SHIMADZU
Excellence in Science

Mass spectrometer radicalisation - OAD-TOF

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Summary

1. Introduction

- Short introduction for Shimadzu QTOF (LCMS-9050)

2. What is OAD?

- Principal technique, Hardware

3. What OAD can do?

- Applications

ANALYTICAL INTELLIGENCE

eco

LCMS-9050

Accelerate Discovery

Sensitivity (S/N) *Reserpine 1 pg	**10000:1**
Mass resolution	**45,000 FWHM**
Mass range	**m/z 10 – 40,000**
Mass accuracy	**< 1 ppm (int. calib.)**
Positive-Negative switching	**500 ms**
Spectrum acquisition frequency	**MS/MS Max 200 Hz**

What is OAD?

Oxygen Attachment Dissociation MS/MS Option Kit

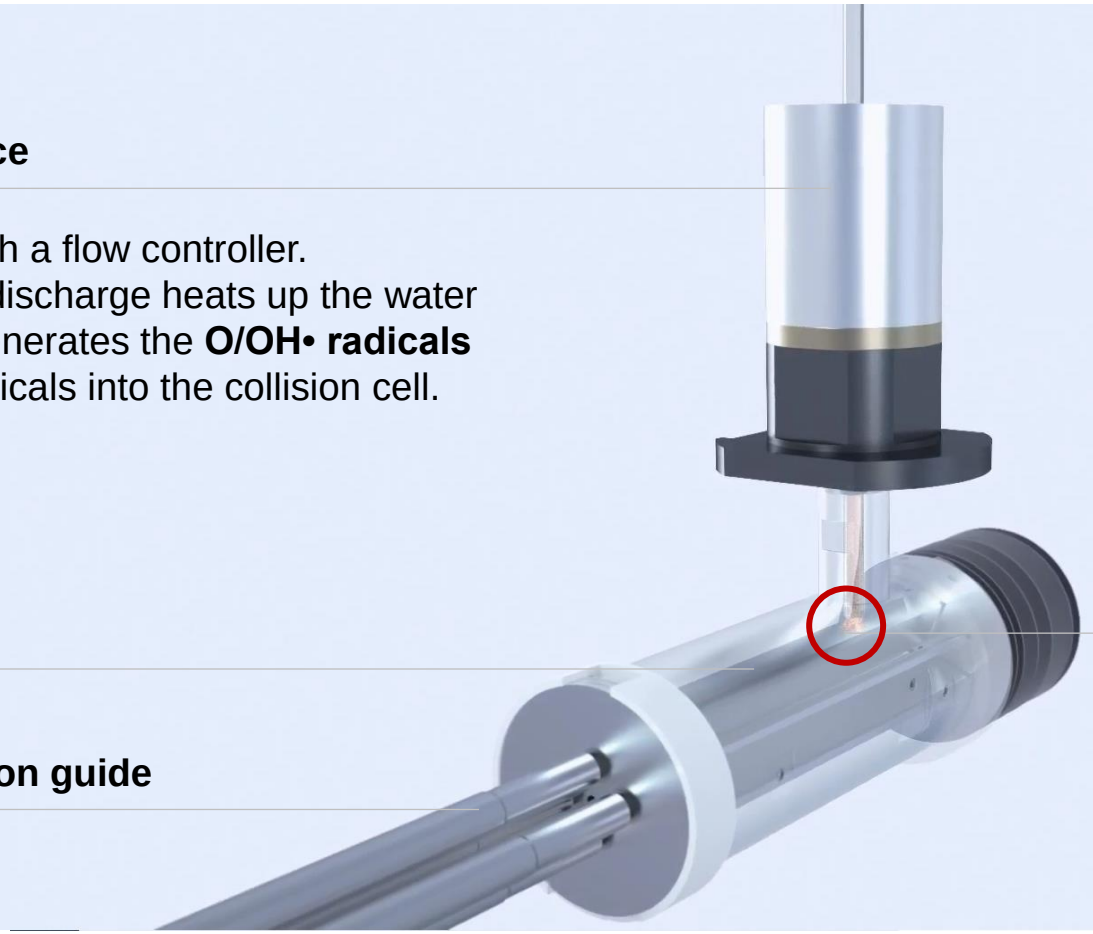
OAD Science fundamentals: the hardware

Uses O/OH• radicals generated by heating water with a microwave discharge

The heated water vapour is an inductively coupled plasma and generates gas phase hydroxyl radicals (OH·) and atomic oxygens (O) which **selectively cleave C=C positions**.

Radical source

Water tank with a flow controller.
A microwave discharge heats up the water vapour and generates the **O/OH• radicals** driving the radicals into the collision cell.



