Supplementary Materials

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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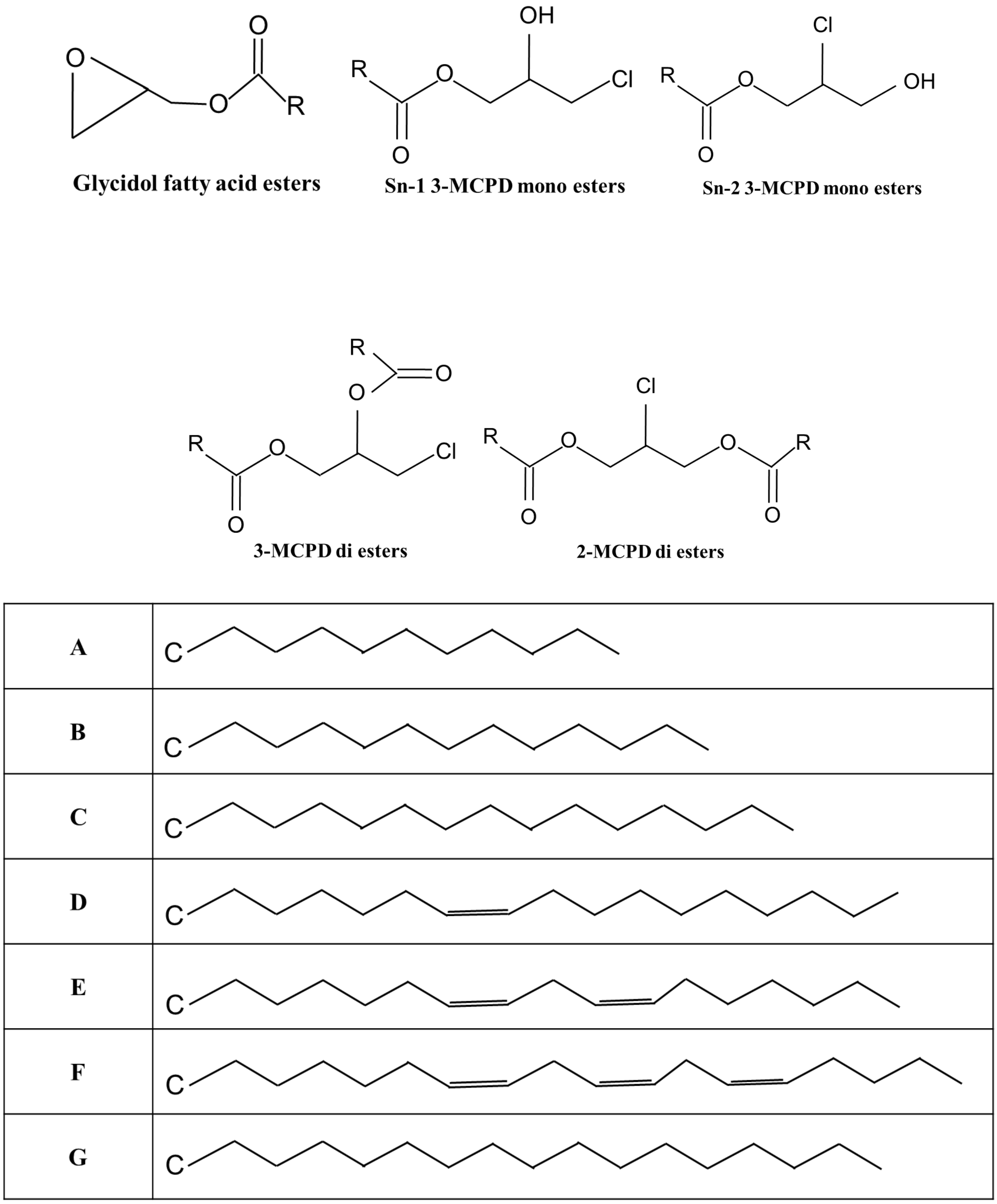

Figure S1. Structures of fatty acid esters of 3-chloro-1,2-propanediol (3-MCPDE), 2-chloro-1,3-propanediol (2-MCPDE), and glycidol (GE).

