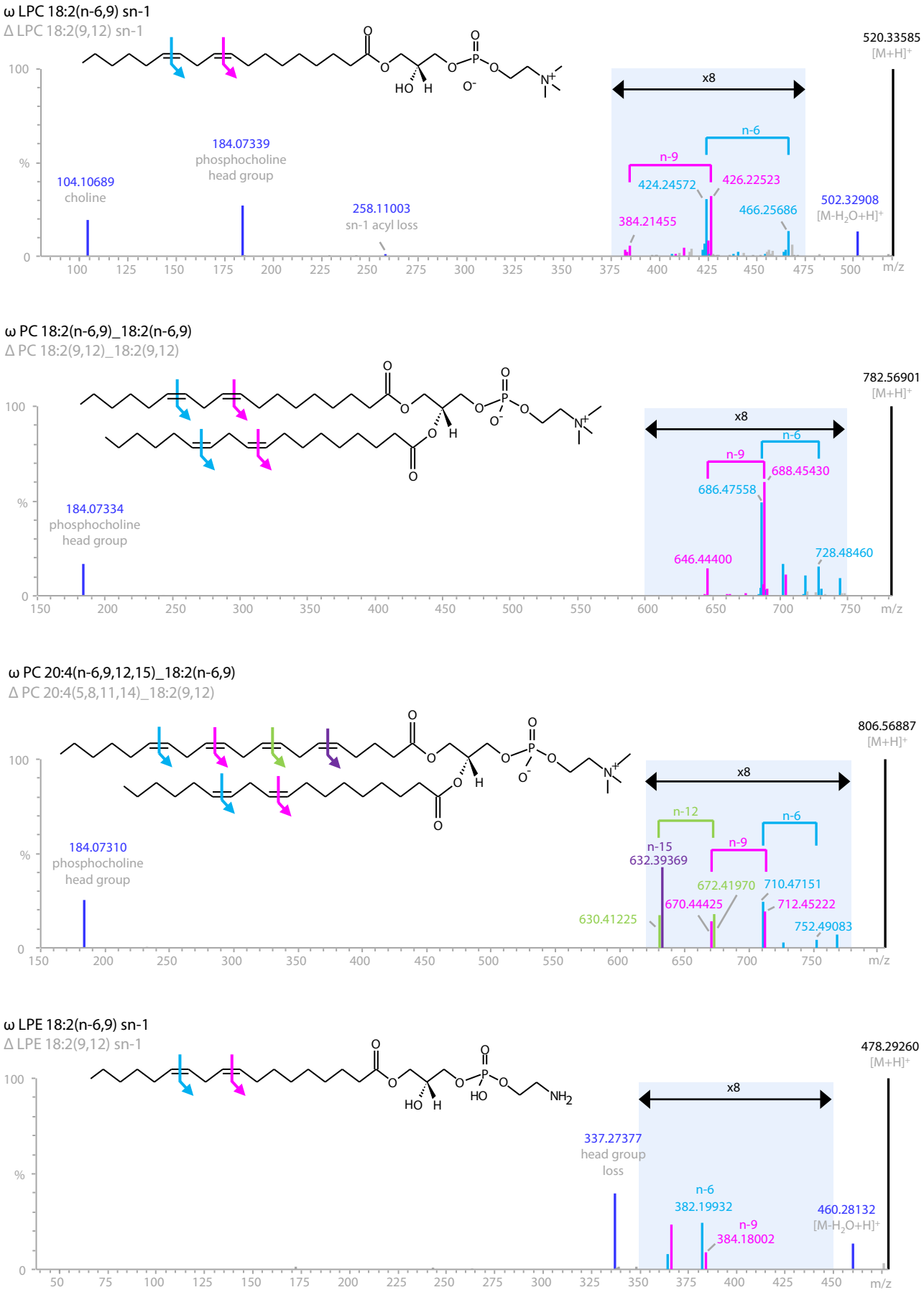

Fig. 4 Structural characterisation of LPC 18:2 sn-1,PC(18:2_18:2), PC(20:4_18:2) and LPE 18:2 sn-1using DDA-MS/MS with simultaneous OAD and CID MS/MS in ESI+. CID specific fragments are highlighted in blue and OAD specific fragments are highlighted in different colours to show the ions corresponding to each C=C double bond in the structure. The omega and delta nomenclature are given for each identification.

Table 2. OAD-MS/MS enabled identification of C=C double bond position(s) in unsaturated lipids found to be significantly diminished in PDAC serum profiles compared to healthy controls by untargeted metabolomics analysis (Log2FC calculated from comparison of 30 PDAC profiles to 30 healthy controls). OAD-MS/MS data were analysed in positive and negative ion mode to confidently identify the double bond positions in each of the lipids.

