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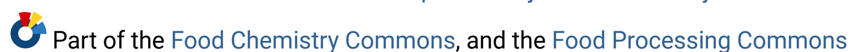
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Cover Page Footnote

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Simultaneous determination of 24 congeners of 2- and 3-monochloropropanediol esters and 7 congeners of glycidyl esters using direct multi-residue analytical LC-MS/MS methods in various food matrices

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Abstract

Glycidyl esters (GEs) and 2- and 3-monochloropropanediol esters (MCPDEs) are emerging process-generated food contaminants known as possible carcinogens. Herein, a direct method is developed and validated for the first time to simultaneously quantify seven GEs and twenty-four MCPDE congeners of processed foods using liquid chromatography-tandem mass spectrometry in a single sequence without ester cleavage or derivatisation, thereby allowing for the simultaneous analysis of numerous food matrices with high accuracy and precision. Our results show levels of GEs varying from <LOQ to 13486 ng/g, whereas those of MCPDEs range from <LOQ to 12019 ng/g, respectively.

Keywords: Food matrix, Glycidyl ester, LC−MS/MS, 3-MCPD ester, 2-MCPD ester

1. Introduction

Glycidyl esters (GEs) and monochloropropanediol esters (MCPDEs), two emerging classes of food-borne process contaminants, are formed in acid-hydrolysed vegetable protein and fat-based matrices exposed to high temperature [1,2]. Many vegetable oils commonly undergo industrial processing to remove components that could negatively impact taste, appearance, odour, shelf stability, and nutritional value [3]. However, industrial processing, particularly high-temperature treatment during deodorisation, is the most important factor for the formation of undesirable process-induced chemical contaminants [4,5]. Apart from deodorisation, gas-frying, char grilling, and baking at approximately 200 °C or higher also seem to result in the formation of MCPDEs and GEs in considerable amounts in edible oils and oil-based food products [6,7]. Studies have demonstrated that the formation of 2- and 3- MCPDE occurs during the chemical reactions of triacylglycerols (TAGs), some diacylglycerols (DAGs) and monoacylglycerols (MAGs) with reactive chlorine donors. DAGs and MAGs have been shown to act as GEs precursors via intramolecular rearrangement [8]. Owing to its epoxide and alcohol functional groups, glycidol can combine with different fatty acid esters to generate seven types of ester contaminants. Monochloropropanediol (MCPD), a type of glycerol chlorohydrin, is produced when the glycerol backbone of lipids replaced with chloride. Its isomers are 3-MCPD(3-chloropropane-1,2-diol) and 2-MCPD (2-chloropropane-1,3-diol) according to the substitution position. MCPD, in a similar way to glycidol, can combine with different fatty acid esters to form MCPDEs: in particular, 28 3-MCPDEs and 35 2-

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MCPDEs isomers are possible. The physical properties, generation mechanism, and food exposure sources of 3- and 2-MCPDEs are different. Furthermore, It had been proposed that 2-MCPDEs, 3- MCPDE, and GEs possibly interconvert in the presence of a chlorine source and high temperatures [9].

The presence of 3-MCPDE, 2-MCPDE, and GEs has been reported in various types of processed foodstuffs and raw materials, especially in refined vegetable oils [2,5,10,11], infant formula, baked goods [8,12] and processed foods [13]. The joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority, USA, and Brazil have delivered scientific opinions on the health risks related to the dietary exposure to 2- and 3- MCPDEs and GEs in certain high-risk foodstuffs such as infant formula [14–17]. The presence of 2- and 3- MCPDEs and GEs in the human diet may raise potential health concerns because these esters can be readily hydrolysed in the gastrointestinal tract into their corresponding free forms, 2- and 3-MCPD and glycidol, which are potentially toxic [14].

In recent decades, glycidol has been direct recognised as a direct alkylating agent and carcinogen in rodents, but no epidemiological or clinical data on glycidol have been reported for humans. However, glycidol has already been identified as a 'possible human carcinogen (group 2A)' by the International Agency for Research on Cancer (IARC) [8,18]. Very little is known about the toxicological effects of 2- and 3-MCPDE and GEs. So far, knowledge of the adverse health effects of these esters is sparse, and the human health significance of these contaminants is hard to evaluate owing to the lack of biological and exposure data [10]. 3-MCPD has shown toxicity to the kidneys and reproductive systems of rats during *in vivo* studies [19]. A tolerable daily intake (TDI) of 2 μg/kg/day for 3-MCPD has been derived by the European Commission's Scientific Committee for Food, whereas the JECFA has established a provisional maximum TDI of 4 μg/kg/day [20,21] Currently, there are very limited toxicological data to establish a maximum TDI value for 2-MCPD [19].

Direct and indirect analytical methods have been developed for the identification of 2- and 3-MCPDE and GEs in foods. Indirect methods, performed by gas chromatography-mass spectrometry (GC/MS) first require the hydrolysis of the esters to release free 2-MCPD, 3-MCPD and glycidol, followed by a derivatisation [20]. Nevertheless, these methods have been shown to produce inaccurate and imprecise results, and their performances are thought to be compromised by the transesterification and derivatisation steps. The disadvantage of transesterification is the potential partial transformation of GEs to 3-MCPDEs during sample preparation in alkaline media, which could result in a bias, whereas the disadvantages of derivatisation are the complicated pre-treatment procedures and overuse of organic solvent. In addition, owing to the lack of information regarding the individual congeners of 2- and 3-MCPDE and GE in foods, it is not possible to identify their potential sources [22]. Recently, a direct method has been developed to characterise all individual esters using liquid chromatography (LC)-MS or LC-MS/MS without the hydrolysis and derivatisation steps [28]. Most of the studies on this subject have established LC-MS direct methods for GEs [18,19,23] and MCPDEs in edible oils. These require removing a large amount of protein, free fatty acids, acylglycerols and TAGs prior to analysis. GEs and MCPDEs are very different in polarity, several purification techniques have been designed, which rely on solid-phase extraction (SPE) clean-up or liquid-liquid extraction. Furthermore, the U.S. Food and Drug

Abbreviations

ACN	acetonitrile
CE	collision energy
DAG	diacylglycerol
DCM	dichloromethane
Et_2O	diethyl ether
EtAc	ethyl acetate
ESI	electrospray ionization
GE	glycidyl ester
HEX	n-hexane
HLB	hydrophilic-lipophilic balance
HPLC	high-performance liquid chromatography
IPA	isopropanol
IS	internal standard
JECFA	joint expert committee on food additives
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MAG	monoacylglycerol
MCPDE	monochloropropanediol ester
MeOH	methanol
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTBE	methyl tert-butyl ether
PP	polypropylene
PTFE	polytetrafluoroethylene
QC	quality control
RSD	relative standard deviation
RT	retention time
SPE	solid-phase extraction
TDI	tolerable daily intake
TGA	triacylglycerol
TFDA	Taiwan Food and Drug Administration
USFDA	United States Food and Drug Administration

Administration (US FDA) has developed the separated pre-treatment processes for the simultaneous analysis of GEs and 3-MCPD monoesters and dieters. A limited number of published studies have dealt with direct methods for analysing these contaminants in edible oil and infant formula [20,24]. The cost for the large number of reagents and materials required to perform the necessary complicated extraction procedures for the analysis of these contaminants in a given sample is also significant [25,26]. For these reasons, no appropriate analytical method has yet been applied to quantify these contaminants in a variety of processed foods. Considering the above reasons, the first aim of this study was to develop a direct analytical method for simultaneous analysis of seven GEs and 24 MCPDEs using LC-MS/MS. Secondly, we aimed at establishing one procedure with environment-friendly yet effective and robust extraction for three categories of food matrixes (oil, low fat content- and high fat content-foods) using two-step SPE clean-ups. Following validation, the established method was applied to assay 7 GEs and 24 MCPDEs in 30 commercially available food products and compared with the results reported in previous studies.

2. Materials and methods

2.1. Reagents and materials

All solvents and reagents were of high-performance LC (HPLC) grade: Acetonitrile (ACN) and methyl tert-butyl ether (MTBE) were obtained from Merck (Darmstadt, Germany); Isopropanol (IPA) and water (HPLC grade) were purchased from J. T. Baker (Phillipsburgh, NJ, USA); n-hexane (HEX) and methanol (MeOH) were manufactured by Burdick and Jackson (Morristown, NJ, USA); ethyl acetate (EtAc) was obtained from DUKSAN Pure Chemicals Ltd. (South Korea). Diethyl ether (Et_2O), Formic acid (ACS reagent), ammonium formate (eluent additive for LC-MS) and Supelclean™ SPE cartridges (LC-C_{18} 1000 mg/6 mL, LC–Si 1000 mg/6 mL, and LC-Si 2000 mg/12 mL) were provided by Sigma–Aldrich (Louis, MO, USA). n-Pentane was obtained from Tedia Brasil (Rio de Janeiro, Brazil). Sodium sulfate anhydrous (ACS grade, size 10–60 mesh) was supplied by Acros (Antwerp, Belgium). Oasis HLB (500 mg/6 mL) cartridges was purchased from Waters (Milford, MA, USA).