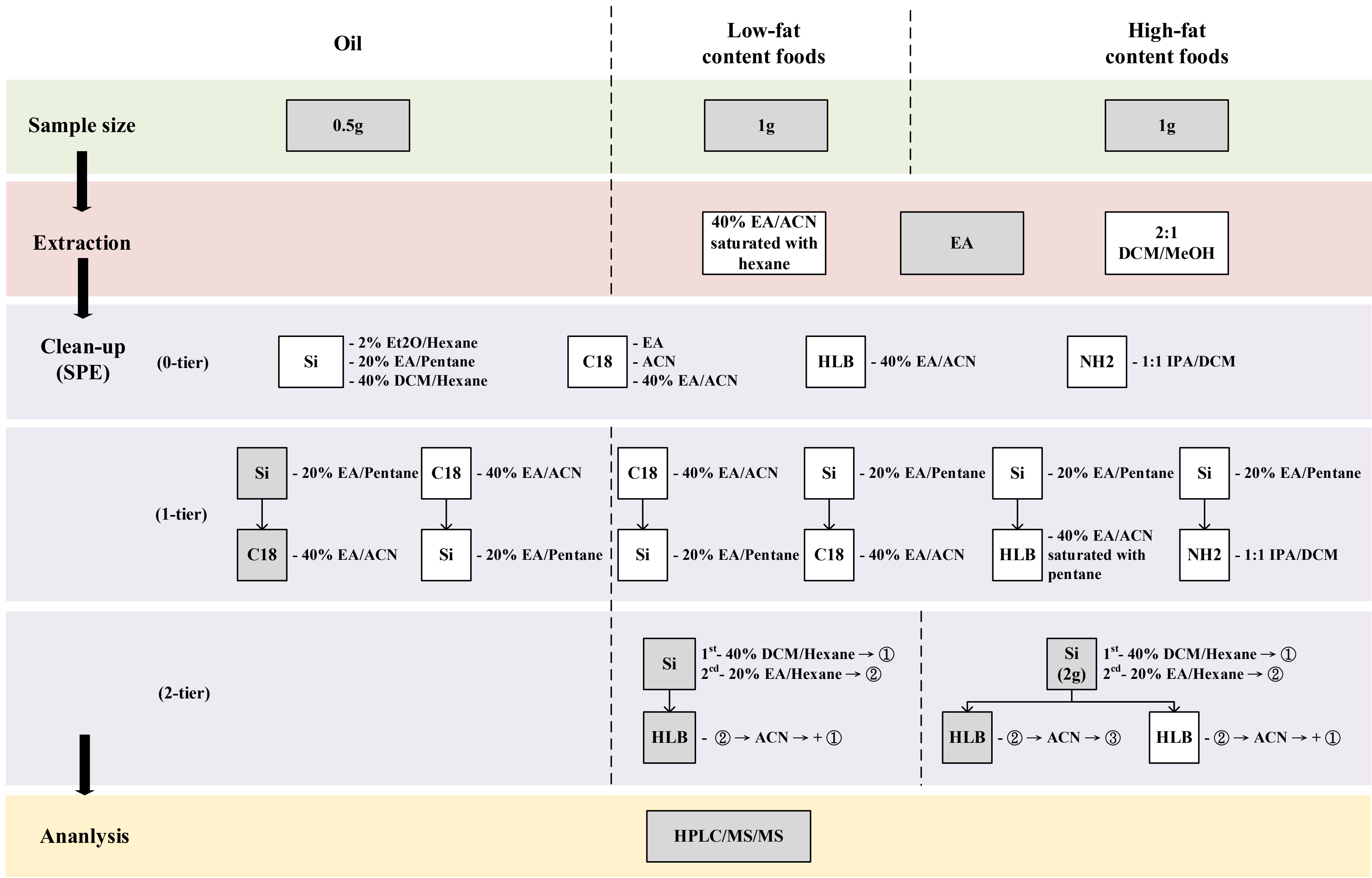

Figure S2. Pre-treatment tests for GEs and 3- and 2-MCPD mono- and di-esters in oils, low-, and high-fat-content foods.

<table>
<tr><th>Test</th><th>Gradient</th><th>TIC</th></tr>
<tr><td>1</td><td>

Time	A(%)	B(%)	Flow rate
0.00	98	2	0.200
3.00	98	2	0.200
3.10	83	17	0.200
10.10	73	27	0.200
14.20	73	27	0.200
14.50	2	98	0.250
20.50	2	98	0.250
20.75	98	2	0.250
26.75	98	2	0.250
27.00	98	2	0.200

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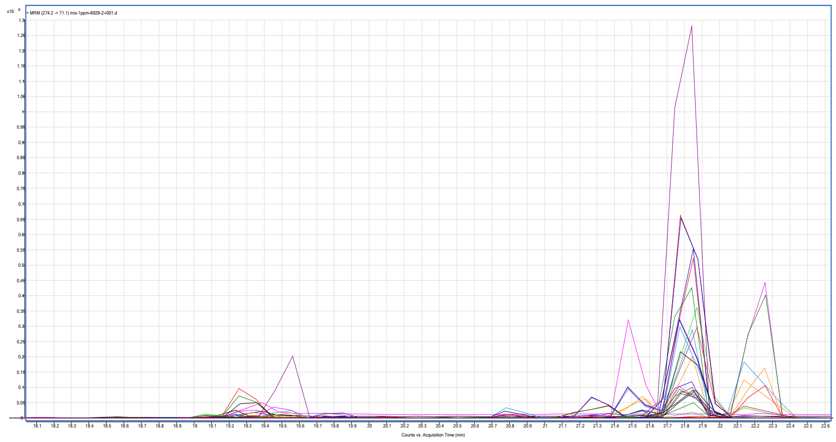

water phase (2 %)

