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Generalized Method to Quantify Glycidol Fatty Acid Esters in Edible Oils

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Abstract We previously reported a method to quantify five species of glycidol fatty acid esters (GEs) in edible oils which used a combination of a double solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS) using fast HPLC. To expand its application, we established a new method using conventional HPLC, which is applicable not only to liquid oils but also to solid ones. The optimized LC-MS conditions using conventional HPLC were useful for standard GEs but not for oil samples because of the insufficient accuracy during sequential runs. Thin-layer chromatographic studies revealed that co-existing diacylglycerols were not sufficiently removed by the original SPE procedure due to excessive amounts of oil applied to the normal-phase SPE, which disturbed the quantitative and stable detection only when conventional HPLC was employed. The amount of oil applied was decreased tenfold (100 mg → 10 mg), which resulted in stable LC-MS measurements. Furthermore, the use of chloroform/acetone, instead of acetonitrile, prior to the SPE treatment expanded its applicability to solid oils, with recovery values ranging from 102.7 to 109.5% for three oil samples (two liquid and one solid oils). This method can form the basis of a standardized method for the quality control of GEs in edible oils.

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Introduction

Since the first report on the occurrence of 3-monochloro-1,2-propanediol (3-MCPD) esters in edible oils [1] which used a gas chromatography-mass spectrometry (GC-MS) method proposed by Divinova et al. [2], Weisshaar has actively studied possible compounds forming 3-MCPD during the GC-MS analysis [3, 4]. Recently, Weisshaar and Perz suggested the existence of relatively high levels of glycidol fatty acid esters (GEs) in the oils [5]. The Federal Institute for Risk Assessment (BfR), a scientific agency of the Federal Republic of Germany, stated its opinion regarding the necessity for a method to quantify GEs in edible oils to provide reliable risk assessment [6]. Thus, rapid development and validation of quantitative methods for GEs have become an important issue for manufacturers and retailers working in the edible oil business.

In the recent report by Weisshaar and Perz [5], two GC-MS procedures were used to estimate levels of GEs in oil. The first procedure determines the level of total 3-MCPD, including 3-MCPD esters and GEs (level A, 3-MCPD in mg/kg oil), whereas the second procedure measures the level of true 3-MCPD, corresponding only to 3-MCPD esters (level B, 3-MCPD in mg/kg in oil). The level of GEs (glycidol in mg/kg oil) can then be estimated as the difference between levels A and B:C = 0.67 × (A − B). This method has been recently adopted as one of the German Society of Fat Science (DGF) standard methods [7]. However, this indirect difference method determines the level as glycidol based on the assumption that 3-MCPD-forming substances (except for 3-MCPD

esters) might originate only from GEs. That method does not directly determine individual GEs possessing various alkyl chains. Therefore, we developed a method to directly quantify five species of GEs (palmitic, C16:0-GE; stearic, C18:0-GE; oleic, C18:1-GE; linoleic, C18:2-GE; linolenic, C18:3-GE) in oils in combination with double solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS) [8]. According to that method, GEs can be detected in the μg range per gram of oil, and can be quantified with acceptable accuracy and without interference of co-existing large amounts of acylglycerols. On the other hand, in that method, the LC-MS requires fast HPLC using a specific column for ultra performance liquid chromatography (UPLC) and a specific LC instrument that can tolerate extremely high pressure [8]. Conventional HPLC would be indispensable for its global use as a standardized method. However, when we tried to use conventional HPLC, the recovery values of standard GEs spiked in the diacylglycerol (DAG)-rich edible oil were much lower than those obtained by the original method [8], and the LC-MS measurements were not stable during sequential runs. Further, a globally generalized method should have applicability not only to liquid oils but also to solid ones.

The aim of this study was to reexamine the original SPE procedure to obtain GE-rich extracts from oils, based on our hypothesis that the low recovery values might be caused by insufficient removal of co-existing acylglycerols (probably DAG) and that those acylglycerols might disturb the quantitative and stable detection by conventional HPLC. In addition, we tried to expand the method so that solid oils can be targeted, by modifying the pretreatment procedure. Herein, we report a more generalized method for the quantification of GEs in oils.