2.2. Standards and QC samples

The dominant congeners of 12 monoesters and 12 diesters of MCPD, which achieved over 95% coverage of total MCPD, were selected based on the previous studies [2,19,24,27,28] and commercial availability. All the following products were provided by Toronto Research Chemicals (Toronto, ON, Canada). MCPD monoester standards: 1-lauroyl-3-chloropropanediol (1-La, CAS No. 20542-96-5), 1-myristoyl-3-chloropropanediol (1-My, CAS No. 30557-03-0), 1-palmitoyl-3-chloropropanediol (1-Pa, CAS No. 30557-04-1), 1-oleoyl-3-chloropropanediol (1-Ol, CAS No. 10311-82-7), 1-linoleoyl-3-chloropropanediol (1-Li, CAS No. 74875-98-2), 1-linolenoyl-3-chloropropanediol (1-Ln, CAS No. 74875-99-3), 1-stearoyl-3-chloropropanediol (1-St, CAS No. 22094-20-8), 2-palmitoyl-3-chloropropanediol (2-Pa, CAS No. 20618-92-2), 2-oleoyl-3-chloropropanediol (2-Ol, CAS No. 915297-48-2), 1-palmitoyl-2-chloropropanediol (1-Pa2, CAS No. 63326-63-6), 1-oleoyl-2-chloropropanediol (1-Ol2, CAS No. 1639207-37-6), 1-linoleoyl-2-chloropropanediol (1-Li2, CAS No. 1639207-38-7). MCPD diester standards: 1,2-bis-linolenoyl-3-chloropropanediol (Ln–Ln, CAS No. 51930-97-3), 1,2-bis-linoleoyl-3-chloropropanediol (Li–Li, CAS No. 7487-96-0), 1,2-bis-oleoyl-3-chloropropanediol (Ol–Ol, CAS No. 69161.73-5), palmitoyl-linoleoyl-3-chloropropanediol (Pa–Li, CAS No. 1246833-87-3), oleoyl-linoleoyl-3-chloropropanediol (Ol-Li, CAS No.1336935-03-5), palmitoyl-oleoyl-3-chloropropanediol (Pa-Ol, CAS No. 1363153-60-9), palmitoyl-stearoyl-3-chloropropanediol (Pa-St, CAS No. 1185060-41-6), oleoyl-stearoyl-3-chloropropanediol (Ol-St, CAS No. 1336935-05-7), 1,2-bis-palmitoyl-3-chloropropanediol (Pa–Pa, CAS No. 51930-97-3), dilinoleoyl-2-chloropropanediol (2Li–Li, CAS No. 1432592-04-5), dipalmitoyl-2-chloropropanediol (2Pa–Pa, CAS No. 169471-41-4), palmitoyl-oleoyl-2-chloropropanediol (2Pa-Ol, CAS No. 1639207-41-2). GE standards: glycidyl laurate (La-GE, CAS No.1984-77-6), glycidyl myristate (My-GE, CAS No.7460-80-2), glycidyl palmitate (Pa-GE, CAS No.7501-44-2), glycidyl oleate (Ol-GE, CAS No.5431-33-4), glycidyl linoleate (Li-GE, CAS No.24305-63-3), glycidyl linolenate (Ln-GE, CAS No.51554-07-5), glycidyl stearate (St-GE, CAS No.7460-84-6). The seven GEs standards currently commercially available and achieved over 86% coverage of total glycidyl [29]. The structures of all these chemicals are provided in Table S1 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal) and Fig. S1 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal).

The internal standards (ISs) of GEs and MCPDEs were as follows: Glycidyl laurate-d5 (La-GE-d5, CAS No. 1329563-35-0), glycidyl myristate-d5 (My-GE-d5, CAS No. 1330180-72-7), glycidyl palmitate-d5 (Pa-

GE-d5, CAS No. 1794941-80-2), glycidyl oleate-d5 (Ol-GE-d5, CAS No. 1426395-63-2), glycidyl linoleate-d5 (Li-GE-d5, CAS No. 1246834-15-0), glycidyl linolenate-d5 (Ln-GE-d5, CAS No. 1287393-54-7), glycidyl stearate-d5 (St-GE-d5, CAS No. 1346598-19-3), 1-oleoyl-3-chloropropanediol-d5 (1-Ol-d5), 1-stearoyl-3-chloropropanediol-d5 (1-St-d5, CAS No. 1795785-84-0), 1,2-bis-linolenoyl-3-chloropropanediol-d5 (Ln-Ln-d5), 1,2-bis-oleoyl-3-chloropropanediol-d5 (Ol-Ol-d5, CAS No. 1246833-00-0), 1,2-bis-palmitoyl-3-chloropropanediol-d5 (Pa-Pa-d5, CAS No. 1185057-55-9), oleoyl-stearoyl-3-chloropropanediol-d5 (Ol-St-d5); these were also purchased from Toronto Research Chemicals (Toronto, Ontario, Canada).

The mixed stock solutions and IS solutions of GEs and MCPDEs were prepared in IPA at a concentration of 5 μg/mL and stored at −20 °C. All of the standard and QC samples were prepared using the stock solutions from the same source and the same concentration. Separate working solutions for standard and QC preparation were made by diluting the stock solution of the analyte with IPA. QC samples (spiked olive oil for edible oils samples, spiked rice cereal extracts for the low-fat samples and spiked infant formula extracts for the high-fat samples) were prepared at concentrations of 10 ng/mL, 50 ng/mL and 100 ng/mL. The standards and QCs were aliquoted into polypropylene tubes and stored in freezers maintained at approximately −80 °C.

2.3. Sample preparation

The improved analytical procedure for the simultaneous determination and separation of seven GEs and 24 MCPDEs (including 2- and 3-MCPD monoesters and diesters) was developed by modifying the previously published methods [2,19,23]. The food samples were purified differently according to their fat contents, and three optimized purification methods were applied to the samples of three categories of foods: (1) edible oils; (2) low-fat-content (≤ 10%) food: cereals, alcohol and beverages, dried spices, soy sauce, processed products of vegetables and seafood; (3) high-fat-content (> 10%) food: animal fats, salad dressings, dairy products (including infant formula, cheese and butter), and processed products of meat and fish.

2.3.1. Edible oils

Edible oil (0.5 g) was weighed in a 15 mL PYREX™ disposable round-bottom threaded culture tube. The sample was spiked with 4 mL of a 20/80 (v/v) EtAc/MTBE mixture and 50 ng IS and sonicated for 2 min for homogenisation. An aliquot of 1 mL of the solution was dried at 55 °C under a stream of nitrogen. A 20/80 (v/v) EtAc/HEX mixture (2 mL) was reconstituted and introduced into a 1 g/6 mL Si SPE cartridge, which was preconditioned with 18 mL of 20% EtAc/HEX. Another 2 mL of 20/80 (v/v) EtAc/HEX was added, and the procedure was repeated three times for a total of 8 mL mixture. The clean-up procedure was repeated once; the eluted fractions were combined and concentrated to dryness with nitrogen at 55 °C. The resulting solution was then reconstituted in 2 mL of a 40/60 (v/v) EtAc/ACN mixture and loaded into a 1 g/6 mL C_{18} SPE cartridge, which was preconditioned with 18 mL ACN. All target compounds were elute from the C_{18} SPE cartridge with 10 mL of 40/60 (v/v) EtAc/ACN. The eluent was concentrated to near dryness, reconstituted with 1 mL IPA, and filtered through a 0.22-mm pore polytetrafluoroethylene (PTFE) membrane for LC-MS/MS analysis.

2.3.2. Low-fat content food

A homogenised food sample (1 g in wet weight, ww) was weighed in a 50-mL polypropylene (PP) conical centrifuge tube; 8 mL LC water was added to the tube, which was vortex for 10 s. Then, 8 mL of EtAc was added to the solution, was further vortexed for 10 min to extract fat from the food sample. Following the extraction, 8 g of Na_2SO_4 was added to remove water. The supernatant was transferred to a new culture tube after centrifugation at 5500 rpm for 15 min. Extraction with EtAc was repeated two more times; the extracts were combined and concentrated to dryness with nitrogen at 55 °C. The residual was dissolved in 10 mL of a 40/60 (v/v) DCM/HEX mixture, and 50 ng IS was used for purification. The sample solution was loaded on a 1000 mg/6 mL Si SPE cartridge that was preconditioned with 5 mL MeOH, 6 mL DCM, and 12 mL 40/60 (v/v) DCM/HEX. The fractionation was achieved with 8 mL DCM/HEX (40/60, v/v) (Fraction 1, F1) and 9 mL EtAc/HEX (20/80, v/v) (Fraction 2, F2). F2 was further concentrated to near dryness and re-dissolved with 2 mL ACN. The sample solution was then introduced into a 500 mg/6 mL HLB SPE cartridge that was preconditioned with 18 mL ACN. The cartridge was eluted with 6 mL ACN (Fraction 3, F3); this fraction was then combined with F1. The mixture solution was concentrated to near dryness at 55 °C, reconstituted with 1 mL of IPA, and filtered through a PTFE membrane (0.22-mm pore size) for LC-MS/MS analysis.

2.3.3. High-fat content food

A homogenized food sample (1 g) was accurately weighted in a 50 mL PP conical centrifuge tube and

the extracted fat was separated from the matrix using 8 mL EtAc and 8 g Na_2SO_4. The extraction was repeated twice. The extracts were combined and dried with nitrogen. The residual was dissolved in 2 mL of a 2% Et_2O/HEX mixture and spiked with 50 ng IS. The sample solution was loaded on a 2000 mg/12 mL Si SPE cartridge that was preconditioned with 24 mL MeOH, 24 mL DCM, and 24 mL 2% Et_2O/HEX. After the first elution, 9 mL 2% Et_2O/HEX was discarded, while the following 16 mL 2% Et_2O/HEX was collected into F1. Finally, the cartridge was eluted with 9 mL 20% EtAc/HEX and collected into F2. This was concentrated to near dryness with nitrogen and redissolved in 1 mL 20% EtAc/HEX. The residues were then introduced on a 1000 mg/6 mL Si SPE cartridge that was preconditioned with 12 mL 20% EtAc/HEX, and eluted with 6 mL 20% EtAc/HEX collected into F3. The resulting solution was concentrated to near dryness with nitrogen and redissolved in 1 mL ACN. The residues were purified through an HLB SPE cartridge (preconditioned with 18 mL ACN). After rinsing the vessel with 2 mL ACN and charging into the cartridge, an aliquot of 2 mL ACN was added directly into the cartridge to collect F4. The cleaned F4 and F1 were then combined and concentrated to near dryness using a gentle stream of nitrogen. The final residues were then reconstituted with 1 mL IPA and filtered through a PTFE membrane (0.22-mm pore size) for LC-MS/MS analysis.