</td></tr>
<tr><td>2</td><td>

Time	A(%)	B(%)	Flow rate
0.00	100	0	0.200
2.10	75	25	0.200
17.10	75	25	0.200
35.0	35	65	0.200
36.0	35	65	0.250
41.0	25	75	0.250
58.0	0	100	0.250
69.0	0	100	0.250

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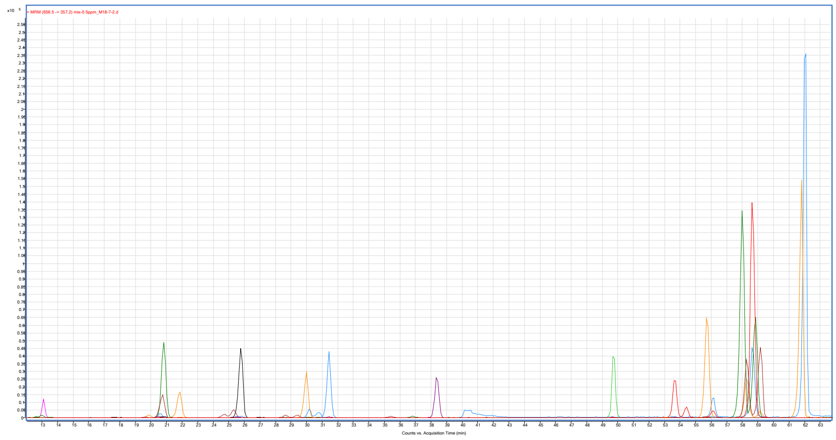

water phase (25 %)

</td></tr>
<tr><td>3</td><td>

Time	A(%)	B(%)	Flow rate
0.00	98	2	0.200
2.00	98	2	0.200
2.10	68	32	0.200
15.00	68	32	0.200
30.20	33	67	0.200
30.5	2	98	0.250
40.50	2	98	0.250
40.75	98	2	0.250
46.75	98	2	0.250
47.00	98	2	0.200

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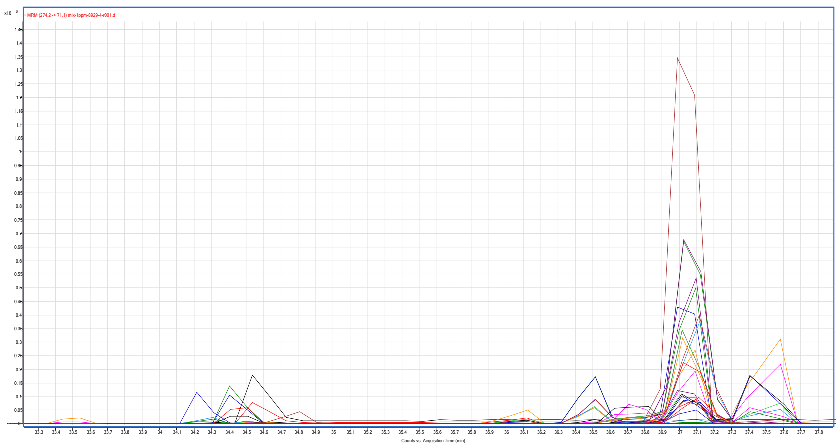

water phase (50 %)

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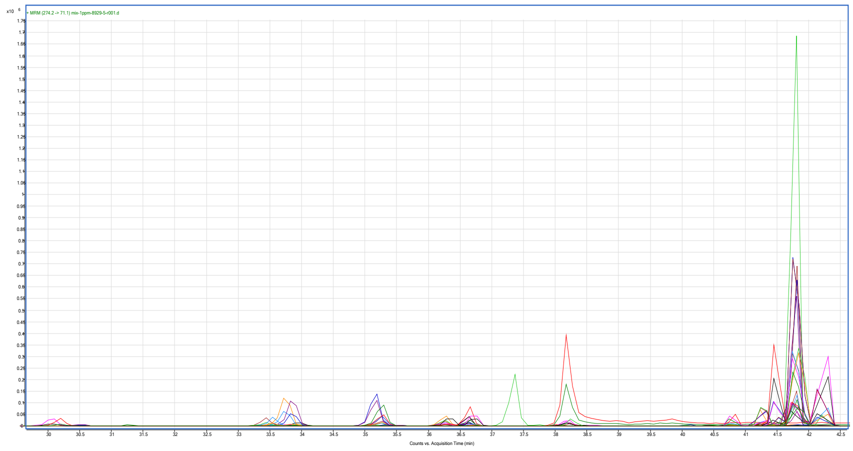
4	Time	A(%)	B(%)	Flow rate	 water phase (100 %)
	0.00	98	2	0.200	
	2.00	98	2	0.200	
	2.10	68	32	0.200	
	20.00	48	52	0.200	
	36.00	15	85	0.200	
	36.25	2	98	0.250	
	42.50	2	98	0.250	
	42.75	98	2	0.250	
	48.75	98	2	0.200	
	49	98	2	0.250	

