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Technical Report

Seed oil conjugated linolenic acid (CLnA) double bond characterization without chemical standards by solvent mediated chemical ionization tandem mass spectrometry

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Abstract:

Polyunsaturated fatty acids with unusual double bond structure are found widely in nature. Their chemical characterization requires methods that differentiate isomers of similar structures. Shimadzu's solvent mediated chemical ionization unit for GC-MS/MS enables use of low volatility CI regents with specific properties. These capabilities are demonstrated here for the covalent-adduct CI methods applied to conjugated trienes in complex seed oil extracts, enabling double bond assignment without chemical standards.

Keywords: Fatty acid methyl esters (FAME), polyunsaturated fatty acids, covalent adduct chemical ionization, seed oils, conjugated linoleic acid, conjugated linolenic acid

1. Double bond diversity in plant oil fatty acids

Edible oils constitute the major source of visible fat used in the food industry. The bulk of unsaturated fatty acids in oils are of a limited number of structures: monoenes such as oleic acid have cis (Z) double bonds in the n-9 or n-7 positions, while polyenes are either in the omega-3 or omega-6 families, in the cis geometry and with all double bonds separated by a methylene (-CH2-) group, called "homoallylic". Many exceptions are known, especially in specialty oils. As unique seed oils are developed for human consumption, structural analysis of unusual fatty acids is increasingly required. Fatty acids with unusual double bond structure may be highly bioactive when consumed by animals or humans, and may represent added value for rare oils. Methods for rapid structural and quantitative analysis would facilitate discovery, development, and quality assurance for such products.

Conjugated diene double bond systems have been of particular interest because of their bioactivity in mammals. Many studies show that the specific effects of particular conjugated linoleic acids (CLAs) depend on their double bond configuration: even a one carbon shift causes dramatic changes in function.

Fatty acids with conjugated triene double bonds are well known and have some known bioactivities but are much less studied than dienes. These conjugated linolenic acids (CLnAs) are present at more than 1% abundance in many plant seed oils. Research on these compounds is limited in part because methods for their analysis are cumbersome, particularly when they are present at low concentrations relative to other fatty acids.

2. FAME structural analysis

Classical methods for determination of double bond position in fatty acids require that the ester be one of a few specialized derivative groups to be amenable to GCMS analysis. Rederivatizing a fatty acid methyl esters (FAME) mixture has numerous disadvantages, particularly for low abundance fatty acids that may be partially lost or difficult to locate in chromatograms of rederivatized sample mixtures. We developed a method for direct structural characterization of FAME in the combined trapping region/chemical ionization (CI) source of older internal ionization ion traps, no longer commercially available. The method, called covalent adduct CI tandem mass spectrometry (CACI-MS/MS) depends on reaction involving CH_3CN, acetonitrile. CACI-MS/MS has not been available for commercial CI mass spectrometers because convention inlet systems do not admit sufficient acetonitrile. Overcoming this limitation would enable structural analysis of most unsaturated FAME, including CLnAs.

We used a Shimadzu GCMS-TQ™8050 NX with instrument parameters and conditions as shown in Table 1. The Shimadzu CI source was installed and CH_3CN was fed by a novel pressurized reagent introduction system (SMCI unit). For CACI-MS/MS, CH_3CN self-reacts in the ion source to form a $CH_2{=}C{=}N{+}{=}CH_2$ reagent ion that reacts with double bonds to yield a cyclic ion 54 daltons above the analyte parent mass.[1, 2] Collisional activation of this [M+54] ion yields 2 or more diagnostic ions that locate the position of the double bond. In practical terms, once the instrument is tuned for this form of solvent mediated CI, the reaction and mass spectra are automatically generated without resorting to unfamiliar procedures. Analytical conditions are shown in Table 1 and a discussion of the method is available in the technical report C146-E403.

1 The University of Texas at Austin

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

3. Conjugated linolenic acids (CLnAs), trienes

The fatty acids of oil-rich honeydew seeds were extracted and converted into a FAME mixture by treatment with sodium methoxide. The region of the chromatogram showing the CLnA elute is presented below. The total ion chromatogram (TIC) shows a series of putative CLnA peaks. CACI-MS/MS product ion scan mass spectra of the [M+54] ion (346 → products) labeled A shows two strong ions representing fragmentation on each side of the double bond system. A structure in Fig. 1 is shown for A. The fragment labeled α carries the carboxyl group while the one labeled ω carries the terminal methyl group. The numbers closest to the structure are the masses of the respective fragments while the other paired numbers are the plus 54 mass units reflecting the addition of the charge-carrying group covalently bound to the former double bonds. It has long been known that an H is transferred to the ion for similar molecules, thus *m/z* 190 and 290 identify the double bond system as located at the 9,11,13 positions.

