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Application News

Kit for Direct Probe Ionization Mass Spectrometer DPiMS™ QT
Optional Kit for Oxygen Attachment Dissociation MS/MS OAD RADICAL SOURCE I
High Performance Liquid Chromatograph Mass Spectrometer LCMS-9050

Identification of Double Bond Positions in Butter Triacylglycerols Using DPiMS QT Kit and OAD-TOF System

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User Benefits

- Qualitative screening with the DPiMS QT offers sample preparation times of approx. 5 min and measurement times of approx. 0.5 min.
- Position-specific fragmentation with the OAD RADICAL SOURCE I allows the identification of double bond positions.
- Fragment ions are detected with high mass accuracy and double bond positions are annotated with high certainty.

■ Introduction

Fats, of which fatty acids are the main constituent component, are one of the most important nutrients in food alongside carbohydrates, proteins, vitamins, and minerals, and have a major impact on biological function. The body stores fats in the form of lipids called triacylglycerols (TGs), which are composed of three fatty acid molecules esterified to a glycerol molecule. Almost all fats obtained from food are TGs, and because the effect of TGs on the body differs depending on the constituent fatty acids, not just the amounts of fat in foods but also the types of fatty acids in dietary TGs are an area of significant interest.

Fatty acids are categorized based on their structure as saturated fatty acids, which have no double bonds, and unsaturated fatty acids, which have double bonds. Unsaturated fatty acids with the same chemical composition are thought to have different effects in the body depending on the position of their double bonds. Because of this, knowing the position of double bonds in dietary lipids is critical to understanding the impact of food on the body.

■ Oxygen Attachment Dissociation (OAD) 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 using an OAD-TOF system equipped with the DPiMS QT probe electrospray ionization kit, which enables probe electrospray ionization (PESI) analysis, to successfully determine the structure of TGs in butter (including double bond positions) by a direct ionization method that uses no chromatographic separation and no retention time data.

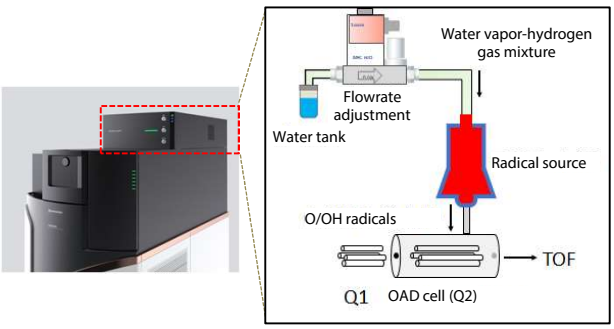

Fig. 1 Principle of OAD

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

Shimadzu's OAD-TOF system, which offers OAD analysis, can perform analysis in both CID and OAD modes and switches easily between each mode via software. The DPiMS QT kit can also be attached to the OAD-TOF system in around 15 seconds.

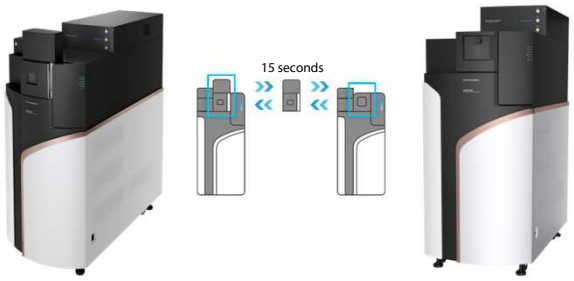

Fig. 2 DPiMS™ QT-Equipped OAD-TOF system (Left) and OAD-TOF system (Right)

■ Analytical Conditions

One of the major advantages of the DPiMS QT kit is that, while sampling and ionization are performed repeatedly at high speed, the very small sampling volumes (several pL) pose minimal risk of instrument contamination, and compared with LC-Q-TOF, analysis can be performed quickly with simple pretreatment.

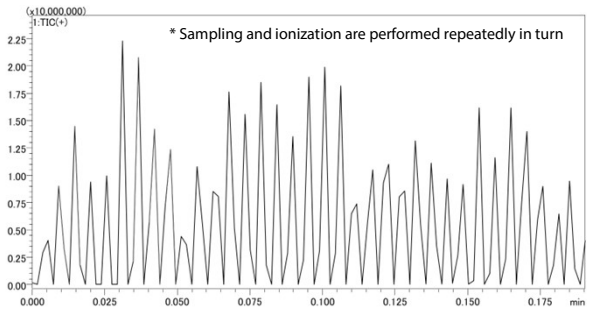

Fig. 3 MS1 Signal Intensity of Butter Extract Acquired with DPiMS QT

The analytical conditions used with this analytical system are shown in Table 1. The OAD-TOF system and DPiMS QT kit combination improved the speed of analysis based on a simple sample pretreatment and an analysis time of just 0.5 min.

