SHIMADZU
Excellence in Science

Application News

High Performance Liquid Chromatograph Mass Spectrometer

Characterisation of Bovine and Buffalo Milk Triacylglycerols at the C=C Double Bond Positional Level Using OAD

Alan Barnes[1], Emily Armitage[1], Sascha Rexroth[2], Georgios Theodoridis[3], Neil Loftus[1]
1 Shimadzu Corporation, Manchester, United Kingdom, 2 Shimadzu Europa GmbH, Duisburg, Germany
3 Aristotle University of Thessaloniki, Greece

User Benefits

- Oxygen Attachment Dissociation (OAD) is a radical-induced dissociation technique which specifically fragments carbon-carbon double bonds and can be used to determine the positions of double bonds (C=C) in complex lipids.
- Acquisition of CID-MS/MS and OAD-MS/MS fragments (OAciD) was applied to the analysis of triacylglycerols (TGs) in milk samples to annotate TGs to the fatty acid (FA) composition level and generate unique OAD fragment ions to determine C=C position.
- Closely eluting TG isomers were differentiated by C=C showing species specific differences at the double bond positional level.

■ Introduction

Worldwide milk consumption is dominated by bovine milk, which accounts for approximately 85% of global milk production, followed by buffalo milk at around 11%. In recent years, consumer preferences have shifted towards higher protein milk products and whole milk, however, milk containing lower fat content is also considered desirable. With a better understanding of lipid composition in milk, alternative products may be developed through processing or animal breeding that favour more desirable characteristics.

Buffalo milk typically contains 7-8% fat compared to 3-4% in bovine milk with >98% total fat estimated to be TGs. Separation of isomeric TGs identified not only relative abundance differences between buffalo and bovine but also different TG isomers were characteristic to a species. As one example TG 28:1 contained five main isomers in buffalo compared to four in bovine. CID-MS/MS successfully identified the fatty acid (FA) chain composition of these isomers, however the two main TG peaks produced identical MS/MS spectra meaning that the structural differences were most likely due to positional differences of the acyl chains or the C=C position.

Recent advancements in alternative fragmentation methods such as oxygen attachment dissociation (OAD) have now provided an opportunity to gain greater understanding of C=C differences. OAD is a radical-induced dissociation technique which specifically fragments C=C double bonds and can be used to determine the positions of double bonds in complex lipids.

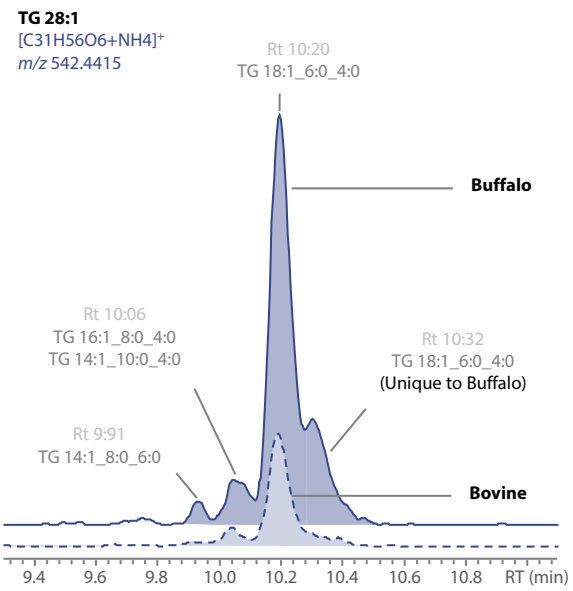

Fig. 1 LC/QTOF analysis of TG 28:1 isomers in bovine and buffalo milk extracts. Five isomers were detected in buffalo milk and identified with four common to bovine, but one later eluting TG unique to buffalo.

OAD-MS/MS and conventional CID-MS/MS can be applied simultaneously in positive or negative ion mode data acquisition on a high-resolution LC/QTOF instrument to generate OAD- and CID-specific fragment ions. This approach was used to characterise C=C positional information in profiling TGs in bovine and buffalo milk.