Collision cell

Quadrupole ion guide

LabSolutions control

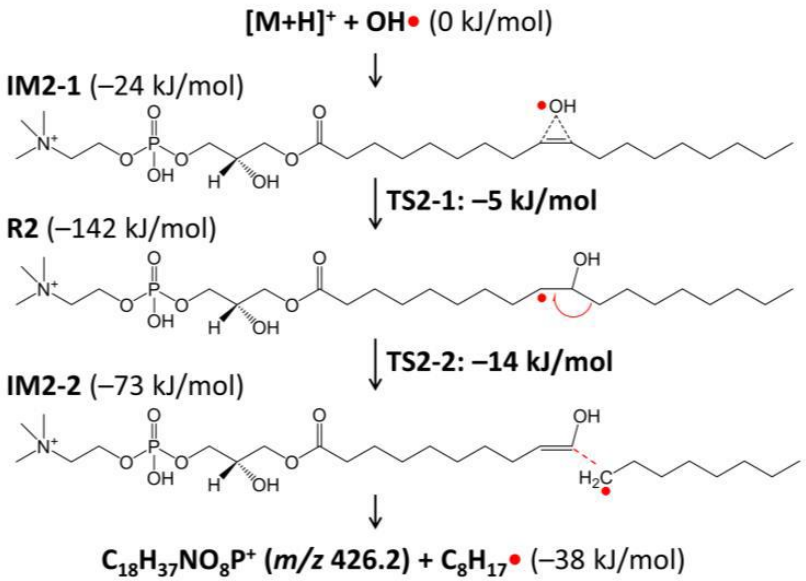
Supports OAD and OAD/CID switching.

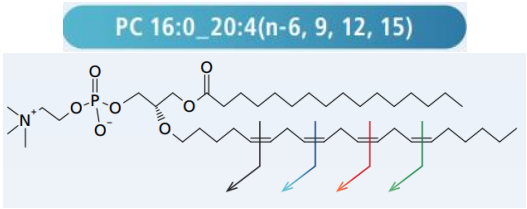
Fragmentation using oxygen radicals

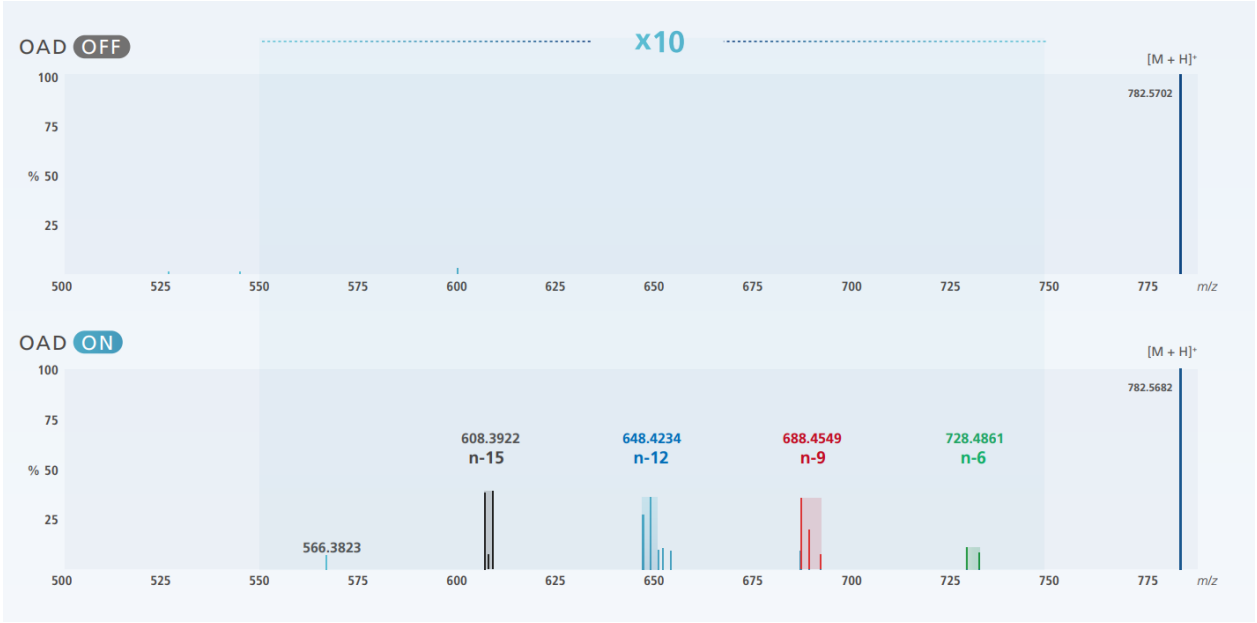
Oxygen radicals react with precursor ions and selectivity cleave C=C bonds

What can OAD do?

The position of double bond can be determined in both positive and negative mode.



