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Technical Report

Double bond localization in unsaturated fatty acid methyl esters (FAME) by solvent mediated chemical ionization (SMCI) tandem mass spectrometry

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Abstract:

Characterization of unsaturated fatty acid structures is an unusually difficult chemical problem because of the similarity of the isomeric structures. Enabled by the Shimadzu solvent mediated chemical ionization unit, we implemented covalent adduct chemical ionization. After a straightforward setup procedure, CACI-MS/MS yields unique diagnostic ions that enable structural characterization of most fatty acid methyl esters (FAME) with no need for standard solutions.

Keywords: Fatty acid methyl esters (FAME), polyunsaturated fatty acids, covalent adduct chemical ionization, structural analysis of fatty acids

1. FAME structural analysis

Triple quadrupole tandem mass spectrometry excels at structural and quantitative analysis because of the specificity of ion fragmentation. Isobaric chemical compounds usually produce fragments unique to their structure upon collisional fragmentation, enabling the isolation of a single analyte from a complex extract of a natural mixture. Isobars that have the same nominal mass but different elemental and/or isotopic compositions can be distinguished by a combination of high mass resolution and exact mass measurements, usually on product ions.

Fatty acid methyl esters (FAME) are among the most studied analyte panels, arguably the first 'omics analyses dating to the 1950s[1]. FAME are usually prepared as the ideal analytical form of fatty acids by conversion from their normal form as esters by hydrolysis and methylation. FAME have excellent analytical properties because they are chemically inactive and volatile, and yield excellent peak shapes and separations when analyzed by GC.

Most FAME of interest in biology are unsaturated, with 1-6 double bonds in mammalian tissue and up to 10 double bonds in marine samples. Double bonds can be present between any of the carbon atoms, and can be in the cis (Z) or trans (E) configuration. The position and geometry of double bonds, along with C chain length, determines the bioactivity of the fatty acid. For instance, 9Z,12Z-octadecadienoic acid (linoleic acid) is a precursor to the key eicosanoid precursor arachidonic acid, while the isomer 9Z,11E-octadecadienoic acid (rumenic acid) is a potent anticarcinogenic factor in rodents, and another isomer 10E,12Z-octadecadienoic acid has no anticarcinogenic activity but inhibits lipid synthesis. Determination of FAME double bond structure is therefore of critical importance.

Surprisingly, determination of FAME double bond structure by tandem mass spectrometry is known as a difficult chemical problem. Electron ionization (EI) results in the rearrangement of double bonds and thus is not suitable for de novo structural analysis. Similarly, conventional proton transfer chemical ionization produces protonated molecular ions but collisional activation of these products does not yield diagnostically useful fragments. Older traditional methods require rederivatization of FAME into other esters with more favorable properties. Among the many disadvantages are the need for specialized chemistry, the possible rearrangement of double bonds during derivatization, and differing chromatographic behavior including changes in retention times and possibly order of elution. A method for direct analysis of FAME avoids all these issues. Spectra presented in this note are derived from mixtures of natural extracts rather than chemically purified standards.

2. Covalent Adduct Chemical Ionization enabled with SMCI unit

In 1999 we described an ion-molecule reaction with all unsaturated FAME that produces a covalently bound adduct ion that, upon collisional activation, yields diagnostic ions identifying double bond position,[2] and in some cases geometry.[3] The method was developed with an internal ionization 3-dimensional ion trap mass spectrometry and widely implemented on those instruments. The 3D ion trap is a particularly suitable device for this purpose because it traps reagent ions from relatively low volatility solvents such as acetonitrile (CH_3CN) which do not accumulate at sufficient levels in conventional Nier-type chemical ionization sources used in quadrupole mass spectrometers interfaced with gas chromatographs. Unfortunately, 3D ion traps with GC inlets ceased to be manufactured several years ago.

To fill this gap, Shimadzu scientists developed solvent mediated chemical ionization (SMCI) which enables covalent adduct chemical ionization (CACI) on a modern triple quadrupole instrument. A small tank of Ar pressurizes a solvent reservoir increasing the flow of gaseous reagent to the CI gas valve and the CI ion source.

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For double bond localization, acetonitrile (CH_3CN) is used. In the CI ion source, CH_3CN undergoes an ion-molecule "self-reaction" to generate a reagent ion of *m/z* 54, 1-methyleneimino-1-ethenylium (MIE).