Experimental Procedure

Reagents

Methanol, acetonitrile, 2-propanol, chloroform, acetone and *n*-hexane, of HPLC grade from Kanto Chemical (Tokyo, Japan), were used to prepare GE-rich extracts from the oil and for the mobile phases in the LC-MS and thin-layer chromatography (TLC). Ultra-pure water prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA) was used in all procedures. All other chemicals used were reagent grade.

Standard Materials

C18:0-GE (purity 96.2%, confirmed using ^{1}H-NMR and GC-FID [8]) was purchased from Tokyo Chemical Industry and was used as a standard material in LC-MS analysis. Other GEs, C16:0-GE (purity 75.6%), C18:1-GE (94.4%), C18:2-GE (89.8%) and C18:3-GE (55.1%), were synthesized in our laboratory [8]. Individual GE solutions at 1,000 μg/mL were prepared using methanol/2-propanol (1:1 vol/vol). Working solutions of standard GEs were then obtained by further dilution of the 1,000 μg/mL stock solutions with methanol/2-propanol (1:1 vol/vol) prior to use.

C18:0-GE was also used as a reference for TLC analysis. Tripalmitin (Sigma-Aldrich, St. Louis, MO, USA), 1,3-dipalmitin, 1,2-dipalmitin and 1-monolinolein (Tokyo Chemical Industry) were used as references for TAG, 1,3-DAG, 1,2-DAG and monoacylglycerols (MAG), respectively. A mixed solution of 0.1% in chloroform (w/v) each was prepared.

Edible Oil Samples

Two commercial edible oil samples were purchased from Japanese markets, and were used for the quantification of GEs. Sample-A is made up chiefly of TAG and consists of 96.1% TAG, 3.9% DAG and 0.0% MAG (palmitic acid 16.4%, stearic acid 2.6%, oleic acid 47.6%, linoleic acid 28.4%, linolenic acid 3.5% others 1.5%). On the other hand, Sample-B contains DAG as the major component (12.5% TAG, 87.0% DAG and 0.5% MAG, and palmitic acid 2.7%, stearic acid 1.1%, oleic acid 37.2%, linoleic acid 49.4%, linolenic acid 8.8% others 0.8%). A refined edible oil sample for the food industry was also used and is designated as Sample-C in this study. Sample-C is solid at room temperature and is TAG-rich (95.2% TAG, 4.8% DAG and 0.0% MAG, and palmitic acid 43.7%, stearic acid 4.4%, oleic acid 39.7%, linoleic acid 10.3%, linolenic acid 0.4% others 1.5%).

TLC Conditions

High performance TLC-plates (silica gel 60, 20 cm × 10 cm, Merck, Darmstadt, Germany) were used for the class separation. After aliquots of the standard solution and concentrated SPE extracts obtained from Sample-A and Sample-B were applied to a plate, it was developed with *n*-hexane/ethyl acetate (70:30 vol/vol). After drying, the plates were sprayed with a 10% copper sulfate and 8% phosphoric acid solution and were then charred by heating at 200 °C for 5 min. By comparison with the reference spots, each spot obtained from the SPE extracts was assigned.

Double SPE Procedure

A new double SPE procedure was developed as follows: about 1 g of the oil was weighed accurately and was

dissolved in 4 mL chloroform, and the solution was then diluted with acetone in a 10-mL volumetric flask. One hundred μL of the chloroform/acetone solution, taken accurately using a glass micro syringe (Hamilton, Reno, NV, USA), was applied to the first reversed-phase SPE using a Sep-Pak Vac RC C18 cartridge 500 mg (Waters, Milford, MA, USA) that had been conditioned with 1 mL methanol and subsequently with 2 mL acetonitrile just prior to use. Two mL acetonitrile was then applied four times to the cartridge, and the acetonitrile applied (total ca. 8 mL) were evaporated to dryness using a nitrogen stream. The dried residues were dissolved in 2 mL chloroform, and the solution was then applied to the second normal-phase SPE, a Sep-Pak Vac RC Silica cartridge 500 mg (Waters), that had been conditioned with 2 mL chloroform just prior to use. Again, 2 mL chloroform was consecutively applied four times to the cartridge, and the chloroform applied (total ca. 10 mL) were dried again using a nitrogen stream. The dried residues (less than 1 mg) were carefully dissolved in 1 mL methanol/2-propanol (1:1 vol/vol). When the concentration of the GE was above the range of calibration lines, the solution was further diluted with methanol/2-propanol.