■ Materials and Methods

Milk samples from bovine and buffalo were prepared by the Folch method (200 µL sample + 50 µL water + 1000 µL solvent - chloroform:methanol 2:1 v/v). The bottom lipid-containing phase was transferred to a glass vial and placed into a speed vac and evaporated to dryness. Samples were reconstituted in 1 mL butanol:methanol:chloroform (3:5:4), then diluted 1:50. Analysis was performed using high-resolution LC/QTOF (LCMS-9050) operating with time-of-flight (TOF) MS (*m/z* 100–1000) followed by data dependent acquisition (DDA) MS/MS in positive ion mode with optimal conditions for simultaneous acquisition of CID and OAD-MS/MS fragments (OAciD). Acquisition conditions summarised in Table 1.

Table 1. Analytical conditions

[UHPLC conditions] (Nexera X2)	
Column	C18 (2.1x100 mm1.7 µm); maintained at 50 °C
Flow Rate	0.4 mL/min
Mobile Phase	A: Water + 10 mM ammonium formate + 0.1% formic acid B: MeOH:IPA (85:15) + 10 mM ammonium formate + 0.1% formic acid C: IPA
Rinse (R0 / R1 / R2)	IPA:MeOH:MeCN:H_2O (1:1:1:1) + 1% formic acid
Gradient	75% B (0-1 min) → 85% B (2.5 min) → 90% B (7.5 min) → 96% B (10-15 min): 100% B (16-32 min) → 10% B, 90% C (32-37 min), 75% B (37-44 min)
Injection	1 µL; autosampler set to 7 °C
[MS conditions] (LCMS-9050)	
Interface	300 °C, 4 kV (ESI+)
Heat block, DL	400 °C, 250 °C
Gas flow rates	Nebulizing gas 3 L/min; Heating gas 15 L/min Drying gas 10 L/min
MS	TOF-MS (*m/z* 100-1000, 50 msec)
MS/MS	DDA-MS/MS (*m/z* 100-1000, top 4, 300 msec), OAciD CE 6-30 V, CID pressure 17 kPa (1.25 second cycle time)

■Results: CID MS/MS

TG annotation by CID-MS/MS can be challenging for coeluting isomers. The sum composition of the TG can be identified by MS accurate mass from which typically only [M+NH4]+ and [M+Na]+ adducts are detected in positive ion mode. Interpretation of CID-MS/MS spectra is greatly enhanced when acquired by targeted MS/MS because extracted ion chromatograms of fragment ions can be plotted to show the peak apex of fragment ions which can be matched to parent ion retention times. For example, TG 28:1 was detected as five isomers (four chromatographic peaks) in buffalo milk (Fig. 1). Chromatographic separation resolved these with the exception of two isomers. Spectral interpretation of MS/MS data under each peak detected fragment ions corresponding to the neutral loss (NL) of FA groups (Fig. 2). The first TG peak was identified as TG 14:1_8:0_6:0 and second peak identified as containing two TGs TG 16:1_8:0_4:0 and TG 14:1_10:0_4:0. The last two peaks contained identical MS/MS spectra, both identified as TG 18:1_6:0_4:0.

TG isomers in milk are widely considered to be challenging to separate and characterise, sometimes requiring alternative separation techniques such as non-aqueous reversed phase (NARP)[1]. These methods typically have very long gradients; however, identification remains limited to the sum composition or constituent FA chains using CID-MS/MS leaving identification of the C=C double bond location unknown.

Characterisation of lipids using OAD has the potential to identify isomeric differences that would not be possible by other techniques. OAciD (simultaneous acquisition of CID- and OAD-MS/MS) enables structural characterisation of lipids through the determination of C=C double bond positions in the FA chains. Chromatographically separated lipids with similar CID-MS/MS can be distinguishable with the addition of OAD-MS/MS specific fragment ions.

