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Development of Gas Chromatography/ Mass Spectrometry for the Determination of Essential Fatty Acids in Food Supplemental Oil Products

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Abstract: A gas chromatography/ mass spectrometric (GC/MS) method was developed as a candidate reference method for the accurate determination of essential fatty acids (linoleic acid, α- and γ-linolenic acids) in food supplemental oil products. Samples were spiked with three internal standards (stearic acid-d_{35}, $^{13}C_{18}$-linoleic acid, and $^{13}C_{18}$-α-linolenic acid). Samples were then subject to saponification, derivatization for methylation, and extraction by organic solvent. For GC/MS measurement, an Agilent HP-88 column, designed for the separation of fatty acid methyl esters, was selected after comparing with other columns as it provided better separation for target analytes. Target analytes and internal standards were detected by selected ion monitoring of molecular ions of their methyl ester forms. The GC/MS method was applied for the measurement of three botanical oils in NIST SRM 3274 (borage oil, evening primrose oil, and flax oil), and measurement results agreed with the certified values. Measurement results for target analytes which have corresponding isotope-labeled analogues as internal standard were calculated based on isotope dilution mass spectrometry (IDMS) approach, and compared with results calculated by using the other two internal standards. Results from the IDMS approach and the typical internal standard approach were in good agreement within their measurement uncertainties. It proves that the developed GC/MS method can provide similar metrological quality with IDMS methods for the measurement of fatty acids in natural oil samples if a proper fatty acid is used as an internal standard.

Key word: Fatty acid, GC/MS, IDMS method, Internal standard method, Botanical oils

Introduction

Interest on polyunsaturated fatty acids has increased during the last decade as they are important essential nutrients in human and widely used for nutritional and medical purpose.[1] Therefore, many food supplemental products based on botanical oils and fish oils are commercially available. Recently, food supplemental products based on borage oil, evening primrose oil, and flax oil became increasingly popular as they are known to be rich with essential fatty acids, such as linoleic acid, α- and γ-linolenic acids.[2] However, contents of essential fatty acids in those commercial products were disputable in many cases. For this reason, reliable analytical methods are required for the purpose of quality control and product-labeling regulations.

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Gas chromatography/mass spectrometry (GC/MS) has been widely used for the analysis of fatty acids in natural oils and food-matrices.[3-5] It requires saponification of sample to release fatty acid from lipid forms and derivatization to fatty acid methyl ester (FAME) forms. High performance liquid chromatography (HPLC) system has been also used for the fatty acid analysis, but it also requires phenacyl derivatization to improve chromatographic retention and separation.[5,6] Later, LC/MS was also applied for the fatty acid analysis.[7,8] However, GC/MS is still preffered for fatty acid analysis in terms of ease of use and high chromatographic separation.

In this study, we established a GC/MS method for the accurate determination of essential fatty acids belonging to ω-3 and ω-6 groups (linoleic acid, α- and γ-linolenic acids) in botanic oils. Figure 1 shows chemical structures of those target analytes. This laboratory, the national metrology institute of Korea, usually chooses isotope dilution mass spectrometry (IDMS) as a prior reference method for quantitative analysis of organic compounds in complex matrices, as the method is capable of overcoming difficulties encountered in correction of recovery in sample preparation processes and demonstrates high accuracy and repeatability.[9,10] However, only a few isotope-labeled essential fatty acids are commercially available so that all essential fatty acids cannot be analyzed by the IDMS approach. In this study, we utilized available isotope-labeled essential fatty acids (stearic acid-d_{35}, $^{13}C_{18}$-linoleic acid, and

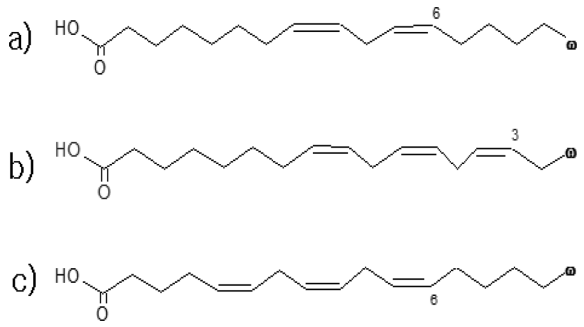

Figure 1. Chemical structures of a) linoleic acid b) α-linolenic acid and c) γ-linolenic acid

