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Quick and Easy Quantification of Petroselinic Acid in Parsley Seed Lipids by Mass Chromatography Using Dimethyl Disulfide Adducts

Akemi KINOSHITA, Kouhei YAMAMOTO, and Akira SHIBAHARA†

Department of Clinical Nutrition, Osaka Prefecture University, 7-30 Habikino 3-chome, Habikino 583-8555, Japan

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1 Introduction

Petroselinic acid (*cis*-6-octadecenoic acid) [18:1(6)], one of the double-bond positional isomers of oleic acid (*cis*-9-octadecenoic acid) [18:1(9)], is the characteristic acyl moiety of seed lipids of the families Umbelliferae (or Apiaceae), Araliaceae, Garryaceae and Cornaceae.[1-3] To illustrate, the content of 18:1(6) amounts to over half of the acyl components in the seed triacylglycerols of coriander (*Coriandrum sativum*, Umbelliferae) and parsley (*Petroselinum rubrum*, Umbelliferae). The biosynthetic mechanism of 18:1(6) in higher plants has been confirmed[4,5] and a series of studies on transgenic plants producing 18:1(6) began.[6] Furthermore, nutritional characteristics of 18:1(6) in dietary triacylglycerols observed in rats has been reported.[7] The effect of 18:1(6) on human nutrition, however, is still unknown.[8]

In seed lipids, 18:1(6) always co-exists with 18:1(9), and, due to their similar chromatographic behavior, determining the presence of these two octadecenoic isomers is difficult.[9]

In this communication, however, we present a new method which can successfully and easily determine the amounts of 18:1(6) and 18:1(9) in lipids. This method uses gas chromatography/mass spectrometry (GC/MS) with a weak-polar capillary column, SPB-50.[10] The SPB-50 column separates the dimethyl disulfide (DMDS) adducts of methyl 18:1(6) and methyl 18:1(9), and therefore, their amounts can be determined.

2 Materials and Methods

2.1 Seeds and chemicals

Parsley seeds were purchased at a local market. Standard 18:1(6), 18:1(9) and 18:1(11) were obtained from Nu-Chek Prep (Elysian, MN), and their purities were over 99%. All other chemicals were analytical reagent grade, and all the solvents were distilled before use.

2.2 Derivatization

Standard fatty acids were methylated with 14% BF_3/methanol and purified by thin-layer chromatography (TLC)[11] for capillary gas chromatography (GC). A single seed of parsley was cut in half with scissors, put into a small glass tube, crushed with a glass rod, and then treated in the same manner as described previously[12] to derive fatty acid methyl esters (FAMEs). These FAMEs were purified by TLC[11] before injection to the capillary GC system. An aliquot of the FAMEs was subjected to the I_2-catalyzed reaction with DMDS[13] with minor modifications (Shibahara et al., manuscript in preparation).

2.3 Capillary GC

FAMEs were analyzed on a fused silica ULBON HR-SS-10 capillary column (50 m × 0.25 mm i.d., 0.25 μm film thickness, chemically bonded type) (Shinwakako, Kyoto, Japan) in a Shimadzu GC-17A gas chromatograph with a split/splitless injector and a flame ionization detector linked to a Shimadzu work station on-line system (Class-GC10). The column temperature was programmed at 100℃ for 2 min isothermally, then to 210℃ at a rate of 6℃/min and held at 210℃ for 40 min. The carrier gas was helium at a split ratio of 1/40 (linear gas velocity: 30.0 cm/sec).

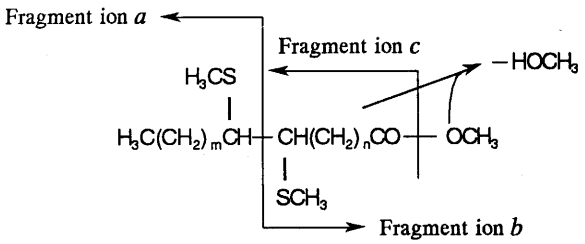
2.4 Capillary GC/MS

DMDS adducts were analyzed on the SPB-50 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco, Bellefonte, PA) linked to a Shimadzu GCMS QP2010 mass spectrometer with a computer on-line system. The column temperature was programmed at 180℃ for 2 min isothermally, then to 300℃ at a rate of 3℃/min and held at 300℃ for 8 min. The carrier gas was helium at a split ratio of 1/10 (linear gas velocity: 35.0 cm/sec). Electron impact mass spectra were measured at an ionizing energy at 70 eV by scanning from 50 to 450 *m/z* (0.5 sec/cycle).