Figure S3. Optimisation for gradient programs using different proportions of water phase.

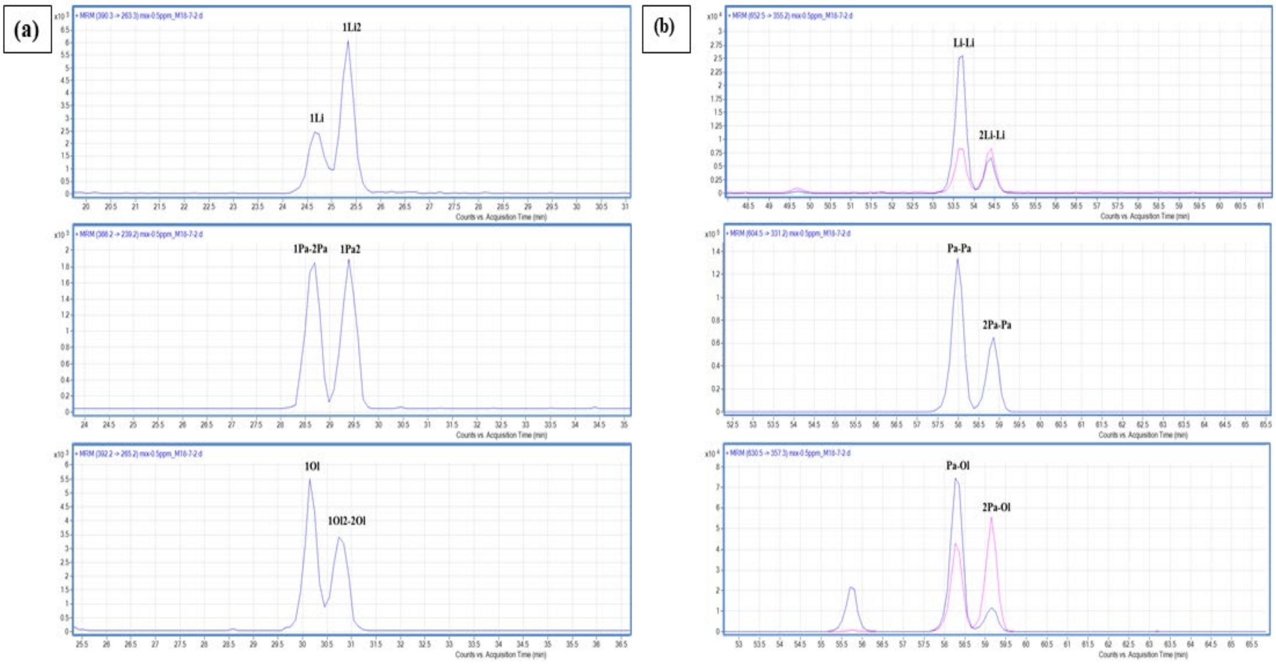


Figure S4. Efficient separation of (a) 2-MCPD and sn1/sn2 3-MCPD Li, Pa, and Ol mono-esters; (b) 2-MCPD and 3-MCPD Li–Li, Pa–Pa and Pa–Ol di-esters.