$^{13}C_{18}$- α-linolenic acid) as internal standards. We compared measurement results with using the three individual internal standards to figure out if a non-IDMS approach with using isotope-labeled analogues of other fatty acids can provide similar metrological quality with the IDMS approach.

Experimental

Materials

Pure substances for linoleic acid, α- and γ-linolenic acids were purchased from Sigma-Aldrich (≥99%, St. Luis, MO, USA). Stearic acid-d_{35}, $^{13}C_{18}$-linoleic acid, and $^{13}C_{18}$-α-linolenic acid were purchased from Sigma-Aldrich, Medical Isotopes Inc. (Pelham, NH, USA), and TRC (North York, ON, Canada) respectively. Those isotope-labeled fatty acids were used as internal standards. All organic solvents used in this study were from Burdick & Jackson (Muskegon, MI, USA). 14% Boron trifluoride in methanol and sodium hydroxide were from Sigma-Aldrich. Pure water was prepared by using a membrane-filtering system and further purified by passing through a Millipore Corp Milli-Q RG purification system. All the solvents and buffer solutions used hereafter in this study were degassed by purging helium before usage. NIST SRM 3274 (borage oil, evening primrose oil, and flax oil) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Calibration Standard Solutions

A standard solution containing the three target analytes (1000 mg/kg level in *n*-hexane) was gravimetrically prepared following the procedures developed and maintained in this laboratory.[11,12] The standard solution was stored at −20°C freezer and used within a week. In a similar way, an internal standard solution containing the three isotope-labeled compounds (1000 mg/kg level in *n*-hexane) was prepared and stored before use. A calibration solution mixture (1:1 ratio of target analytes and their internal standards) was prepared by gravimetrically mixing similar amounts of the standard solution and the internal standard solution, which was used as a calibrant for GC/MS measurement of oil samples.

Sample Preparation

0.1 g of oil sample was taken into a bottle and gravimetrically diluted by adding appropriate amount of *n*-hexane (0.1% butylated hydroxyl toluene as an antioxidant) to make target analytes in 1000 mg/kg level in the diluent. 0.3 mL of the diluent was taken into a reaction vial and spiked with similar amount of the internal standard solution to make 1:1 isotope ratio for each target analytes if a corresponding isotope-labeled analogue is available or 1:1 ratio of a target analyte and its internal standard. 5 mL of 0.5 mol/L of sodium hydroxide in methanol was added into the vial. The vial was purged with argon gas and heated to 100 °C for 10 minutes for saponification. Then, 5 mL of 14% boron trifluoride in methanol was added into the vial and heated to 100 °C for 5 minutes for methylation of free fatty acids. 10 mL of saturated sodium chloride solution was added into the vial and FAMEs was extracted by 5 mL of *n*-heptane and petroleum ether. After removing residual water with sodium sulfate, the extraction solution was concentrated to 0.6 mL under nitrogen gas and subject to GC/MS measurement. The same preparation processes were applied to the calibration solution mixture for methyl esterification of target analytes and their internal standards.

GC/MS Analysis

The GC/MS system used in this study was an Agilent GC/MSD (model No. 5973, Palo Alto, CA, USA). In the initial stage of this study, a non-polar DB-5MS colum (J & W Scientific, length 60 m, i.d. 0.25 mm, thickness 0.25 μm), a polar DB-1701 column (J & W Scientific, length 60 m, i.d. 0.25 mm, thickness 0.5 μm), and a HP-88 FAMEs column (Agilent, length 60 m, i.d. 0.25 mm, thickness 0.2 μm) were tested for their chromatographic performance for FAMEs analysis. The HP-88 column was chosen and used throughout this study [see results and discussion section]. Helium was used as carrier gas at a flow rate of 1.0 mL/min. Its injection port was kept at 250 °C in splitless mode. The temperature of the GC oven started at 180 °C and maintained for 25 minutes and was ramped to 230 °C at the rate of 20 °C/min and held for 10 minutes. The interface to the mass spectrometer was maintained at 250 °C .