LC-MS Conditions

An Agilent 1100 Series LC-MS SL (single quadrupole) system equipped with an atmospheric pressure chemical ionization source, ChemStation software (Agilent Technologies, Palo Alto, CA, USA) and an L-column ODS 4.6 mm ID × 150 mm with 5 μm packing materials (Chemical Evaluation and Research Institute, Tokyo, Japan) was used as a LC-MS instrument to provide the conventional HPLC analysis. The mobile phases A (acetonitrile/methanol/water 17:17:6 vol/vol) and B (2-propanol) were consecutively time-programmed: A 98% (B 2%) at the start (0.0 min), a linear gradient of A 98–55% (B 2–45%) between 0.0 and 15.0 min, an isocratic elution of A 0% (B 100%) from 15.1 to 25.0 min, and finally an isocratic elution of A 98% (B 2%) from 25.1 to 35.0 min, at a flow rate of 1 mL/min. The injection volume was 20 μL of each solution, and the column temperature was maintained at 40 °C. Atmospheric pressure chemical ionization was performed with the following settings: ionization; positive ion mode, vaporizer temperature; 500 °C, heater temperature of nitrogen gas; 350 °C, flow of heated dry nitrogen gas; 5.0 L/min, nebulizer gas pressure; 0.241 MPa, corona current; 8.0 μA, fragmentor voltage; 150 V. For the selected ion monitoring measurement with unit mass resolution, each of the protonated molecular ions $[M + H]^+$ was used: *m/z* 313 for C16:0-GE, *m/z* 341 for C18:0-GE, *m/z* 339 for C18:1-GE, *m/z* 337 for C18:2-GE and *m/z* 335 for C18:3-GE.

Quantification of GEs in the Oils and Recovery Tests

To quantify GEs in the oils, calibration lines were generated by the injection of standard GE solutions in the range between 5 and 1,000 ng/mL (5, 10, 50, 200 and 1,000 ng/mL). Limit of detection (LOD) and limit of quantification (LOQ) were defined as signal to noise (S/N) = 3 and 10, respectively [9], based on a 20 μL injection of the standard GE solution at a concentration of 10 ng/mL. The oil samples were each analyzed in triplicate. The means ± SD of levels are expressed as microgram weights of GEs to gram weight of oil (μg/g). Recovery values were obtained from the ratio of the difference between quantified levels of the GEs in the spiked oil and those in the non-spiked oil to the known levels spiked (100 μg of each GE). After the solvent was evaporated, each oil sample (ca. 1.0 g) was added with accurate weighing, implying that the spiked levels of GEs corresponded to 100 μg/g each in oil samples. The means ± SD of recovery values are expressed on the basis of triplicate analyses.

Results and Discussion

LC-MS Conditions Using Conventional HPLC

LC using a flow rate of 1 mL and an L-column ODS with 4.6 mm ID and 5 μm packing materials was adopted for the conventional HPLC separation in LC-MS analysis. The composition of the two mobile phase system was the same as originally reported [8], but the time program for the reversed-phase gradient was slightly modified to reduce the total run time. Accompanied by the changes in LC conditions and the instrument used, the MS parameters in the positive ion APCI were optimized to detect GEs with maximal S/N (data not shown). Under those LC-MS conditions, the five species of GEs were completely separated and could be detected in each of the selected ion monitoring chromatograms within 15 min (Fig. 1).