$CH_3CN + CH_2CN^+ \rightarrow CH_2=C=N^+=CH_2$ (*m/z* 54)

The MIE ion reacts with double bonds in FAME to yield an ion 54 daltons above the molecular mass of the parent ion. Collisionally activated dissociation of the [M+54] causes fragmentation at the site of the former double bond in a predictable way.[4] Antiparallel addition of MIE to the double bond yields a fragment at the other side of the molecule, thus localizing the site of the former double bond.[5]

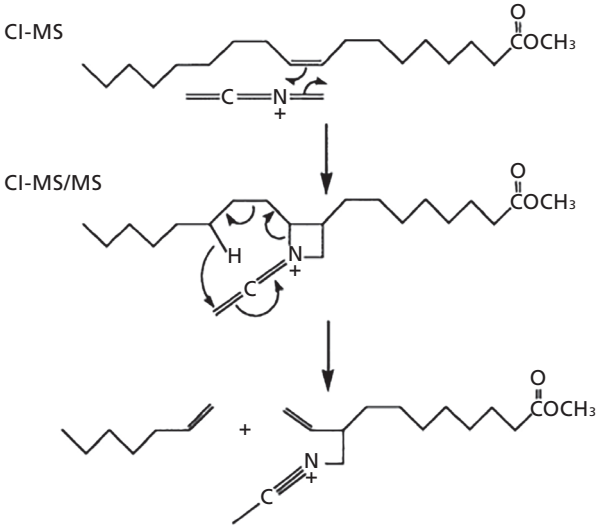

Fig. 1 Reaction of MIE with a monoene

3. Analytical condition

The method was implemented using a Shimadzu GCMS-TQ™8050 NX with instrument parameters and conditions shown in Table 1. An example that shows clear distinction between isomers shown below for monounsaturated FAME Me18:1n-9 (methyl oleate) and Me18:1n-7, both of molecular mass 296. MS/MS of the [M+54] ion at *m/z* 350 yields unique ions that definitively distinguish the two isomers. The diagnostic ions containing the alpha (α) C at the ester and the terminal methyl known as the omega (ω) C are labeled. Diagnostic ions (α, ω) for 18:1n-9 and 18:1n-7 appear at (252, 208) and (280, 180) respectively and clearly distinguish the two isomers.

Table 1 Analytical conditions

GC-MS:	GCMS-TQ™8050 NX
Autosampler:	AOC-20i+s
Column:	BPX-70 (20 m x 0.22 mmI.D., df = 0.25 µm)
Glass Insert:	Split-less Deactivated Liner w/ Low Wool
GC	
Inj. Temp.:	250 °C
Inj. Mode:	Splitless
Column Oven Temp.:	80 °C, 25 °C/min to 170 °C (4 min), 7 °C/min to 240 °C (10 min)
Flow Control:	Linear velocity (47.2 cm/sec)
MS	
Interface Temp.:	240 °C
Ion Source Temp.:	240 °C
Ionization Mode:	SMCI
Data Acq. Mode:	Product ion scan
Event Time:	0.3 sec