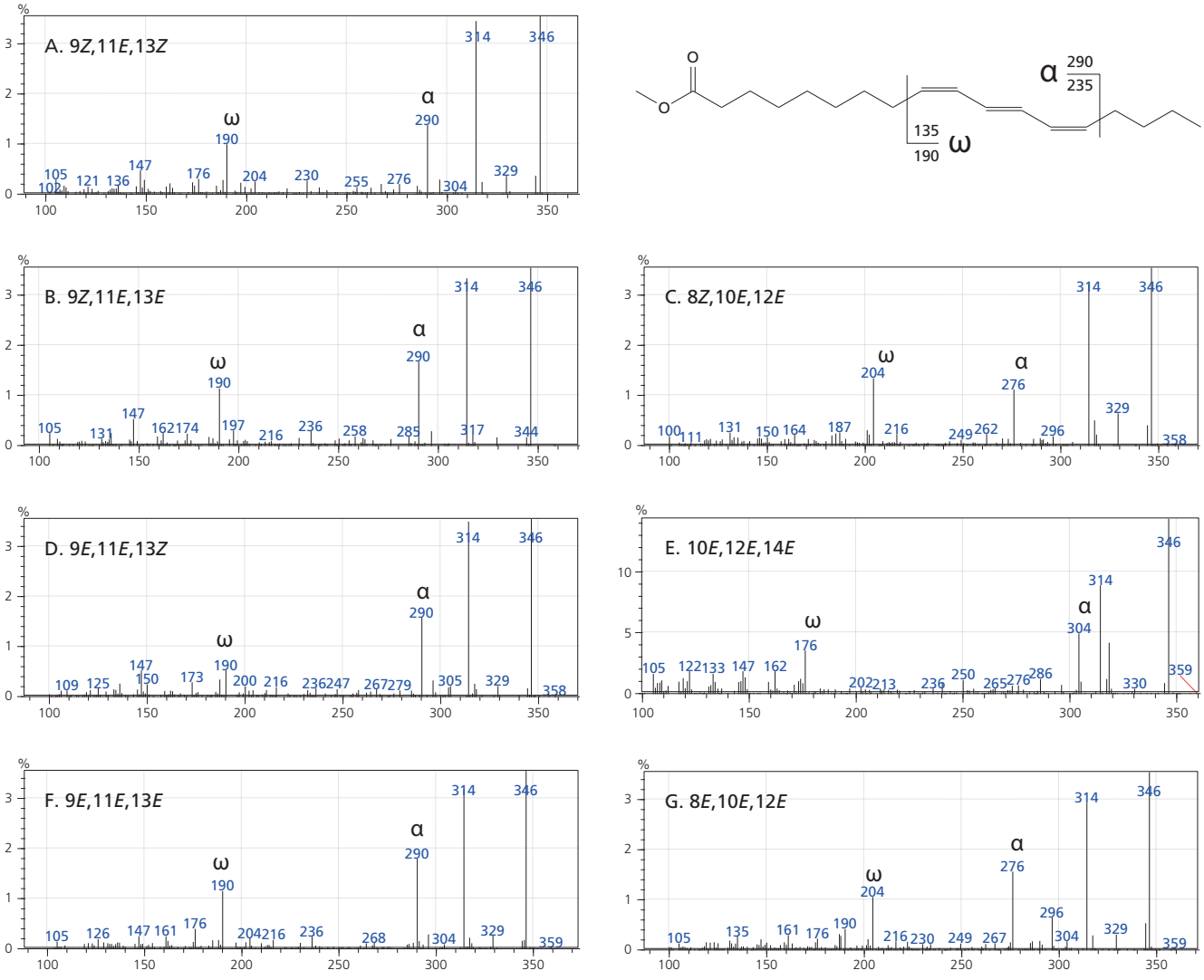

Fig. 1 CACI-MS/MS product ion scan mass spectra of CLnA

The chromatogram in Fig. 2 presents traces for the sum of the two diagnostic ions for each of three positional isomers. Peaks labeled A, B, D, and F show prominent peaks for the two diagnostic ions for the 9.11.13 isomer, and differ only in double bond geometry (Z, E; cis, trans). Peaks C and G show prominent peaks for the diagnostic ions for the 8,10,12 isomer. Peak E, while small, yields a peak for the 10,12,14 isomer that is not present in the chromatograms for the other diagnostic ions. Examination of mass spectra confirm these assignments.