The performance of this LC-MS technique was evaluated through analysis of the standard GEs (Table 1). The relative standard deviation (RSD) for retention times and areas during six consecutive runs were in the range of 0.02–0.04% and 0.6–3.2%, respectively (Table 1), which was superior to our previous results 0.18–0.23% and 4.3–7.0% [8]. The LOD and LOQ ranged from 0.67 to 1.0 ng/mL and from 2.2 to 3.3 ng/mL, respectively (Table 1). The sensitivity was approximately three times higher than in the original technique [8]. All calibration lines of the GEs tested showed R^2 (correlation of determination) = 1.0000 between 5 and 1,000 ng/mL (Table 1). Although the concentration range differed slightly from the original method [8], the linearity was better. Therefore,

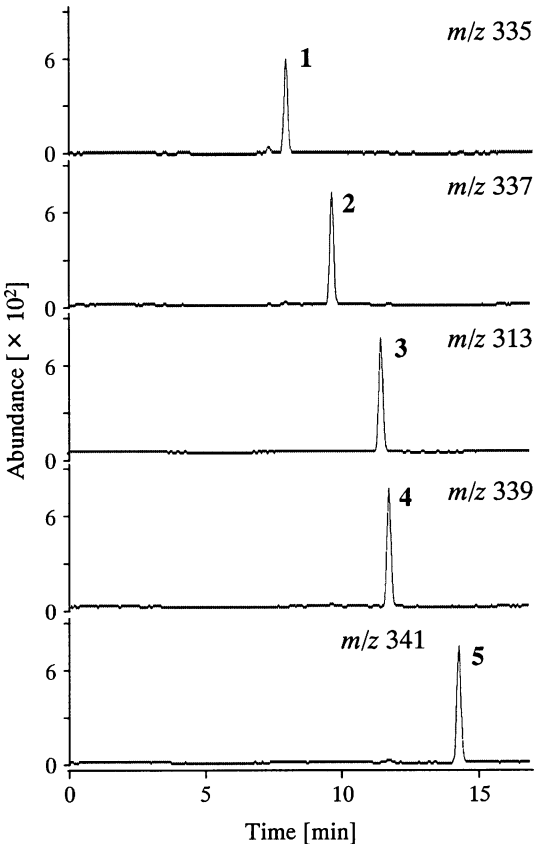

Fig. 1 LC-MS selected ion monitoring chromatograms of five species of standard GEs at a concentration of 200 ng/mL each. *Peaks 1* C18:3-GE, *2* C18:2-GE, *3* C16:0-GE, *4* C18:1-GE, *5* C18:0-GE

compared with the original LC-MS technique using a fast HPLC instrument [8], this LC-MS technique is more appropriate to quantify the GEs.

Based on these satisfactory results for the standard GEs, we used the LC-MS technique with conventional HPLC to determine recovery values for known levels of the standard GEs spiked to Sample-A (TAG-rich oil) and Sample-B (DAG-rich oil). The recovery values of Sample-B were less than 50% for a few species of GEs (data not shown), which meant less reproducibility of this method, although those of Sample-A were ca. 70%. In addition, the LC-MS measurements were not stable during sequential runs. Therefore, we judged that the analytical conditions should be changed into those that would provide accuracy and stability for sequential runs of oil samples even under conventional HPLC conditions. We hypothesized that co-existing acylglycerols (probably DAG) might not be sufficiently removed from oil samples, and that the fast HPLC might work well even when acylglycerols were present in the GE-rich extracts, while those acylglycerols might disturb the quantitative and stable detection of GEs when conventional HPLC was employed. To test that hypothesis, the effects of the double SPE procedure in our original method [8] on the removal of co-existing acylglycerols were reexamined in detail.

