# Determination of double bond positions of unsaturated fatty acids by a chemical ionization mass spectrometry computer system

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**Abstract** After stereospecific oxidation, trimethylsilylated methyl esters of mono- and diunsaturated fatty acids were analyzed by combined gas–liquid chromatography–chemical ionization mass spectrometry. The positions of original double bonds were deduced from the fragment ions produced by the cleavage of the carbon–carbon bond between two trimethylsilyl ethers. These fragment ions were recorded at *m/e* 187 and 259 in the case of 16:1(n-7), at *m/e* 187 and 287 in the case of 18:1(n-7), at *m/e* 215 and 259 in the case of 18:1(n-9), and at *m/e* 172 and 259 in the case of 18:2(n-6), respectively. The diastereoisomers of monounsaturated fatty acids can be discriminated by comparing the intensities of the fragment ions at *m/e* 253 and 285 in the case of 16:1 and at *m/e* 281 and 313 in the case of 18:1. The diastereoisomers of diunsaturated fatty acids may also be distinguished from each other by comparing the intensities of the fragment ions formed by the loss of trimethylsilyl function from the characteristic ions. Mono- and diunsaturated fatty acids in liver may be dominantly in *cis*-18:1(n-1) and *cis-cis*-18:2(n-6) as determined by a mass chromatographic technique.

**Supplementary Key Words** trimethylsilylated methyl ester · stereospecific oxidation

In electron impact (EI) mass spectrometry, it has been quite difficult to determine the position of double bonds in unsaturated fatty acids, because the positional isomers of only monounsaturated fatty acids give almost identical spectra (1). In 1965, isopropylidene derivatives of monounsaturated fatty acids after stereospecific oxidation were analyzed by McCloskey and McClelland (2). Similar studies have been performed with a number of unsaturated fatty acids using EI mass spectrometry (3–10). However, in the case of polyunsaturated fatty acids, only the position of a double bond close to the *omega and carboxyl-terminus* of the molecule can be deduced by EI mass spectrometry.

Recently, chemical ionization (CI) mass spectrometry has successfully been applied to the determination of double bond positions in alkenes (11). This report describes the use of a combined gas–liquid chromatography–CI mass spectrometry computer system for the identification of the positional and diastereoisomers of trimethylsilylated methyl esters of mono- and diunsaturated fatty acids. A preliminary account of this study has been published elsewhere (12).

## MATERIALS AND METHODS

### Materials

Methyl esters of palmitoleic(*cis*-9-hexadecenoic), palmitelaidic(*trans*-9-hexadecenoic), oleic(*cis*-9-octadecenoic), elaidic(*trans*-9-octadecenoic), *trans*-vaccenic (*trans*-11-octadecenoic), *cis*-vaccenic(*cis*-11-octadecenoic), linolenic(*cis-cis*-9,12-octadecadienoic), and linolelaidic(*trans-trans*-9,12-octadecadienoic) acids were obtained from Sigma Chemical Co. St. Louis, MO. Those fatty acids were more than 99% pure as judged by capillary gas–liquid chromatography. Preparations of methyl esters of fatty acids from human liver were carried out as described elsewhere (13). Osmium tetroxide was obtained from E. Merck, Darmstadt, Germany.

### Stereospecific oxidation and trimethylsilylation of mono- and diunsaturated fatty acids

The stereospecific oxidation was carried out essentially as described by Wolff, Wolff, and McCloskey (14). Mono- and diunsaturated fatty acids (0.5 mg of each) were dissolved in 0.2 ml of dioxane–pyridine 8:1(v/v) and 1 mg of osmium tetroxide (1% solution in dioxane, freshly prepared) was added. After

Abbreviations: GLC, gas–liquid chromatography; CI, chemical ionization; *cis* 16:1(n-7), palmitoleic acid; *trans* 16:1(n-7), palmitelaidic acid; *cis* 18:1(n-9), oleic acid; *trans* 18:1(n-9), elaidic acid; *cis* 18:1(n-7), *cis*-vaccenic acid; *trans* 18:1(n-7), *trans*-vaccenic acid; *cis* 18:2(n-6), linoleic acid; *trans* 18:2(n-6), linoleelaidic acid.

2 hr at room temperature, a mixture of 5 ml of methanol and 1.5 ml of 16% $Na_2SO_3$ solution was added and the mixture was shaken for 1 hr. After centrifugation to remove $Na_2SO_3$, the suspension was evaporated to dryness. The residue was resuspended in ether and evaporated to dryness (two times). The final residue was dissolved in a small volume of ether–chloroform. It was then transferred into a screw-capped culture tube (13 × 100 mm) and the solvent was evaporated to dryness under nitrogen. The residue was completely dried over $P_2O_5$ in a desiccator and was converted into the corresponding trimethylsilyl derivatives according to the procedure of Carter and Gaver (15).

