SHIMADZU
Excellence in Science

Application News

No. M294

Gas Chromatography Mass Spectrometry

Analysis of Double Bond Position in Unsaturated Fatty Acid Methyl Esters by SMCI Method

The compounds called fatty acids are measured in wide-ranging areas from foods to the biological samples. Analysis using the gas chromatograph (GC) is a suitable measurement technique, as GC has a high separation capability in the chromatogram. In this technique, the fatty acids are first derivatized by methyl esterification to produce inactivated fatty acid methyl esters (FAME) with ample volatility. However, because localization of the double bond position in unsaturated FAMEs is difficult, even when using mass spectrometry is used, research on this issue was limited.

To overcome this difficulty, an analysis technique based on chemical ionization utilizing acetonitrile was developed [1], and expanded the possibilities of double bond localization. Solvent mediated chemical ionization (SMCI) is a soft ionization method using an organic solvent, and use of the new Shimadzu SMCI unit makes it possible to introduce acetonitrile, which is necessary in this double bond localization technique, into the analytical instrument.

This article introduces the results of an analysis of unsaturated FAMEs by the SMCI method. For details of the analytical method and real sample analysis, refer to Shimadzu Technical Reports C146-0396 and C146-0397.

R. Kitano

Samples and Analysis Conditions

A 50 ng/mL mixed standard solution of methyl oleate, methyl linoleate, and methyl linolenate was prepared, and measurement was carried out under the conditions in Table 1 using the SMCI method.

Table 1 Instrument and Analysis Conditions

Instrument		
GCMS	:	GCMS-TQ™ 8040 NX
Autosampler	:	AOC-20i+s
Column	:	BPX-70 (L: 25 m, df: 0.25 µm, ID: 0.22 mm)
Insert liner	:	Split-less deactivated liner w/low wool
GC conditions		
Injection temp.	:	250 °C
Injection volume	:	1 µL
Injection mode	:	Split-less
Carrier gas control	:	Linear velocity (46.2 cm/s)
Column oven temp.	:	80 °C → (15 °C/min) → 170 °C (4 min) → (7 °C/min) → 240 °C (10 min)
MS conditions		
Interface temp.	:	240 °C
Ion source temp.	:	230 °C
Ionization method	:	SMCI (acetonitrile)
Measurement mode	:	Scan, product ion scan (CE = 6 V)
Event time	:	0.5 s

Scan Mass Spectra

With the conventional CI method, mainly the peak [M+1] originating from the addition of protons to the molecular weight is observed. However, with the SMCI method using acetonitrile, the peak [M+54] is obtained by addition of the reactant ions produced by the interaction with acetonitrile. Since the reactant ions are added selectively to the double bond, the adduct ions can be confirmed from mass spectra of unsaturated FAMEs, as shown in Fig. 1. Double bond localization is done by conducting a product ion scan analysis for this [M+54] ion.

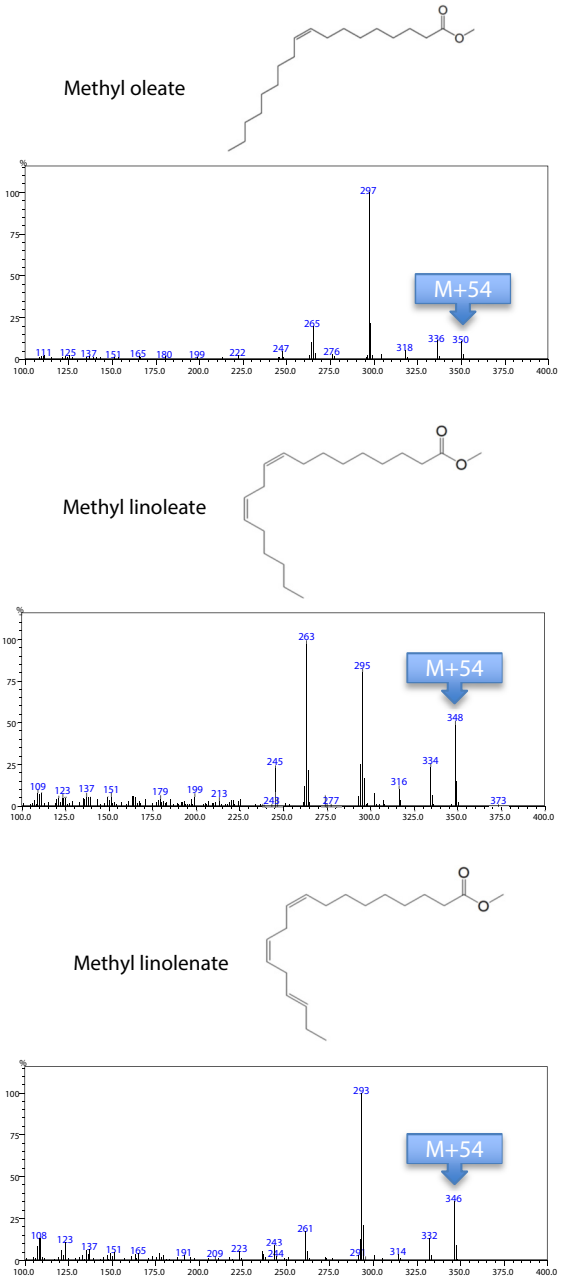

Fig. 1 SMCI Scan Mass Spectra

Product Ion Scan Mass Spectra

Fig. 2 shows the results of the product ion scan for the precursor ions, the [M+54] confirmed in Fig. 1. Two distinctive ions can be confirmed in each of the unsaturated FAMEs, and it is possible to trace back to the double bond position from these values. The reactant ions originating from acetonitrile are added to the double bond, and cleavage due to CID occurs at the corresponding locations. Because the reactant ions are added from two directions, two distinctive ions can be confirmed. These correspond to the peaks indicated by the red numbers in the mass spectra.