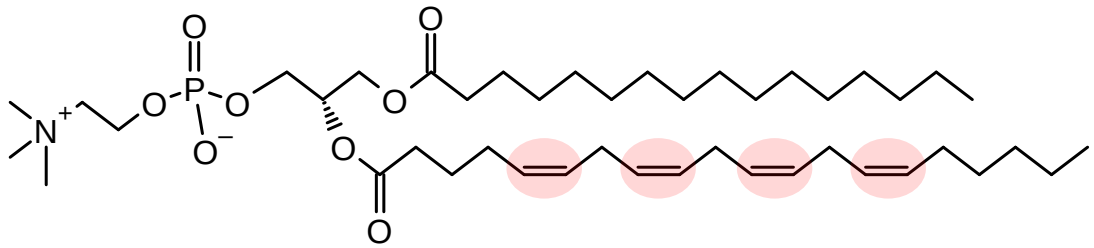




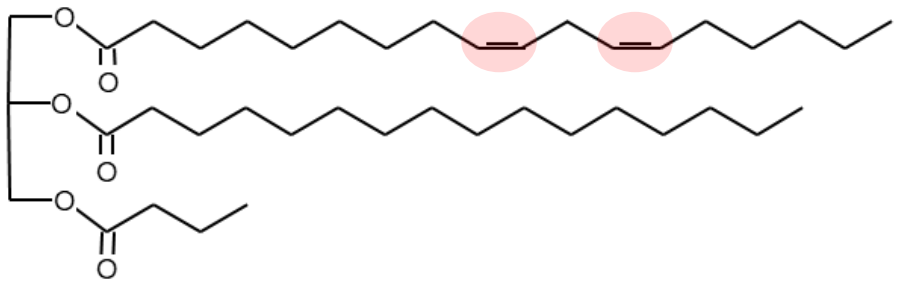
Which Compounds Can be the Target for OAD ?

Lipids

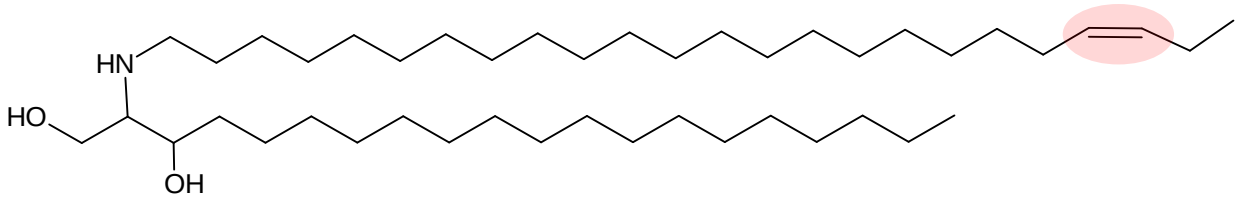
Phosphatidylcholine (PC)