New Double SPE Procedure

To evaluate the efficacy of removal of co-existing acylglycerols in the original SPE procedure [8], we used TLC by which fat/oil classes such as TAG, 1,3-DAG, 1,2-DAG, MAG and GEs can be separated and visualized on TLC plates, as shown in Fig. 2, lane a. First, GE-rich extracts prepared from Sample-A and from Sample-B using the original SPE method [8] were analyzed using TLC. The results revealed that large amounts of co-existing TAG and small amounts of co-existing 1,3-DAG and 1,2-DAG in Sample-A were not observed on the TLC plates (Fig. 2, lanes b vs. c). In the case of Sample-B, on the other hand, large amounts of 1,3-DAG and 1,2-DAG remained in the extracts, despite the fact that TAG and MAG were not detected (Fig. 2, lanes d vs. e). Since the first reversed-phase SPE was designed to remove hydrophobic TAG from

Table 1 Percentages of relative standard (RSD%) during consecutive runs, limit of detection (LOD), limit of quantification (LOQ) and calibration line obtained from standard glycidol fatty acid esters (GEs)

GE	RSD%[a]		LOD[b]	LOQ[b]	Calibration line[c]	
	Retention time	Area	(ng/mL)	(ng/mL)	Equation	R^2
C16:0-GE	0.03	0.6	1.0	3.3	$y = 162.9x - 36.7$	1.0000
C18:0-GE	0.02	1.4	0.67	2.2	$y = 144.9x - 19.3$	1.0000
C18:1-GE	0.03	2.1	0.83	2.8	$y = 169.8x - 17.7$	1.0000
C18:2-GE	0.03	3.2	0.83	2.8	$y = 124.9x - 43.8$	1.0000
C18:3-GE	0.04	1.1	0.67	2.2	$y = 86.8x + 140.1$	1.0000

[a] Obtained by six consecutive runs of the standard GE solution at a concentration of 200 ng/mL

[b] Defined as signal to noise = 3 for LOD and 10 for LOQ [9] based on 20 μL injection of the standard GE solution at a concentration of 10 ng/mL

[c] Calculated from the equation $y = Ax + B$, where x is the injected concentration (ng/mL), y is the peak area, A is the slope, and B is the intercept in the range between 5 and 1,000 ng/mL (5, 10, 50, 200 and 1,000 ng/mL)