The abundance of all CLnA total about 0.2% by weight thus the range of each identified CLnA is <0.1% by weight.

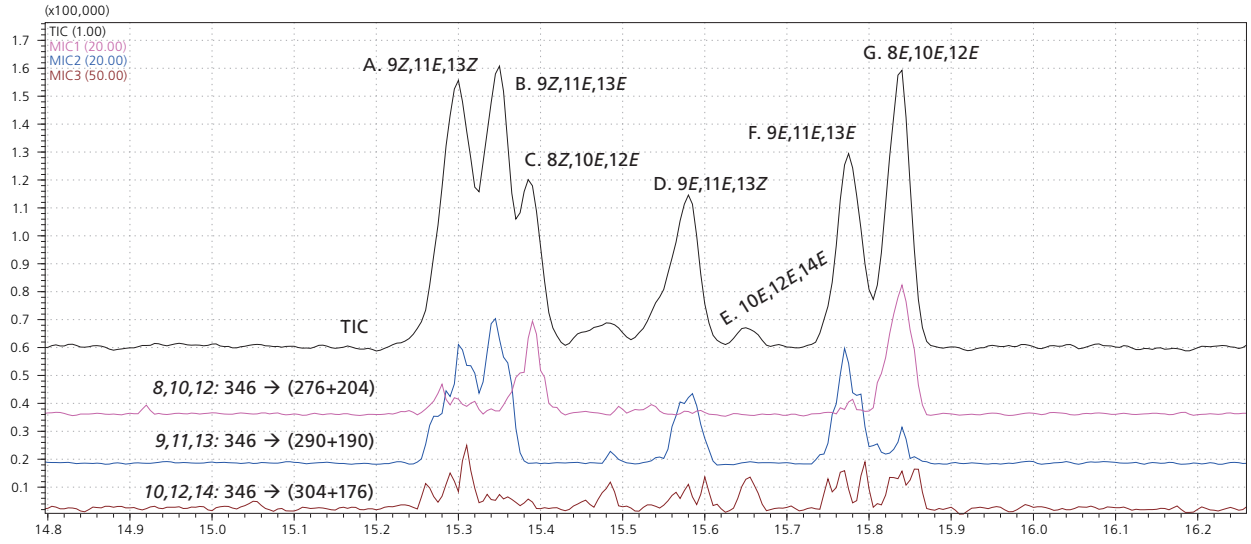

Fig. 2 Total ion current and summation mass chromatogram (MIC) of CLnA

The CACI-MS/MS implemented on the Shimadzu GCMS-TQ™ 8050 NX is quantitative and enables differentiation of biological replicates. Two chromatograms of the CLnA region of CLnAs for two different seed samples are shown in Fig. 3. From inspection it is evident that the relative abundances of CLnA A, B, and C are different, with A the largest peak in sample 1 and B the largest in sample 2. More dramatic is the difference in abundance for CLnA E, where sample 1 is several times more abundant than sample 2.