The mass spectrometer was operated under electron impact ionization condition with a source temperature of 250°C. For roughly screening samples, scan mode was used. For quantitative analysis, the MS was operated in selected ion monitoring (SIM) mode with detecting molecular ions of methylated ester forms of linoleic acid at *m*/*z* 294, α- and γ-linolenic acids at *m*/*z* 292, stearic acid-d_{35} at *m*/*z* 333, $^{13}C_{18}$-linoleic acid at *m*/*z* 312, and $^{13}C_{18}$-α-linolenic acid at *m*/*z* 310.

For the calculation of GC/MS measurement results, the equation used for IDMS methods can be found in our previous articles.[12,13]

Results and Discussion

Comparison of GC columns

Figure 2 shows chromatograms of a standard solution containing the three target analytes from using three different GC columns. In cases of the non-polar DB-5MS column and the

polar DB-1701 column, the three analytes are congested in a narrow time zone and at least two of three analytes are slightly overlapped. In the case of the HP-88 FAMEs column which was specially designed for FAMEs analysis, the three analytes are well separated. Therefore, we chose the HP-88 FAMEs column to be used for the analysis of essential unsaturated fatty acids.

Screening of Three Botanical Oil Samples

Three botanic oil products included in NIST SRM 3274 (borage oil, evening primrose oil, and flax oil) were tested by the GC/MS method (in scan mode) to survey the overall contents of fatty acids in those products. Figure 3 shows total ion chromatograms of the three oil samples. Five fatty acids were dominant in those oil products. All three botanic oils contained linoleic acid as a dominant component. γ-Linolenic acid was detected as a dominant component in borage oil and evening primrose oil, but it was a minor component in flax oil. α-Linolenic acid was dominant in flax oil while it was a minor component in borage oil and evening primrose oil.

Quantitative Analysis

Among the three target analytes, we decided to determine only the dominant components in each oil samples. Table 1 summarizes the measurement results of the three target analytes in those botanical oils. The certified values listed in the table were obtained by NIST from their GC/MS method with using stearic acid-d_{35} as an internal standard.[2] In this study, GC/MS measurement results were separately calculated based on each of three individual internal standards. As shown in Table 1, our GC/MS measurement results for the three target analytes in the oil samples, calculated based on stearic acid-d_{35} as an internal standard, were in good agreement with the values certified by NIST within their uncertainties. It indicates that overall measurement procedure adopted in this study is at least equivalent to the NIST method. As $^{13}C_{18}$-linoleic acid is included as an internal standard, linoleic acid contents in borage oil and evening primrose oil sample in SRM 3274 were calculated with the IDMS approach. The IDMS results are in good agreement with the values certified by NIST within their uncertainties. Also, the IDMS results are in good agreement with the results calculated based on either $^{13}C_{18}$-α-linolenic acid or stearic acid-d_{35} as an internal standard except the case of borage oil with using stearic acid-d_{35} as an internal standard. Even in this case, the value from using stearic acid-d_{35} is only 2% higher than value from the IDMS approach, and its uncertainty range is slightly off from that of the IDMS results. We note that the difference may be due to inhomogeneity of the sample rather than due to difference in measurement. In the case of α-linolenic acid in flax oil, the IDMS results and the results based on the other two individual internal standards agree with each other and with the certified value from NIST. The non-IDMS results were more like typical internal standard methods, and isotopic analogues of other fatty acid (not the target analytes) were used as they are not present in natural oil