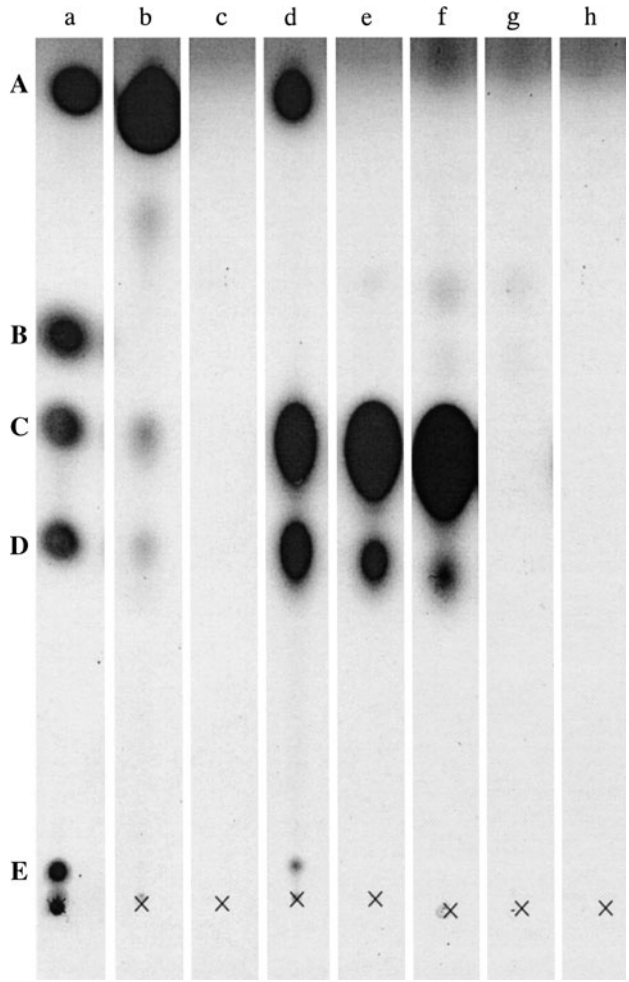

Fig. 2 TLC analysis of fats/oil classes in edible oils and in their extracts. Reference spots: *A* tripalmitin (representative TAG); *B* C18:0-GE (GEs); *C* 1,3-dipalmitin (1,3-DAG); *D* 1,2-dipalmitin (1,2-DAG); *E* 1-monolinolein (MAG). *Lanes a* references, *b* Sample-A, *c* extract prepared from Sample-A based on the original SPE procedure [8], *d* Sample-B, *e* extract prepared from Sample-B (100 mg) based on the original SPE procedure [8], *f* same as e except for the application amount for the second normal-phase SPE being one-half (50 mg), *g* same as e except for the application amount being one-fifth (20 mg), *h* same as e except for the application amount being one-tenth (10 mg)

the oil [8], these results demonstrate that the first SPE works completely even when the TAG-rich oil is applied. On the other hand, the second normal-phase SPE did not work to remove co-existing 1,3-DAG and 1,2-DAG in the DAG-rich oil (Sample-B), although the second SPE was designed to remove polar DAG and MAG from the oil [8].

The incomplete removal of 1,3-DAG and 1,2-DAG from the DAG-rich Sample-B might be caused by an overloading of the oil samples (100 mg as the crude oil) applied to the normal-phase SPE, because small amounts of 1,3-DAG and 1,2-DAG were completely removed in the case of the TAG-rich Sample-A (Fig. 2, lane c). Therefore, we examined the influence of decreased amounts of Sample-B for the second normal-phase SPE on the removal of 1,3-DAG and 1,2-DAG. In the case of one-half the amount of the original (=50 mg), the removal was not sufficient, and was similar to when 100 mg was applied (Fig. 2, lanes e and f). However, one-fifth (=20 mg) and one-tenth (=10 mg) were considerably effective, as seen in the disappearance of the 1,3-DAG and 1,2-DAG spots in the TLC chromatograms (Fig. 2, lanes g and h). This indicates that if the amount of oil applied is decreased to one-fifth or less, large amounts of co-existing DAG can be completely removed even from the DAG-rich oil.

To verify this, we did recovery tests using decreased oil samples to one-tenth of the original (100 mg → 10 mg). The recovery values of standard GEs spiked in the oil samples were as follows: 82.2–94.6% for Sample-A, and 94.5–105.9% for Sample-B (data not shown). These values are closer to 100% and the RSD values are lower than our original recovery values [8], implying the superior trueness and precision. The method using conventional HPLC did not cause any chromatographic problems even during sequential runs of oil samples. Therefore, the results demonstrate that decreasing the amount of oil sample (10 mg), which leads to the complete removal of co-existing DAG during the double SPE, is effective for accurately and robustly quantifying GEs in oil under conventional HPLC conditions.

Expansion of Applicability into Solid Oil

The newly developed SPE procedure was useful for liquid oils such as Sample-A and Sample-B, but it could not work for solid oils such as Sample-C. This was due to the fact that Sample-C was not entirely dispersed in acetonitrile, and the GEs seemed not to be extracted quantitatively. The first step of the sample preparation (dispersion of 100 mg oil in acetonitrile) was only designed to extract the GEs from liquid oils [8]. Therefore, to enhance the extraction efficacy from solid oils, we performed solubility tests using acetone and chloroform that are more powerful solvents for hydrophobic materials than acetonitrile, considering that the use of a too powerful solvent might cause the failure to remove co-existing TAG in the first reversed-phase SPE. The results indicated that the three oil samples were substantially dissolved when more than 2 vol of chloroform is contained in 10 vol of a mixed acetone/chloroform solvent. On the other hand, TLC experiments demonstrated that co-existing TAG are completely removed in the first reversed-phase SPE if the acetone/chloroform solution consists of less than 6 vol of chloroform and if the applied volume is 100 μL or less (data not shown).