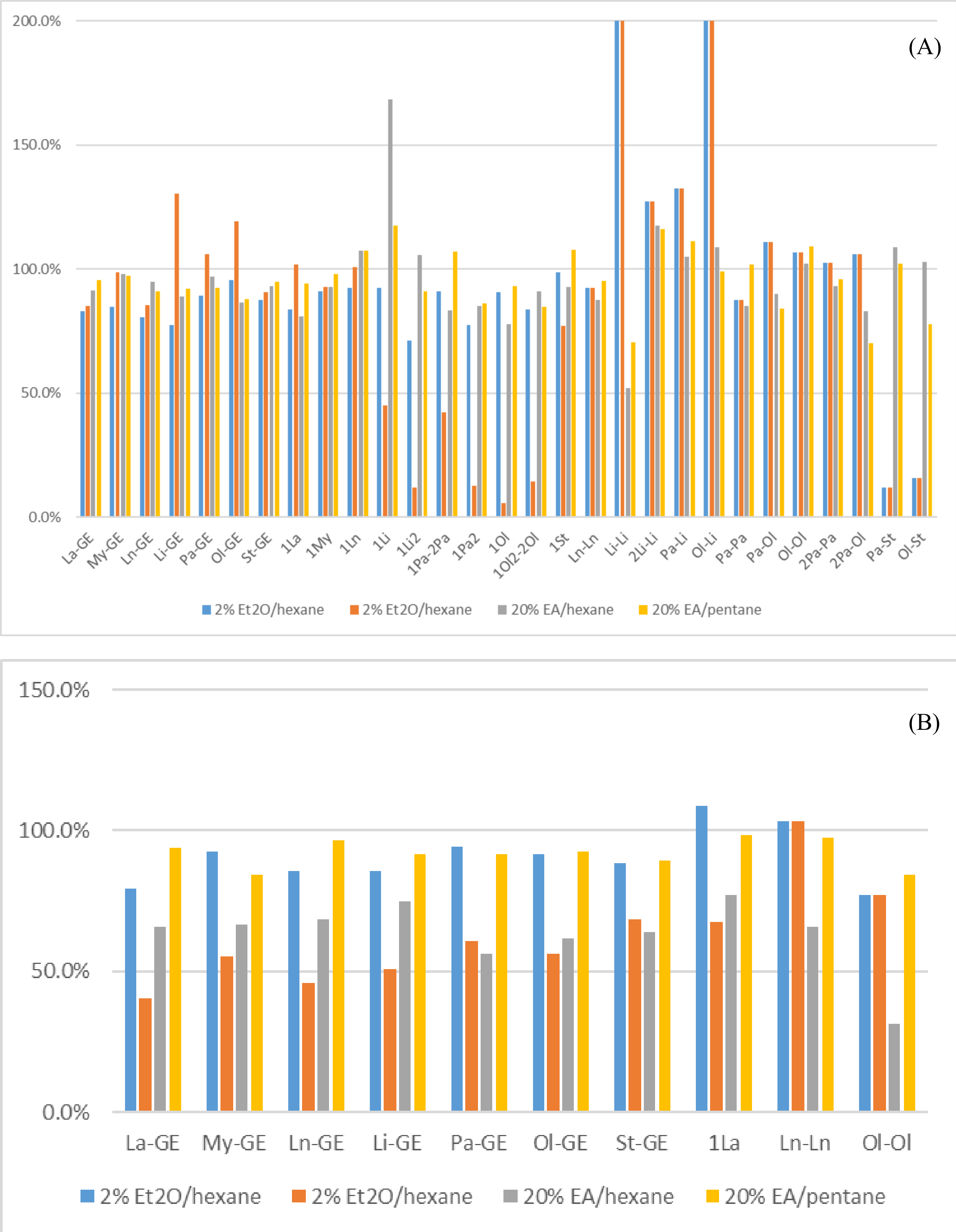


Figure S5. Elution solvent tests for a Si SPE cartridge using an edible oil sample: (A) recoveries of spiked standards (50 ng/mL); (B) recoveries of labelled standards.