2.4. LC-MS/MS analysis

The simultaneous analysis of 3-MCPDEs, 2-MCPDEs, and GEs was performed using an Agilent 1200 HPLC series system, an Agilent 6410B tandem mass spectrometry, and an Agilent Pursuit XRs C_{18} 2.0 × 150 mm, 3.0 μm particle size analytical column. The column was held at 30 °C and the injection volume was 5 μL. The MCPD diesters and monoesters and GEs were separated using the following mobile phases: phase A consisted of a mixture of 2 mM ammonium formate and 0.05% formic acid in methanol/water (75/25, v/v), and whereas the phase B was composed of IPA with 2 mM ammonium formate and 0.05% formic acid. The chromatographic conditions to separate and quantify GEs and MCPDEs were as follows: 100% A with an initial flow rate of 0.2 mL/min for the first 2 min, followed by 75% A/25% B at 2.1 min, holding at 75% A until 15 min, a linear ramp to 55% A at 35 min, stepping to 35% A at 36 min, holding at 35% A until 41 min, a linear ramp to 25% A at 58 min, stepping to 0% A at 58.1 min, holding at 0% A until 69 min, and stopping the controller. The chromatographic separations of seven GEs and 24 MCPDEs are shown in Fig. 1.

The tandem mass spectrometer equipped with an electrospray ionization (ESI) source, was operated in positive ion mode (ESI+) and used for MS/MS analysis of all the compounds. The two most abundant and stable ion transitions resulting from the loss

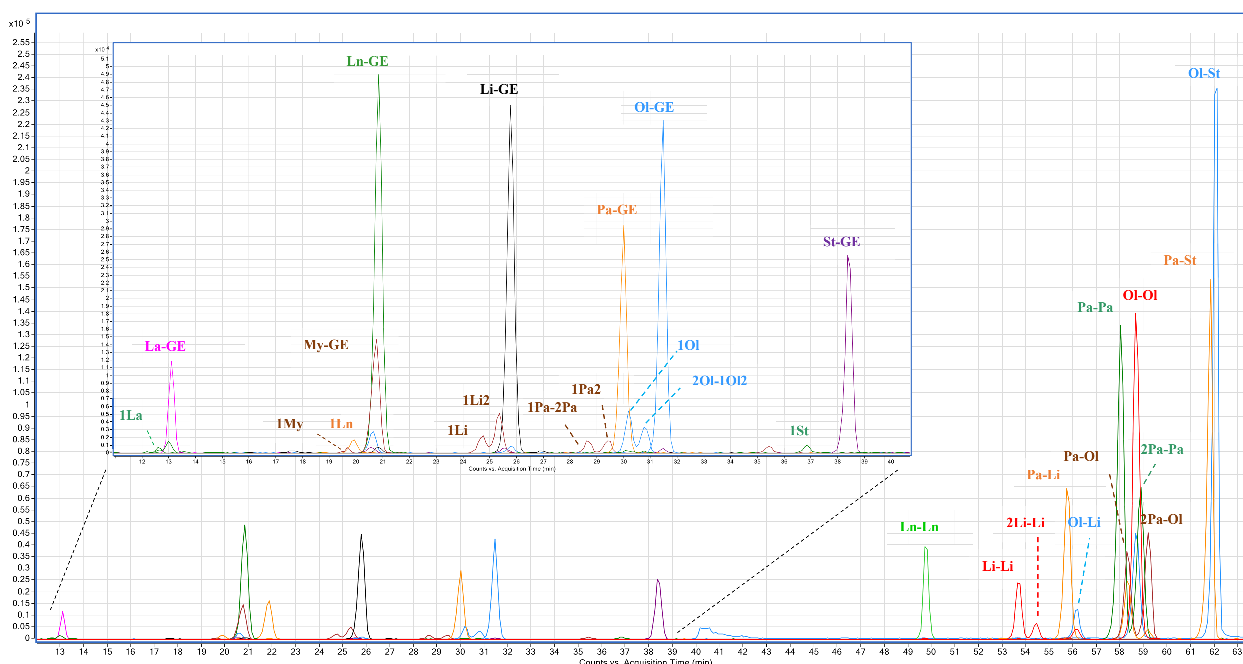

Fig. 1. LC-MS/MS data for seven GEs and 24 MCPDEs using the chromatographic separation system for a standard spiked sample (100 ng/mL).

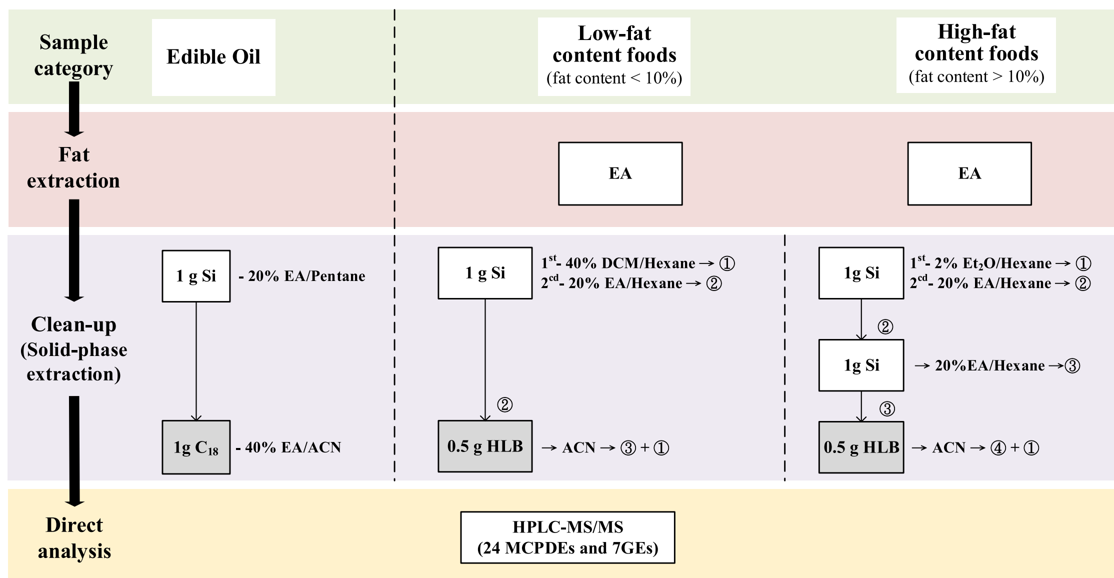

Fig. 2. Optimised sample pre-treatment of GEs and 3- and 2-MCPD mono- and di-esters for oils, low-, and high-fat-content foods.

of each fatty acid for GEs and MCPDEs were acquired as quantifiers and qualifiers, respectively. These transitions were monitored, as well as the same transition for the Cl-37 isotope for confirmation [19,23,24,28,31]. The source parameters, such as fragmenter and collision energy (CE), were optimized for each target compound. Q1 and Q3 were set at unit resolution. The capillary voltage was set at 4000 V; the desolvation temperature was set at 350 °C; the cone gas flow rate was 10 L/min; the nebuliser pressure was 40 psi. The individual MS/MS transitions, approximate retention times (RTs), IS, fragmenter, and CE for each compound are shown in Table 1, with the quantitation ions listed first, followed by one or two confirmatory ions for each compound. The RTs for each analyte were determined by analysing a mixed standard under the conditions described above in the standard multiple reaction monitoring (MRM) mode (scheduled MRM). A representative MRM scan is shown in Table 1.

2.5. Method validation

The method was validated for analysis in edible oils, low-fat-content food, and high-fat-content food (Fig. S2 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)), according to the guideline form International Conference on Harmonisation (ICH) Q2 (R1) [30] and Taiwan Food and Drug Administration (TFDA) [31] for calibration curve, precision, accuracy, selectivity, recovery, instrument detection limit, and method detection limit. All glassware was cleaned with acetone and n-hexane to remove possible background contamination.

2.5.1. Calibration curves

Calibration curves were obtained by plotting peak area ratios (analyte/IS) versus nominal concentrations. Optimal calibration models were determined by statistical analysis on ten-point calibration curves (1, 5, 10, 25, 50, 100, 250, 500, 750, and 1000 ng/mL with 50 ng/mL IS) of targeted GEs, 2-MCPDEs and 3-MCPDEs in IPA, while studying the linear relationship over a broad range (Table 2). The calibration model selection of the ratio of the chromatographic peak area to that of the corresponding IS was based on each weighting factor of $1/x^2$. Selected calibration models were evaluated by back-calculation of all the calibrators in which the obtained back-calculated concentrations of the calibrators should be within ± 20% of the nominal value. Fit correlation was $R^2 \geq 0.995$.

2.5.2. Precision, accuracy and robustness

Recovery was estimated using the low-concentration, median-concentration, and high-concentration spiked samples, by comparing the initial concentrations of the spiked standards. Precision was indicated as the percentage of relative standard deviation (RSD) assayed with the variabilities among intraday and interday tests being less than 20%. The intraday precision was evaluated by analysing three sample matrices of edible oils, low fat content food (cereal), and high fat content food (infant formula)

Table 1. Optimised LC-MS/MS parameters for seven GEs and 24 MCPDEs.