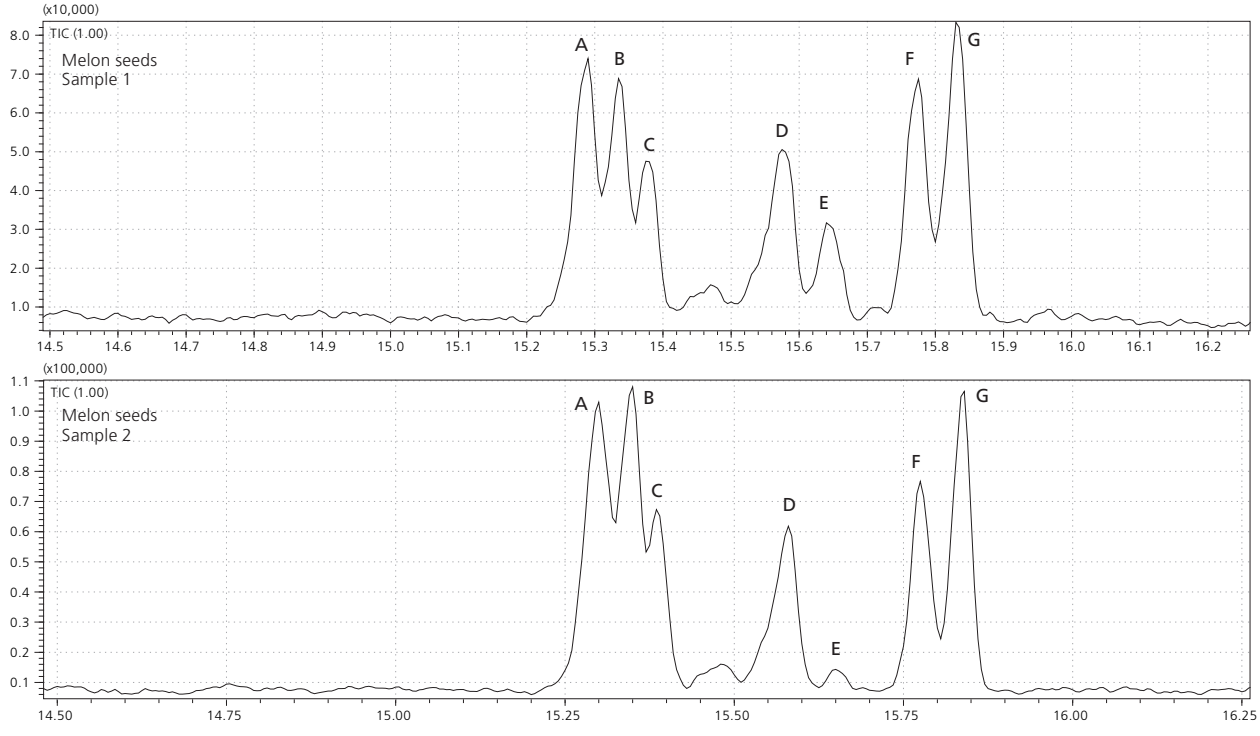

Fig. 3 Total ion current of CLnA

4. Conclusions

Shimadzu's unique solvent mediated chemical ionization unit enables implementation of the CACI-MS/MS technique, the only means for localization of double bonds in unsaturated FAME without resort to rederivatization and unfamiliar chromatography. Unlike the older internal ionization ion traps used for CACI-MS/MS, the triple quadrupole excels at MRM for the best sensitivity and quantitative analysis. More information on the application of this technique to CLnAs is available elsewhere.[3] With the high separation resolving power of capillary gas chromatography and structurally rich mass spectra now available from this system, the solvent mediated CI system is unparalleled for structural characterization of unsaturated FAME and represents the unrivaled state-of-the-art.

References

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Optional unit for the GCMS-NX series

SMCI Unit

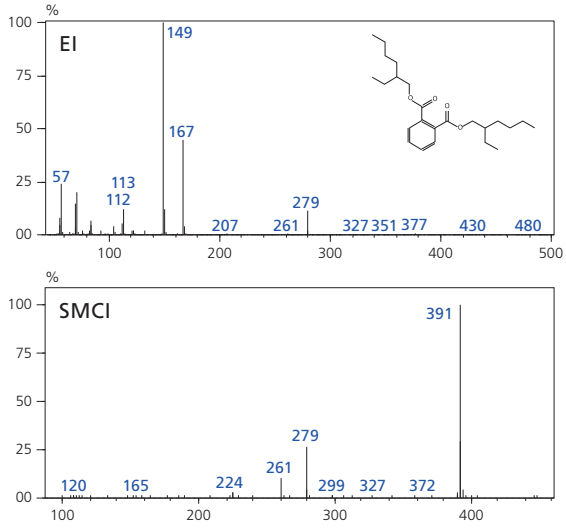
SMCI stands for Solvent Mediated Chemical Ionization, a soft ionization method for GCMS. The headspace reagent gas from the sample bottle is introduced into the GCMS ionization unit to be ionized, which then causes chemical ionization (CI) of the target molecule via protonation.* Previous CI methods 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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