### Chemical ionization mass spectrometry

A Shimadzu LKB 9000A combined gas–liquid chromatography(GLC)–mass spectrometer equipped with a CI source was used. The data processing system included a GCMS-PAC 300 DG consisting of an OKITAC 4300 minicomputer with 12K core, a typewriter, an incremental plotter, a magnetic disk, and an interface to the GLC–mass spectrometer. A glass column (1 m × 3 mm, ID) packed with 2% OV-7 on Chromosorb W(80–100 mesh) was used. The column temperature was maintained at 210°C, and the flow rate of helium gas was 30 ml/min. The ion source temperature was kept at 200°C. Isobutane was used as the reagent gas. The pressure of the CI source was 0.9 torr. All of the mass spectra were obtained at an electron energy of 500 eV, emission current of 400$\mu$A, and accelerating voltage of 3.5 KV.

## RESULTS AND DISCUSSION

### Mass spectra of trimethylsilylated methyl esters of mono-unsaturated fatty acids

**Fig. 1** shows the typical CI mass spectra of trimethylsilylated methyl esters of oleic and elaidic acids after stereospecific oxidation. The ions $(M - 1)^+$ and $(M + 73)^+$ were recorded at *m/e* 473 and 547 by the ion molecular reaction in the CI source (16, 17). The ion at *m/e* 473 had an intensity of approximately 1% of the total ion current. These ions and the ions *m/e* 459 and 442 corresponding to *m/e* $(M - 15, M - CH_3)^+$ and $(M - 31, M - OCH_3)^+$ clearly indicate the molecular weight. Recognizable ions at *m/e* 385 and 295 were formed by the subsequent losses of trimethylsilanol from a protonated molecule $MH^+$ which may be an unstable species. Generalized formulae showing the fragmentations are shown in **Fig. 2.** The fragments *a* and *b* in the generalized formulae are

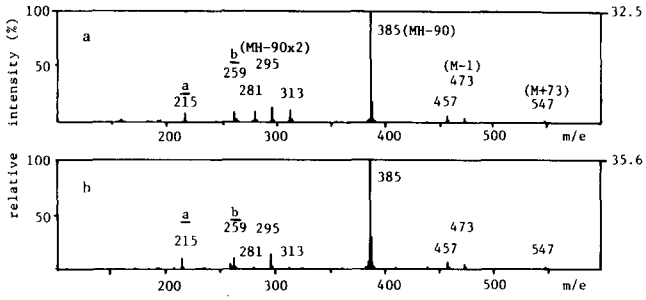


**Fig. 1.** Chemical ionization mass spectra of methyl 9,10-(ditrimethylsilyloxy)oleic acid(top) and methyl 9,10-(ditrimethylsilyloxy) elaidic acid(bottom).

formed by cleavage of the carbon–carbon bond between the two trimethylsilylethers on either side of the double bond in the molecule and are helpful in directly locating the position of the original double bond. These fragment ions are shifted to *m/e* 187 and 287 in the case of trimethylsilylated methyl esters of vaccenic acid [18:1(n-7)]. The fragment ion may be produced by the loss of the trimethylsilyl radical from the fragment ion at *m/e* 385. The ion at *m/e* 281 is probably produced by the loss of methanol. These fragment ions at *m/e* 313 and 281 provide information for the discrimination of diastereoisomers of octadecenoic acids. When the *cis-* or *trans*-configuration of the double bond in the original fatty acid is trimethylsilylated after stereospecific oxidation, it may be converted to the *threo-* or *erythro*-configuration. As shown in Fig. 1, relative intensities of these fragment ions at *m/e* 313 and 281 derived from methyl esters of oleic acid are more than quadruple those of the corresponding fragment ions from methyl esters of elaidic acid. Similar results are obtained in the mass spectra of methyl esters of vaccenic acid. In the case of the trimethylsilylated methyl esters of hexadecenoic acids, it may also be possible to locate the position of the original double bond by comparing the intensities of the fragment ions at *m/e* 187 and 259, and to discriminate the diastereoisomers by comparing the intensities of the fragment ions at *m/e* 253 and 285.

