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Novel Pre-fractionation Method of *Trans* Fatty Acids by Gas Chromatography with Silver-Ion Cartridge Column

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Abstract: We developed a novel pre-separation method of *trans* fatty acids (TFAs) using a silver-ion cartridge column and GC. As a preliminary study, a mixture of fatty acid methyl esters consisting of saturated, *cis*-unsaturated, and *trans*-unsaturated fatty acids was dissolved in dichloromethane and loaded onto a Bond Elut SCX ion-exchange cartridge column that was converted to the silver-ion form. The column was then eluted with dichloromethane to obtain the saturated fatty acids, dichloromethane/ethyl acetate (90/10) for the *trans* mono-ene, dichloromethane/ethyl acetate (65/35) for the *cis* mono-ene, dichloromethane/acetone (60/40) for the *trans* di-ene, and acetone/acetonitrile (80/20) for the others. Satisfactory separation of the *cis*/*trans* isomers was confirmed by GC analysis. To generalize this technique, the elution conditions of the ready-to-use Discovery Ag-ION SPE cartridge column were also optimized. Both cartridge columns had good separation, recovery, and repeatability. Peer laboratory verification was carried out between two laboratories using different production lots of the ready-to-use cartridge column, and the robustness of the product and reproducibility of the method were found to be satisfactory. This technique is therefore a powerful tool not only for routine analyses of TFAs in oils, fats, and foods but also for detailed analyses of TFAs in various research fields.

Key words: *trans* fatty acid, pre-fractionation, gas chromatography, silver-ion chromatography

1 INTRODUCTION

Partial hydrogenation of edible fats/oils inevitably creates geometrical and positional isomers of unsaturated fatty acids. Their *trans* isomers, called *trans* fatty acids (TFAs), have been reported to raise serum levels of low-density lipoprotein cholesterol and to increase the risk of coronary heart disease[1]. The World Health Organization (WHO) recommends that TFA intake be limited to less than 1% of the total energy intake[2]. In 2003, Denmark became the first country to ban the sale of fats and oils with greater than 2% industrially produced *TFAs*[3]. In that same year, the U.S. Food and Drug Administration (FDA) issued a regulation requiring manufacturers to list TFA on the Nutrition Facts panel of foods and some dietary supplements[4]. Thus, the influence of TFAs on our health has led to an increased interest in the development of an accurate and rapid method for the determination of the total TFA content of foods.

To analyze TFAs, several official organizations such as AOAC International (AOAC) and the American Oil Chemists' Society (AOCS) have employed infrared spectroscopy (IR)[5-8] and gas chromatography (GC)[9-12]. The IR method is simple to perform but lacks sensitivity and information about fatty acid composition. The GC method that uses a long capillary column (50 to 100 m) coated with highly polar stationary phases has also been widely used for TFA analysis. However, even with this type of column, several peaks of *cis*/*trans* isomers sometimes overlap each other on a GC chromatogram, which is mostly due to excess amounts of *cis* isomers in the sample. To separate these *cis*/*trans* isomers, a pre-separation by silver-ion thin-layer chromatography (Ag-TLC)[13] or silver-ion high-performance liquid chromatography (Ag-HPLC)[14] has been employed. However, these procedures are notoriously time consuming

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and require refined skills. Christie reported[15] the separation of fatty acid methyl esters (FAMEs) using the silver-ion solid-phase extraction (Ag^+-SPE) technique, in which the main focus was not the separation of *cis*/*trans* isomers but the separation of FAMEs according to the number of double bonds.

Cis double bonds offer more steric accessibility than their *trans* counterparts and can therefore form stronger polar complexes in the stationary phase. As a result, TFAs are more weakly retained on Ag^+-SPE than their *cis* counterparts. Differences in retention strength between classes of FAMEs and silver counter-ions can be exploited, allowing for FAME fractionation prior to GC analysis.