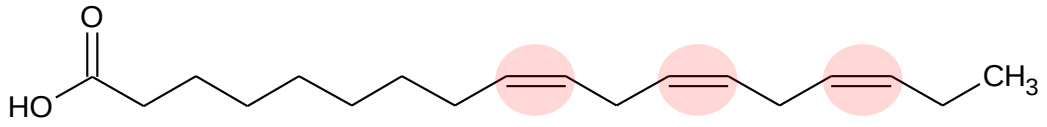
Triacylglycerol



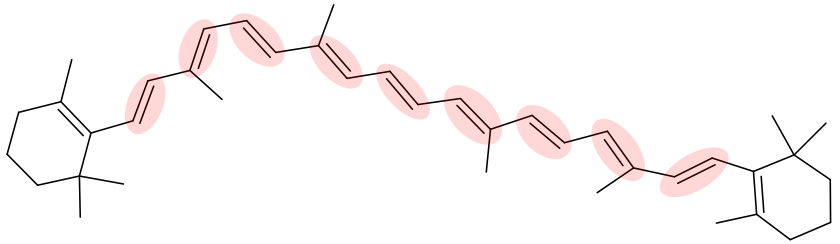
Ceramide



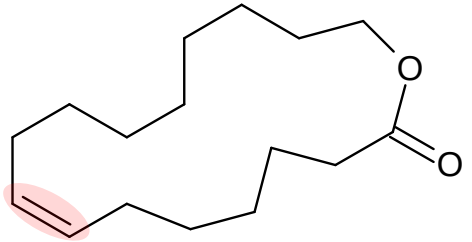
Fatty acids



Carotenoids



Lactones*

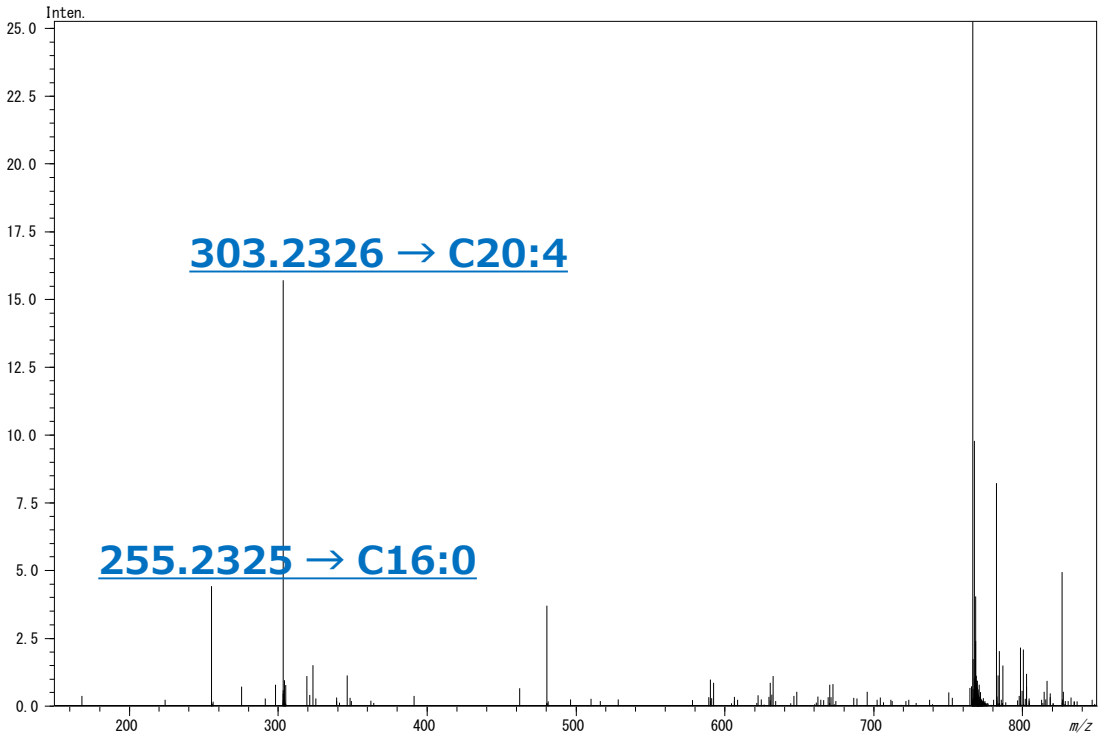


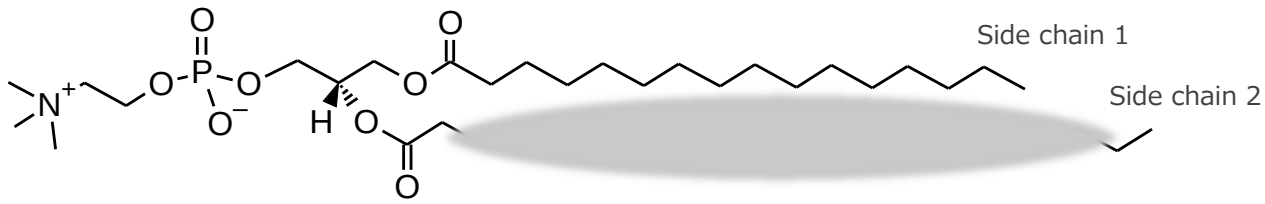
* Hydrolysis is required

Positive-Negative Simultaneous analysis x CID x OAD

Structural analysis of phospholipid (PC 36:4) in mouse liver

MS/MS spectrum by CID (Negative mode)





?

CID can provide

- Number of carbon for side chain
  →C16 (side chain 1), C20 (side chain 2)

- Number of double bond
  → 4 (side chain 2)