Compound	RT	Q1 (*m/z*)	Q3 (*m/z*)	DP	CE	Internal standard	RT	Q1 (*m/z*)	Q3 (*m/z*)	DP	CE
Glycidyl esters											
La-GE	13.1	274.2	57.1	80	21	La-GE-d5	12.9	279.2	57.1	220	29
	13.1	274.2	71.1	80	17		12.9	279.2	71.1	220	17
My-GE	20.7	302.2	57.1	81	21	My-GE-d5	20.4	307.2	57.2	91	21
	20.7	302.2	71.1	81	17		20.4	307.2	71.2	91	21
Pa-GE	30.0	330.3	57.1	96	25	Pa-GE-d5	20.8	335.3	57.1	148	29
	30.0	330.3	85.1	96	21		20.8	335.3	85.1	148	21
Ol-GE	31.4	356.3	55.1	91	49	Ol-GE-d5	31.2	361.3	55.1	101	41
	31.4	356.3	57.1	91	25		31.2	361.3	57.1	101	29
Li-GE	25.8	354.3	95.1	101	25	Li-GE-d5	25.5	359.3	81.1	96	29
	25.8	354.3	57.1	101	25		25.5	359.3	95.1	96	25
Ln-GE	20.8	352.3	95.1	96	21	Ln-GE-d5	20.6	357.3	95.1	96	21
	20.8	352.3	55.1	96	45		20.6	357.3	55.1	96	41
St-GE	38.4	358.3	57.1	101	25	St-GE-d5	38.1	363.3	57.1	96	29
	38.4	358.3	85.1	101	21		38.1	363.3	71.1	96	21
Mono-MCPDEs											
1-La	12.6	310.2	183.1	80	8	1Ol-d5	30.0	397.2	265.3	91	9
	12.6	310.2	57.1	80	25		30.0	397.2	247.2	91	13
	12.6	312.2	183.2	81	5		30.0	399.2	265.2	96	9
1-My	19.7	338.2	211.3	96	5	1Ol-d5	30.0	397.2	265.3	91	9
	19.7	338.2	57.2	96	25		30.0	397.2	247.2	91	13
	19.7	340.2	211.3	86	5		30.0	399.2	265.2	96	9
1-Pa	28.7	366.2	239.2	91	9	1Ol-d5	30.0	397.2	265.3	91	9
	28.7	368.2	239.3	91	9		30.0	397.2	247.2	91	13
	–	–	–	–	–		30.0	399.2	265.2	96	9
1-Ol	30.2	392.2	265.2	101	9	1Ol-d5	30.0	397.2	265.3	91	9
	30.2	392.2	55.1	101	54		30.0	397.2	247.2	91	13
	30.2	394.2	265.3	96	9		30.0	399.2	265.2	96	9
1-Li	24.7	390.3	263.3	101	8	1Ol-d5	30.0	397.2	265.3	91	9
	24.7	390.3	245.2	101	9		30.0	397.2	247.2	91	13
	24.7	392.3	263.3	96	8		30.0	399.2	265.2	96	9
1-Ln	19.9	388.3	261.2	101	5	1Ol-d5	30.0	397.2	265.3	91	9
	19.9	388.3	81.1	101	33		30.0	397.2	247.2	91	13
	19.9	390.3	261.2	101	9		30.0	399.2	265.2	96	9
1-St	36.9	394.2	267.3	101	9	1St-d5	37.2	399.2	267.3	101	9
	36.9	394.2	57.2	101	33		37.2	399.2	57.1	101	41
	36.9	396.2	267.3	96	9		37.2	401.2	267.1	106	9
2-Pa	28.7	366.2	239.3	91	5	1Ol-d5	30.0	397.2	265.3	91	9
	28.7	368.2	239.4	90	5		30.0	397.2	247.2	91	13
	–	–	–	–	–		30.0	399.2	265.2	96	9
2-Ol	30.8	392.2	265.3	96	9	1Ol-d5	30.0	397.2	265.3	91	9
	30.8	392.2	55.1	96	54		30.0	397.2	247.2	91	13
	30.8	394.2	265.2	101	9		30.0	399.2	265.2	96	9
1-Pa2	29.4	366.2	239.2	96	8	1Ol-d5	30.0	397.2	265.3	91	9
	29.4	368.2	239.2	91	5		30.0	397.2	247.2	91	13
	–	–	–	–	–		30.0	399.2	265.2	96	9
1-Ol2	30.8	392.2	265.2	91	9	1Ol-d5	30.0	397.2	265.3	91	9
	30.8	392.2	55.1	91	50		30.0	397.2	247.2	91	13
	30.8	394.2	265.2	106	9		30.0	399.2	265.2	96	9
1-Li2	25.3	390.3	263.3	101	4	1Ol-d5	30.0	397.2	265.3	91	9
	25.3	390.3	245.2	101	9		30.0	397.2	247.2	91	13
	25.3	392.3	263.3	101	8		30.0	399.2	265.2	96	9
Di-MCPDEs											
Li–Li	53.7	652.5	355.2	168	21	Pa-Pa-d5	59.1	609.5	336.2	153	17
	53.7	652.5	81.1	168	54		59.1	609.5	57.2	153	50
	53.7	654.5	357.3	159	21		59.1	611.5	338.3	148	17
Ol–Ol	58.7	656.5	357.2	158	17	Ol-Ol-d5	58.5	661.5	362.3	148	17
	58.7	656.5	95.1	158	45		58.5	661.5	95.1	148	50
	58.7	658.5	359.2	158	17		58.5	663.5	364.3	158	17
Pa–Li	55.7	628.5	355.2	148	17	Pa-Pa-d5	59.1	609.5	336.2	153	17
	55.7	628.5	331.3	148	13		59.1	609.5	57.2	153	50
	55.7	630.5	357.3	143	17		59.1	611.5	338.3	148	17

(*continued on next page*)

Table 1. (continued)

Compound	RT	Q1 (*m/z*)	Q3 (*m/z*)	DP	CE	Internal standard	RT	Q1 (*m/z*)	Q3 (*m/z*)	DP	CE
Ol-Li	56.1	654.5	355.2	158	21	Ln-Ln-d5	49.7	653.5	358.3	143	17
	56.1	654.5	357.3	158	17		49.7	653.5	93.1	143	50
	56.1	656.5	357.3	153	21		49.7	655.5	360.3	148	21
Pa-Ol	58.3	630.4	357.2	148	17	Pa-Pa-d5	59.1	609.5	336.2	153	17
	58.3	630.4	331.2	148	13		59.1	609.5	57.2	153	50
	58.3	632.4	359.3	158	13		59.1	611.5	338.3	148	17
Pa-St	61.8	632.5	359.3	143	17	Ol-St-d5	62.4	663.5	364.3	163	13
	61.8	632.5	331.2	143	17		62.4	663.5	57.1	163	50
	61.8	634.5	361.3	158	13		62.4	663.5	362.3	163	17
Ol-St	62.0	658.5	359.3	158	17	Ol-St-d5	62.4	663.5	364.3	163	13
	62.0	658.5	357.2	158	17		62.4	663.5	57.1	163	50
	62.0	660.5	361.3	153	17		62.4	663.5	362.3	163	17
Pa–Pa	58.0	604.5	331.2	148	13	Pa-Pa-d5	59.1	609.5	336.2	153	17
	58.0	604.5	57.1	148	54		59.1	609.5	57.2	153	50
	58.0	606.5	333.3	148	13		59.1	611.5	338.3	148	17
Ln–Ln	49.8	648.5	353.2	153	21	Ln-Ln-d5	49.7	653.5	358.3	143	17
	49.8	648.5	93.1	153	49		49.7	653.5	93.1	143	50
	49.8	650.5	355.2	153	21		49.7	655.5	360.3	148	21
2Li–Li	54.4	652.5	355.2	158	17	Ln-Ln-d5	49.7	653.5	358.3	143	17
	54.4	652.5	81.1	158	50		49.7	653.5	93.1	143	50
	54.4	654.5	357.2	143	17		49.7	655.5	360.3	148	21
2Pa–Pa	58.8	604.5	331.2	143	13	Ol-Ol-d5	58.5	661.5	362.3	148	17
	58.8	604.5	57.1	143	54		58.5	661.5	95.1	148	50
	58.8	606.5	333.2	143	13		58.5	663.5	364.3	158	17
2Pa-Ol	59.2	630.5	331.2	148	13	Pa-Pa-d5	59.1	609.5	336.2	153	17
	59.2	630.5	357.3	148	17		59.1	609.5	57.2	153	50
	59.2	632.5	333.2	163	13		59.1	611.5	338.3	148	17

Collision energy (CE); Declustering potential (DP).

on the same day, whereas the interday precision was estimated over 7 d. Robustness studies.

The robustness test was assessed for the developed method by making minor changes in the optimized values of LC-MS parameters and sample preparation. Following five variables were evaluated for this purpose: Organic solvent concentration (± 5%), Buffer concentration (± 0.5 mM/± 0.05%), the flow rate of the mobile phase (± 0.05 mL/min), extraction time (± 5 min), and matrix effect (12 different sample matrix). The standard concentration of 50 ng/mL was used in this study, and the peak areas and recovery rate obtained under these conditions were calculated and statistically analyzed.

2.6. Application

To objectively evaluate the performance of our three optimized pre-treatment methods and a novel LC-MS/MS trace analysis method, 59 food samples categorised into 12 groups obtained from Taiwanese supermarkets were preliminarily applied to analyse the individual 31 congeners of GEs and 3- and 2-MCPDEs.

3. Results and discussion

3.1. Method validation

3.1.1. Chromatography and LC-MS/MS method

To simultaneously and accurately identify the individual congeners of GEs and 3- and 2-MCPDEs via LC-MS/MS, we first needed to perform a quantitative and qualitative selection of ion pairs with high specificity and ensure instrumental stability and sensitivity. In accordance with MacMahon et al. [32], a Pursuit XRs C18 column (Agilent) was employed for the chromatographic separation of the target analytes. The mobile phases were also optimized using different proportions of water phase (2%, 25%, 50%, and 100%) and selected gradient programs (Fig. S3 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)). A mixture of 2 mM ammonium formate and 0.05% formic acid in 75/25 MeOH/H_2O, provided the optimum chromatographic performance for all analytes, with adequate peak shape and sharpness. Considering the separation periods for GE/3-MCPD monoesters and 3- and 2-MCPD diesters [19,31], the seven GEs and 24 MCPDEs were successfully separated by the column

Table 2. Calibration curve, IDLs (ng/mL), LODs and LOQs (ng/g) for GEs and MCPDEs.