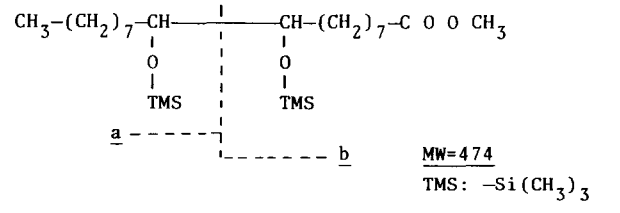


**Fig. 2.** Generalized formula showing the fragmentations of octadecenoic acid. Fragmentation due to elimination of trimethylsilanol: *m/e* 475$(MH)^+$—*m/e* 385—*m/e* 295.

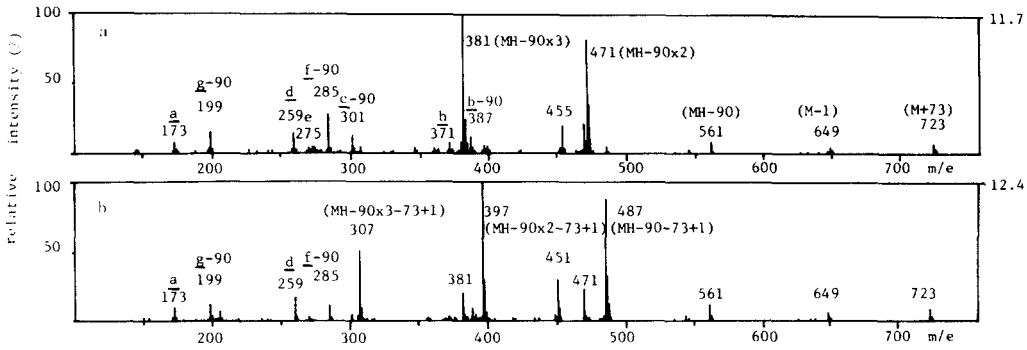


**Fig. 3.** Chemical ionization mass spectra of methyl 9,10,12,13-(tetratrimethylsilyloxy)linoleic acid(top) and methyl 9,10,12,13-(tetratrimethylsilyloxy)linolelaidic acid (bottom).

## Mass spectrometry of trimethylsilylated methyl esters of octadecadienoic acids

Typical CI mass spectra of the trimethylsilylated methyl esters of linoleic and linolelaidic acids after stereospecific oxidation are shown in **Fig. 3.** The ions corresponding to $(M - 1)^+$ and $(M + 73)^+$ are recorded at *m/e* 649 and 723. The presence of the fragment ions at *m/e* 635 and 619 corresponding to $(M - 15)^+$ and $(M - 31)^+$ also indicate their molecular weights. After stereospecific oxidation, the *cis-*, *cis-* and *trans-*, *trans*-configurations of the double bond in the original fatty acids may be converted to the corresponding *threo-*, *threo-* and *erythro-*, *erythro*-configurations. Simplified formulae showing the fragmentation are shown in **Fig. 4.** Significant ions at *m/e* 561, 471, and 381 are formed by the subsequent losses of the trimethylsilanols from a protonated molecule $MH^+$. The fragment ions that are produced by the loss of the trimethylsilyl radical from these fragment ions are recorded at *m/e* 487, 397, and 307, respectively. These six ions provide information for the discrimination of the diastereoisomers of octadecadienoic acids.

The fragment ions at *m/e* 561, 471, and 381 clearly demonstrate high intensities in the *threo-*, *threo-* isomers of octadecadienoic acid [*cis* 18:2]. On the other hand, in the case of *erythro-*, *erythro*-isomers of octadecadienoic acid (*trans* 18:2), the fragment ions at *m/e* 487, 397, and 307 are always demonstrated with high intensities. Moreover, the recognizable ions at *m/e* 259 and 173, representing the *d* and *a* in the generalized formulae in Fig. 4, are produced by cleavage of the carbon–carbon bond between the trimethylsilyl ethers and are helpful in directly locating the positions of the double bonds. Whereas the fragments *b*, *c*, *f*, *g*, and *h*, in Fig. 4 are unstable, and ions of (b − 90), (c − 90), (f − 90), (g − 90), and (h − 90) are formed from these ions by losing a trimethylsilanol moiety. It is, therefore, possible to deduce from these ions the positions of the original double bonds.