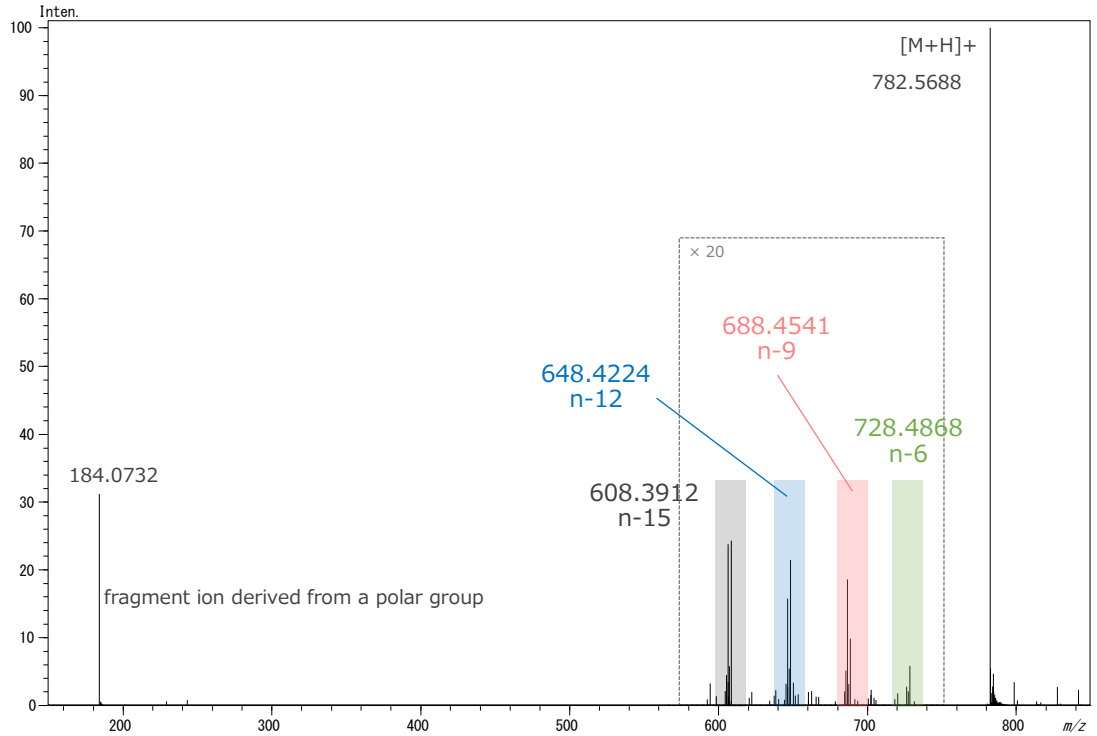
→ PC 16:0_20:4

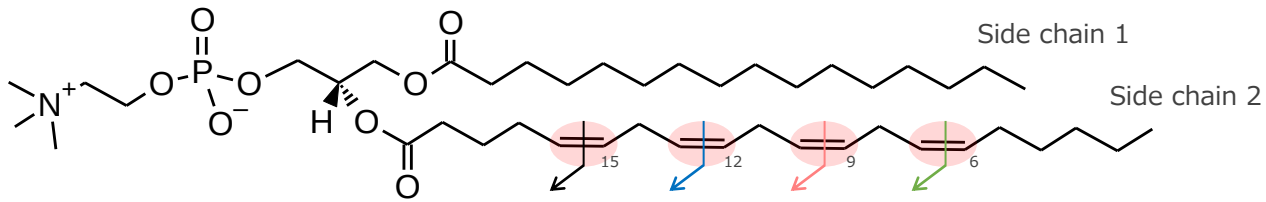
We still don't know where the double bonds are on side chain 2…

Positive-Negative Simultaneous analysis x CID x OAD

Structural analysis of phospholipid (PC 36:4) in mouse liver

MS/MS spectrum by OAD (Positive mode)





→ PC 16:0_20:4 (n=6, 9, 12, 15)

The position of double bond can be determined!

Comprehensive and detailed structural analysis is possible.

The selective fragmentation power of OAD

OAD delivers positive identification even with multiple C=C positional isomers

OAD is not limited to single bond cleavages, it can be applied to complex lipid chemistries and unlike OzID it is not influenced by the presence of adduct ions (efficiency of ozonolysis is influenced by the distance for the adduct to double bond position).

Lipid annotation.

PG 22:6(n-3,6,9,12,15,18)/22:6(n-3,6,9,12,15,18)