Compounds	Calibration curve				IDL (ng/mL)	LOD (n = 7) (ng/g)			LOQ (n = 7) (ng/g)		
	Range (ng/mL)	RSD (%)	Slope	R		Oils	Low-fat food	High-fat food	Oils	Low-fat food	High-fat food
Glycidyl esters											
La-GE	1–1000	3%	0.022191	0.999	0.39	2.21	6.89	13.8	6.71	20.9	41.9
My-GE	1–1000	6%	0.029356	0.999	0.26	2.55	7.95	15.9	7.73	24.1	48.3
Ln-GE	1–1000	3%	0.021861	0.999	0.31	1.85	5.74	11.5	5.59	17.4	34.9
Li-GE	1–1000	2%	0.026004	0.999	0.29	1.14	3.56	7.13	3.46	10.8	21.6
Pa-GE	1–1000	10%	0.023365	0.999	0.33	1.98	6.17	12.3	6.00	18.7	37.4
Ol-GE	1–1000	6%	0.028412	0.999	0.37	2.87	8.94	17.9	8.70	27.1	54.3
St-GE	1–1000	4%	0.030789	0.999	0.27	1.93	6.01	12.0	5.84	18.2	36.5
Mono-MCPDEs											
1La	1–750	8%	0.025612	0.998	0.28	1.44	4.49	9.01	4.37	13.6	27.3
1My	1–750	13%	0.025188	0.998	0.51	2.60	8.12	16.3	7.88	24.6	49.2
1Ln	1–750	11%	0.024476	0.997	0.66	2.89	9.04	18.1	8.78	27.4	54.8
1Li	1–750	8%	0.028675	0.999	0.61	1.74	5.41	10.9	5.28	16.4	32.9
1Li2	1–750	14%	0.048533	0.997	0.57	1.54	4.79	9.60	4.67	14.5	29.1
1Pa/2Pa	1–750	15%	0.022296	0.998	0.65	3.66	11.4	22.9	11.1	34.6	69.3
1Pa2	1–750	11%	0.023210	0.998	0.62	2.97	9.27	18.6	9.02	28.1	56.3
1Ol	1–750	2%	0.027829	0.997	0.53	3.01	9.37	18.8	9.12	28.4	56.9
1Ol2/2Ol	1–750	11%	0.025494	0.998	0.67	2.32	7.22	14.5	7.03	21.9	43.9
1St	1–750	14%	0.010248	0.999	0.66	5.02	15.6	18.0	15.2	47.3	54.6
Di-MCPDEs											
Ln–Ln	1–750	3%	0.024652	0.999	0.24	2.91	9.08	18.2	8.81	27.5	55.0
Li–Li	1–750	7%	0.027750	0.999	0.45	1.73	5.38	10.8	5.24	16.3	32.7
2Li–Li	1–750	7%	0.024439	0.999	0.44	2.48	7.72	15.4	7.50	23.4	46.8
Pa–Li	1–750	5%	0.027664	0.999	0.20	2.87	8.94	17.9	8.70	27.1	54.3
Ol-Li	1–750	7%	0.029860	0.999	0.42	2.14	6.67	13.3	6.47	20.2	40.4
Pa–Pa	1–750	1%	0.037827	0.999	0.15	1.71	5.31	10.6	5.15	16.1	32.2
2Pa–Pa	1–750	2%	0.026540	0.999	0.25	2.39	7.46	14.9	7.25	22.6	45.3
Pa-Ol	1–750	4%	0.026690	0.999	0.18	0.683	2.14	4.26	2.07	6.48	12.9
2Pa-Ol	1–750	12%	0.027103	0.999	0.28	1.11	3.43	6.89	3.35	10.4	20.9
Ol–Ol	1–750	3%	0.026968	0.998	0.18	1.35	4.22	8.45	4.10	12.8	25.6
Pa-St	1–750	6%	0.022373	0.999	0.16	1.14	3.53	7.06	3.44	10.7	21.4
Ol-St	1–750	7%	0.026942	0.999	0.18	0.795	2.49	4.95	2.41	7.53	15.0

RSD: Relative standard deviation; IDL: Instrument detection limit; LOQ: Limit of quantitation.

and detected within 69 min in this study (Fig. 1). Numerous separation tests for the sn-2 3-MCPD monoester isomers (e.g. 1Pa/2Pa/1Pa2 and 1Ol1/1Ol2/2Ol) could not be achieved by MS because these isomers are detected by the same MRM pairs. The separations of sn1/sn2 in all analytes were verified with the individual standard. Thus, it was possible to accurately quantify only their combined concentrations (1Pa–2Pa and 1Ol2–2Ol). Moreover, the suggested MeOH proportion in the water phase and modifiers (ammonium formate and formic acid) both enabled the adequate separation of MCPD monoester (1Li/1Li2, 1Pa–2Pa/1Pa2, and 1Ol/1Ol2–2Ol) (Fig. S4a (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)) and diester (Li–Li/2Li–Li, Pa–Pa/2Pa–Pa, and Pa–Ol/2Pa–Ol) (Fig. S4b (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)) isomers.

For MS/MS analysis, the fragmentation of the most abundant molecular ion $[M + NH_4]+$ was chosen. The optimized results of fragmentation, precursor/product ions, and the corresponding IS were shown in Table 1. The selection of product ions was based on the intensity of the transitions and related to the unsaturation degree of the fatty acid chain. The instrument conditions of this study was similar to those of Leigh and MacMahon [24,27]. Moreover, we had a larger number of more MCPDE ISs than those available in other studies, which was useful to correct for the loss of analyte during sample preparation, injection, and ionization. The seven GEs congers of and 24 3- and 2-MCPDE congers were successfully quantified simultaneously in a single injection.

3.1.2. Optimized clean-up method for the edible oils

The extraction methods were performed in olive oil samples, and 20% MTBE/EtAc was chosen as the solvent according to the previous studies [19,23,31].

Table 3. Spike recovery rate for GEs and MCPDEs using the optimised multi-phase extraction and clean-up systems.

Analyte	Spike level (ng/mL)	Edible Oils			Low fat content food			Spike level (ng/mL)	High fat content food		
		Average recovery (%, n = 15)	RSD_{intra}[a] (%, n = 15)	RSD_{inter}[b] (%, n = 15)	Average recovery (%, n = 15)	RSD_{intra} (%, n = 15)	RSD_{inter} (%, n = 15)		Average recovery (%, n = 15)	RSD_{intra} (%, n = 15)	RSD_{inter} (%, n = 15)
Glycidyl esters											
La-GE	10	91.3	5.33	3.80	80.7	8.50	6.91	20	103	3.53	2.84
	50	93.8	1.51	3.21	112	0.16	0.48	50	106	2.10	2.70
	100	92.4	1.32	1.35	77.4	3.03	2.68	100	94.6	1.05	2.71
My-GE	10	88.6	5.16	8.04	82.3	11.6	11.6	20	110	3.26	5.05
	50	97.6	1.47	1.20	83.9	0.89	1.12	50	114	1.01	3.31
	100	99.4	4.04	2.88	101	2.23	2.98	100	104	2.14	3.84
Ln-GE	10	87.5	1.02	3.50	77.2	7.53	6.71	20	90.6	2.16	3.86
	50	90.1	1.20	3.12	82.1	3.00	5.14	50	96.1	2.92	2.15
	100	89.7	3.12	3.05	85.2	0.33	1.21	100	90.4	1.18	1.23
Li-GE	10	90.6	6.79	7.38	79.0	3.75	3.52	20	94.5	4.42	3.60
	50	95.2	2.65	4.20	108	3.69	3.92	50	103	2.94	4.35
	100	95.1	3.48	3.06	83.4	1.23	1.66	100	92.2	2.05	3.78
Pa-GE	10	87.8	4.11	6.55	77.1	5.06	6.12	20	102	3.42	4.11
	50	92.0	2.56	1.75	100	3.19	2.48	50	106	0.20	3.68
	100	93.3	0.05	1.94	83.9	3.74	3.68	100	95.9	2.61	1.99
Ol-GE	10	92.6	4.25	13.1	77.5	4.00	3.07	20	101	3.90	4.28
	50	82.4	7.17	10.3	97.0	0.88	0.88	50	106	3.85	4.32
	100	85.4	0.16	2.29	80.8	1.45	2.98	100	97.4	0.11	2.71
St-GE	10	95.8	0.90	6.74	82.1	10.2	1.83	20	95.2	5.14	3.50
	50	96.8	4.24	3.26	82.2	11.3	1.84	50	106	2.76	4.33
	100	99.3	2.87	2.57	85.1	1.84	3.62	100	94.6	1.79	3.14
Mono-MCPDEs											
1La	10	89.8	13.5	8.60	92.1	6.47	12.7	20	94.7	4.31	3.98
	50	98.0	2.59	4.76	100	2.30	4.56	50	103	0.36	1.78
	100	101	3.48	5.02	86.3	2.02	4.86	100	89.5	0.54	0.42
1My	10	93.1	12.7	10.8	107	2.25	3.44	20	96.9	0.74	9.45
	50	99.2	2.80	3.48	96.0	9.90	8.51	50	109	0.98	9.06
	100	109	7.88	4.63	101	0.26	5.37	100	100	1.01	0.82
1Ln	10	95.7	2.41	6.92	87.9	13.5	19.3	20	101	4.75	7.65
	50	104	1.73	4.52	88.0	1.00	0.90	50	111	3.67	2.81
	100	104	0.83	4.02	87.9	2.23	9.02	100	101	3.37	6.08
1Li	10	122	1.51	5.00	91.2	13.9	13.2	20	97.6	4.30	6.45
	50	123	1.50	6.59	86.0	0.67	0.96	50	110	6.98	6.25
	100	124	0.26	3.88	102	6.79	12.2	100	94.8	3.38	6.67
1Li2	10	89.4	6.09	4.54	82.6	8.50	10.5	20	95.0	9.68	9.89
	50	95.2	1.14	5.49	77.0	0.32	6.02	50	105	5.69	7.41
	100	89.9	3.97	4.32	82.7	2.31	6.26	100	94.2	0.17	5.29
1Pa/2Pa	10	103	1.08	4.44	84.3	12.3	11.5	20	91.9	1.22	5.46
	50	110	0.40	3.69	93.0	0.96	6.43	50	100	8.91	6.91
	100	112	0.80	4.14	90.8	2.68	4.27	100	88.5	5.73	3.81
1Pa2	10	88.8	8.86	6.58	88.4	8.57	11.2	20	98.7	5.36	3.41
	50	88.4	5.93	4.48	88.0	1.54	4.69	50	108	0.72	6.49
	100	89.9	6.38	6.69	84.0	0.17	5.98	100	92.5	1.36	5.80