Table 1 Analytical Conditions for DPiMS QT and OAD-TOF system

System:	DPiMS QT + LCMS-9050 + OAD RADICAL SOURCE I
Polarity:	Positive
DL Temp.:	250 °C
Heat Block Temp.:	50 °C
Interface Voltage:	3.0 kV
CE:	20 V (CID), 5 V (OAD)
TOF-MS:	*m/z* 100-1000
Measurement Time:	0.5 min

■ Butter Pretreatment

Fig. 4 shows the sample pretreatment used to analyze the TGs in a commercially available butter. 200 mg of butter was weighed and transferred to a 1.5 mL microtube, 1 mL of 20/80 (v/v) ultrapure water/isopropanol was then added to the microtube and the mixture agitated ultrasonically at 40 °C for 5 min. After separating the mixture by centrifugation, 10 µL of supernatant was recovered, instilled onto a liquid sample plate specifically designed for DPiMS, and analyzed.

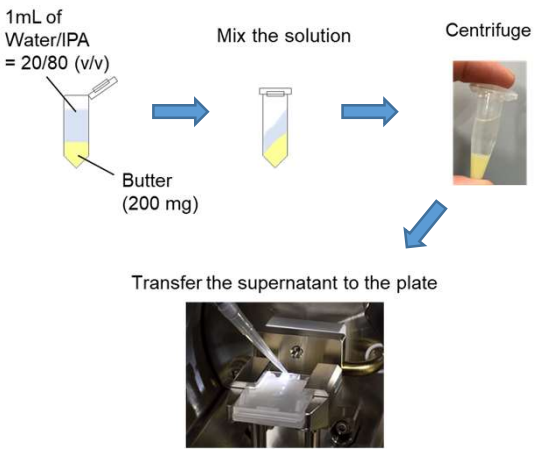

Fig. 4 Butter Preparation Workflow

Table 2 List of TG Candidates in Butter Extract Obtained with MS-DIAL

MS1 (*m/z*)	Adduct	Predicted Structure
493.3504	$[M+Na]^+$	TG 24:0
521.3825	$[M+Na]^+$	TG 26:0
535.3969	$[M+Na]^+$	TG 27:0
549.4146	$[M+Na]^+$	TG 28:0
563.4274	$[M+Na]^+$	TG 29:0
575.4286	$[M+Na]^+$	TG 30:1
577.4457	$[M+Na]^+$	TG 30:0
591.4584	$[M+Na]^+$	TG 31:0
603.4599	$[M+Na]^+$	TG 32:1
605.4765	$[M+Na]^+$	TG 32:0
631.4913	$[M+Na]^+$	TG 34:1
633.5084	$[M+Na]^+$	TG 34:0
657.507	$[M+Na]^+$	TG 36:2
659.5223	$[M+Na]^+$	TG 36:1
661.5384	$[M+Na]^+$	TG 36:0
673.5362	$[M+Na]^+$	TG 37:1
685.5381	**$[M+Na]^+$**	**TG 38:2**
687.5538	**$[M+Na]^+$**	**TG 38:1**
689.5681	$[M+Na]^+$	TG 38:0
713.5682	$[M+Na]^+$	TG 40:2
715.5845	$[M+Na]^+$	TG 40:1

■ Identification of Candidate Butter TGs

Fig. 5 shows the results of MS1 analysis of the butter extract obtained with the DPiMS QT. The data file obtained from analysis (file extension: .lcd) was transferred to MS-DIAL[2)] (ver. 5.1.230517) and lipids were identified based on precursor ion *m/z* data. The candidate TGs in the butter extract that were identified using MS-DIAL are shown in Table 2 and the settings used for this identification are shown in Fig. 6. TG 38:2 and TG 38:1 were selected from Table 2 for the purpose of this example analysis, CID and OAD analysis were performed to obtain detailed structural data, and the resulting MS/MS spectra were used for characterization.