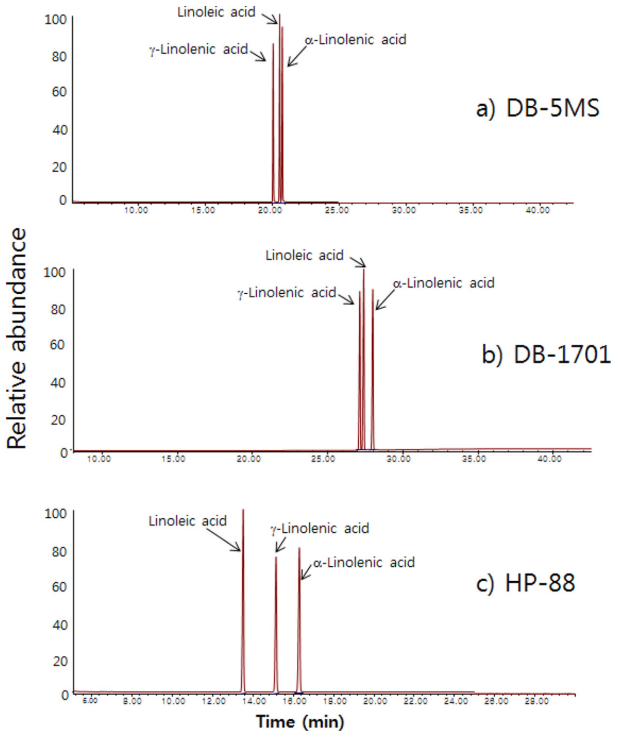

Figure 2. Total ion chromatograms of a standard solution containing the three target analytes with three different GC columns. (a) and (b) were obtained with GC conditions optimized to obtain best separation among the three analytes. Optimized GC conditions for (c) is described in experimental section.

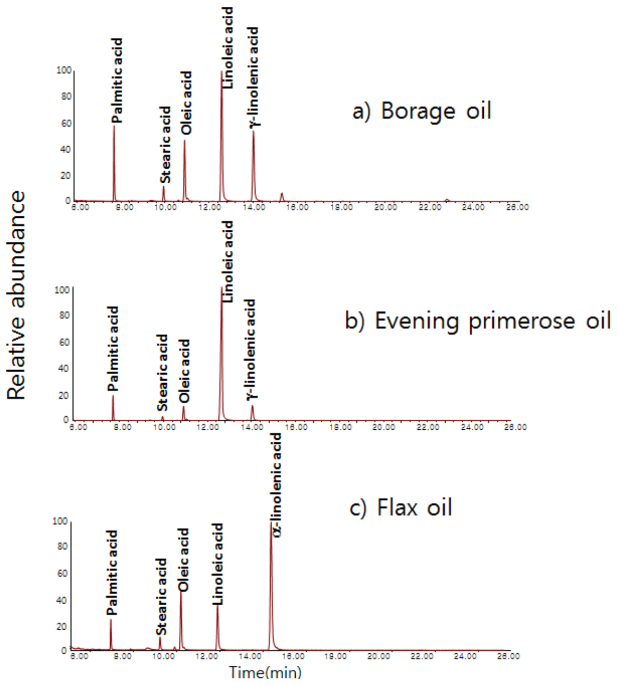

Figure 3. Total ion chromatograms (scan mode) of (a) borage oil, (b) evening primrose oil, and (c) flax oil.

Table 1. Measurement results of linoleic acid, α-linolenic and γ-linolenic acid in NIST SRM 3274