1Ol	10	94.2	0.72	15.2	89.6	3.55	15.5	20	96.5	4.52	5.35
	50	99.9	0.31	8.90	94.0	4.06	8.94	50	106	1.15	3.24
	100	102	7.90	6.14	82.6	1.41	10.5	100	89.8	2.36	2.12
1Ol2/2Ol	10	80.5	0.17	7.29	90.8	1.63	2.56	20	96.4	5.78	8.09
	50	87.1	9.33	6.13	84.0	2.36	3.88	50	102	3.40	5.44
	100	85.8	1.43	4.82	81.0	2.59	5.00	100	94.4	1.04	4.88
1St	10	90.3	11.4	7.33	88.6	7.38	15.7	20	91.4	0.43	11.4
	50	106	8.16	7.21	101	3.46	1.81	50	106	0.32	9.55
	100	110	10.0	7.72	98.5	11.8	10.6	100	93.8	4.34	3.06
Di-MCPDEs											
Ln−Ln	10	88.7	6.59	6.04	85.1	5.23	4.21	20	88.7	2.12	6.01
	50	95.1	1.24	3.67	99.2	1.05	2.56	50	97.5	0.01	5.35
	100	95.9	4.84	3.68	89.4	0.76	7.56	100	90.3	0.61	1.52
Li−Li	10	93.6	1.07	18.7	95.4	1.69	6.74	20	116	4.80	3.23
	50	97.4	7.97	10.7	113	1.69	8.99	50	115	2.81	5.80
	100	83.0	0.82	3.21	97.4	2.79	6.93	100	115	0.15	3.84
2Li−Li	10	91.8	8.08	12.8	95.8	1.95	6.16	20	115	9.20	5.46
	50	118	11.0	6.55	91.0	1.76	4.38	50	120	4.02	3.30
	100	113	6.73	4.89	94.1	9.15	6.90	100	108	1.56	2.13
Pa−Li	10	106	11.3	7.74	91.0	6.15	8.96	20	70.2	2.33	4.89
	50	106	7.03	6.59	110	0.56	3.55	50	79.1	5.17	5.99
	100	104	0.08	5.80	88.5	4.31	3.12	100	81.5	2.61	2.90
Ol-Li	10	108	7.50	5.16	84.9	4.25	9.34	20	89.1	1.39	4.49
	50	102	9.51	9.19	94.0	3.55	4.19	50	99.0	1.85	1.99
	100	97.2	11.6	9.02	91.1	5.10	4.39	100	91.8	0.18	1.57
Pa−Pa	10	89.9	7.57	5.08	108	3.55	2.38	20	100	2.83	3.60
	50	97.3	10.0	9.01	89.9	3.49	3.19	50	109	3.17	2.89
	100	95.8	1.50	4.62	117	2.85	2.27	100	100	3.63	3.16
2Pa−Pa	10	84.8	0.62	6.51	94.7	2.16	1.39	20	79.5	3.75	5.01
	50	80.3	3.12	6.57	90.0	0.89	3.08	50	86.5	0.52	2.64
	100	90.0	2.59	2.79	98.6	1.82	8.00	100	81.6	2.62	5.69
Pa-Ol	10	110	17.4	14.5	88.3	4.05	3.55	20	85.0	3.32	4.02
	50	103	11.4	9.99	99.4	2.02	1.95	50	91.4	1.28	2.73
	100	97.8	2.58	2.45	92.6	0.11	4.75	100	84.3	0.69	0.84
Ol−Ol	10	91.4	13.1	9.98	85.1	1.65	3.61	20	71.5	4.57	3.88
	50	95.5	7.85	5.61	84.6	1.53	2.52	50	78.4	1.03	5.45
	100	99.4	2.11	3.24	92.0	1.00	5.27	100	72.0	1.34	4.37
2Pa-Ol	10	80.9	0.42	0.69	86.2	7.35	6.84	20	115	8.18	8.54
	50	85.7	2.35	13.9	93.4	0.78	2.31	50	115	0.62	7.18
	100	81.5	0.16	0.34	89.4	3.37	10.4	100	117	9.99	7.39
Pa-St	10	94.1	11.1	7.00	89.6	1.67	2.33	20	72.7	2.53	3.61
	50	98.9	6.43	5.28	95.0	1.90	1.38	50	82.6	0.07	5.48
	100	96.2	1.26	2.88	103	1.31	1.14	100	74.1	1.26	5.82
Ol-St	10	84.8	2.73	4.99	84.6	5.55	3.48	20	79.1	5.17	5.99
	50	95.0	3.08	4.19	98.8	1.76	1.95	50	81.5	2.61	2.90
	100	81.0	0.19	1.86	95.5	4.27	4.89	100	89.1	1.39	4.49

[a] RSD_{intra}, intra-day precision.
[b] RSD_{inter}, inter-day precision.

A multi-step purification approach using SPE was required to avoid potential contaminates in the extracts, including TAGs, DAGs, and MAGs. We tested several SPE cartridges types (e.g., Si, C_{18}, and HLB), purification solvents (e.g., ACN, EtAc, and their mixture), and different elution volumes in the clean-up procedure. Using the Si SPE cartridge (Fig. S5 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)), the poor elution (5.86–102% and 12.1–504% of spiked standards in mono- and/or di-MCPDEs, respectively) was occurred when applying both the combined method (eluting solvent: 2% Et2O/HEX) for the simultaneous determination of GEs, and 2- and 3-MCPDEs and the separated method published by the USFDA, indicating that the exiting interferences (e.g. TAGs, DAGs, and MAGs) were not removed. Nevertheless, replacing Et_2O with EtAc and HEX with n-pentane could increase the recoveries of spiked standards, especially for di-MCPDEs. The resulting recoveries of spiked and labelled internal standards were 87.9–97.3% and 84.6–96.4% for GEs, 84.7–118% and 98.4% for mono-MCPDEs, and 70.1–116% and 84.3–97.5% for di-MCPDEs, respectively. Using the C_{18} SPE cartridge (Fig. S6 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)), ACN provided a more effective removal of non-polar interferences (TAGs) and could elute GEs and mono-MCPDEs separately. EtAc provided a more effective removal of polar interferences (DAGs and MAGs) and could elute di-MCPDEs separately. However, 40% EtAc/ACN afforded the mutual elution of the GEs (83–99%), mono-MCPDEs (76–104%), and di-MCPDEs (72–117%) by the C_{18} SPE cartridge. In this study, only 7 mL 40% EtAc/ACN was used for elution with the C_{18} SPE cartridge, compared with the 14 mL ACN used in the USFDA method (MacMahon et al., 2013); our method, therefore, allows for reducing the usage of eluting solvent. However, all analytes were eluted simultaneously by one C_{18} SPE cartridge, whereas a Si SPE one was unable to achieve complete purification, resulting in ion suppression caused by co-eluted TAGs. To reduce matrix effects (Table S2 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)), we decided to adsorb first the polar matrix continually with two Si SPE cartridges and then the non-polar matrix with one C_{18} SPE cartridge. With this procedure, the sample clean-up was more effective when the two Si SPE was performed before the C_{18} SPE because 3-MCPD diesters are less polar than most components in the oil matrix, including most TAGs and all DAGs and MAGs. All recoveries of GEs and 2- and 3-MCPDEs were higher than 75% for spiked standards and higher than 85% (except Ol-St) for labelled internal standards.

3.1.3. Optimized clean-up method for the fat-containing foods

The chemical composition of fat-containing foods was significantly more complex (owing to the presence lipids, fats, carbohydrates, proteins, and nutritional additives) than that of the refined oils; therefore, fat extraction was necessary to reduce the matrix effect. To assess the efficiency of the fat extraction during method development, we compared three different solvents or buffers (EtAc, 40% EtAc/ACN saturated with HEX, and DCM/MeOH (2/1, v/v)) for fat extraction in processed foods, which were calculated using the nutrition labels on each infant formula sample (Table S3 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)). EtAc was the most effective, with fat recoveries of 89%, in agreement with the results of Leigh and MacMahon [27]. Because several SPE cartridges had been used in previous sample clean-ups, a different clean-up procedure based on SPE was applied to this sample and validated accordingly. A two-stage SPE clean-up procedure with appropriate eluting solvents was employed for both the low-fat (containing less than 10% fat, e.g. rice cereal) and high-fat (containing more than 10% fat, e.g., infant formula) content foods using Si, C_{18}, and HLB SPE cartridges [13,20,23,27]. The average recoveries obtained with C_{18}–Si, Si–C_{18}, or Si–NH_2 SPE cartridges showed poor effectiveness for mono- and di-MCPDEs; in particular, the recovery of di-MCPDEs exhibited evident matrix effects (Table S4 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)). Subsequently, the coupled Si and HLB cartridges used for the final cleanup procedure in the two-stage SPE method provided satisfactory recoveries of spiked standards (80.6–93.0% of GEs, 105–127% of mono-MCPDEs and 87.9–105% of di-MCPDEs) and internal standards (all labelled compounds >78%). This first elution step based on Si SPE cartridge achieved an almost complete sample clean-up because the chosen conditions were such that the GEs or MCPDEs were eluted, whereas part of the polar matrix and fats remained in the cartridge. First, 40% DCM/HEX was used to collect the di-MCPDEs and separate the interferences (e.g. TAGs) with the similar polarities. Then, 20% EtAc/HEX was then used to collect the GEs, mono-MCPDEs, and potential interferences

(e.g. TAGs and fatty acids). The extract was subsequently introduced into the HLB SPE cartridge and purified with ACN to enrich the GEs and mono-MCPDEs.

A three-stage SPE clean-up procedure and multistep washing process were applied for the high-fat-content foods building on the two-stage SPE clean-up procedure used for the low-fat-content foods. As shown in Table S5 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal), poor recoveries of mono- and di-MCPDEs were achieved by all the combinations of the selected SPE cartridges (C_{18}–Si–Si, Si–Si–C_{18}, and Si–HLB–Si) owing to the low recoveries of internal standards. We hypothesised that the high-fat-content foods may contain more TAGs and free fatty acids than expected, resulting in the suppression of MCPD diester recovery. The Si–Si–HLB cartridge combination provided effective recoveries of both spiked (91.5–106% of GEs, 82.6–117% of mono-MCPDEs and 74.3–120% of di-MCPDEs) and internal standards (all labelled compounds >85%) and was selected as the final clean-up system for high-fat-content foods. A 2-g Si SPE cartridge was first eluted with the optimized volume (9 mL) of 2% Et_2O/HEX to remove the interferences of lowest polarity (e.g. fatty acids) and then discarded. An additional 16 mL of 2% Et_2O/HEX (F1) was used to collect the di-MCPDEs and the following 9 mL of 20% EA/HEX (F2) was used to collect the GEs and mono-MCPDEs. In the second stage, the F2 was further introduced into a 1-g Si SPE cartridge and eluted with 6 mL 20% EA/HEX (F3) to remove the interferences of similar polarity (e.g. DAGs and MAGs). In the third stage, F2 was finally purified using a 500-mg HLB SPE cartridge (retaining GEs and mono-MCPDEs) and eluted with 6 mL ACN to reduce the interferences of lower polarity (e.g. TAGs). The published direct LC-MS/MS methods from MacMahon, Begley, and Diachenko [19], MacMahon et al. [32] and Leigh and MacMahon [27] required at least two separated pre-treatments and at least two injections to obtained GEs and 2- and 3-MCPDEs from edible oil or infant formula. Instead, our optimized clean-up method using a single validated procedure and a single injection provide the effective, robust, and highly accurate determination of GEs and MCPDEs in high-fat-content food.