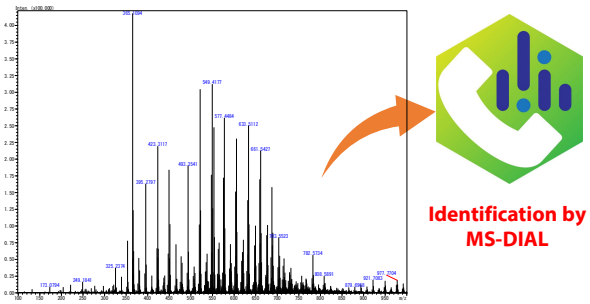

Fig. 5 MS Spectrum of Butter Extract Acquired with DPiMS QT

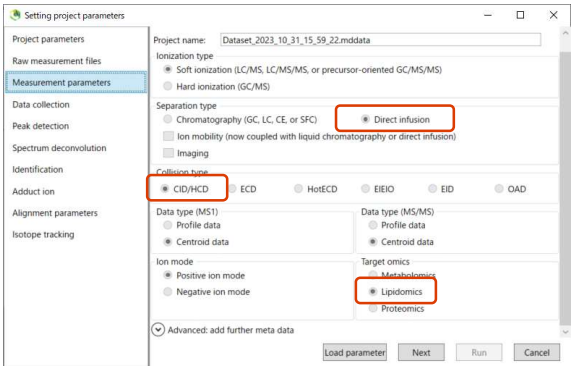

Fig. 6 Settings for PESI Data Analysis by MS-DIAL

■ Determining Lipid Subclass by CID Analysis

TG 38:1 and TG 38:2 in the butter extract were subjected to MS/MS analysis with the DPiMS QT in CID mode and the chain length of constituent fatty acids and number of double bonds were identified based on neutral loss. Fig. 7 shows each MS/MS spectrum with the fatty acid chain length and number of double bonds noted above the spectral peak of each fatty acid that was identified.

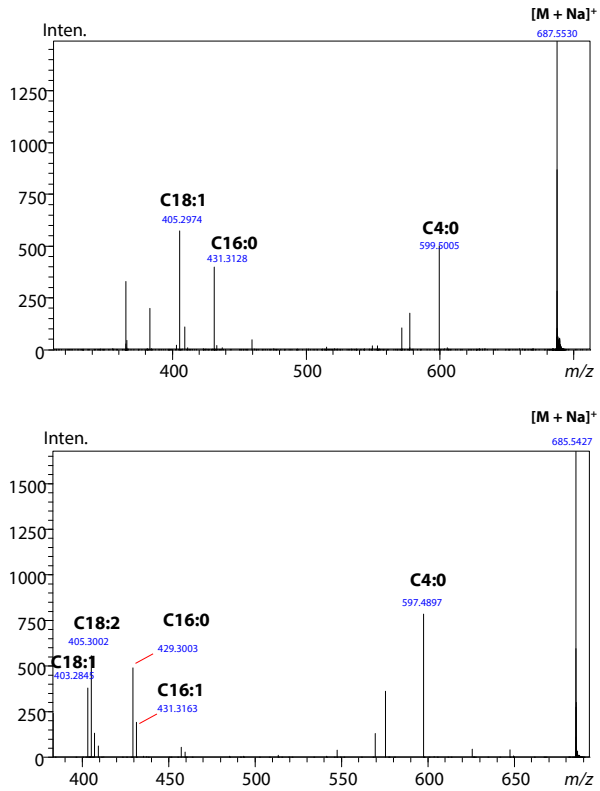

Fig. 7 MS/MS Spectrum of TG 38:1 (Top) and TG 38:2 (Bottom) in Butter Extract Acquired in CID Mode

■ Identifying Double Bond Positions in Fatty Acids by OAD

OAD analysis identifies double bond positions by causing position-specific fragmentation near double bonds. Butter extract TG 38:1 and TG 38:2 were subjected to MS/MS analysis in OAD mode and the positions of double bonds in each constituent fatty acid were identified. Fig. 8 shows an example ion cleavage by OAD. The OAD method causes cleavage of the C-C bond adjacent to the double bond, allowing identification of the position of the double bond. Table 3 shows the relationship between double bond position and the position-specific fragments obtained from OAD analysis of lipids that contain unsaturated fatty acids. The TG 38:1 and TG 38:2 MS/MS spectra obtained from analysis are shown in Fig. 9 and Fig. 10, respectively. Furthermore, because the neutral loss (NL) results obtained in CID mode for TG 38:2 suggested a mixture of two compounds with the same precursor ion, the double bond positions were determined in both compounds.