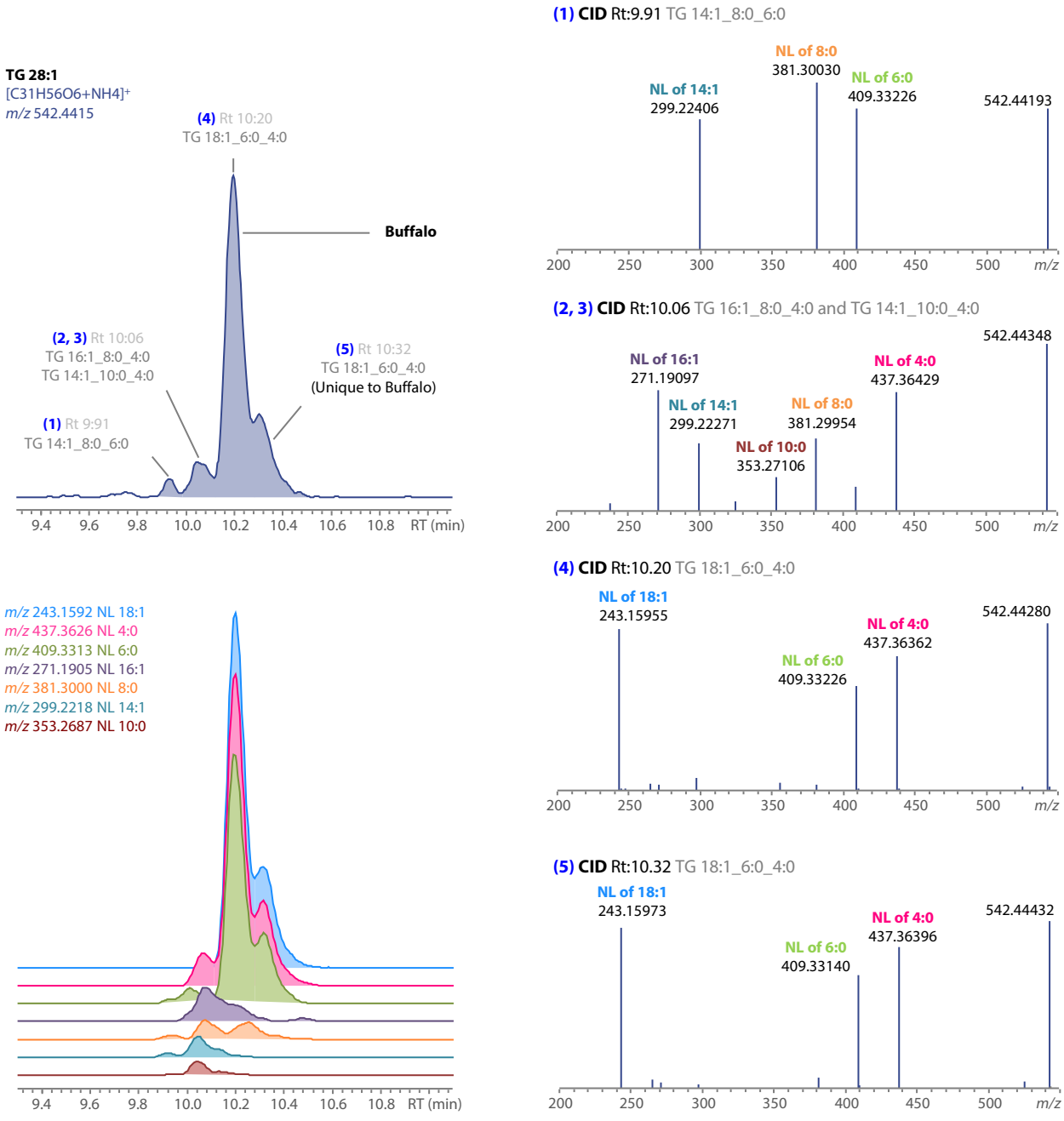

Fig. 2. Characterisation of TG 28:1 isomers in buffalo milk. Five isomers were detected in buffalo milk. CID-MS/MS spectra for each of the four peaks and neutral loss (NL) mass chromatograms are presented for the buffalo milk extract, including the fourth peak which was absent in the bovine milk profile.