In this study, by applying the above technique for a mixture of *cis*/*trans* isomers, we developed a novel, simple, and accurate pre-separation method of TFAs using a silver-ion cartridge column prior to GC analysis. Validation studies for the proposed procedure were also carried out between two laboratories.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

2.1.1 Reagents

A strong cation exchange column, Bond Elut SCX, packed with a silica-based benzenesulfonic acid medium, was purchased from Varian (Palo Alto, CA). Another column, Discovery Ag-ION SPE, which was packed with a silica-based benzenesulfonic acid medium coated with silver ions, was purchased from Supelco (Bellefonte, PA). Stearic acid methyl ester (18:0, >99.5%), elaidic acid methyl ester (18:1 *t*, >99.0%), oleic acid methyl ester (18:1 *c*, >99.0%), linoleic acid methyl ester (18:2 *c*,*c*, >98.5%), linolenic acid methyl ester (18:3 *c*,*c*,*c*, >99.0%), and "linoleic acid methyl ester *cis*/*trans*-isomers," consisting of *trans*-9,*trans*-12-octadecadienoic acid (18:2 *t*,*t*), *cis*-9,*trans*-12-octadecadienoic acid (18:2 *c*,*t*), *trans*-9,*cis*-12-octadecadienoic acid (18:2 *t*,*c*), and *cis*-9,*cis*-12-octadecadienoic acid (18:2 *c*,*c*), were purchased from Sigma-Aldrich (St. Louis, MO). "Linolenic acid methyl ester isomer mix" (>98.0%), consisting of *trans*-9,*trans*-12,*trans*-15-octadecatrienoic acid (18:3 *t*,*t*,*t*), *trans*-9,*trans*-12,*cis*-15-octadecatrienoic acid (18:3 *t*,*t*,*c*), *trans*-9,*cis*-12,*trans*-15-octadecatrienoic acid (18:3 *t*,*c*,*t*), *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid (18:3 *c*,*t*,*t*), *cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid (18:3 *c*,*c*,*t*), *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid (18:3 *c*,*t*,*c*), *trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid (18:3 *t*,*c*,*c*), and *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid (18:3 *c*,*c*,*c*), was purchased from Supelco (Bellefonte, PA). Heptadecanoic acid methyl ester (17:0, >99.0%) was purchased from Merck (Whitehouse Station, NJ). Boron trifluoride reagent (14%) for methyl esterification for GC analysis was purchased from Wako Pure Chemical (Osaka, Japan). Other reagents used for this study were of analytical grade.

2.1.2 Standard mixtures of FAME

For the spike test, the standard mixtures of FAMEs were prepared as follows. The *cis* isomers mixture was prepared by mixing equal volumes of oleic acid methyl ester, linoleic acid methyl ester, and linolenic acid methyl ester. The *trans* isomers mixture was prepared by mixing equal volumes of elaidic acid, "linoleic acid methyl ester *cis*/*trans*-isomers," and "linolenic acid methyl ester isomer mix." The ratios of each isomer in the *trans* isomers mixture were as follows: 18:1 *t*, 33%; 18:2 *t*,*t*, 17%; 18:2 *c*,*t*, 7%; 18:2 *t*,*c*, 7%; 18:3 *t*,*t*,*t*, 10%; sum of 18:3 *t*,*t*,*c*, 18:3 *t*,*c*,*t*, and 18:3 *c*,*t*,*t*, 15%; and sum of 18:3 *c*,*c*,*t*, 18:3 *c*,*t*,*c*, and 18:3 *t*,*c*,*t*, 7% (3% of 18:2 *c*,*c* and 1% of 18:3 *c*,*c*,*c* were also included).

2.1.3 Oil and food samples

Olive oil (Japanese Pharmacopoeia) was purchased from Wako Pure Chemical. Shortening, butter, margarine, chocolate, pie, and cookies were purchased from supermarkets in Aichi, Japan in 2007.

2.2 Methods

2.2.1 Gas chromatography analysis

GC analysis of the FAMEs was performed on a Shimadzu GC-1700 equipped with a flame ionization detector and a capillary column (SP2340 [60 m × 0.25 mm i.d.]; Supelco). The column oven was temperature programmed to increase from 150℃ to 220℃ at a rate of 1.3℃/min. Helium was used as the carrier (0.5 mL/min) and make-up gas. Temperatures of both the injector and detector were 210℃. Heptadecanoic acid methyl ester was added to the test solution as an internal standard.