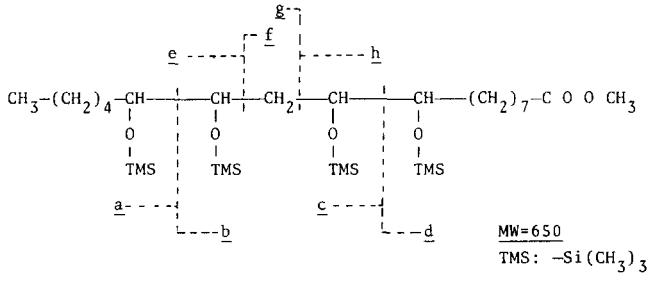


**Fig. 4.** Generalized formula showing the fragmentations of octadecadienoic acid. *a*) Fragmentation due to elimination of trimethylsilanol: *m/e* $651(MH)^+$—*m/e* 561—471—381. *b*) Fragmentation due to elimination of trimethylsilyl radical: *m/e* $561(MH - 90)^+$—*m/e* 487, *m/e* 471—397, *m/e* 381—*m/e* 307.

## Chromatography of trimethylsilylated methyl esters of mono-unsaturated fatty acids in human liver

It has been reported that GLC separations of *erythro-* and *threo*-isomers of fatty acid vicinal diols could be achieved by the trifluoroacetyl or isopropylidene derivatives because of differences in their physical properties (18, 19). In 1970, Hites and Biemann (20) reported a new, simple method of chromatography for the identification of minor biological samples that could not separated by GLC. We applied this technique to identify fatty acid methyl esters from human liver. **Fig. 5** shows a mass chromatogram of the trimethylsilylated methyl esters of mono-unsaturated fatty acids from human liver, which were purified by thin layer chromatography on silica gel plates impregnated with silver nitrate (13). As shown in Fig. 5, five peaks were recorded on the total ion monitor (peaks 1–5). However, a mass chromatogram of the eight fragment ions for *m/e* 287, 187, 259, 215, 281, 313, 253, and 285 were further separated into six components. Peaks 1 and 2 can be identified as 16:1(n-7) from the facts that the ions $(M - 1)^+$ and $(M + 73)^+$ were recorded at *m/e* 445 and 519 in their mass spectra,

and that the recognizable fragment ions at *m/e* 187 and 259 representing the position of the original double bond were detected. Moreover, peaks 1 and 2 can also be identified as *trans* 16:1(n-7) and *cis* 16:1(n-7), respectively, because the fragment ions at 253 and 285, which discriminate the diastereoisomers of hexadecenoic acids, are dominant in peak 2 rather than peak 1. Peaks 3, 4, and 5 can be identified as 18:1, because the ions $(M - 1)^+$ and $(M + 73)^+$ are recorded at *m/e* 473 and 547 in their mass spectra. The diastereoisomers of 18:1 can be discriminated by a mass chromatogram of the fragment ions at *m/e* 281 and 313. Peaks 3 and 4 can be identified as *trans*-forms and peak 5 is identified as *cis*-form, because these ions are more intense in peak 5. A mass chromatogram of the fragment ions at *m/e* 215, 259 and 187, 287 representing 18:1(n-9) and 18:1(n-7) which are positional isomers of the double bond of 18:1, shows

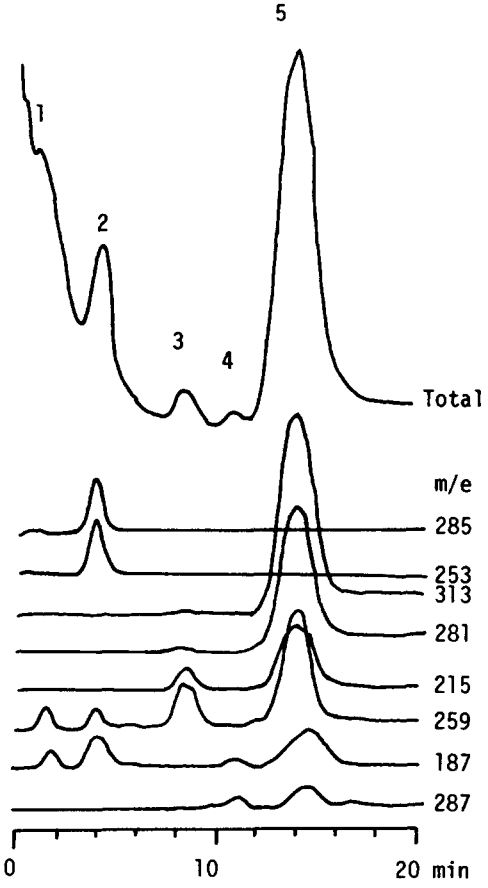


**Fig. 5.** Mass chromatogram of trimethylsilylated methyl esters of monounsaturated fatty acids from human liver. Five peaks are recorded on the total ion monitor (peaks 1–5). A mass chromatogram of the eight fragment ions for *m/e* 287, 187, 259, 215, 281, 313, 253, and 285 were separated into six components. Peak 1 can be identified as *trans* 16:1, peak 2 as *cis* 16:1, peak 3 as *trans* 18:1(n-9), peak 4 as *trans* 18:1(n-7), peak 5 as *cis* 18:1(n-9) and *cis* 18:1(n-7), respectively. The sensitivities of the mass chromatograms for *m/e* 215, 259, 187, and 287 are elevated twofold.