■Results: OAD MS/MS

For abundant TGs, a combined OAciD-MS/MS acquisition of sodium ion adduct precursors generates data containing both CID fragment ions relating to the FA NL information in addition to C=C bond information from OAD. Low abundant TGs benefit from separate OAD/CID-MS/MS. Fragment ion chromatograms from the precursor ion *m/z* 547.3969 show OAD specific ions from the [C31H56O6+Na]+ precursor ion (Fig. 3). OAD-MS/MS spectra include specific fragmentation for the $C_{x-1}H_{2x-2}O_{-1}$ ions providing evidence of structurally different FAs. Fragment ion peak apex retention times (Rts) of OAD ions were matched to those of CID FA NL fragment ion Rts to enable complete annotation of the main TG 28:1 isomers detected in buffalo milk.

OAD analysis identified C=C positions in three distinct locations: n-5, n-7 and n-9. The first TG 28:1 isomer was very low intensity and both OAD and CID data were needed to identify this as TG 14:1(n-5)_8:0_6:0.

Combining the fragment information derived from OAciD on the chimeric MS/MS spectrum for the peak at 10.06 min (2, 3), present in both bovine and buffalo milk extracts, it was possible to identify the presence of TGs structurally characterised as TG 16:1(n-7)_8:0_4:0 and TG 14:1(n-5)_10:0_4:0.

The unique TG detected in buffalo was structurally characterised as TG 18:1(n-7)_6:0_4:0, differentiating it from TG 18:1(n-9)_6:0_4:0 which was present in both buffalo and bovine milk extracts.