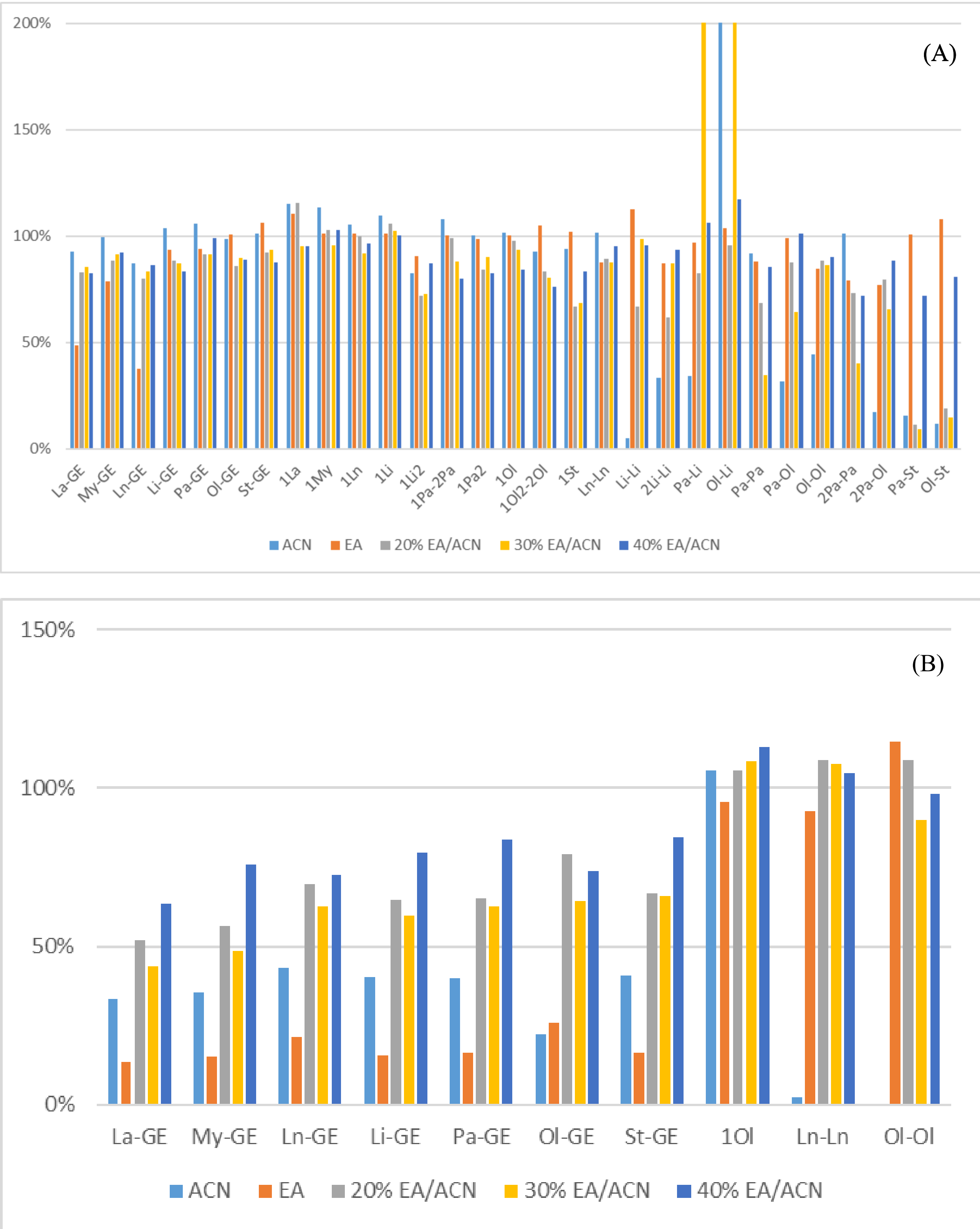


Figure S6. Elution solvent tests for a C_{18} SPE cartridge using an edible oil sample: (A) recoveries of spiked standards (50 ng/mL); (B) recoveries of labelled standards.

