SHIMADZU

TP 257

Characterization of Unsaturated Fatty Acids in Negative OAD-MS/MS using LCMS-9050

Hidenori Takahashi, Mami Okamoto, Yuta Miyazaki, Yohei Arao, Natsuyo Asano
Shimadzu Corporation, Kyoto, Japan

1. Introduction to OAD-MS/MS

- While low-energy CID-MS/MS is one of the most effective fragmentation techniques for structural analysis, it may not be ideally suited for the analysis of certain isomers.
- Several novel fragmentation techniques have been proposed to complement low-energy CID-MS/MS.

Table 1. Example of proposed novel fragmentation techniques

Electron-based fragmentation
EIEIO, ECD(Electron Capture Dissociation) by Zubarev et al. (1996)
Anion-based fragmentation
ETD(Electron Transfer Dissociation) by Syka et al. (2004)
Photon-based fragmentation
IRMPD (Infrared), UVPD (Ultraviolet), BRID (Blank body infrared)

- We have introduced neutral radical-based fragmentation techniques to structural analysis of biomolecules, peptides and lipids, since 2016.

Neutral radical-based *Takahashi et al., *Anal. Chem.* 2018, 90, 12, 7230.

Charge-neutral radical-induced dissociation is available in both positive and negative ion modes!

O• **OAD (Oxygen Attachment Dissociation)-MS/MS**
$[M+H]^+ + O^\bullet \rightarrow [M + H + O]^{+\bullet} \rightarrow$ *fragments*

H• **HAD (Hydrogen Abstraction Dissociation)-MS/MS**
$[M+H]^+ + H^\bullet \rightarrow M^{+\bullet} + H_2 \rightarrow$ *fragments*

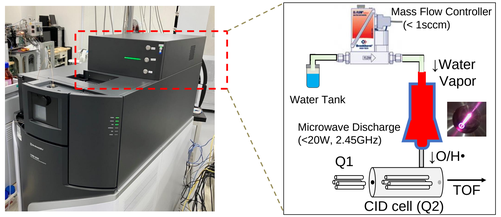


Fig. 1. Shimadzu LCMS-9050 (Q-TOF) with OAD unit.

2. Lipid MS/MS: CID vs. OAD vs. HAD Comparison

- **CID-MS/MS** does not provide detailed structural information within carbon chains. Instead, CID selectively cleaves labile polar head groups (*m/z* 184).

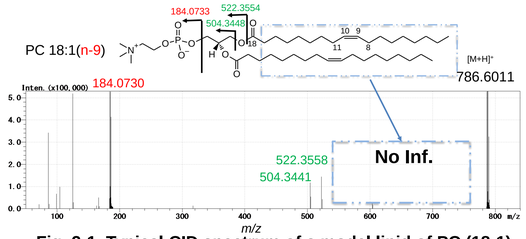


Fig. 2-1. Typical CID spectrum of a model lipid of PC (18:1).

- **OAD-MS/MS** clearly provides C=C positional information. Atomic oxygen selectively oxidizes and cleaves at C=C.

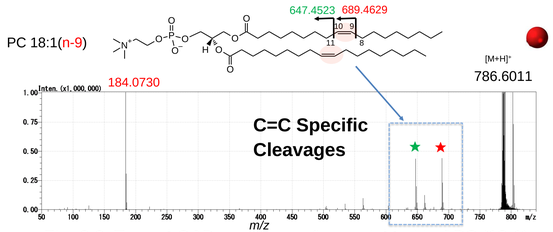


Fig. 2-2. Typical OAD spectrum of a model lipid of PC (18:1).

- **HAD-MS/MS** provides sequential structural information within carbon chains. The sensitivity of HAD is lower than OAD for C=C position assignment, since fragment ions become too complicated in a complex sample mixture.

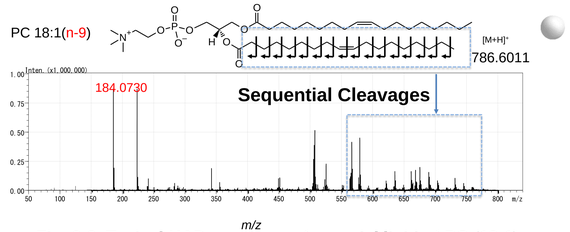


Fig. 2-3. Typical HAD spectrum of a model lipid of PC (18:1).