In mono- and bivalent unsaturated FAMEs, ions generated by cleavage on each side of the double bond can be observed, and in a trivalent unsaturated FAME, the two end double bonds existing with methylene group, cleavage occurs between the two double bonds. Thus, designated ions can be observed corresponding to the number of double bonds and their coordination sites.

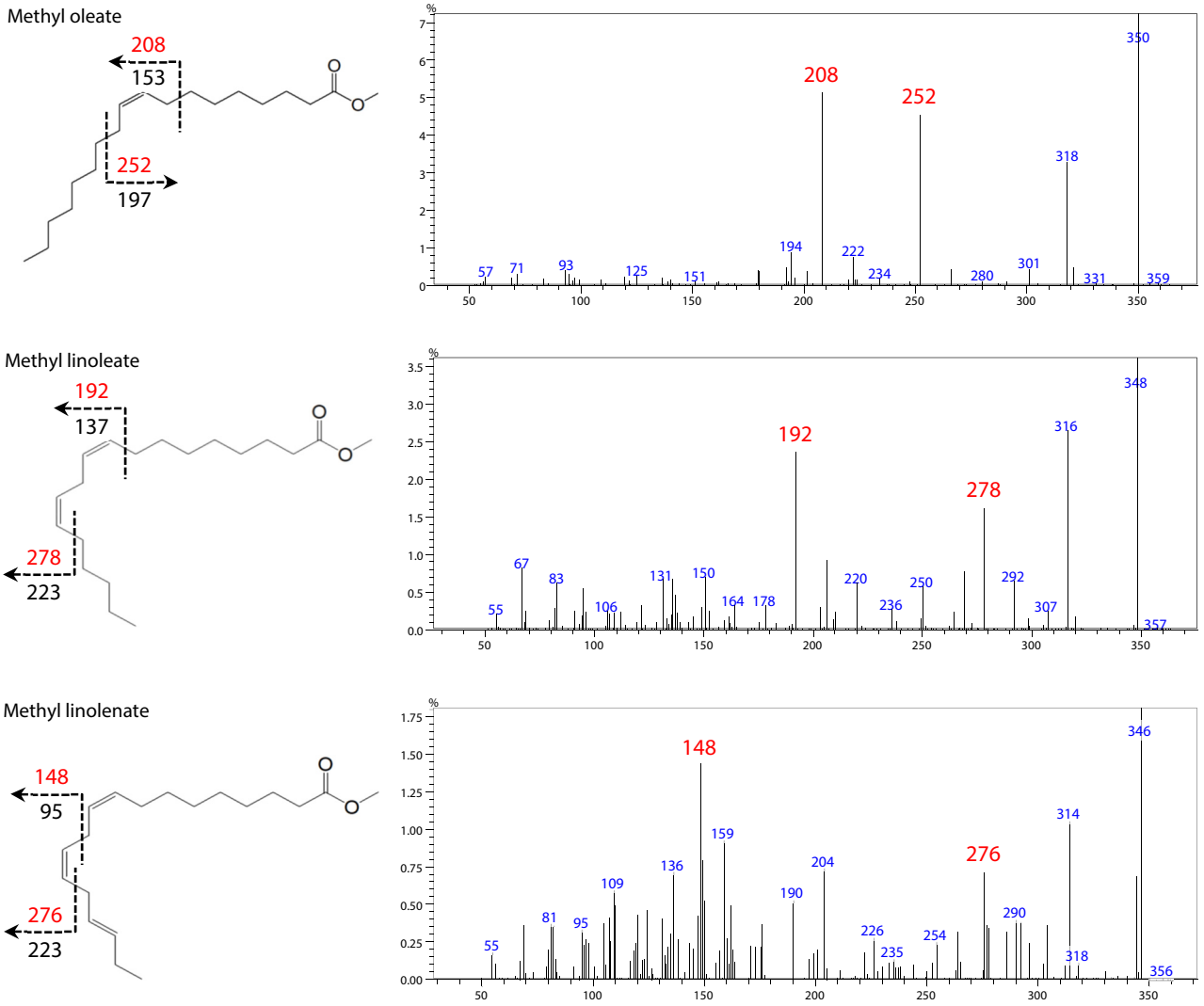

Fig. 2 SMCI Product Ion Scan Mass Spectra
(The red numbers (upper numbers) in the structural formulas are the values with the reactant ion observed in mass spectra, and the black numbers (lower numbers) are the values without the reactant ion.)

Conclusion

By using the SMCI method, it is possible not only to confirm the molecular weight of unsaturated fatty acid methyl esters, but also to identify the positions of their double bonds, which had been difficult to analyze and required special derivatization when using the conventional techniques. SMCI can also be applied to compounds for which standards do not exist, and is useful in structural analysis of unknown compounds.

<Reference>

(1) Van Pelt, C.K. and J.T. Brenna, *Acetonitrile chemical ionization tandem mass spectrometry to locate double bonds in polyunsaturated fatty acid methyl esters.* Anal Chem, 1999, 71(10): p. 1981-9.

First Edition: May 2020

Shimadzu Corporation
www.shimadzu.com/an/