3 Results and Discussion

3.1 Fragmentation of the DMDS adducts

Electron impact ionization of the DMDS adducts of methyl monoenoate gave the recognizable molecular ion and the set of key fragment ions *a*, *b* and *c* derived from the frag-



Scheme 1 Fragmentation pattern of dimethyl disulfide adducts derived from methyl monoenoate.

† Corresponding author. E-mail: shibahar@rehab.osakafu-u.ac.jp

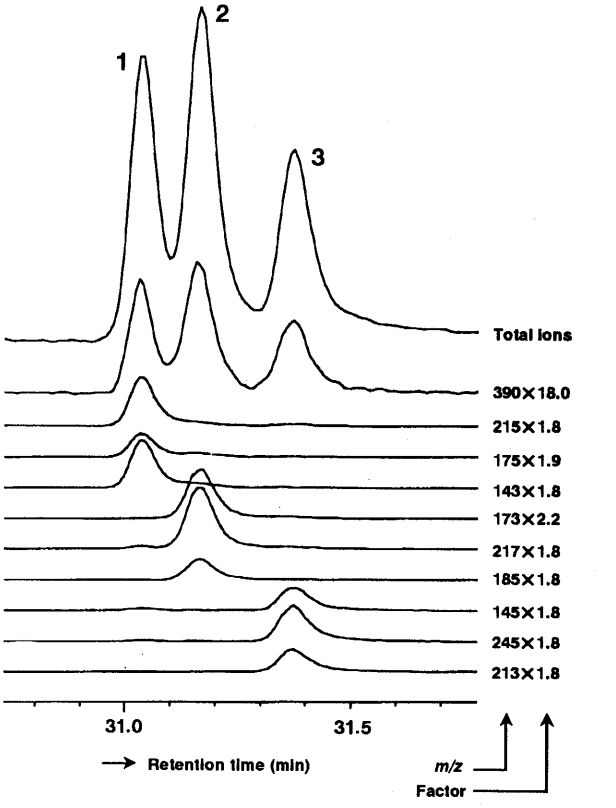


Fig. 1 Mass chromatogram of dimethyl disulfide adducts derived from a mixture of authentic methyl 18:1(6), 18:1(9) and 18:1(11).
1=methyl 6,7-bis(methylthio)octadecanoate derived from methyl 18:1(6), 2=methyl 9,10-bis(methylthio)-octadecanoate derived from methyl 18:1(9), 3=methyl 11,12-bis(methylthio)octadecanoate derived from methyl 18:1(11). The ion at *m/z* 390 is the molecular ion of the dimethyl disulfide adducts of methyl 18:1(6), 18:1(9) and 18:1(11). Explanation of the other ions (a set of ions at *m/z* 215, 175 and 143, a set of ions at *m/z* 173, 217 and 185, and a set of ions at *m/z* 145, 245 and 213) is given in the text. For readability, we magnified the mass chromatographic responses by using different numerical factors of magnification.