OAD identifies multiple C=C locations

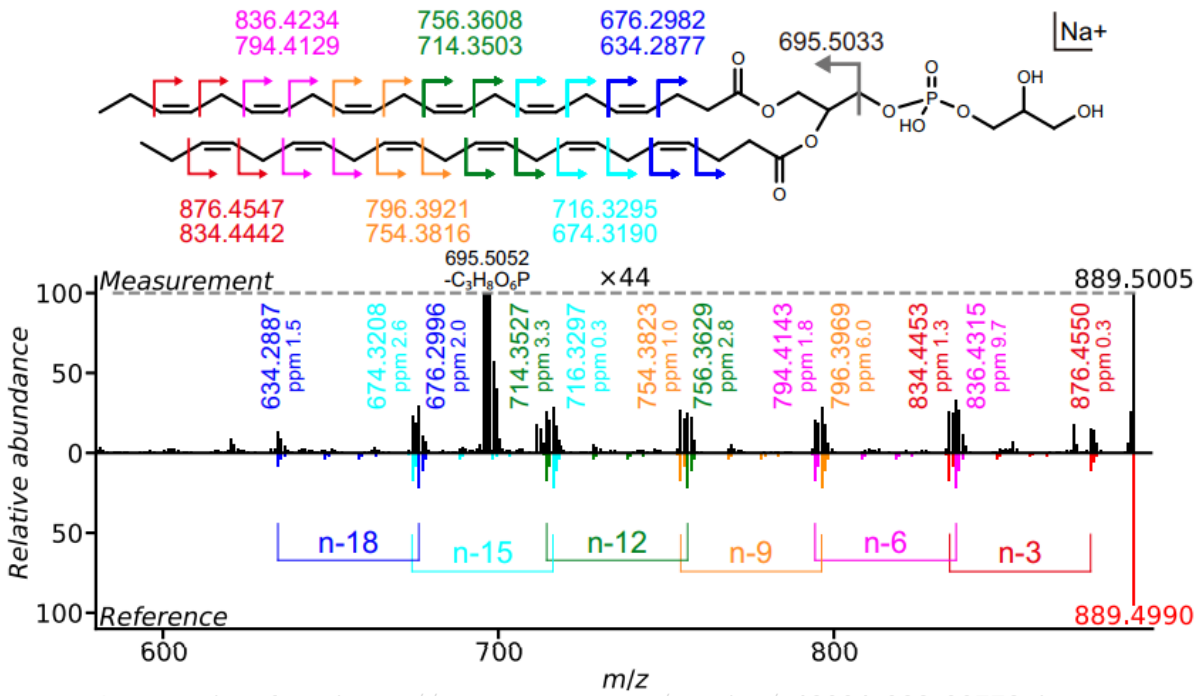
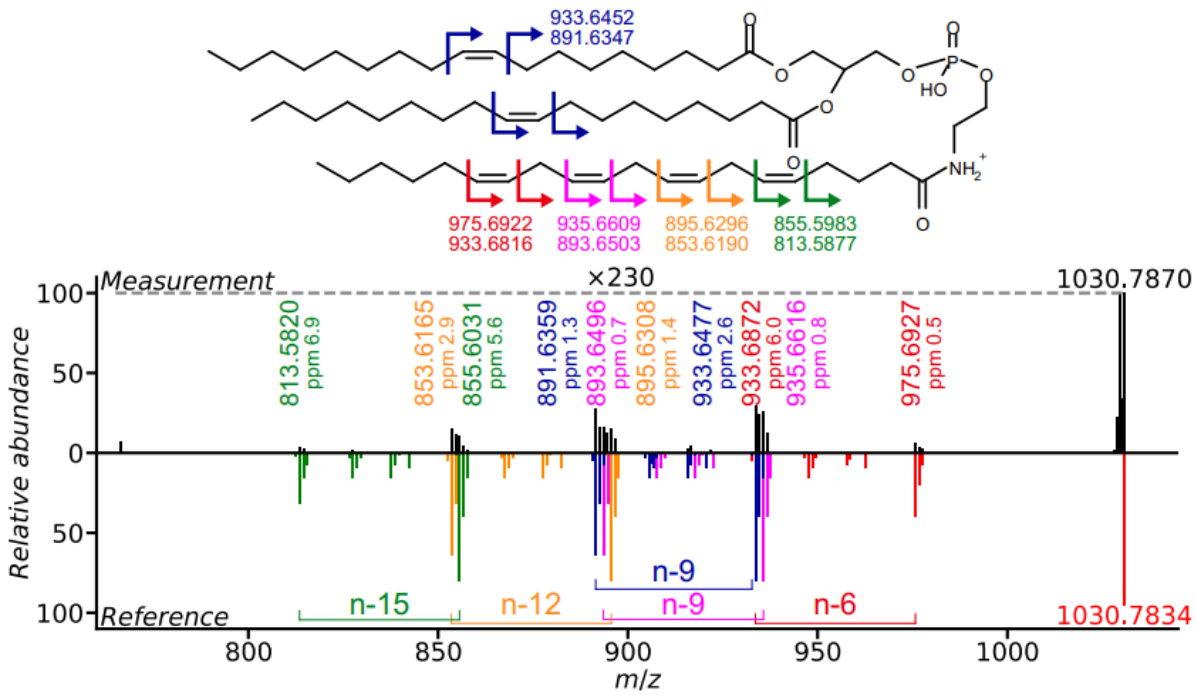


Image taken from https://www.nature.com/articles/s42004-022-00778-1

Lipid annotation.

PE-N(FA 20:4(n-6,9,12,15)) 18:1(n-9)/18:1(n-9)

OAD MS/MS identifies multiple C=C locations



The added value of OAD in lipid annotation/identification

LC-MS/MS can be processed in MS-DIAL which performs peak picking and MS/MS assignment of spectra irrespective of fragmentation method

MS-DIAL performs CID-spectral annotation to the molecular species level, then applies MS-RIDD to identify diagnostic fragment pairs in the OAD spectra to resolve the C=C double bond positions. Mapping results provides structural identification.