3.2. Method performance

3.2.1. Linearity

The internal calibration curves were established by the least-squares linear regression with the weighting factor of 1/x using at least six points of concentration levels (ranged from 1 to 1000 ng/mL for GEs and 1–750 ng/mL for mono- and di-MCPDEs, Table 2) and comprised across the entire range of concentrations in oil, low-fat-, and high-fat-content food samples. Linearity was verified in a wide working range for all samples with correlation coefficients generally higher than 0.997. The calibration curves and linearity in this study were comparable to those of other studies. Moreover, none of the targeted analytes showed accuracy greater than the recovery specification of 20% for each point of the calibration curve. The RSDs of slopes for different regression lines were found to not exceed 15% relative errors, which validated the results (Table 2).

3.2.2. Sensitivity

In this study, two types of limits were evaluated to determine instrument sensitivity (instrument detection limit [IDL]) and method sensitivity (limit of detection [LOD] and limit of quantification [LOQ]). The IDLs were calculated from three times the standard deviation of replicate analysis of seven solvent blanks with the 99% confidence interval. The LODs were estimated by LC–ESI–MS/MS in the MRM mode using $LOD = 3.3 \times s/m$ (where s is the residual standard deviation of a regression line and m is the slope of the calibration curve). The LOQ for each analyte was calculated as $LOQ = 10 \times s/m$. The estimated values for LOD and LOQ, together with repeatability, were provided in Table 2. The LODs ranged from 0.26 to 0.39 ng/mL of GEs, 0.28–0.67 ng/mL of mono-MCPDEs and 0.15–0.45 ng/mL of di-MCPDEs. For oil, the LOQs ranged 3.46–8.70 ng/g of GEs, 4.37–15.2 ng/g of mono-MCPDEs and 2.07–8.81 ng/g of di-MCPDEs. For low fat content food, the LOQs ranged 10.8–27.1 ng/g of GEs, 14.5–47.3 ng/g of mono-MCPDEs and 10.4–27.5 ng/g of di-MCPDEs. For high fat content food, the LOQs ranged 21.6–54.3 ng/g of GEs, 27.3–69.3 ng/g of mono-MCPDEs, and 12.9–55.0 ng/g of di-MCPDEs. Given the above purification procedures, these LOD and LOQ values were considered adequate and were generally similar to or better than those reported in previous studies using the same detection technique according to the methods developed by the USFDA [19,27,31], Japan [23], or Canada [18].

3.3. Precision (repeatability), accuracy (recovery), and robustness

Recoveries and precision were tested during the entire procedure by analysis of the targeted GEs, mono-MCPDEs and di-MCPDEs in three main categories of food matrices. Recoveries adjusted with an

Table 4. Occurrences of GEs and MCPDEs in selected food samples among 12 categories.

Food category	Average (range) (ng/g)				
	Edible oils (n = 19)	Infant formulas (n = 10)	Cereals (n = 3)	Condiment (n = 3)	Meat products (n = 3)
Glycidyl esters					
La-GE	15.1 (ND[a] -<8.00[b])	35.4 (ND-71.1)	32.7 (ND-56.8)	78.1 (ND-140)	38.4 (ND-73.7)
My-GE	59.7 (ND-147)	ND	26.6 (ND-32.1)	64.8 (ND-147)	ND
Ln-GE	26.3 (ND-55.8)	ND	79.5 (ND-204)	84.4 (ND-219)	196 (ND-434)
Li-GE	1712 (603—3912)	24.8 (<50.0)	53.5 (ND-68.7)	120 (<25.0—177)	49.6 (<24.6—113)
Pa-GE	3965 (1395—1410)	ND	109 (ND-202)	64.3 (ND-101)	111 (ND-251)
Ol-GE	6108 (2425—13,486)	32.3 (ND-<50.0)	124 (ND-332)	39.0 (ND-63.3)	ND
St-GE	439 (171—939)	37.2 (ND-118)	22.6 (ND-<25.0)	49 (ND-74.0)	29 (ND-50.9)
Mono-MCPDEs					
1La	ND	<24.6	ND	17.2 (ND-<25.0)	17.3 (ND-<25.0)
1My	ND	ND	ND	36.1 (ND-59.8)	ND
1Ln	ND	ND	ND	ND	ND
1Li	309 (221—393)	ND	25.2 (ND-34.6)	84.4 (80.5—98.6)	22.0 (ND-<25.0)
1Pa/2Pa	430 (245—632)	59.3 (ND-74.4)	73.6 (ND-104)	87.5 (ND-120)	83 (ND-140)
1Ol	657 (426—808)	ND	45.0 (ND-47.7)	119 (ND-206)	40.2 (ND-64.1)
1Ol2/2Ol	116 (68.4—200)	ND	24.0 (ND-25.5)	ND	ND
1St	78.0 (ND-122)	103 (ND-165)	ND	155 (ND-309)	95.3 (ND-199)
1Li2	76.1 (51.6—105)	<50.0	13.7 (ND-<25.0)	ND	ND
1Pa2	137 (112—170)	39.9 (ND-63.9)	30.5 (ND-35.8)	38.0 (ND-58.8)	44.4 (ND-77.4)
Di-MCPDEs					
Ln-Ln	ND	ND	ND	ND	ND
Li-Li	408 (298—596)	19.1 (ND-<50.0)	16.3 (ND-<25.0)	105 (ND-283)	ND
Pa-Li	1570 (1117—2280)	ND	96.7 (ND-176)	37.4 (ND-58.3)	36.4 (ND-55.4)
Ol-Li	5537 (3766—8201)	56.2 (ND-80.1)	64.5 (ND-94.1)	177 (ND-351)	54.5 (<50.0—114)
Pa-Pa	1138 (800-1268)	ND	133 (ND-247)	150 (ND-238)	58.2 (ND-143)
Pa-Ol	8196 (5972—12019)	18.7 (ND-<50.0)	293 (<12.3—842)	63.8 (<25.0—142)	12.6 (ND-<50.0)
Ol-Ol	2124 (1482—3061)	33.9, <50—52.3	99.6 (ND-274)	46 (<25.0—63.0)	33.4 (<50.0—50.8)
Pa-St	149 (105—180)	ND	29.4 (<25.0—38.9)	94.7 (ND-137)	58.6 (<50.0—126)
Ol-St	481 (380—684)	24.8, ND-<50.0	103 (<25.0—199)	177 (<25.0—1005)	354 (ND-716)
2Li-Li	226 (183—273)	ND	42.7 (ND-93.3)	46.6 (ND-93.4)	ND
2Pa-Pa	673 (494—803)	ND	46.6 (ND-90.0)	87.8 (ND-149)	ND
2Pa-Ol	2537 (1227—3760)	ND	113 (ND-322)	70.3 (<25.0—122)	15.1 (ND-<50)

Food category	Average (range) (ng/g)						
	Seafood products (n = 3)	Snacks (n = 3)	Composite foods (n = 3)	Dairy products (n = 3)	Beverage (n = 3)	Vegetable products (n = 3)	Bean products (n = 3)
Glycidyl esters							
La-GE	60.6 (ND-141)	129 (ND-283)	91.9 (ND-235)	ND	81.3 (ND-162)	43.7 (ND-55.4)	ND
My-GE	44.6 (ND-86.2)	88.3 (ND-283)	58.3 (69.8—293)	ND	ND	39.7 (ND-70.2)	ND
Ln-GE	358 (ND-1039)	742 (ND-283)	316 (ND-913)	ND	228 (ND-347)	177 (ND-285)	ND
Li-GE	116 (<25.0—298)	629 (499—702)	173 (129—202)	15.4 (ND-<50.0)	87.0 (ND-166)	47.0 (ND-66.8)	85.3 (<25.0—162)
Pa-GE	197 (32.8—117)	1311 (961—1847)	432 (369—492)	20.6 (ND-<50.0)	125 (ND-231)	196 (ND-321)	103 (<25.0—221)