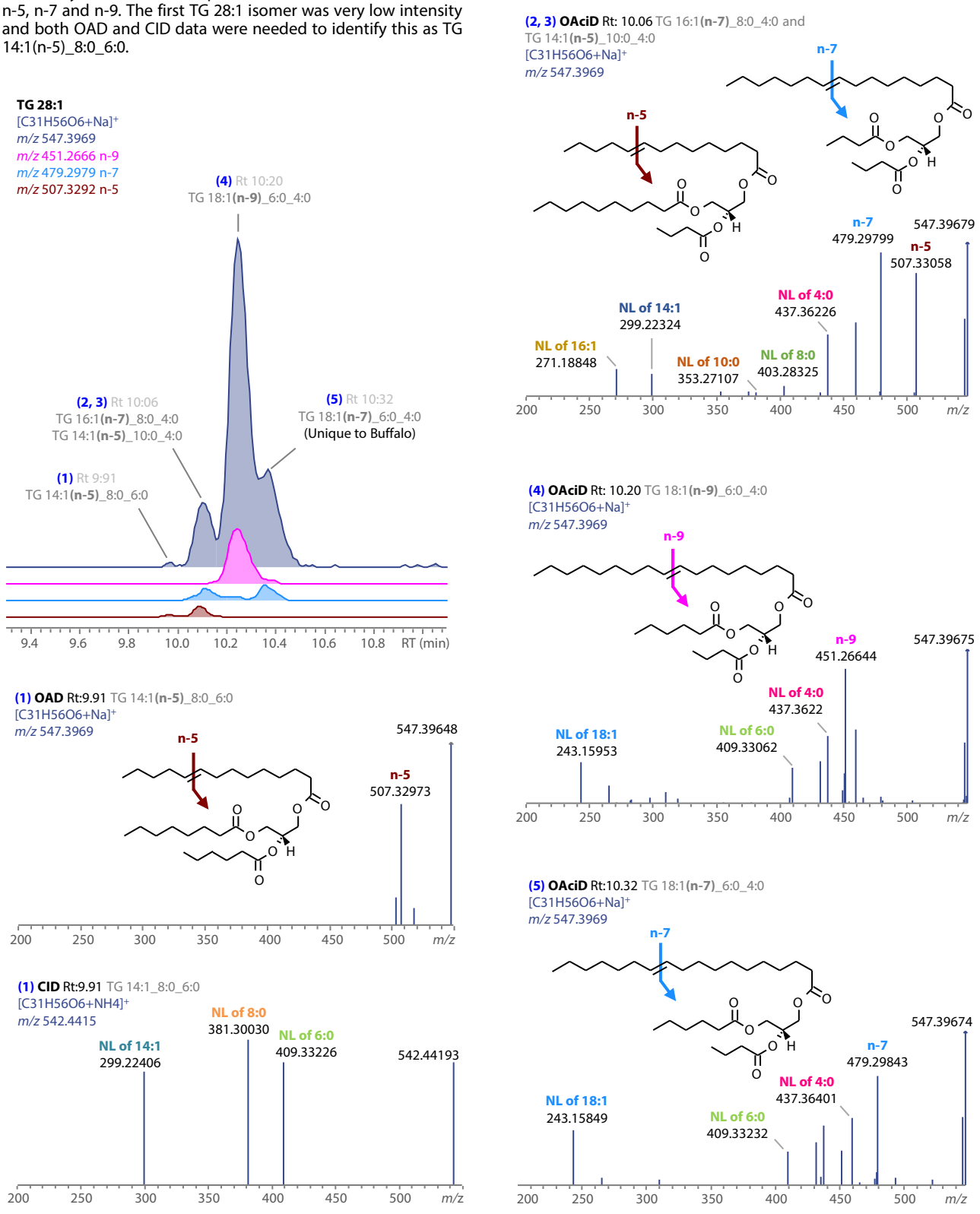

Fig. 3. Structures, mass chromatograms and OAciD-MS/MS spectra for TG 28:1 isomers in buffalo milk. (1) TG 14:1(n-5)_8:0_6:0 characterised by OAD- and CID-MS/MS. (2-5) OAciD-MS/MS on the sodium adduct precursors generated both CID- and OAD-specific fragment ions enabling the annotation of TG 16:1(n-7)_8:0_4:0, TG 14:1(n-5)_10:0_4:0, TG 18:1(n-9)_6:0_4:0 and TG 18:1(n-7)_6:0_4:0. Spectral intensity was magnified to display OAD fragment ions.