Based on these findings, the SPE procedure was finally developed as described in Sect. "Experimental Procedure". When this procedure was performed together with LC-MS

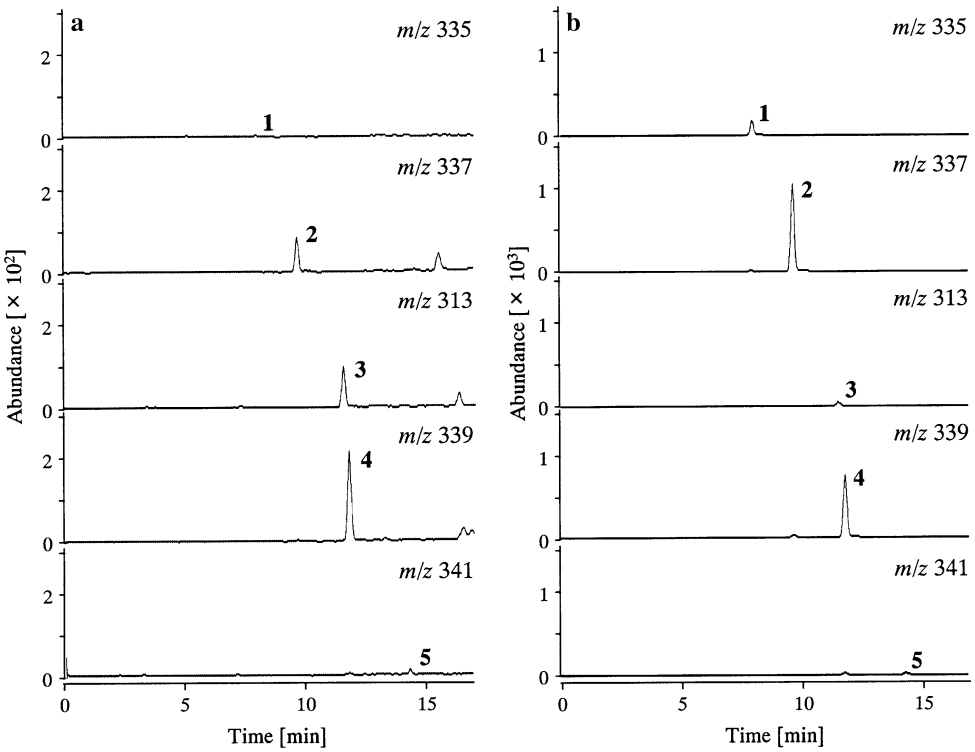

Fig. 3 LC-MS selected ion monitoring chromatograms of GEs in edible oils. **a** Sample-A, **b** Sample-B. *Peaks* as defined for Fig. 1

using conventional HPLC, the LC-MS measurements for oil samples provided better shaped-peaks and better baselines, even during sequential runs of oil samples, as exemplified in Fig. 3. Thus, this procedure did not cause any chromatographic problems even under conventional HPLC conditions. The recovery values of the standard GEs spiked into the three edible oil samples were as follows: 102.7–108.2% with RSD 1.2–3.1% for Sample-A, 105.8–109.7% with RSD 1.3–2.4% for Sample-B, and 105.3–109.5% with RSD 1.6–4.0% for Sample-C (Table 2). Although all average recovery values were a little above 100%, we judged that this method has sufficient accuracy for quantitative analysis.

The GE compositions in the edible oils, which were calculated from the mean levels in Table 2, seemed almost the same as the corresponding fatty acid compositions (Sample-A; C16:0-GE 20.6%, C18:1-GE 52.2% and C18:2-GE 27.2%: Sample-B; C16:0-GE 2.0%, C18:0-GE 1.0%, C18:1-GE 35.9%, C18:2-GE 49.4% and C18:3-GE 11.7%: Sample-C; C16:0-GE 30.6%, C18:0-GE 4.6%, C18:1-GE 48.6% and C18:2-GE 16.2%). In order to estimate the LOD and LOQ for oil samples, the concentrations corresponding to $S/N = 3$ and 10, respectively [9], were calculated using chromatographic data of standard GEs-spiked oil samples. The LOD were 1.0–1.2 ng/mL for C16:0-GE; 0.67–0.76 ng/mL for C18:0-GE; 0.91–1.1 ng/mL for C18:1-GE; 0.85–1.0 ng/mL for C18:2-GE; 0.73–0.94 mg/mL for C18:3-GE, whereas the LOQ for oil sample solutions were the same or slightly higher than LOQ for standard solutions (Table 1) as follows: 3.4–3.8 ng/mL for C16:0-GE; 2.2–2.5 ng/mL for C18:0-GE; 3.0–3.5 ng/mL for C18:1-GE; 2.8–3.4 ng/mL for