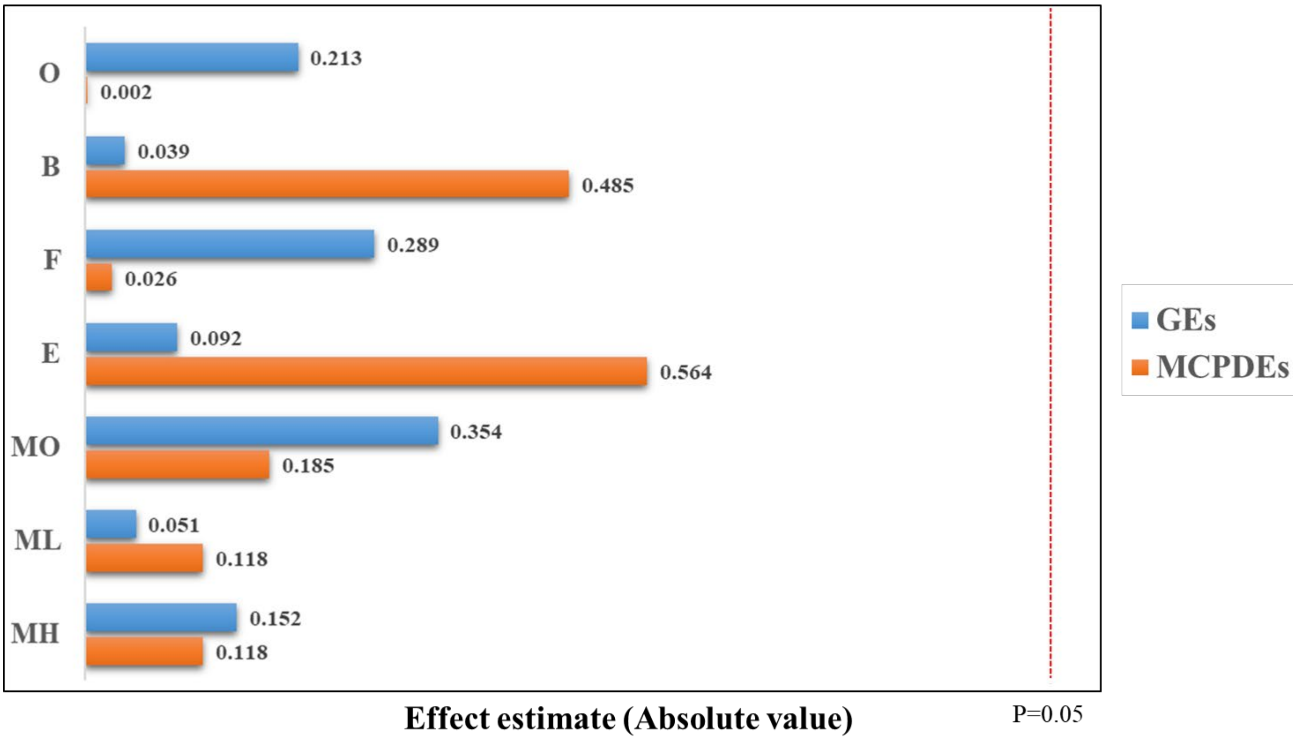


Figure S7 Factor effects of method robustness. The bar indicates the magnitude and the bias of the effects. The effect is the change in response due to the change of a factor. It is the average response at the high level minus the average response at the spiked level (50 ng/mL). There are both main effects and interaction effects.

Table S1. Structures of fatty acid esters of 3-chloro-1,2- propanediol (3-MCPDE), 2-chloro-1,3-propanediol (2-MCPDE), and glycidol (GEs).

Glycidyl esters

Compound name	**Abbreviation**	**R =**
Glycidyl laurate	La-GE	A
Glycidyl Myristate	My-GE	B
Glycidyl Palmitate	Pa-GE	C
Glycidyl Oleate	Ol-GE	D
Glycidyl Linoleate	Li-GE	E
Glycidyl Linolenate	Ln-GE	F
Glycidyl Stearate	St-GE	G

3-MCPDE

Compound name	**Abbreviation**	**R =**	
3-MCPD mono-esters			
1-Lauroyl-3-chloropropanediol	1-La	A	
1-Myristoyl-3-chloropropanediol	1-My	B	
1-Palmitoyl-3-chloropropanediol	1-Pa	C	
1-Oleoyl-3-chloropropanediol	1-Ol	D	
1-Linoleoyl-3-chloropropanediol	1-Li	E	
1-Linolenoyl-3-chloropropanediol	1-Ln	F	
1-Stearoyl-3-chloropropanediol	1-St	G	
2-Palmitoyl-3-chloropropanediol	2-Pa	C	
2-Oleoyl-3-chloropropanediol	2-Ol	D	
3-MCPD di-esters			
Palmitoyl-linoleoyl-3-chloropropanediol	Pa-Li	C	E
Oleoyl-linoleoyl-3-chloropropanediol	Ol-Li	D	E
Palmitoyl-oleoyl-3-chloropropanediol	Pa-Ol	C	D
Palmitoyl-stearoyl-3-chloropropanediol	Pa-St	C	G
Oleoyl-stearoyl-3-chloropropanediol	Ol-St	D	G
1,2-Bis-palmitoyl-3-chloropropanediol	Pa-Pa	C	C
1,2-Bis-linoleoyl-3-chloropropanediol	Li-Li	E	E
1,2-Bis-oleoyl-3-chloropropanediol	Ol-Ol	D	D
1,2-Bis-linolenoyl-3-chloropropanediol	Ln-Ln	F	F

2-MCPDE

Compound name	**Abbreviation**	**R =**	
2-MCPD mono-esters			
1-Palmitoyl-2-chloropropanediol	1-Pa2	C	
1-Oleoyl-2-chloropropanediol	1-Ol2	D	
1-Linoleoyl-2-chloropropanediol	1-Li2	E	
2-MCPD di-esters			
Dilinoleoyl-2-chloropropanediol	2Li-Li	E	E
Dipalmitoyl-2-chloropropanediol	2Pa-Pa	C	C
Palmitoyl-oleoyl-2-chloropropanediol	2Pa-Ol	C	D

Table S2. Spike recovery rate of GEs and MCPDEs using multi-SPE purification in edible oil (n = 6).