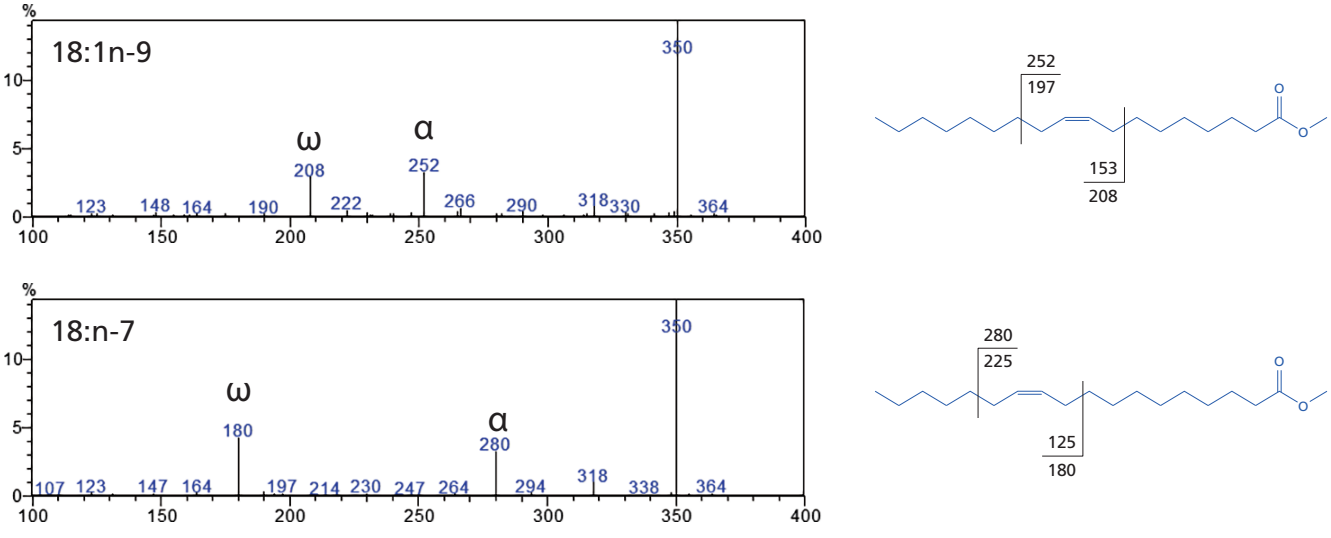

Fig. 2 CACI-MS/MS product ion scan mass spectra of monoene FAME isomers

4. Polyunsaturated FAME

In single stage CACI-MS, all FAME yield a series of 4-5 ions that are routinely used to distinguish FAME from other compounds. Single stage CACI-MS of methyl linoleate (methyl 9Z,12Z-octadecaenoic acid) and its conjugated isomer methyl 10E,12Z-octadecaenoic acid both have the same five peaks, [MH-50], [MH-32], [MH], [M+54-32], and [M+54], listed from low to high *m/z*. These peaks are of particular utility for low abundance compounds, enabling rapid differentiation of FAME from non-FAME compounds such as contaminants from sample preparation or the injector. For the important case of dienoic FAME, the abundance of the loss of 32 (CH_3OH) from [M+54] is greater for the conjugated FAME; thus, a ratio of [M+54]/[M+54-32] greater than 1 is characteristic of a conjugated FAME.[3, 6]

The most commonly analyzed polyunsaturated FAME in human and mammalian samples are the omega-3 and omega-6 fatty acids. CA-CI-MS/MS product ion scan mass spectra for FAME of the two major isomers alpha-linolenic acid (methyl 9Z,12Z,15Z-octadecatrienoate) and gamma-linolenic acid (methyl 6Z,9Z,12Z-octadecatrienoate). The two diagnostic ions stand out above the non-specific ion series in the two spectra.

A useful feature of the CACI-MS/MS product ion scan spectra is that the ω diagnostic ions are identical for all polyunsaturated FAME of a series, that is, the ω diagnostic ion is always *m/z* 148 for omega-3 FAME, while *m/z* 190 is the diagnostic ion for omega-6 polyunsaturated FAME. This feature is particularly useful for identifying low abundance members of each series.