Table 2 Levels of glycidol fatty acid esters (GEs) in edible oil samples and recovery values obtained by a newly developed method

		Level (μg/g)[a]	Recovery (%)[a]	
Edible oil	GE	Mean ± SD	Mean ± SD	RSD%
Sample-A	C16:0-GE	1.9 ± 0.04	106.3 ± 1.3	1.2
	C18:0-GE	<0.5[b]	103.5 ± 1.4	1.3
	C18:1-GE	4.8 ± 0.17	106.7 ± 1.3	1.2
	C18:2-GE	2.5 ± 0.17	108.2 ± 1.6	1.5
	C18:3-GE	<0.5[b]	102.7 ± 3.1	3.1
Sample-B	C16:0-GE	5.8 ± 0.15	106.3 ± 1.8	1.7
	C18:0-GE	3.1 ± 0.10	105.8 ± 1.4	1.3
	C18:1-GE	106 ± 0.9	106.5 ± 2.6	2.4
	C18:2-GE	146 ± 0.8	107.2 ± 2.0	1.9
	C18:3-GE	34.7 ± 0.95	109.7 ± 2.6	2.3
Sample-C	C16:0-GE	10.0 ± 0.14	106.7 ± 1.7	1.6
	C18:0-GE	1.5 ± 0.05	107.0 ± 3.3	3.1
	C18:1-GE	15.9 ± 0.48	109.5 ± 4.1	3.7
	C18:2-GE	5.3 ± 0.13	106.2 ± 4.2	4.0
	C18:3-GE	<0.5[b]	105.3 ± 1.7	1.6

[a] Analyzed in triplicate

[b] Determined by the lowest concentration of the standard solution for calibration and dilution degrees (100-fold dilution) in the oil sample preparation

C18:2-GE; 2.4–3.1 mg/mL for C18:3-GE. Since the oil sample solutions were prepared by dilution of GE-rich fractions obtained from 10 mg oil with 1 mL methanol/2-propanol, the LOQ for the oil samples were determined as 0.22–0.38 μg/g oil. However, those values are just a rough estimate. To obtain precise LOD and LOQ values for oil samples, further verification would be needed.

It should be emphasized that we have developed a generalized method using conventional HPLC, which can be used not only for liquid oils but also for solid ones. This method can form the basis of a standardized method for the quality control of GEs in edible oils. However, we think that this method should be improved from the viewpoint of its practical use. The validity of this method remains unknown regarding its compatibility as a routine method that every analyst can use in different laboratories without any difficulties. The further improvement may be required as a routine method. Also, the further simplification and time reduction may be desired to monitor manufacturing processes of edible oils. Chloroform may be replaced with other organic solvents from the toxicological and eco-friendly viewpoints if possible. Instead of the linear gradient system, isocratic or step gradient separation in HPLC may be useful. Furthermore, if an internal standard (e.g. glycidol heptadecanoic acid ester) is used, the recovery values that are currently above 100% might be closer to 100%.

Conclusion

The original method that used both double SPE and LC-MS with a fast HPLC instrument [8] has been changed into a more generalized method. Thus, the modified SPE procedure (oil amounts applied, 100 mg → 10 mg) enabled us to use a conventional HPLC instrument for the quantification of GEs in edible oils because co-existing DAG could be completely removed. Also, by a change of organic solvent used in the first step of sample preparation (acetonitrile → acetone/chloroform), the method was applicable not only to liquid oils but also to solid ones. This generalized method can be globally useful for manufacturers and retailers working in the edible oil business.

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