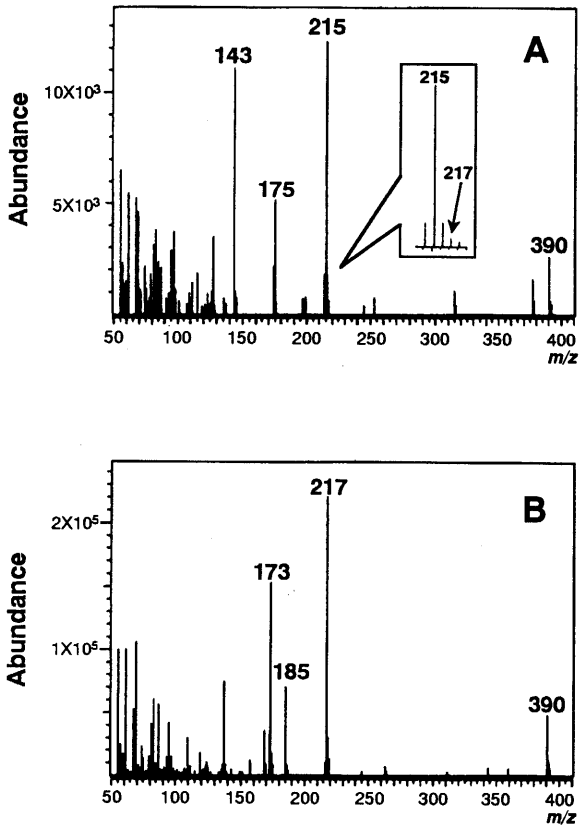


Fig. 2 Mass spectra of dimethyl disulfide adducts of methyl 18:1(6) (A) and methyl 18:1(9) (B).
Spectra A and B are taken at the peak tops of peaks 1 and 2, respectively, in Fig. 1. Mass spectral assignments are given in the text.

mentation, as illustrated in Scheme 1. The key fragment ions *a* and *b* came from the cleavage between the methylthio-substituted carbons; the key fragment ion *c* was yielded due to the loss of methanol from the key fragment ion *b*.[14]

3.2 Analytical process

We prepared a FAME mixture of authentic 18:1(6), 18:1(9) and 18:1(11) (36:40:24, by weight), converted the mixture to the DMDS adducts, and analyzed them on the SPB-50 column in GC/MS. Fig. 1 shows the mass chromatogram traced with the total ions, the molecular ion (*m/z* 390) and the key fragment ions *a*, *b* and *c* (cf. Scheme 1).

3.2.1 Mass spectra of DMDS adducts

Mass spectra taken from the top of peaks 1 and 2 in Fig. 1 are shown in Fig. 2A and 2B. In mass spectrum A (Fig. 2A), the set of the fragment ions at *m/z* 215, 175 and 143 corresponds to the key fragment ions *a*, *b* and *c* (Scheme 1) (the molecular ion at *m/z* 390), indicating that the structure of the adducts was methyl 6,7-bis(methylthio)octadecanoate. Thus its original double-bond was at the Δ-6 position in the C18 chain. In mass spectrum B (Fig. 2B), the set of the fragment ions at *m/z* 173, 217 and 185 corresponds to the key fragment ions *a*, *b* and *c* (Scheme 1) (the molecular ion at *m/z* 390), indicating that the structure of the adducts was methyl 9,10-bis(methylthio)octadecanoate. Thus its original double-bond was at the Δ-9 position in the C18 chain.

On the basis of these elucidations, we can clearly locate the original double-bonds of the methyl monoenoates. In addition, we can determine the ratio of the isomeric DMDS adducts in the sample by measuring the peak area ratio of the key fragment ions *a*, *b* and *c* on the mass chromatogram.

3.2.2 Calibration curves

To make the calibration curve for determining the amount of 18:1(6), we prepared eleven kinds of standard mixtures containing known amounts of methyl 18:1(6) and methyl 18:1(9). These standard mixtures were methylthiolated and mass chromatographed to trace the key fragment ions *a*, *b* and *c* (cf. Scheme 1).

The value V_a indicating the peak area ratio of the key fragment ion *a* was calculated with the following formula: V_a = peak area of *m/z* 215 / (peak area of *m/z* 215 + peak area of *m/z* 173) × 100, where the ions at *m/z* 215 and *m/z* 173 represent the DMDS adducts of methyl 18:1(6) and methyl 18:1(9), respectively. We plotted the values of V_a and the amounts of 18:1(6) of the eleven standard mixtures to get the calibration curve shown in Fig. 3A. For each standard mixture, the value for V_a is in direct proportion to the amount of 18:1(6). These results prove that our method can be used to successfully determine the contents of 18:1(6) and of 18:1(9).