At Supelco, the GC analysis of FAMEs was performed on an Agilent HP 5890 equipped with a flame ionization detector and a capillary column (SP2560 [75 m × 0.18 mm i.d.]; Supelco). The column oven temperature was 180℃. Hydrogen was used as the carrier (40 cm/s). The temperature of both the injector and detector were 220℃.

2.2.2 Extraction of lipids from food samples and methyl esterification

Total lipids were extracted from the oily food samples by the method of Folch *et al.*[16]. The extracted lipids were methyl-esterified by a modified AOCS official method[17] as follows. Briefly, about 50 mg of the lipids in 2 mL of chloroform were mixed with 1.5 mL of a 0.5 M NaOH-methanol solution and heated at 100℃ for 9 min. Then 2 mL of a 14% boron trifluoride-methanol solution was added and the temperature maintained at 100℃ for 7 min. FAMEs obtained by this methyl esterification procedure were extracted with n-hexane.

2.3 Preliminary study

We first optimized separation conditions for a self-made

SPE column as a preliminary study.

2.3.1 Self-made Ag^+-cartridge column chromatography

A Bond Elut SCX column was converted to the silver ion (Ag^+) form for use in the preliminary study by flushing with 2 mL of acetonitrile followed by 1 mL of 20 mg/mL silver nitrate in acetonitrile-water (9:1 v/v). The column was then flushed with 5 mL each of acetonitrile, acetone, and dichloromethane, respectively. The prepared Ag^+-cartridge column was wrapped with aluminum foil to exclude light prior to use.

FAMEs (0.5 mg) were dissolved in a small volume of chloroform and loaded onto the Ag^+-cartridge column, then eluted with each solvent under gravity as shown in Table 1. The eluates were defined as fractions (abbreviated as "Fr.") 1 to 8.

2.3.2 Elution patterns of FAMEs

As mentioned above, fifteen 18-carbon FAME compounds, 18:0, 18:1 *c*, 18:1 *t*, 18:2 *c*,*c*, 18:2 *c*,*t*, 18:2 *t*,*c*, 18:2 *t*,*t*, 18:3 *c*,*c*,*c*, 18:3 *c*,*c*,*t*, 18:3 *c*,*t*,*c*, 18:3 *t*,*c*,*c*, 18:3 *c*,*t*,*t*, 18:3 *t*,*c*,*t*, 18:3 *t*,*t*,*c*, and 18:3 *t*,*t*,*t*, were applied to the column to be separated. Equal volumes of oleic acid, the *cis* isomers mixture, and the *trans* isomers mixture (described in the "Standard mixture of FAME" section) were combined and then applied to the column to be separated. The FAMEs were analyzed by GC and the recovery of FAME in each fraction was calculated.

2.3.3 Recovery test of FAMEs added to oil

To confirm the accuracy of the proposed procedure, a recovery test of the *trans* FAMEs was carried out using actual oil. The *trans* isomers mixture, which included the 13 *trans* FAMEs, was added to an olive oil at a ratio of 1 to 10, and was fractionated by the proposed procedure. Then each fraction obtained for *trans* FAMEs was analyzed by GC to calculate the recovery.

2.3.4 Comparison between novel and Ag-TLC methods

To compare the results from the proposed procedure with those from the conventional method, levels of TFAs were determined in a beef fat and in two kinds of shortening made from vegetable oil and vegetable/animal oil by both the proposed method and the conventional Ag-TLC method[12]. A TLC plate coated with silica gel (200×200 mm, Merck) was dipped into a 20% (w/v) silver nitrate solution in acetonitrile. The plate was dried and then activated at 110℃ for 30 min. The FAMEs in hexane were applied about 3 cm from the bottom edge of the plate and developed with hexane/ethyl acetate/xylene (90:5:5, v/v/v). FAME bands were detected under UV light after spraying the plate with 0.1% (w/v) of 2′,7′-dichlorofluorescein in ethanol, and each band was scraped off separately based on the description[12]. The corresponding *trans*-FAMEs in the scraped bands were extracted with diethylether and analyzed by GC according to the aforementioned manner.

In this study, TFA was defined as all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated carbon-carbon double bonds in the *trans* configuration.

2.4 Practical study

2.4.1 Preparation of Ag^+-cartridge column

As a practical study, a Discovery Ag-ION SPE, a ready-to-use Ag^+-cartridge column, was used. The column was pre-conditioned with 4 mL of acetone and 8 mL of hexane, sequentially.

FAMEs dissolved in chloroform were loaded onto the Ag^+-cartridge column and then eluted with each solvent under gravity as shown in Table 2. The eluates were defined as Fr. 1′ to 5′.

2.4.2 Elution patterns and recovery test of FAMEs

Similar to the preliminary study, elution patterns and a recovery test for the FAMEs were also explored for the Discovery Ag-ION SPE.

2.4.3 Peer laboratory verification

Peer laboratory verification of the proposed method was carried out between two laboratories: Japan Food Research Laboratories and Supelco. We focused on the reproducibility and robustness for the elution patterns of FAMEs because this step was the crucial part of our method. Between the two laboratories, recoveries of the *trans*

Table 1 Composition of eluates for Bond Elut SCX.

Fraction No.	Eluate Volume (ml)	Ratio (%)		
		Dichloromethane	Ethylacetate	Methanol
1	5	100	0	0
2	5	99	1	0
3	5	95	5	0
4	5	30	70	0
5	5	20	80	0
6	5	0	100	0
7	5	0	80	20
8	5	0	0	100

Table 2 Composition of eluates for Discovery Ag-ION SPE.

Fraction No.	Eluate Volume (ml)	Ratio (%)			
		Dichloromethane	Ethylacetate	Acetone	Acetonitrile
1'	6	100	0	0	0
2'	8	90	10	0	0
3'	6	75	25	0	0
4'	6	60	0	40	0
5'	6	0	0	80	20

Table 3 Recoveries of fatty acid methyl esters by Bond Elut SCX.

Fr. No.	Recovery (%)												
	18:0	18:1 *t*	18:1 *c*	18:2 *t,t*	18:2 *c,t*	18:2 *t,c*	18:2 *c,c*	18:3 *t,t,t*	18:3 *t,c,t t,t,c*	18:3 *c,t,t c,c,t*	18:3 *c,t,c*	18:3 *t,c,c*	18:3 *c,c,c*
1	98	–	–	–	–	–	–	–	–	–	–	–	–
2	–	96	–	–	–	–	–	–	–	–	–	–	–
3	–	–	98	–	–	–	–	–	–	–	–	–	–
4	–	–	–	101	100	99	–	–	–	–	–	–	–
5	–	–	–	–	–	–	48	90	–	–	–	–	–
6	–	–	–	–	–	–	51	8	–	–	–	–	–
7	–	–	–	–	–	–	–	–	95	97	100	97	–
8	–	–	–	–	–	–	–	–	–	–	–	–	97

– : not detected

Fr. No.: fraction number

FAMEs of each fraction were evaluated using different production lots of Ag-Ion SPE.

2.4.4 Analysis of various food samples

TFA contents in various commercial oily/fatty foods such as shortening, butter, margarine, chocolate, pie, and cookies were analyzed by the proposed method.

3 RESULTS AND DISCUSSION

3.1 Preliminary study

3.1.1 Elution pattern of FAMEs using Bond Elut SCX

The recoveries and elution patterns of fifteen 18-carbon FAMEs using a Bond Elut SCX are shown in Table 3.

The saturated FAME was found to be eluted in Fr. 1. Each *trans* isomer of mono-ene, di-ene, and tri-ene unsaturated FAME was eluted in Fr. 2, Fr. 4, and Fr. 7, respectively, except for the all *trans* isomer (18:3 *t,t,t*). *Cis* isomers of mono-ene, di-ene, and tri-ene FAME were eluted in Fr. 3, Fr. 5, and Fr. 8, respectively. Therefore, trans isomers of FAMEs were separated from the *cis* isomers with the self-prepared Ag^+ column, with one exception. The 18:3 *t,t,t* isomer was coeluted with the corresponding *cis* isomers like linoleic acid (18:2 *c,c*) in Fr. 5. Because 18:3 *t,t,t* and 18:2 *c,c* were clearly separated on the GC chromatogram, as exemplified by the difference in retention time of 1.5 min, the proposed procedure was found to be suitable for the TFA separation.

3.1.2 Recovery test for FAMEs added to oil

In the recovery test, each fraction for *trans* FAMEs added to olive oil was analyzed by GC, and the test was repeated five times. The recoveries and relative standard deviation (RSD) of each *trans* FAME are indicated in Table 4. In the cases of Fr. 4 and 7, the total recovery in each fraction is also shown. The average recoveries of the total *trans* mono-ene, di-ene, and tri-ene were satisfactory, as exemplified by their values of 101.7%, 101.4%, and 96.3%, respectively. The repeatability for the three *trans* isomers was also satisfactory according to the RSDs of 1.1%, 1.6%, and 3.1%, respectively. Several recoveries were near 90% or over 120%, which was probably due to the low concentration of tri-ene isomers in the FAME mixture. Therefore, the described fractionation was found to be effective for the actual oil.

3.1.3 Comparison between novel and Ag-TLC methods

Chromatograms of shortenings made from animal and vegetable oils are shown in Fig. 1. As shown in the chromatograms, complete *cis*/*trans* separation was achieved

Table 4 Recoveries of *trans* fatty acid methyl esters added to olive oil.

Fr. No.	Fatty acid	Recovery (%)						RSD(%)
		1*	2*	3*	4*	5*	Mean	
2	18:1*t*	100.4	101.4	102.3	100.8	103.6	101.7	1.1
4	18:2*t,t*	98.0	103.3	102.3	102.3	103.1	102.6	2.7
	18:2*c,t*	103.4	97.6	97.6	99.8	101.8	100.0	2.3
	18:2*t,c*	96.6	97.4	100.7	100.6	102.6	99.6	2.3
	Total	98.9	100.7	100.9	103.6	102.7	101.4	1.6
7	18:3*t,c,t t,t,c*	91.2	96.7	98.9	91.0	90.6	93.7	3.7
	18:3*c,t,t c,c,t*	94.9	100.9	103.4	91.0	91.8	96.4	5.1
	18:3*c,t,c*	100.0	92.6	97.1	95.0	103.3	97.6	3.8
	18:3*t,c,c*	107.4	106.7	105.3	97.4	125.2	108.4	8.4
	Total	95.0	98.6	100.7	92.0	95.3	96.3	3.1

RSD: relative standard deviation
Fr. No.: fraction number
*: repetition number

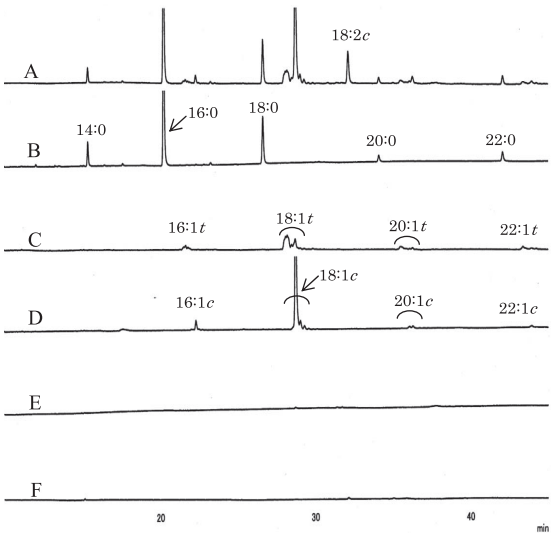

Fig. 1 Chromatogram of shortening composed of vegetable and animal oil with and without pre-fractionation by Bond Elut SCX.
A: without Ag-column pre-fractionation; B: fraction 1 for saturated FAME; C: fraction 2 for *trans* mono-ene FAME; D: fraction 3 for *cis* mono-ene FAME; E: fraction 4 for *trans* di-ene FAME; F: fraction 7 for *trans* tri-ene FAME. The fraction number is defined in Table 1.

not only for the 18-carbon FAMEs but also for FAMEs with other numbers of carbon atoms. Peak identification of the *trans* FAMEs whose standards were not available was carried out according to the official method[11, 12]. Comparative results between the proposed method and the conventional Ag-TLC method are shown in Table 5. The 20:1 *t* isomer shows a large relative difference between the two methods, but is present in less than 2 g/100 g; this is probably due to the accumulated errors during the quantification of several minor positional isomers. Near agreement in the separation patterns for the two methods allowed us to propose that our novel procedure should be an acceptable alternative to the official TLC method. Additionally, the operation time of this method is much shorter than that of the Ag-TLC method, especially when the sample number is large. Therefore, the proposed method should be superior to conventional separation methods, particularly in high throughput situations.

3.2 Practical study

3.2.1 Elution Pattern of FAMEs using Discovery Ag-ION SPE

Recoveries and elution patterns of fifteen 18-carbon FAMEs using the Discovery Ag-ION SPE are shown in Table 6.

Because some properties of the Ag^+ resin were different between the ready-to-use and hand-prepared columns, the optimized elution conditions and patterns were also different. As shown in Table 6 saturated and *trans*/*cis* isomers of mono-ene unsaturated FAMEs were separated in Fr. 1' to 3' in a similar manner as in the preliminary study.

In the cases of di-ene and tri-ene, *trans* isomers of di-ene and 18:3 *t,t,t* were found in Fr. 4', and *cis* isomers of both di-ene and tri-ene and *trans* isomers of tri-ene (except for 18:3 *t,t,t*) were found in Fr. 5'. Unlike the preliminary study, di-ene and tri-ene isomers were not completely separated. However, as in the case of the preliminary study, the *trans*-18:3 isomers were clearly separated from cis-18:2 and *cis*-18:3 on the GC chromatogram, thereby showing the satisfactory applicability of the ready-

Table 5 Comparison between Novel and Ag-TLC methods.

Fatty acid	Fatty acid contents (g/100g)					
	Shortening (vegetable)		Shortening (vegetable and animal)		Beef Fat	
	Novel	Ag-TLC	Novel	Ag-TLC	Novel	Ag-TLC
16:0	22.4	26.6	21.1	22.3	22.2	20.8
16:1*trans*	−	−	1.3	1.3	−	−
16:1*cis*	−	−	1.3	0.9	0.2	0.2
18:0	5.0	5.4	6.1	6.1	14.1	12.0
18:1*trans*	8.1	8.6	7.3	6.8	0.3	0.3
18:1*cis*	32.6	36.7	22.1	22.8	3.4	3.8
20:1*trans*	−	−	2.8	1.4	−	−
22:1*trans*	−	−	0.8	0.6	−	−

− : not detected

Table 6 Recoveries of fatty acid methyl esters by Discovery Ag-ION SPE.

Fr. No.	Recovery (%)												
	18:0	18:1 *t*	18:1 *c*	18:2 *t,t*	18:2 *c,t*	18:2 *t,c*	18:2 *c,c*	18:3 *t,t,t*	18:3 *t,c,t t,t,c*	18:3 *c,t,t c,c,t*	18:3 *c,t,c*	18:3 *t,c,c*	18:3 *c,c,c*
1'	99	−	−	−	−	−	−	−	−	−	−	−	−
2'	−	99	5	−	−	−	−	−	−	−	−	−	−
3'	−	−	94	4	−	−	−	−	−	−	−	−	−
4'	−	−	−	98	102	100	1	85	−	−	−	−	−
5'	−	−	−	−	−	−	99	19	100	99	101	105	100

− : not detected

Fr. No.: Fraction number

Table 7 Recoveries of *trans* fatty acid methyl esters added to olive oil.

Fatty acid	Recovery (%)								RSD (%)
	1*	2*	3*	4*	5*	6*	7*	Mean	
18:1 *trans*	99	101	100	100	101	99	99	100	0.8
18:2 *trans*	97	98	97	96	96	98	100	97	0.8
18:3 *trans*	97	98	96	99	98	99	97	98	1.1

RSD: relative standard deviation

*: repetition number

to-use conditions for TFA analysis. Additionally, because the number of fractions was smaller than in the preliminary study, the GC operation time could be shortened by about two-thirds.

3.2.2 Recovery test for FAMEs added to oil

The recoveries and the relative standard deviation (RSD) of the *trans* FAMEs added to olive oil are indicated in Table 7. This test was repeated seven times. As in the case of the preliminary study, the average recoveries of the *trans* mono-ene, di-ene, and tri-ene were satisfactory, as exemplified by the values of 100%, 97%, and 98%, respectively. The repeatability for the three *trans* isomer classes was also satisfactory according to RSDs of 0.8%, 0.8%, and 1.1%, respectively.

Therefore, the practical procedure using the ready-to-use column was also found to be effective for the actual oil.

3.2.3 Peer laboratory verification

Peer laboratory verification was carried out between two laboratories using different production lots of Discovery Ag-ION SPE. As shown in Table 8, the robustness of the

Table 8 Recoveries of fatty acid methyl esters for peer laboratory verification.

Lab. No	Fr. No.	Recovery (%)												
		18:0	18:1 *t*	18:1 *c*	18:2 *t,t*	18:2 *c,t*	18:2 *t,c*	18:2 *c,c*	18:3 *t,t,t*	18:3 *t,c,t* *t,t,c*	18:3 *c,t,t* *c,c,t*	18:3 *c,t,c*	18:3 *t,c,c*	18:3 *c,c,c*
A	1'	101	−	−	−	−	−	−	−	−	−	−	−	−
	2'	−	100	4	−	−	−	−	−	−	−	−	−	−
	3'	−	1	95	6	−	−	−	−	−	−	−	−	−
	4'	−	−	−	94	100	100	−	100	−	−	−	−	−
	5'	−	−	−	−	−	−	100	−	100	100	100	100	100
B	1'	101	−	−	−	−	−	−	−	−	−	−	−	−
	2'	−	96	2	−	−	−	−	−	−	−	−	−	−
	3'	−	4	97	4	−	−	−	−	−	−	−	−	−
	4'	−	−	2	96	100	100	−	94	−	−	−	−	−
	5'	−	−	−	−	−	−	100	6	100	100	100	100	100

− : not detected
Fr. No.: Fraction number
Lab. A: Japan Food Research Laboratories
Lab. B: Supelco
Different production lots were used in the each laboratory.
Recoveries were shown as an average of four runs in Lab. A, and of three runs in Lab. B.

Table 9 *Trans* fatty acid contents in various foods.

Fatty acid	*Trans* fatty acid contents (g/100g)					
	Shortening	Butter	Margarine	Chocolate*	Pie	Cookie
18:1*trans*	11.4	3.40	9.22	8.60	3.23	0.81
18:2*trans*	0.45	−	0.17	0.19	−	−
18:3*trans*	0.29	−	−	0.50	−	−
Total	12.1	3.40	9.39	9.29	3.23	0.81

− : less than 0.05 g/100g
*: Chocolate did not contain a cacao-fat but substituted-fat

method was confirmed for the elution pattern and the recoveries of each fraction from two separate laboratories. The robustness of the method was also confirmed for 2 lots of Ag-Ion SPE. In addition to the robustness for the product, the reproducibility of the method was found to be satisfactory.

3.2.4 Analysis of various food samples

To evaluate method performance, several food samples were analyzed using the ready-to-use column. As shown in Table 9, TFAs were detected from all food and oil samples tested, and their types and amounts were varied.

Though the foods and oils showed wide variations in composition and amount of TFAs, good separation of *cis*/*trans* isomers was achieved with the Ag^+-column for not only pure oil but also for oil extracted from food. These results allowed us to ensure that the new method was applicable to various food samples.

In Fig. 2, a magnified chromatogram for 18:1 isomers with or without the column fractionation is shown. This demonstrated the removal effect of major cis isomers by the fractionation. In this case, 18:1 *t* FAME content gave a nearly 10% higher value with fractionation than without fractionation, probably due to the removal of the overlap of *cis* isomers. A similar phenomenon was observed even using a 100-m column for GC that was employed in both the AOAC and AOCS methods. Therefore, in some cases, the results from these official methods without Ag pre-fractionation could give lower *trans*-FAMEs content than those from the proposed method.