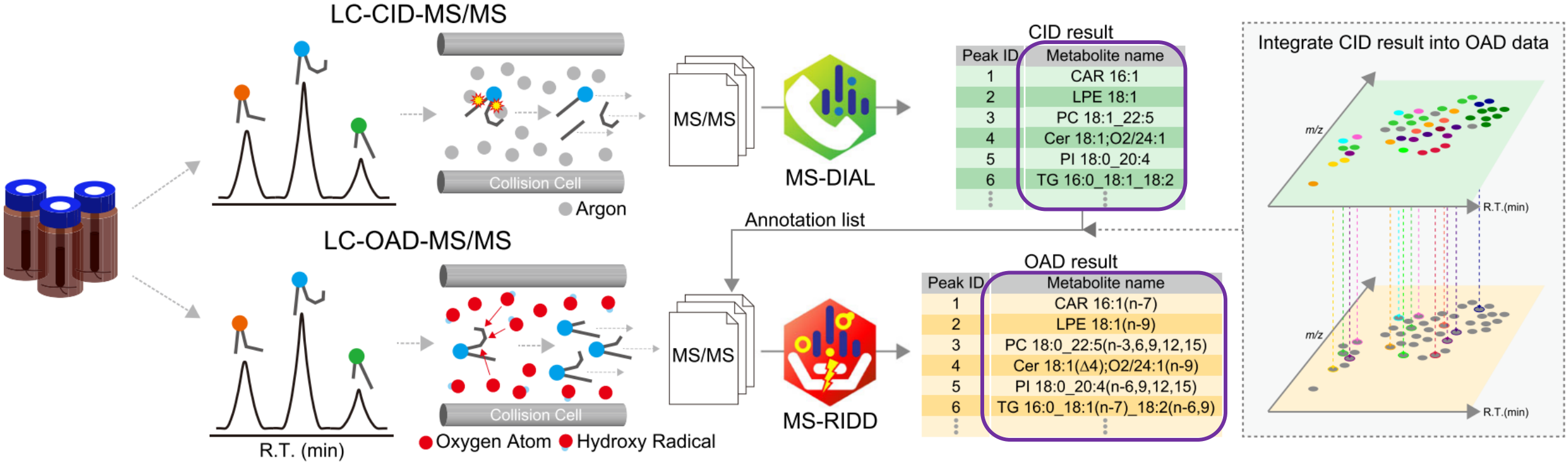


Image taken from https://www.nature.com/articles/s42004-022-00778-1

Unique Combination with OAD

Ultra High Performance Liquid Chromatograph

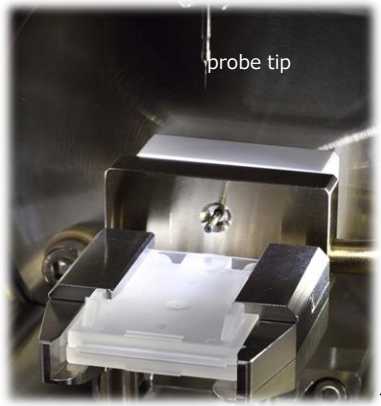
Nexera シリーズ

Supercritical Fluid Extraction / Chromatograph System

Nexera UC

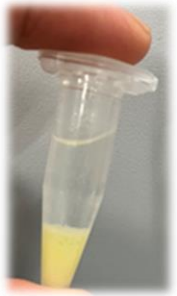
Kit for Direct Probe Ionization Mass Spectrometer

DPiMS QT

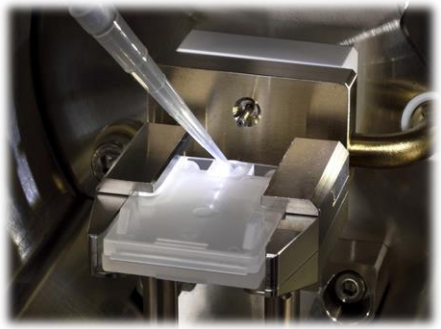


PESI (DPiMS) x OAD

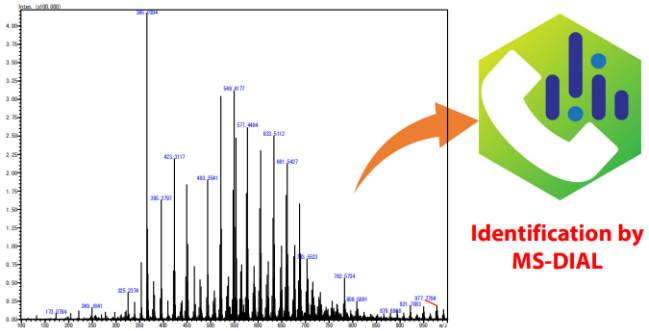
Analysis of lipids in butter by PESI-OAD-TOF



Extract with water and IPA



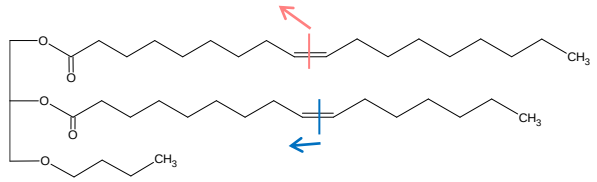
Drop the supernatant on the sample plate