Annotation	Formula	Detected m/z	Detected RT	Theoretical m/z	Ion	log2(FC)
LPC 18:2(n-6,9) sn-1	C26H50NO7P	520.3408	6.76	520.33977	[M+H]+	-0.63
LPC 18:2(n-6,9) sn-2	C26H50NO7P	520.3401	6.48	520.33977	[M+H]+	-0.84
LPC 18:3(n-3,6,9) sn-1	C26H48NO7P	518.3246	5.95	518.32412	[M+H]+	-0.85
LPC 18:3(n-3,6,9) sn-1	C26H48NO7P	562.3152	5.98	562.31504	[M+HCOO]-	-0.63
LPC 20:5(n-3,6,9,12,15) sn-1	C28H48NO7P	542.3241	5.98	542.32412	[M+H]+	-0.85
LPC 20:5(n-3,6,9,12,15) sn-1	C28H48NO7P	586.3159	6.01	586.31504	[M+HCOO]-	-0.59
LPC 20:5(n-3,6,9,12,15) sn-2	C28H48NO7P	542.3241	5.77	542.32412	[M+H]+	-0.89
LPC 20:5(n-3,6,9,12,15) sn-2	C28H48NO7P	586.3153	5.79	586.31504	[M+HCOO]-	-0.65
LPE 18:1(n-9) sn-1	C23H46NO7P	480.3085	7.75	480.30847	[M+H]+	-0.81
LPE 18:1(n-9) sn-1	C23H46NO7P	478.294	7.78	478.29391	[M-H]-	-0.70
LPE 18:1(n-9) sn-2	C23H46NO7P	480.3086	7.43	480.30847	[M+H]+	-0.74
LPE 18:1(n-9) sn-2	C23H46NO7P	478.294	7.46	478.29391	[M-H]-	-0.71
LPE 18:2(n-6,9) sn-1	C23H44NO7P	478.2922	6.70	478.29282	[M+H]+	-1.10
LPE 18:2(n-6,9) sn-1	C23H44NO7P	476.2786	6.73	476.27826	[M-H]-	-0.93
LPE 18:2(n-6,9) sn-2	C23H44NO7P	478.2926	6.43	478.29282	[M+H]+	-1.06
LPE 18:2(n-6,9) sn-2	C23H44NO7P	476.2784	6.45	476.27826	[M-H]-	-0.97
PC 14:0_18:2(n-6,9)	C40H76NO8P	730.5378	19.75	730.53813	[M+H]+	-0.71
PC 14:0_18:2(n-6,9)	C40H76NO8P	774.5289	19.86	774.52906	[M+HCOO]-	-0.65
PC 15:0_18:2(n-6,9)	C41H78NO8P	744.5533	21.41	744.55378	[M+H]+	-0.84
PC 15:0_18:2(n-6,9)	C41H78NO8P	788.5447	21.53	788.54471	[M+HCOO]-	-0.84
PC 16:0_20:5(n-3,6,9,12,15)	C44H78NO8P	780.554	20.31	780.55378	[M+H]+	-0.76
PC 16:0_20:5(n-3,6,9,12,15)	C44H78NO8P	824.5455	20.41	824.54471	[M+HCOO]-	-0.65
PC 18:0_18:2(n-6,9)	C44H84NO8P	786.6005	27.07	786.60073	[M+H]+	-0.67
PC 18:0_20:5(n-3,6,9,12,15)	C46H82NO8P	808.585	23.66	808.58508	[M+H]+	-0.93
PC 18:0_20:5(n-3,6,9,12,15)	C46H82NO8P	852.576	23.81	852.57601	[M+HCOO]-	-0.89
PC 18:1(n-9)_18:2(n-6,9)	C44H82NO8P	784.585	23.51	784.58508	[M+H]+	-0.70
PC 18:1(n-9)_18:2(n-6,9)	C44H82NO8P	828.577	23.64	828.57601	[M+HCOO]-	-0.66
PC 18:2(n-6,9) /18:2(n-6,9)	C44H80NO8P	782.5702	20.74	782.56943	[M+H]+	-1.54
PC 18:2(n-6,9)/18:2(n-6,9)	C44H80NO8P	826.5614	20.85	826.56036	[M+HCOO]-	-1.42
PC 18:2(n-6,9)_20:4(n-6,9,12,15)	C46H80NO8P	806.569	20.24	806.56943	[M+H]+	-0.99
PC 18:2(n-6,9)_20:4(n-6,9,12,15)	C46H80NO8P	850.561	20.35	850.56036	[M+HCOO]-	-0.97
SM 18:1(n-14);O2/16:0	C39H79N2O6P	703.5764	19.16	703.57485	[M+H]+	-0.60
SM 18:2(n-4,14);O2/16:0	C39H77N2O6P	745.5501	17.72	745.55013	[M+HCOO]-	-0.76

■ Conclusion

OAD-MS/MS is a novel fragmentation method providing complementary structural information to CID in positive and negative ion 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 characterise lipids found to be potential biomarkers of pancreatic cancer in a previous study using high resolution metabolomics analysis by UHPLC-CID-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 supports the automated processing and identification of lipids in OAD-MS/MS data acquired using the LCMS-9050.

■ References

1. Uchino, H., Tsugawa, H., Takahashi, H., & Arita, M. (2022). Computational mass spectrometry accelerates C = C position-resolved untargeted lipidomics using oxygen attachment dissociation. Communications Chemistry, 5(1).

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