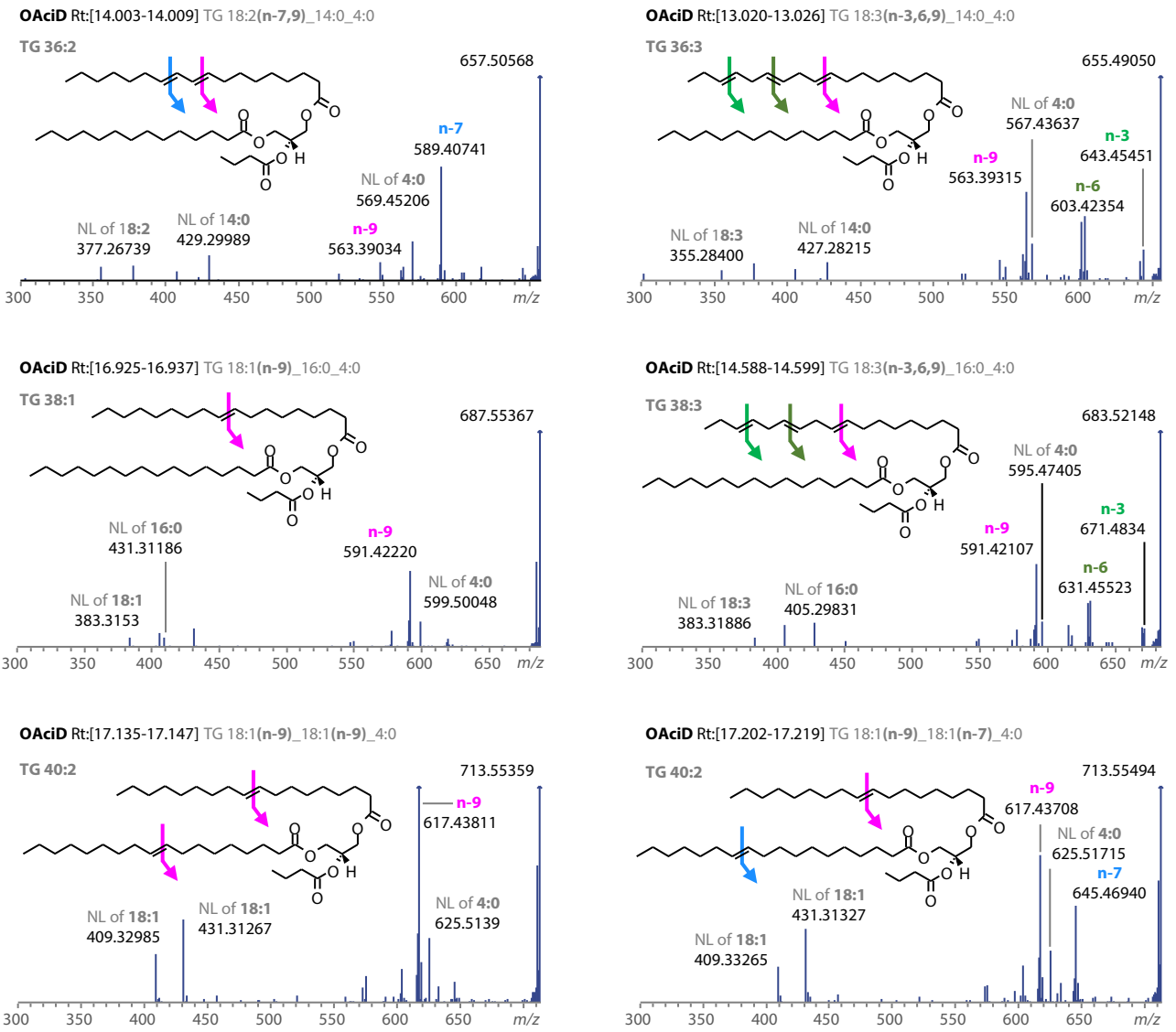

Fig. 4 Examples of higher mass TGs annotated using OAciD combined fragmentation. Coeluting TG 40:2 was identified containing TG 18:1(n-9)_18:1(n-9)_4:0 and TG 18:1(n-9)_18:1(n-7)_4:0. Spectral intensity was magnified to display OAD fragment ions.

Using OAciD fragmentation, CID ions provided evidence for the FA composition while OAD specific fragment ions showed the potential to separate coeluting isomers at the MS/MS level. Using this approach, 30 unsaturated TGs were structurally characterised in the milk extracts (examples of higher mass TGs are shown in Fig. 4).

In higher organisms, FA double bond positions are typically found in the n-3, n-6 or n-9 positions however in ruminant species vaccenic acid is also commonly observed due to the influence from the ruminant gut microbiome. The differences detected between buffalo and bovine species for TG 18:1(n-7)_6:0_4:0 in buffalo may therefore reflect the physiological differences in the digestive tracts and gut microbiomes.

■ Conclusion

OAciD-MS/MS was used to generate fragmentation data sufficient to annotate TGs at the fatty acid composition level as well as determining the C=C double bond position(s).

OAciD enabled highly specific fragmentation resulting in selective detection and high reporting confidence.

TGs in a complex food commodity could be annotated with far greater specificity using OAciD-MS/MS compared to CID-MS/MS.

■ References

1. Comprehensive Characterization of Bovine Milk Lipids: Triglycerides (2020) Liu Z., Li C., Pryce J., Rochfort S., ACS Omega, 5, 12573−12582

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

05-SCA-210-099-EN First Edition: Oct. 2026

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to comps that are not available in your country. Please contact us to check the availability of these comps 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 comps/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 and Nutrition
- Food Metabolomics
- Life Science
- Lipidomics

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry