**NOTE**

**Modified Gas Chromatographic Method to Determine Monoacylglycerol and Diacylglycerol Contents in Edible Fats and Oils**

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**Abstract:** Monoacylglycerol (MAG) and diacylglycerol (DAG) are minor components of edible fats and oils, and they relate to the quality of these foods. The AOCS official method Cd 11b-91 has been used to determine MAG and DAG contents in fats and oils. There are, however, difficulties in the determination of MAG and DAG using this analytical procedure. Therefore, we improved this method by modifying the trimethylsilyl derivatization procedure and replacing the internal standard (IS) material. In our modified method, TMS-HT (mixture of hexamethyldisilazane and trimethylchlorosilane) was used for derivatization of MAG and DAG, which was followed by liquid-liquid extraction with water and n-hexane solution containing the IS, tricaprin. Using the modified method, we demonstrated superior repeatability in comparison with that of the AOCS method by reducing procedural difficulties. The relative standard deviation of distearin peak areas was 1.8% or 2.9% in the modified method, while it was 5.6% in the AOCS method. In addition, capillary columns, such as DB-1ht and DB-5ht could be used in this method.

**Key words:** monoacylglycerol, diacylglycerol, capillary gas chromatography, trimethylsilyl derivatization

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**1 INTRODUCTION**

Edible fats and oils mostly consist of triacylglycerol (TAG) and a small amount of diacylglycerol (DAG); however, some contain more than 5% DAG, lowering their value in terms of quality. Rice bran oil and palm oil contain relatively large amounts of DAG because of high lipase activity. Therefore, it is necessary to reduce DAG in edible fats and oils. However, using an oil refining process does not easily remove DAG from crude fats and oils. These products often contain small amounts of MAG, which can be added to processed foods for texture adjustment. Considerable amounts of MAG may promote smoking in edible fats and oils during deep- and pan-frying. MAG and DAG may be precursors of 3-monochloro-1,2-propanediol (3-MCPD) and its fatty acid esters, which are likely toxic and carcinogenic to humans. Thus, it is undesirable for cooking oils to contain DAG and MAG, and it is important to know their amounts in these oils.

The AOCS has recommended two official methods (Cd 11b-91 "Determination of Mono- and Diglycerides by Capillary Gas Chromatography (GC)") and Cd 11d-96 "Mono- and Diglycerides Determination by HPLC-ELSD") for the determination of MAG and DAG contents in edible fats and oils. In the AOCS official method Cd 11d-96, DAG and MAG
are separated from fats and oils using normal-phase high-performance liquid chromatography (HPLC) and then measured with an evaporative light scattering detector (ELSD) \(^{60}\). The analytical procedure is simple but precise calibration is required, and the response of the ELSD does not show linear correlation with the concentration. The detection sensitivity of ELSD depends on the chemical structure of the analyte. Additionally, it is likely that capillary GC is more popular than ELSD-HPLC, and Cd 11b-91 (hereafter referred to as the AOCS method) is likely used by many laboratories. In the AOCS method, \(N\),\(O\)-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in pyridine are used to convert DAG and MAG into more volatile trimethylsilyl ether derivatives, which are then measured by capillary GC. We analyzed palm oil twice according to the AOCS method, and found the estimated DAG content to be 9.4 or 12.6 \(\text{g/100 g}\) (Table 4). This poor repeatability was considered to be because of the difficulty in adding very small volumes of reagent, such as BSTFA, TMCS, and pyridine, even with the use of a micropipette. In particular, accurate addition of 0.1 mL of pyridine, containing an internal standard (IS) material, is difficult.

Therefore, we sought to improve the AOCS method by modifying the trimethylsilyl (TMS) derivatization procedure and replacing the IS material. We also attempted to use 100% dimethylpolysiloxine, as well as 5% phenyl- and 95% dimethylpolysiloxine columns.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

1-Stearoyl-rac-glycerol (monostearin) (purity \(\geq 99\%\)), glyceryl-1,3-distearate (distearin) (purity \(\geq 99\%\)), tetradecane (purity \(\geq 99\%\)), and tricaprin (purity \(\geq 99\%\)) were purchased from Sigma-Aldrich (St. Louis, USA). BSTFA (purity >95\%), TMCS (purity >98\%), and the mixture of hexamethyldisilazane and trimethylchlorosilane (TMS-HT) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Pyridine (JIS special grade) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Sample preparation for the repeatability test

Monostearin and distearin (10.0 \(\pm 0.5 \text{ mg}\)) were diluted to 20 mL using chloroform-methanol (1:1, v/v) in a volumetric flask. Two milliliters of the prepared solution was transferred to a glass test tube equipped with a screw-cap. The solvent was evaporated at 60°C under a nitrogen stream to obtain the dried residue. The experiment was repeated six times.

2.3 Sample preparation for the linearity test

Monostearin and distearin (12.5 \(\pm 0.5 \text{ mg}\)) were diluted to 25 mL using chloroform-methanol (1/1, v/v) in a volumetric flask. Two series of the monostearin and distearin solution (0.5, 1.0, 2.0, 4.0, and 6.0 mL) were transferred into separate glass test tubes, each equipped with a screw-cap. The solvent was evaporated at 60°C under a nitrogen stream to obtain the dried residue.

2.4 Sample preparation for the quantitative estimation of MAG and DAG in palm oil

Palm oil (30.0 \(\pm 1.0 \text{ mg}\)) was placed in a glass test tube equipped with a screw-cap.

2.5 TMS derivatization procedure

Approximately 0.3 mL of TMS-HT was added to these glass test tubes using a glass syringe. They were kept at room temperature for approximately 30 min for complete TMS derivatization.

To prepare the IS solution for the modified method, 10.0 \(\pm 0.5 \text{ mg}\) tricaprin was diluted to 20 mL using \(n\)-hexane in a volumetric flask. One milliliter of this IS solution was added to the glass test tubes using a volumetric pipette and mixed well. Water (2 mL) was then added and mixed with the solution, which was kept aside until phase separation. The upper layer from each glass test tube was transferred to a new GC vial.

2.6 Analytical conditions for GC

Each sample was analyzed by gas chromatograph (Agilent Technologies 6890, USA). The capillary columns were DB-1ht (15 m \(\times 0.32 \text{ mm} \times 0.10 \mu \text{m}\), Agilent Technologies) or DB-5ht (15 m \(\times 0.32 \text{ mm} \times 0.10 \mu \text{m}\), Agilent Technologies). Helium was used as a carrier gas at a fixed flow rate of 5 mL/min. The column oven temperature was programmed to be 80°C initially and raised at a rate of 10°C/min until it reached 360°C, the final temperature, which was maintained for 15 min. The temperatures of the injector and detector were 320°C and 350°C, respectively. One microliter was injected by split injection mode at a split ratio of 50:1.

2.7 Statistical analysis

The relative response factor \(R_F\) was calculated according to the following equation:

\[
R_F = \frac{A_i \times C_i}{A_d \times C_d},
\]

where \(A_i\) and \(A_d\) are the peak areas and \(C_i\) and \(C_d\) are the concentrations of the IS and an acylglycerol such as monostearin and distearin, respectively. The \(R_F\) was calculated by the repeatability of the peak area (Table 2).

The repeatability was verified by the relative standard deviation (RSD) of each peak area when repeating the measurement of monostearin and distearin six times. The linearity was verified by the correlation between each peak area and the concentration of monostearin and distearin.

Concentrations of monostearin and distearin were esti-
estimated according to the following equation:

\[
\text{Estimated concentration} = \frac{(A_t \times R_F \times C_l)}{A_i}
\]

The relative error of monostearin and distearin was calculated according to the following equation:

\[
\text{Relative error} = \left( \frac{\text{Estimated concentration} - \text{Known concentration}}{\text{Known concentration}} \right) \times 100
\]

3 RESULTS and DISCUSSION

Gas chromatograms of monostearin, distearin, and tricaprin (IS) on DB-1ht and DB-5ht columns are shown in Fig. 1. Monostearin, tricaprin, and distearin were eluted from the DB-1ht column at 14.1, 18.1, and 22.1 min, respectively. They were also eluted from the DB-5ht column at 14.6, 18.8, and 22.7 min, respectively. Tetradecane, an IS used in the AOCS method, was eluted at 3 min on the gas chromatogram, while monostearin and distearin were eluted at 14.6 and 22.7 min, respectively (data not shown). Among other acylglycerols, monopalmitin, monoolein, dipalmitin, and diolein were eluted from the DB-1ht column at 12.8, 13.9, 21.0, and 21.8 min, respectively (data not shown). They were also eluted from the DB-5ht column at 13.2, 14.3, 21.7, and 22.5 min, respectively (data not shown). The separation of individual acylglycerols was sufficient on both columns, indicating that they were both effective for the determination of MAG and DAG.

Results of the linearity analyses for monostearin, distearin, and tricaprin (IS) by the modified method using DB-1ht and DB-5ht columns are shown in Fig. 2. A good correlation coefficient \(R^2 > 0.997\) was obtained between the peak area and the concentration of monostearin, distearin, and the IS in the range of 0.25 to 3.00 mg/mL. Similar results were obtained for other MAG such as monopalmitin and monoolein, and DAG such as dipalmitin and diolein (data not shown). Therefore, this GC analytical condition was expected to be effective for the determination of MAG and DAG contents in edible fats and oils. Meanwhile, a greater slope was obtained for monostearin compared with distearin and the IS; indicating that monostearin could be detected at a higher sensitivity than distearin and the IS.

Table 1 depicts the \(R_F\) values of monostearin and distearin against each IS. The \(R_F\) of monostearin against tricaprin was 0.77 (DB1-ht) and 0.71 (DB-5ht) in the modified method, while it was 0.66 against tetradecane using the AOCS method. The \(R_F\) of distearin against tricaprin was 0.89 (DB1-ht) and 0.92 (DB-5ht) in the modified method, while it was 0.79 against tetradecane using the AOCS method. Thus, the \(R_F\) values obtained using the modified method were closer to 1.00 than those obtained using the AOCS method. These results may be explained by a difference in molecular weight between tetradecane and tricaprin. The relative ratio of molecular weight of monostearin to tricaprin used in the modified method is 0.64, while that of monostearin to tetradecane used in the AOCS method is 1.81. Moreover, the molecular weight of distearin is three times \(3.37\) that of tetradecane. For the same reason, the retention time of tricaprin was closer to those of acylglycerols than that of tetradecane.
tetradecane. Therefore, proper $R_F$ for each acylglycerol should be used in this GC analysis.

Table 2 demonstrates the repeatability of the analysis for monostearin and distearin using the modified method of DB-1ht and DB-5ht columns, and the AOCS method of DB-5ht column. The experiment was repeated six times. The RSD was greater than 5.6% for the peak area of distearin using the AOCS method. The poor repeatability of the AOCS method could be because of the inability to accurately transfer small amounts of reagents such as 0.1 mL of TMCS containing the IS and 0.2 mL of BSTFA using micropipette. On the other hand, the repeatability was improved using the modified method, because sufficient amounts of the reagents could be added, such as 0.3 mL of TMS-HT using a glass syringe and 1.0 mL of n-hexane containing the IS using a volumetric pipette. As a result, the RSD of distearin peak areas were 1.8% (DB-1ht) and 2.9% (DB-5ht) using the modified method, while it was 5.6% using the AOCS method. Additionally, the RSD of the IS peak areas were 1.4% (DB-1ht) and 2.9% (DB-5ht) using the modified method, while it was 14.0% using the AOCS method. The largest RSD obtained using the AOCS method could be the result of difficulty in the accurate addition of 0.1 mL of the pyridine reagent containing the IS. Therefore, the modified method improved the repeatability of the AOCS method. Moreover, the 100% dimethylpolysiloxane capillary column (DB-1ht) was found to be available in addition to the 5% phenyl- and 95% dimethylpolysiloxane capillary column (DB-5ht) using the modified method.

As shown in Table 2, using the modified method, the peak areas of distearin and monostearin were smaller than those obtained using the AOCS method when certain concentrations of monostearin and distearin were analyzed. This might be caused by the liquid-liquid extraction with water and n-hexane after TMS derivatization in the modified method. Some TMS groups in monostearin and distearin might be dislocated during the liquid-liquid extraction.

### Table 1

| Materials | Relative response factor ($R_F$) | Modified method | AOCS method | Molecular weight |
|-----------|---------------------------------|----------------|-------------|-----------------|
|           |                                 | DB-1ht         | DB-5ht      |                 |
|           | Mean ± SD<sup>a</sup> | $RSD$ (%)<sup>b</sup> | Mean ± SD  | $RSD$ (%) |
| Monostearin | 0.77 ± 0.03  | 2.3            | 0.71 ± 0.05 | 3.6    | 0.66 ± 0.05  | 3.6        | 358.56    |
| Distearin  | 0.89 ± 0.02  | 1.8            | 0.92 ± 0.03 | 2.9    | 0.79 ± 0.07  | 5.6        | 669.09    |
| Tricaprin<sup>c</sup> | 1.00    | 1.00           | 1.00        |         |             |            | 554.85    |
| Tetradecane<sup>d</sup> | 1.00    |               |             |         |             |            | 198.39    |

<sup>a</sup> SD: standard deviation  
<sup>b</sup> RSD: relative standard deviation  
<sup>c</sup> Tricaprin: IS of the modified method  
<sup>d</sup> Tetradecane : IS of the AOCS method

### Table 2

| Materials | Modified method | AOCS method |             |
|-----------|----------------|-------------|-------------|
|           | DB-1ht         | DB-5ht      | DB-5ht      |
|           | Mean ± SD<sup>a</sup> | $RSD$ (%)<sup>b</sup> | Mean ± SD  | $RSD$ (%) |
| Monostearin | 263 ± 6  | 2.3          | 258 ± 9    | 3.6     | 1024 ± 37 | 3.6       |
| Distearin  | 228 ± 4  | 1.8          | 215 ± 6    | 2.9     | 854 ± 48  | 5.6       |
| Tricaprin<sup>c</sup> | 203 ± 3  | 1.4          | 182 ± 5    | 2.9     |           |           |
| Tetradecane<sup>d</sup> |       |              |             |         | 676 ± 94  | 14.0      |

<sup>a</sup> SD: standard deviation  
<sup>b</sup> RSD: relative standard deviation  
<sup>c</sup> Tricaprin : IS of the modified method  
<sup>d</sup> Tetradecane : IS of the AOCS method

Materials concentration are 1.0 mg/mL.
Determination of Monoacylglycerol and Diacylglycerol

J. Oleo Sci. 66, (6) 601-606 (2017)

Table 3  Known and estimated concentrations and the calculated relative errors of monostearin and distearin.