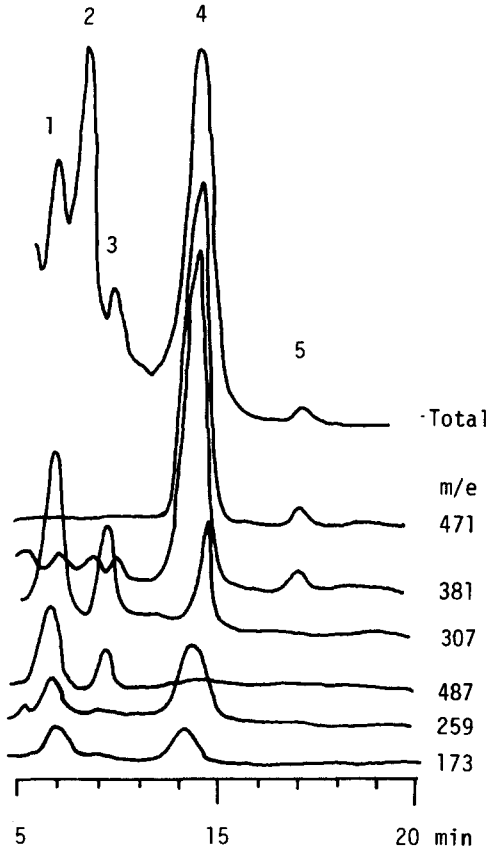


**Fig. 6.** Mass chromatogram of trimethylsilylated methyl esters of diunsaturated fatty acids from human liver. Five peaks are recorded on the total ion monitor (peaks 1–5). A mass chromatogram of the six fragment ions for *m/e* 471, 381, 487, 307, 259, and 173 shows that peak 1 can be identified as *trans*-, *trans*-9, 12-18:2, and peak 4 as *cis*-, *cis*-9, 12-18:2, respectively.

that peaks 3 and 4 can be 18:1(n-9) and 18:1(n-7), respectively, and peak 5 contains two components of 18:1(n-9) and 18:1(n-7). It is concluded that peak 3 can be identified as *trans* 18:1(n-9), peak 4 as *trans* 18:1(n-7), and peak 5 as *cis* 18:1(n-9) and *cis* 18:1(n-7), respectively, and that monounsaturated fatty acids from human liver are dominant. Thus, even small peaks of *trans* 16:1(n-7), *trans* 18:1(n-9) and *trans* 18:1(n-7) and that of *cis* 18:1(n-7), which are overlapped with the large amounts of *cis* 18:1(n-9), can be easily determined.

### Chromatography of trimethylsilylated methyl esters of diunsaturated fatty acids in human liver

A mass chromatogram of trimethylsilylated methyl esters of diunsaturated fatty acids from liver is shown in **Fig. 6.** Five peaks are recorded on total ion monitor (peaks 1–5). In the mass spectra of peaks 1, 3, 4, and 5, the ions *m/e* 647 $(M - 1)^+$ and 723 $(M + 73)^+$, indicating the molecular weight of 18:2, are recorded as the highest ions. A mass chromatogram of six frag-

ment ions for *m/e* 173, 259, 307, 381, 471, and 487 show that peaks 1 and 3 can be identified as *trans-*, *trans*-isomers, because of the presence of the ions *m/e* 307 and 487; the peaks 4 and 5 as *cis-*, *cis*-isomers, because of the presence of the ions *m/e* 471 and 381; and peaks 1 and 4 as 18:2(n-6), because of the presence of the ions *m/e* 173 and 259. Therefore, peaks 1 and 4 can be identified as *trans* 18:2 (n-6) and *cis* 18:2(n-6), respectively. However, we could not distinctly identify peaks 3 and 5, which were probably diastereo- and positional isomers of 18:2. The ion *m/e* 307 of peak 4, representing the *trans-*, *trans*-isomers of 18:2, may originate from the fragment ion due to impurities, because a mass chromatogram of the ion *m/e* 485 showed no peak with the same retention time.

It may be interesting that the *trans*-isomers in human liver can be detected as minor components on a mass chromatogram. However, the *trans*-configurations in unsaturated fatty acids in human liver should be unstable species,[1] so we are unable to discuss their quantitation.

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