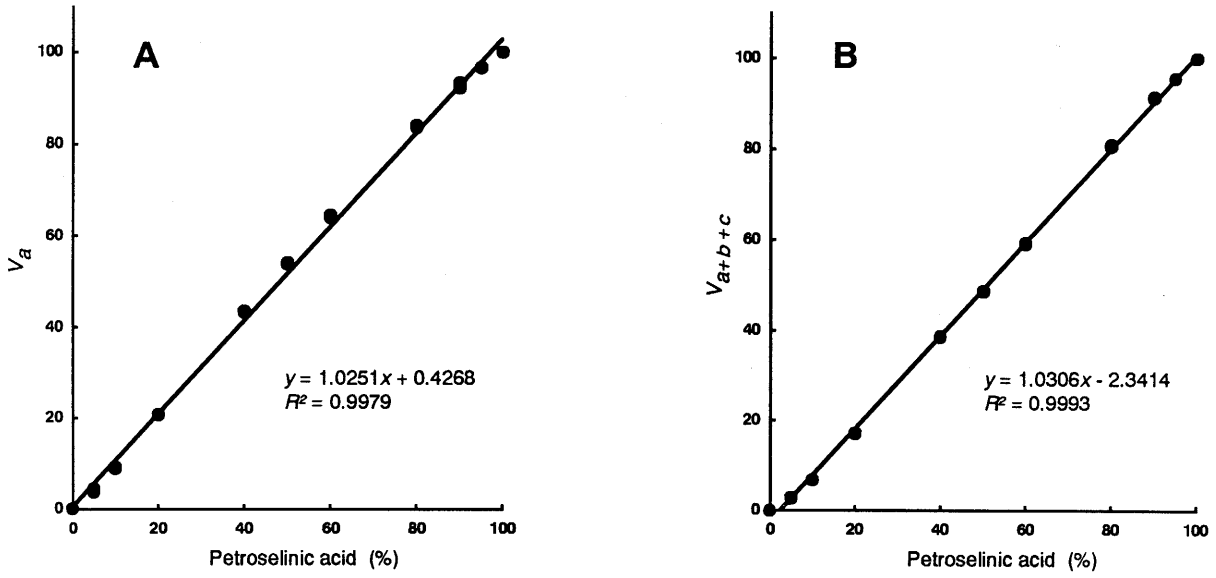


Fig. 3 Calibration curves for determining the amounts of 18:1(6).
In calibration curve A, the amounts of 18:1(6) of the eleven standard mixtures are plotted on the *x*-axis, and the values V_a on the *y*-axis. The value V_a indicating the peak area ratio of the key fragment ion *a* is calculated with the following formula: V_a = peak area of *m/z* 215 / (peak area of *m/z* 215 + peak area of *m/z* 173) × 100, where the ions at *m/z* 215 and *m/z* 173 represent the dimethyl disulfide adducts of methyl 18:1(6) and methyl 18:1(9), respectively. Explanation for the calibration curve B is given in the text. Each plot in each curve is from duplicate determinations of the eleven standard mixtures, and so, twenty-two points (not averaged) are plotted.

Similarly the value V_b (indicating the peak area ratio of the key fragment ion *b*) and the value V_c (indicating the peak area ratio of the key fragment ion *c*) were calculated. We plotted the values of V_b and the values of V_c and the amounts of 18:1(6) of the eleven kinds of standard mixtures. Unlike the values of V_a, the values of V_b and V_c were not in direct proportion to the amounts of 18:1(6) (data not shown). This is probably due to the different amounts of the key fragment ion *c* (which is the secondary ion from the key fragment ion *b* when methanol is lost) from the C6 chain and from the C9 chain. Using the value V_{b+c} (calculated from the key fragment ions *b* plus *c*) (data not shown) gave a calibration curve resembling that in Fig. 3A. Fig. 3B shows the calibration curve using the value V_{a+b+c} (calculated from the key fragment ions *a* plus *b* plus *c*). The increase in proportion was linear with the increasing amounts of 18:1(6) of the eleven standard mixtures.

The calibration curves (Fig. 3A and 3B) were constant in repeated experiments, and no statistical treatment was employed. These results also prove that our new GC/MS method quantifies 18:1(6) and 18:1(9).

3.2.3 Advantage of using the SPB-50 capillary column

During this research, if we had used a non-polar DB-5ms capillary column (which we used in our previous work[15]) instead of using the SPB-50 capillary column, the DMDS adducts of methyl 18:1(6) and 18:1(9) would not have separated on the mass chromatogram. Calculating the ratio of the DMDS adducts, therefore, would have been tedious to determine because of the presence of naturally-occurring isotopes (having the same mass numbers as the key fragment ions *a*, *b* or *c*). For example, we would not have been able to distinguish the key fragment ion *b* at m/z 217 of the DMDS adducts of methyl 18:1(9) (Fig. 2B) from the naturally-occurring isotope peak at *m/z* 217 of the key fragment ion *a* at *m/z* 215 of the DMDS adducts of methyl 18:1(6) (Fig. 2A). During the present research, we learned that if we use the weak-polar SPB-50 capillary column, the three kinds of the DMDS adducts of methyl 18:1(6), 18:1(9) and 18:1(11) clearly separate, as shown in Fig.1. We can ignore the naturally-occurring isotope peaks on the mass chromatogram.