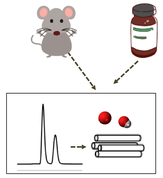
3. Negative OAD-MS/MS in lipid analysis

Table 2. Analytical Condition

HPLC condition
System: Shimadzu Nexera X3
Flow rate: 0.3 mL/min
Mobile phase A: 1:1:3 (v/v/v) ACN:MeOH: water with ammonium acetate (5mM) and 10nM EDTA
Mobile phase B: 100% IPA with ammonium acetate (5mM) and 10nM EDTA.
Column: Acquity UPLC Peptide BEH C18 (50 ×2.1mm; 1.7µm; Waters, USA)
Gradient: 0min 0% (B); 1min 0% (B); 5min 40% (B); 7.5min 64% (B); 12min 64% (B); 12.5min 82.5% (B); 19 min 85% (B); 20min 95% (B); 20.1min 0% (B); and 25min 0% (B).

MS condition
System: Shimadzu LCMS-9050
Mode: OAD
Polarity: Negative ESI
CE: +10 V
MS/MS: Auto-MS/MS Top 10

Sample: Lipid extract from a mouse's brain with the addition of several commercially available fatty acid standards. The method for preparing the lipid extract adheres to the procedure described in Uchino.H et al. *Commun Chem.* 5, 162 (2022).



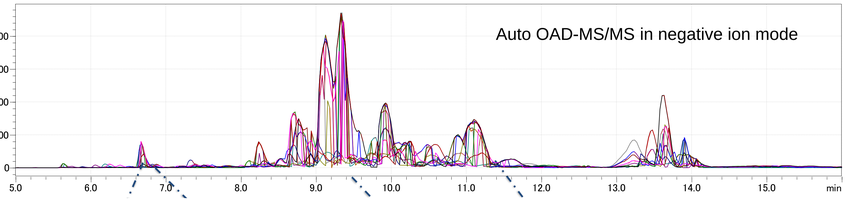
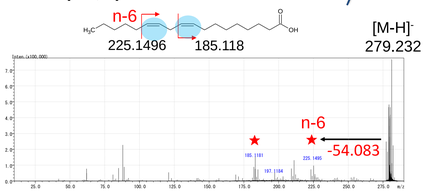
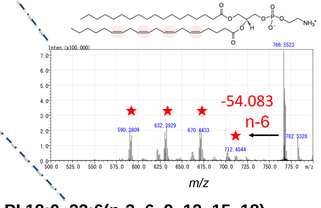


Fig. 3. Example of negative-OAD spectrum at several retention time.

FA 18:2(n-6, 9) Linoleic acid

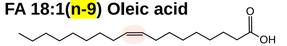


PE 18:0_20:4(n-6, 9, 12, 15)

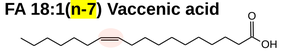


Co-eluted isomers

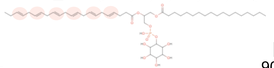
FA 18:1(n-9) Oleic acid

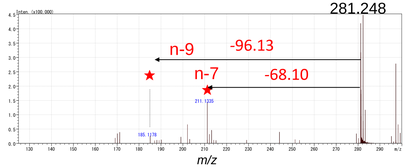


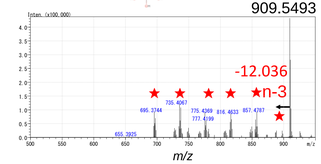
FA 18:1(n-7) Vaccenic acid



PI 18:0_22:6(n-3, 6, 9, 12, 15, 18)







4. Conclusions

- OAD was successfully integrated into the QTOF of LCMS-9050.
- OAD has demonstrated its effectiveness as a powerful tool for negative MS/MS fragmentation.
- Without any need for derivatization, OAD can accurately assign the C=C bond positions in lipids and fatty acids.