Ol-GE	86.7 (31.1−116)	1619 (ND-3032)	398 (ND-679)	ND	ND	ND	115 (ND-247)
St-GE	49 (ND-75.0)	185 (140−258)	144 (50.1−229)	20.3 (ND-<50.0)	17.2 (ND-<25.0)	18.4 (ND-<25.0)	20.3 (ND-<25.0)
Mono-MCPDEs							
1La	14.8 (ND-<24.8)	ND	ND	17.3 (ND-<50.0)	15.0 (ND-<25.0)	15.0 (ND-<25.0)	17.2 (ND-<25.0)
1My	24.3 (ND-24.4)	3.8 (ND-61.7)	ND	ND	32.3 (ND-62.5)	ND	ND
1Ln	ND	ND	ND	ND	ND	ND	ND
1Li	142 (102−193)	118 (54.6−192)	46.4 (24.8−63.9)	ND	38.8 (ND-71.0)	27.6 (ND-33.1)	ND
1Pa/2Pa	216 (116−198)	152 (142−171)	81.8 (ND-107)	54.9 (ND-96.0)	56.1 (ND-117)	ND	ND
1Ol	257 (158−334)	215 (124−325)	84.8 (ND-143)	ND	59.9 (ND-113)	47.9 (ND-64.7)	ND
1Ol2/2Ol	56.8 (<25.0−119)	133 (67.1−210)	44.0 (ND-58.1)	ND	48.2 (ND-112)	ND	ND
1St	46.1 (ND-45.1)	73.5 (ND-127)	ND	62.7 (ND-94.4)	ND	ND	ND
1Li2	33.1 (<25.0−63.6)	21.3 (ND-<25.0)	17.7 (ND-<25.0)	ND	13.7 (ND-<25.0)	13.7 (ND-<25.0)	ND
1Pa2	60.0 (ND-118)	51.7 (ND-66.3)	ND	37.8 (ND-57.5)	34.8 (ND-62.4)	ND	ND
Di-MCPDEs							
Ln-Ln	ND	ND	ND	ND	ND	ND	ND
Li-Li	28.6 (ND-62.3)	52.5 (ND-79.8)	82.7 (ND-135)	36.9 (<50.0−61.0)	ND	ND	152 (ND-335)
Pa-Li	164 (73.9−299)	236 (156−311)	327 (264−374)	198 (ND-432)	37.8 (ND-59.6)	80.8 (43.5−102)	54.9 (ND-54.9)
Ol-Li	426 (130−992)	162 (111−256)	347 (163−704)	ND	54.7 (ND-124)	87.2 (<25.0−212)	304 (<25.0−483)
Pa-Pa	191 (102−242)	425 (346−479)	680 (492−981)	ND	86.5 (ND-227)	201 (59.7−353)	32.9 (ND-67.0)
Pa-Ol	635 (ND-1445)	984 (<25.0−1691)	1292 (ND-1948)	18.6 (ND-<50.0)	17.6 (ND-<25.0)	8.31 (ND-<12.2)	143 (<25.0−378)
Ol-Ol	231 (32.8−117)	306 (84.2−330)	347 (81.2−489)	ND	26.5 (<25.0−54.6)	50.2 (<25.0−113)	72.4 (<25.0−139)
Pa-St	81.7 (32.8−117)	164 (<24.6−401)	111 (69.8−198)	15.4 (ND-<50.0)	81.5 (50.1−108)	48.6 (<25.0−62.7)	ND
Ol-St	336 (70.0−818)	1076 (111−2960)	375 (145−835)	ND	357 (<25.0−756)	268 (ND-28.7)	24.7 (<25.0)
2Li-Li	ND	ND	ND	146 (ND-280)	ND	ND	61.4 (ND-138)
2Pa-Pa	88.1 (32.8−117)	163 (ND-283)	204 (69.8−293)	ND	ND	24.6 (ND-28.7)	34.6 (ND-59.1)
2Pa-Ol	278 (ND-317)	386 (<24.9−714)	474 (ND-709)	ND	13.4 (ND-<25.0)	29.7 (ND-73.5)	76.2 (ND-194)

[a] "ND" indicated the detected concentration of the food sample below the LOQ and the calculation assigned the half value of LOQ.
[b] "< the lowest concentration of calculation curve" indicated the detected concentration of the food sample below the lowest concentration of calculation curve and above the LOQ and the calculation assigned the half value of the lowest concentration of calculation curve.

IS were determined by comparing the outcomes from the spiked test samples with those of the standard solutions (considered 100% recovery) at high (100 ng/mL), medium (50 ng/mL), and low (10 ng/mL) levels minus the amount found in the (unspiked) quality controls (QCs). The relative recoveries of GEs (82.4–99.3%, 77.1–112% and 90.4–114%), mono-MCPDEs (80.5–124%, 77.0–107% and 88.5–111%) and di-MCPDEs (80.9–118%, 84.6–117% and 70.2–120%) for edible oil (olive oil), low fat content sample (cereal) and high fat content sample (infant formula), respectively, ranged between 70 and 130% and were overall satisfactory. Our recovery data of different spiked samples were compared to those reported in previous studies pertaining to edible oil (MacMahon et al.: 98.0–113% for GEs, 76.8–115% for mono-MCPDEs, and 87.7–117% for di-MCPDEs; Yamazaki et al.: 93–117% for mono-MCPDEs and 66–136% for di-MPDE) [19,23,31] and those of spiked infant formula samples (Leigh et al.: 92.9–107% for GEs, 93.1–106% for mono-MCPDEs and 88.7–108% for di-MCPDEs) [27]. The recoveries of all the investigated congeners of GEs, mono-MCPDEs, and di-MCPDEs had an RSD lower than 20% indicating good method precision.

Intraday precision was assayed by analysing three categories of the selected sample matrices on the same day, whereas interday precision was estimated over 7 d. The respective intraday and interday RSDs of spiked olive oil samples were in the range of 0.05–7.17% and 1.20–10.3% for GEs, 0.26–11.4% and 3.48–15.2% for mono-MCPDEs, and 0.08–17.4% and 0.34–18.7% for di-MCPDEs; those of spiked cereal samples were 0.16–11.6% and 0.48–11.6% for GEs, 0.17–13.9 and 0.90–19.3% for mono-MCPDEs, and 0.11–9.15% and 1.14–10.4% for di-MCPDEs; those of spiked infant formula samples were 0.11–5.14% and 1.23–5.05% for GEs, 0.17–9.68% and 0.42–9.89% for mono-MCPDEs, and 0.01–9.20% and 0.84–8.54% for di-MCPDEs. Our relative errors were similar or lower than those obtained by past studies for edible oil (MacMahon et al.: 1.3–9.7% for GEs, 5.00–11.9% for mono-MCPDEs and 1.8–16.0% for di-MCPDEs; Yamazaki et al.: 10.6–25.5% for mono-MCPDEs and 5.50–23.5% for di-MCPDEs) [19,23,31] and those of infant formula (Leigh et al.: 1.0–6.9% for GEs, 1.5–8.0% for mono-MCPDEs and 1.1–9.5% for di-MCPDEs) [27]. One instrument blank and two procedural blanks were analysed as part of the OC in each batch, and all results were below half the LOQ. We could conclude that the developed method can fulfil the accuracy precision, and sensitivity requirements for analysing our targeted GEs, mono-MCPDEs and di-MCPDEs in three food categories.

The design variables parameter settings of the center point reflect those of the method robustness, which should be validated (Table S6 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)). The results obtained in the twelve runs to the standard solution and food samples were demonstrated. The calculation of the standardized effects [33] of the five factors and their interpretations are given in Fig. S7 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal). It can be seen that five factors are not significant for the established experimental dominions. So, these results prove that these methods are robust for the selected food samples among 12 categories.

3.4. Applicability in 12 categories of food samples

We employed the newly validated and reliable quantitation method to target GEs and mono- and di-MCPDEs in 59 food samples pertaining 12 categories (Table 4), including edible oils, infant formulas, cereals, condiments, meat products, seafood products, snacks, composite foods (e.g. fried rice, pizza, burgers, instant noodles and soup), dairy products, beverages, vegetable products and bean products, purchased from Taiwan's primary supermarkets to assess the performance and applicability of the method. One blank control and two QCs were included in each analytic batch. The recoveries of targeted GEs, mono- and di-MCPDEs for two spiked QC samples were 73–124% and for labelled internal standards were 53–101% (except Ol-St <10% in edible oils). The relative percentage differences for the two spiked QC samples in each analytic batch were all <15%. For GEs, mono-MCPDEs and di-MCPDEs, at least one compound was detected in all the samples tested (100%). The highest concentration of GEs found was 13486 ng/g (Ol-GE in palm oil), which was consistent with the data reported by MacMahon et al. [2], but higher than the values found by Blumhorst, Venkitasubramanian, and Collison [34]. The predominant GEs found in the 12 categories of food samples were Ol–GE (average concentration: 39.0–6108 ng/g), followed by Li–GE (15.4–1712 ng/g). The concentrations of La–GE, Li–GE, Ol–GE and St–GE in infant formulas were similar to those obtained by Leigh and MacMahon [24]. In addition to edible oils, high concentrations of seven GEs were present in snack samples (88.3–1619 ng/g) and composite

foods (58.3–432 ng/g). At least one congener of mono-MCPDEs was found in all samples tested (100%), and the highest concentration (808 ng/g) of 1Ol was found in palm oil. For di-MCPDEs, at least one compound was detected in all the samples tested (100%). The highest concentration of di-MCPDEs found was 12019 ng/g of Pa–Ol in palm oil. The predominant mono-MCPDEs found in the 59 samples were 1Ol (ND–657 ng/g), and 1Li (ND–309 ng/g), whereas the predominant di-MCPDEs were Pa–Ol (12.6–8196 ng/g), and Ol–Li (ND–5537 ng/g). The characteristics of mono- and di-esters of 3-MCPDEs and 2-MCPDEs in edible oils and infant formulas were in agreement with the findings reported by Leigh and MacMahon [24] and Yamazaki et al. [23], although Ln–Ln was not found in any of the food samples in the current study. The concentration of 2-/3-MCPDEs and GEs in refined oil were significantly higher than non-refined oil, especially in palm oil. The formation of GEs and 3-MCPDEs can occur at high-temperature treatment (>200 °C) during deodorisation of edible oils [4–8]. In addition, higher concentrations of seven GEs and 24 MCPDEs were present in snack samples and composite foods that labeled containing palm oil than those not contained. Palm oil, as a widely used ingredient, might be the main contributor of these food-borne process contaminants in the selected processed food products. Further studies are necessary in order to explore the formation mechanism and the reduction strategy in the production of refined edible oil and the processed foods.

4. Conclusions

This is the first published report on a new and direct LC-MS/MS method for simultaneously quantifying seven GEs and 24 2- and 3-MCPDE congeners in various processed foods without ester cleavage and derivatization. The combinations of labelled internal standards, effective extraction and straightforward and robust multi-phase SPE clean-up systems allowed for the effective analysis of numerous samples in different matrices in a single validated procedure with high accuracy and precision. The method was developed and validated in our laboratory; its applicability was tested using a variety of 12 categories of processed foods. Our optimized methods not only significantly reduced time and cost by approximately 30–60% for each sample, thereby providing substantially better results compared to other existing approaches (Table S7 (https://www.jfda-online.com/cgi/editor.cgi?article=3442&window=additional_files&context=journal)). Additional processed foods from the commercial supermarkets should be characterized to identify the possible contaminants and conduct a health risk assessment of the dietary intake of GEs and 2- and 3-MCPDEs.

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