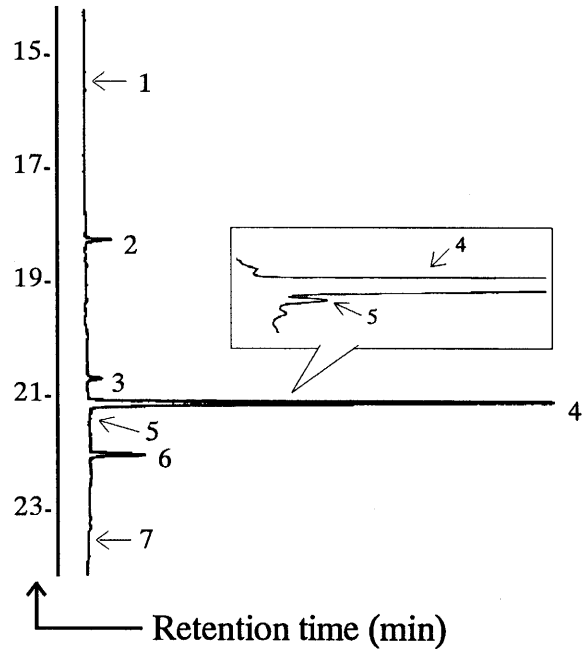


Fig. 4 Capillary gas chromatogram of fatty acid methyl esters prepared from a single seed of parsley (cf. Table 1, seed no. 2).
1=14:0, 2=16:0, 3=18:0, 4=18:1(6) + 18:1(9), 5=18:1(11), 6=18:2(9,12), 7=18:3(9,12,15). Peak assignments were determined by comparing their retention times to those of authentic standards and our laboratory standard.

Table 1 Major fatty acid composition of individual seeds of parsley

Fatty acid (wt%)	Seed no. 1	2	3	4	5	6
14:0	3.0	0.6	1.5	4.1	2.1	10.8
16:0	5.1	2.3	5.6	3.8	3.8	4.0
18:0	2.6	2.6	2.3	2.0	2.5	1.7
18:1(6)	54.5	67.9	63.6	64.8	72.3	61.6
18:1(9)	16.7	10.1	11.0	6.4	6.3	8.2
18:1(11)	0.7	0.6	0.5	0.4	0.4	0.4
18:2(9,12)	12.0	10.9	9.7	8.5	9.0	9.9
18:3(9,12,15)	1.1	0.5	0.4	0.3	0.3	0.3
Others	4.3	4.5	5.4	9.7	3.3	3.1
$\frac{18:1(6)}{\text{Total } 18:1} \times 100$	75.8	86.4	84.7	90.5	91.5	87.7

3.3 Application of the new GC/MS method to quantify 18:1(6) in parsley seed lipids

We applied this method for quantifying 18:1(6) in parsley seed lipids. Fig. 4 shows a gas chromatogram taken from analyzing the FAMEs prepared from a single seed of parsley. All the peaks numbered in Fig. 4 were confirmed by comparing their retention times to those of authentic standards and our laboratory standard mixture. After obtaining the data on fatty acid composition [in which the percentages of 18:1(6) and of 18:1(9) are still unknown, and the percentage of 18:1(11) is known] by capillary GC, an aliquot of the FAMEs was converted to the DMDS adducts and analyzed by GC/MS. Then on the basis of mass chromatographic tracing, the amounts of 18:1(6) and 18:1(9) were determined from the calibration curve A (Fig. 3A).

Table 1 summarizes the fatty acid composition of individual seeds of parsley. Six analyses were carried out with six seeds (one analysis per one seed). The seeds contained varying amounts of 18:1(6): the maximum amount was 72.3% (seed no. 5), and the minimum amount was 54.5% (seed no. 1). The amounts of other fatty acids similarly varied; and especially, the amounts of 14:0 and 18:1(9) widely varied, ranging from 0.6% to 10.8% and from 6.3% to 16.7%, respectively. Even though these seeds came from one package from a local market, the seeds contained different amounts of 18:1(6). This means that high-yielding 18:1(6) parsley plants can be specified.

Plant lipid researchers will greatly benefit from our quick and easy method for determining the amount of 18:1(6).

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