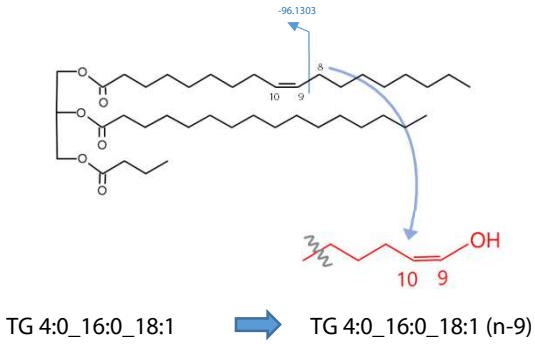

Fig. 8 Example Lipid Fragmentation by OAD

Table 3 NL Predictions by OAD Analysis

First Double Bond	n-	NL [Da]	First Double Bond	n-	NL [Da]
n-6	6	-54.0833	n-9	9	-96.1303
	9	-94.1157		12	-136.1626
	12	-134.1481		15	-178.1951
n-7	7	-68.0990	-	-	-

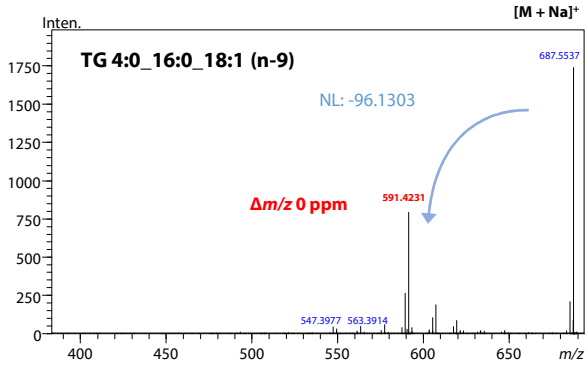

Fig. 9 Butter TG 38:1 MS/MS Spectrum Obtained in OAD Mode and Identification of Double Bond Position

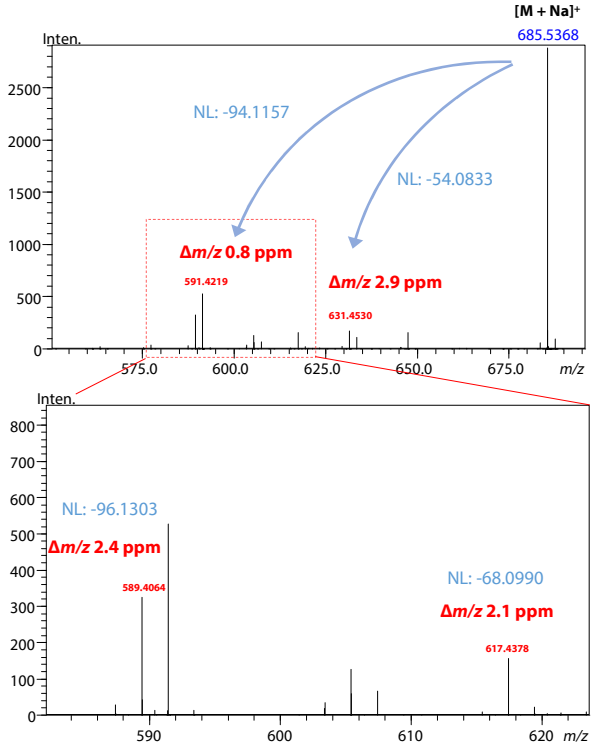

Fig. 10 MS/MS Spectrum of Butter TG 38:2 Obtained in OAD Mode

Fig. 11 shows the structures of TG 38:1 and TG 38:2 determined based on the analyses performed in CID mode and OAD mode.

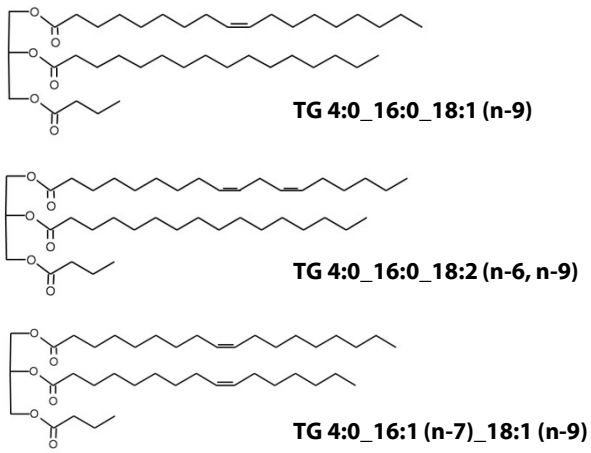

Fig. 11 Structures of TG 38:1 and TG 38:2 in Butter Determined by CID and OAD Analysis

■ Conclusion

Combining the DPiMS QT kit with the OAD-TOF system allowed rapid structural characterization of TGs in butter, including the position of double bonds. The excellent mass accuracy of the LCMS-9050 allowed easy verification of neutral loss and highly accurate structural characterization.

<References>

1) H. Uchino, *et al.*, *Communications Chemistry*, **5**, 162 (2022)

2) H. Tsugawa, *et al.*, *Nature Methods*, **12**, 523-526 (2015)

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