| Acylglycerol | Modified method |          |          |          | AOCSS method |          |          |
|--------------|-----------------|----------|----------|----------|---------------|----------|----------|
|              | DB-1ht          | DB-5ht   | DB-5ht   |          |               | DB-5ht   |          |
|              | Known concentration (mg/mL) | Estimated concentration (mg/mL) | Relative error (%) | Known concentration (mg/mL) | Estimated concentration (mg/mL) | Relative error (%) | Known concentration (mg/mL) | Estimated concentration (mg/mL) | Relative error (%) |
| Monostearin  | 0.262           | 0.282    | 7.6      | 0.246    | 0.252         | 2.4      | 0.280    | 0.267         | 4.7      |
|              | 0.524           | 0.565    | 7.9      | 0.492    | 0.492         | 0.1      | 0.560    | 0.528         | 5.7      |
|              | 1.048           | 1.101    | 5.0      | 0.984    | 0.972         | 1.3      | 1.120    | 1.029         | 8.1      |
|              | 2.096           | 2.177    | 3.9      | 1.968    | 1.948         | 1.0      | 2.240    | 2.072         | 7.5      |
|              | 3.144           | 3.298    | 4.9      | 2.952    | 2.933         | 0.7      | 3.360    | 3.078         | 8.4      |
| Distearin    | 0.250           | 0.266    | 6.5      | 0.244    | 0.244         | 0.0      | 0.250    | 0.238         | 4.7      |
|              | 0.500           | 0.528    | 5.5      | 0.488    | 0.490         | 0.4      | 0.500    | 0.480         | 3.9      |
|              | 1.000           | 1.053    | 5.3      | 0.976    | 0.972         | 0.4      | 1.000    | 0.961         | 3.9      |
|              | 2.000           | 2.083    | 4.1      | 1.952    | 1.960         | 0.4      | 2.000    | 1.960         | 2.0      |
|              | 3.000           | 3.060    | 2.0      | 2.928    | 2.972         | 1.5      | 3.000    | 2.871         | 4.3      |

and then underivatized monostearin and distearin could be transferred to the water phase. As a result, the concentration of TMS-derivatized monostearin and distearin were reduced to a certain extent, although their composition was invariable. Therefore, the liquid-liquid extraction did not influence the linearity and repeatability of the modified method.

Table 3 depicts the known and estimated concentrations and the calculations for the relative error of monostearin and distearin. Using the modified method of DB-1ht and monostearin concentrations between 0.262 and 3.144 mg/mL, the relative error ranged from 3.9 to 7.9. Using the modified method of DB-5ht and the concentrations between 0.246 and 2.952 mg/mL, the relative error ranged from −1.3 to 2.4. On the other hand, using the AOCS method and the concentrations between 0.280 and 3.360 mg/mL, the relative error ranged from −8.4 to −4.7. Similarly, using the modified method of DB-1ht and distearin concentrations between 0.250 and 3.000 mg/mL, the relative error ranged from 2.0 to 6.5. Using the modified method of DB-5ht and the concentrations between 0.244 and 2.928 mg/mL, the relative error ranged from −0.4 to 1.5. Using the AOCS method and the concentrations between 0.250 and 3.000 mg/mL, the relative error ranged from −4.7 to −2.0. These results suggested that the modified method could be useful for quantitative determination of monostearin and distearin.

Table 4 demonstrates the quantitatively estimated MAG and DAG contents in palm oil obtained using the modified and AOCS methods. In this experiment, MAG and DAG were confined to acylglycerols containing palmitic, stearic, oleic, linoleic, and linolenic acids (data not shown). DAG was detected in palm oil using these methods, while MAG was not. The DAG content, as estimated by the modified method, was approximately 6.3 g/100 g (DB-1ht) and 7.5 g/100 g (DB5-ht), whereas the content was 9.4 or 12.6 g/100 g using the AOCS method. The modified method had a smaller margin of error compared with the AOCS method. Although the GC columns had some influence on the quantitative determination of DAG content, the modified method had superior repeatability.

4 CONCLUSION

In this study, we improved the AOCS method by modifying the TMS derivatization procedure and replacing the internal standard material. Moreover, the 100% dimethylpolysiloxane capillary column was found to be available in addition to the 5% phenyl- and 95% dimethylpolysiloxane capillary column. As a result, the accuracy of repeatability in determining the monostearin, distearin, and DAG contents in palm oil was improved using the modified method, which could be effective for the determination of MAG and DAG contents in edible fats and oils.
ACKNOWLEDGEMENT

The authors thank Dr. Yasushi Endo, Dr. Masakazu Yamaoka, and Mr. Yoshiaki Hirata for their helpful suggestions.

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