4 CONCLUSION

A novel pre-separation method of *cis*/*trans* isomers

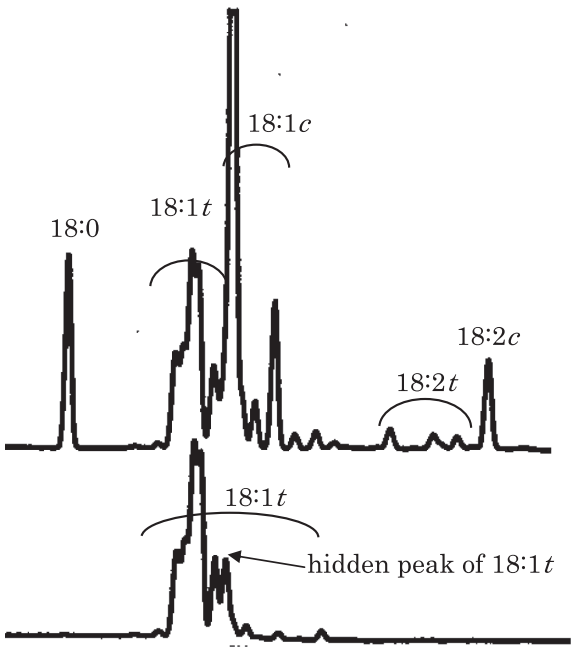

Fig. 2 Chromatogram of shortening with and without pre-fractionation. The chromatogram of the shortening is magnified. The upper chromatogram was obtained without pre-fractionation. The lower is the mono-ene *trans* fraction by pre-fractionation. On the upper chromatogram, peak identification of 18:1 *t* and 18:1 *c* was carried out according to the official method.

using a silver-cartridge column was developed. *Trans* isomers of mono-, di-, and tri-unsaturated fatty acid methyl esters were almost completely separated by the present fractionation method.

In Ag-ion SPE, silver ions are anchored onto an SCX SPE functional group as counter-ions. As the FAME sample passes through the cartridge, the SCX-silver counter-ion acts as an electron acceptor to form polar complexes with the double bonds of unsaturated FAMEs. The strength of these interactions increases with the number of double bonds, and saturated fatty acids are only weakly retained. *Cis* double bonds offer more steric accessibility than their *trans* counterparts and can therefore form stronger polar complexes with the stationary phase. As a result, TFAs are more weakly retained on Ag-Ion SPE than *cis* fatty acids. Differences in retention strength between classes of FAMEs and silver counter-ions can be exploited allowing for FAME fractionation prior to GC analysis.

The proposed method is considered to be a good alternative to conventional separation methods, especially when a large number of samples must be tested. We expect that it will not only be useful for routine analyses but also for detailed analyses of TFAs in a wide range of research fields.

References

1) Mensink, R. P.; Katan, M. B. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Engl. J. Med.* **323**(7), 439-445(1990).
2) World Health Organization Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation. *WHO Technical Report Series No.916*, Geneva(2008).
3) Astrup, A. The trans fatty acid story in Denmark. *Atheroscler* Suppl. **7**, 43-46(2006).
4) Moss, J. Labeling of trans fatty acid content in food, regulations and limits-the FDA view. *Atheroscler* Suppl. **7**, 57-59(2006).
5) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Cd* 14-95(1997).
6) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Cd 14d-99*(1999).
7) Official Methods of Analysis. *AOAC International, Method 965.34*(1997).
8) Official Methods of Analysis, *AOAC International, Method 2000.10*(2000).
9) Official Methods of Analysis. *AOAC International, Method 996.06*(2001).
10) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Ce 1f-96*(2002).
11) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Ce 1h-05*(2005).
12) Japan Oil Chemists' Society, Standard Methods for the Analysis of Fats, Oils and Related Materials(in Japanese). *Tentative method 17-2007*(2007).
13) Japan Oil Chemists' Society, Standard Methods for the Analysis of Fats, Oils and Related Materials(in Japanese). *Tentative method 8-2003*(2003).
14) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Ce 1g-96*(1997).
15) Christie, W. W. Solid-phase extraction columns in the analysis of lipids. *Advances in Lipid Methodology-One*. Oily Press. Ayr. pp. 1-17(1992).
16) Folch, J.; Lees, M.; Sloane Stanley, G. H. A. Simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509 (1957).
17) American Oil Chemists' Society, Official Methods and Recommended Practices. *AOCS, Official Method Ce 1b-89*(1997).