Sample	Analyte	Certified value by NIST (% in kg/kg)[a]	Measured in this study	
			Internal standard used	Measurement value[a] (% in kg/kg)
Borage oil	Linoleic acid	37.40 ± 3.5	Stearic acid-d_{35}	39.85 ± 0.78
			$^{13}C_{18}$-linoleic acid	38.27 ± 0.36
			$^{13}C_{18}$-α-linolenic acid	38.85 ± 0.38
	γ-Linolenic acid	25.1 ± 2.4	Stearic acid-d_{35}	26.47 ± 0.87
			$^{13}C_{18}$-linoleic acid	25.43 ± 0.78
			$^{13}C_{18}$-α-linolenic acid	25.81 ± 0.62
Evening primrose oil	Linoleic acid	74.20 ± 2.4	Stearic acid-d_{35}	75.75 ± 0.53
			$^{13}C_{18}$-linoleic acid	74.00 ± 1.04
			$^{13}C_{18}$-α-linolenic acid	73.83 ± 1.58
	γ-Linolenic acid	9.99 ± 0.41	Stearic acid-d_{35}	10.51 ± 0.12
			$^{13}C_{18}$-linoleic acid	10.65 ± 0.12
			$^{13}C_{18}$-α-linolenic acid	9.95 ± 0.20
Flax oil	Linoleic acid	17.10 ± 1.10	Stearic acid-d_{35}	16.39 ± 0.34
			$^{13}C_{18}$-linoleic acid	16.41 ± 0.51
			$^{13}C_{18}$-α-linolenic acid	15.97 ± 0.34
	α-linolenic acid	57.90 ± 3.00	Stearic acid-d_{35}	60.15 ± 1.43
			$^{13}C_{18}$-linoleic acid	60.33 ± 2.21
			$^{13}C_{18}$-α-linolenic acid	57.64 ± 1.75

[a] The number after "±" is the expanded uncertainty of the value in 95% level of confidence.

samples. It is noticeable that results from IDMS method and those from internal standard method are in good agreement and provides similar degree of measurement uncertainties. As γ-linolenic acid does not have isotope-labeled analogues as internal standard in this study, measurement results were calculated using the three individual internal standards. Results of γ-linolenic acid from using all three internal standards agreed to each other within their uncertainties.

Conclusions

We developed a GC/MS method for the accurate determination of fatty acids in oil samples. Validity of the method was evaluated by comparing measurement results of NIST SRM with the certified values. Also the method was further evaluated by comparing results calculated by the IDMS approach and those from the typical (non-IDMS) internal standard approach. Results from the two approaches with the GC/MS method were in good agreement within their uncertainties. It proves that the developed GC/MS method can provide similar metrological quality with IDMS methods for the measurement of fatty acids in natural oil samples if a proper fatty acid with similar chemical and physical properties with target analytes is used as an internal standard. We note that this conclusion may be limited to the application of the method to refined natural oils which have relatively simple matrices. Further test may be required to apply the non-IDMS method to samples with more complex matrices.

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References

1. Stránský, K. S.; Zarevúcka, M.; Wimmer, Z. *Food Chem.* **2005**, 92, 569.
2. Sanders, L.; Schantz, M.; Sharpless, K.; Wise, S. *Lipid Technol*. **2009**, 21, 7.
3. Christie, W. *Industrial Crops Products* **1999**, 10, 73.
4. Traitler, H.; Winter, H.; Richli, U.; Ingenbleek, Y. *Lipids* **1984**, 19, 923.
5. Brondz, I. *Anal. Chim. Acta* **2002**, 465, 1.
6. Chen, S.-H.; Chuang, Y.-J. *Anal. Chim. Acta* **2002**, 465, 145.
7. Laakso, P. *J. AOCS*, **1997**, 74, 1291.
8. Lacaze, J.-P. C. L.; Stobo, L. A.; Turrell, E. A.; Quilliam, M. A. *J. Chromatography A* **2007**, 51, 1145.
9. De Leenheer, A. P.; Thienpont, L. M. *Mass Spectrom. Rev.* **1992**, 11, 249.
10. De Bièvre, P. *Anal. Proc*. **1993**, 30, 328.
11. Shin, H.; Kim, B.; Lee, J. *Food Chem.* **2013**, 138, 1109.
12. Lee, J.; Jang, E.-S.; Kim, B. *Anal. Chim. Acta* **2013**, 787, 132.
13. Lim, Y. O.; Kim, B. J.; Ahn, S.; Kim, J. *Food Chem*. **2011**, 126, 1393.