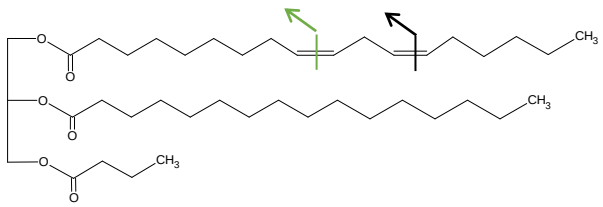
Identification by MS-DIAL

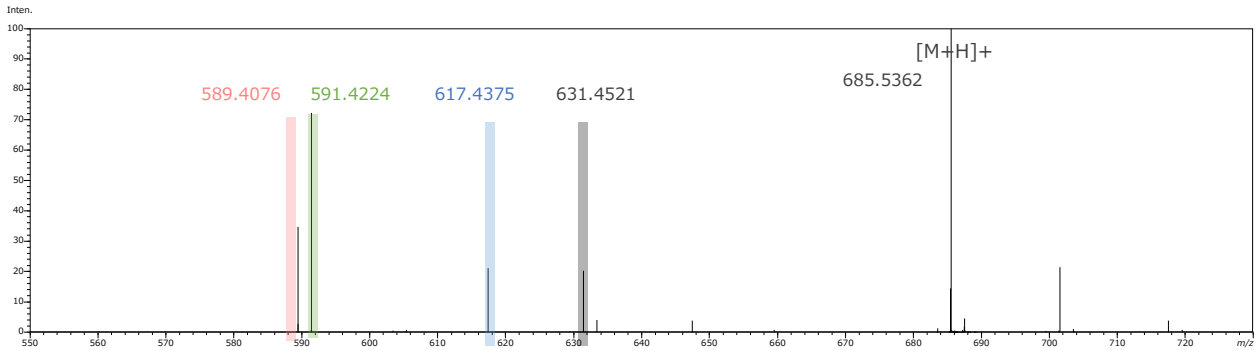
Fig. 5 MS Spectrum of Butter Extract Acquired with DPiMS QT

TG 18:1(n-9)_16:1(n-7)_4:0



TG 18:1(n-6,n-9)_16:0_4:0

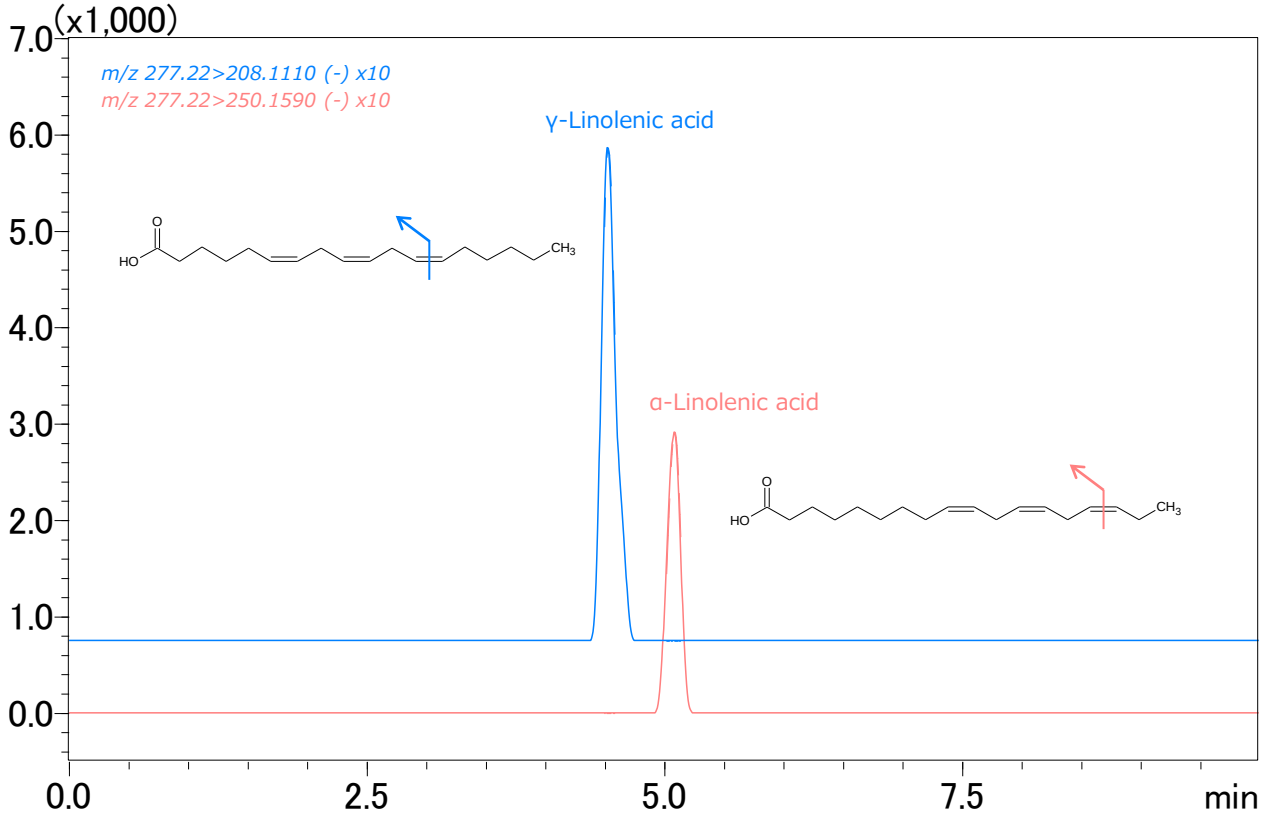




The position of double bond can be identified easily and quickly.

SFC × OAD

Free fatty acid analysis by SFC-OAD-TOF



SFC and OAD combination gives complete separation for isomers that can't be separated by LC, and specifically identify the compounds by OAD.

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