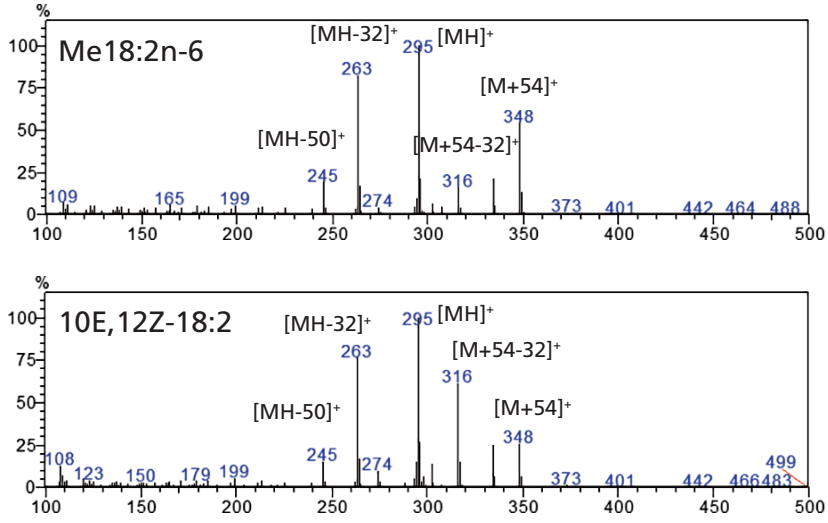

Fig. 3 CACI-MS scan mass spectra of polyunsaturated FAME isomers

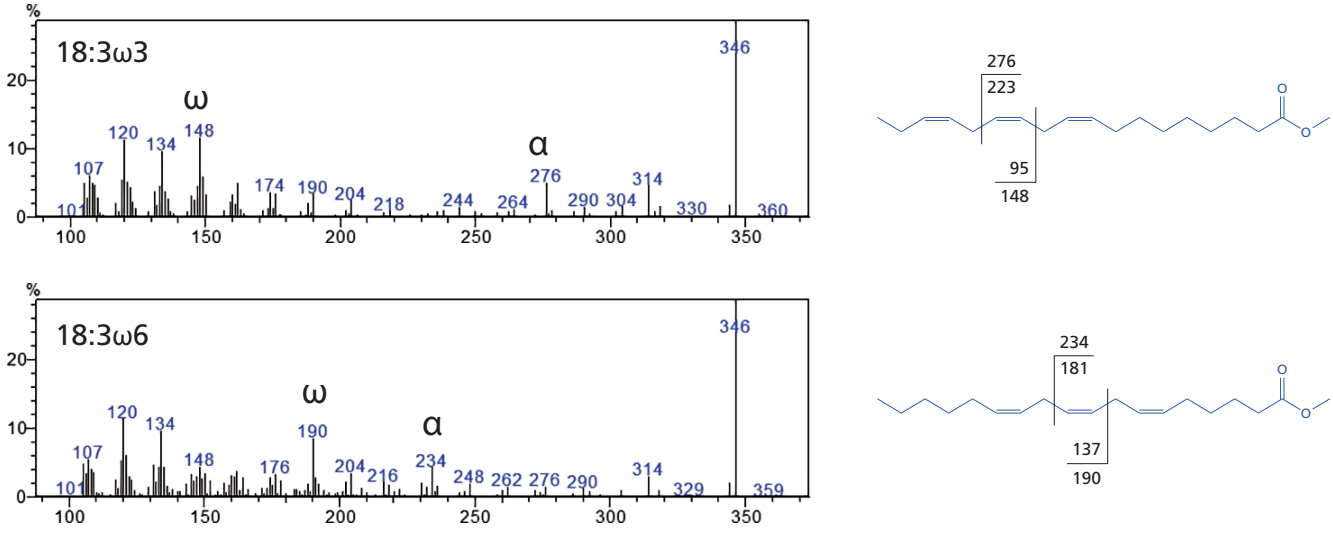

Fig. 4 CACI-MS/MS product ion scan mass spectra of alpha-linolenic and gamma-linolenic acids

5. Conclusions

Shimadzu's solvent mediated chemical ionization unit enables rapid and simple analysis of FAME mixtures by facilitating CA-CI-MS/MS. Single stage and tandem MS spectra are similar to classical spectra obtained with instruments no longer commercially available. It enables unambiguous assignment of chemical structure to FAME without chemical standards, which are not available for most FAME.

References

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Optional unit for the GCMS-NX series

SMCI Unit

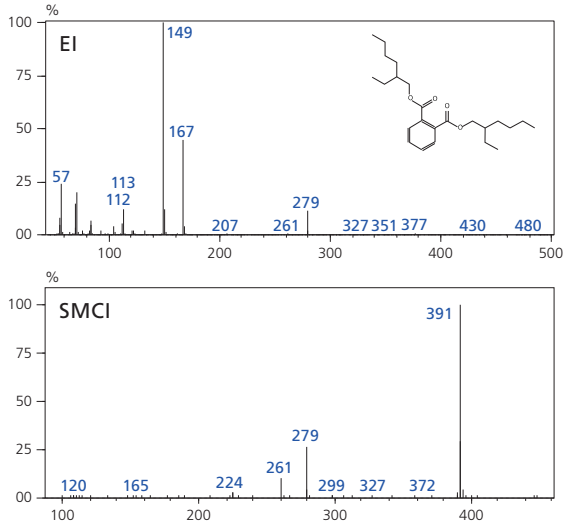
SMCI stands for Solvent Mediated Chemical Ionization, a soft ionization method for GCMS. The headspace reagent gas from the sample bottle is introduced into the GCMS ionization unit to be ionized, which then causes chemical ionization (CI) of the target molecule via protonation.* Previous CI methods have required the use of flammable reagent gas cylinders, but SMCI can be carried out with a general organic solvent such as methanol or acetonitrile, together with nitrogen or argon gas. This results in greater safety and lower running costs.

* Patent pending

SMCI unit + GCMS-QP2020 NX

For more details of SMCI Unit, visit our website.

SMCI can obtain the same results as previously-existing CI methods, but is less dependent on the compound. For example, it has been difficult to verify the molecular weight of phthalate esters using EI or previously-existing CI method, whereas SMCI can identify the quasi-molecular ions.



The mass spectrum of bis(2-ethylhexyl) phthalate (MW=390) obtained using different ionization methods

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