Muti-SPE	C18-Si			C18-Si-Si			**Si-Si-C18**		
	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.
Glycidyl esters									
La-GE	93.3	0.05	61.7	85.5	0.21	66.0	94.6	4.92	86.3
My-GE	102	16.6	72.2	88.2	0.88	75.0	96.2	3.25	90.2
Ln-GE	89.9	3.09	69.1	80.2	6.40	71.6	91.8	4.69	94.1
Li-GE	95.0	14.5	73.1	89.2	3.06	72.2	95.7	2.81	89.3
Pa-GE	95.6	1.42	68.0	103	7.85	67.2	96.7	0.26	91.6
Ol-GE	103	2.87	63.8	84.4	0.02	67.5	95.8	4.15	92.8
St-GE	97.7	10.4	60.9	89.5	2.07	67.7	102	5.08	88.8
Mono-MCPDEs									
1La	94.7	9.48	-	86.5	22.4	-	81.1	5.26	-
1My	94.1	16.6	-	98.7	34.6	-	91.4	6.09	-
1Ln	113	20.5	-	82.0	1.26	-	96.4	13.8	-
1Li	114	10.6	-	91.7	6.33	-	109	9.39	-
1Li2	97.9	5.87	-	88.9	25.1	-	99.4	6.17	-
1Pa/2Pa	91.6	10.8	-	99.7	3.38	-	84.4	14.2	-
1Pa2	82.9	10.4	-	80.9	13.8	-	81.1	2.75	-
1Ol	85.9	2.91	81.1	96.6	10.5	76.8	90.3	6.18	102
1Ol2/2Ol	88.1	12.8	-	73.6	16.0	-	86.2	19.1	-
1St	94.2	3.85	102	87.3	25.5	82.6	105	13.8	103
Di-MCPDEs									
Ln-Ln	83.4	5.50	103	78.6	1.03	104	90.7	12.7	97.7
Li-Li	39.7	2.48	-	72.4	9.76	-	75.2	4.82	-
2Li-Li	79.4	18.6	-	100	5.09	-	102	4.77	-
Pa-Li	136.	1.63	-	82.4	0.45	-	109	12.5	-
Ol-Li	153	6.15	-	106	0.54	-	129	3.70	-
Pa-Pa	84.2	2.35	60.7	78.4	7.20	83.5	85.6	1.46	99.8
2Pa-Pa	76.5	4.25	-	70.6	0.78	-	89.4	5.41	-
Pa-Ol	100	3.82	-	99.3	2.49	-	99.1	2.24	-
2Pa-Ol	91.7	3.06	-	117	9.34	-	103	6.18	-
Ol-Ol	79.1	10.7	52.6	80.1	0.32	80.2	101	2.59	91.7
Pa-St	89.5	0.94	-	81.7	2.51	-	107	19.1	-
Ol-St	89.2	0.32	5.0	83.8	5.15	7.8	90.3	8.60	9.9

[a] 50 ng/mL was spiked into the edible oil. Reco.: recovery; RSD: relative standard deviation.

Table S3. Comparison of fat recovery in various extraction solvents.

	Extraction system		
Solvent	EA	40% EA/ACN saturated with hexane	2:1 DCM/MeOH
Food matrix	Infant formula (Fat content=28%)		
Extraction efficiency	89%	71%	68%
Extract appearance	Transparent	Turbid	Turbid
Reference	Dubois, et al. 2019, Leigh and MacMahon 2016	This study	Becalski, et al. 2015

Table S4. Spike recovery rate of GEs and MCPDEs using multi-SPE purification in low-fat-content food (rice cereal, n = 6).

Muti-SPE	C18-Si			Si-C18			Si-NH2			Si-HLB		
	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.
Glycidyl esters												
La-GE	80.0	7.24	56.6	90.3	1.82	34.0	100	22.9	76.5	80.6	3.19	78.4
My-GE	92.3	10.9	61.5	97.5	3.26	57.2	101	33.5	97.2	85.6	2.63	86.7
Ln-GE	80.8	2.70	74.5	73.2	6.71	62.2	91.1	29.3	102	85.2	3.52	86.1
Li-GE	85.7	10.9	71.7	89.9	8.40	64.8	97.2	20.7	90.0	83.3	1.85	92.2
Pa-GE	94.7	12.7	27.2	103	14.2	23.4	119	29.7	70.3	93.0	1.37	79.5
Ol-GE	86.1	1.65	39.2	91.9	1.97	30.9	91.0	17.7	82.0	88.7	0.95	81.0
St-GE	85.7	6.36	37.1	75.2	5.18	39.1	100	23.1	91.8	89.1	2.22	79.9
Mono-MCPDEs												
1La	88.7	5.94	-	117	4.01	-	90.0	26.7	-	127	4.54	-
1My	95.4	27.1	-	118	7.30	-	81.1	21.2	-	116	7.40	-
1Ln	84.9	14.3	-	157	2.27	-	100	1.85	-	124	7.31	-
1Li	84.6	7.29	-	151	6.01	-	101	25.6	-	108	10.9	-
1Li2	80.5	17.3	-	132	11.4	-	100	27.9	-	107	12.8	-
1Pa/2Pa	88.3	13.2	-	133	13.1	-	88.8	28.9	-	113	8.04	-
1Pa2	81.5	33.5	-	85.1	11.1	-	91.0	15.8	-	111	9.43	-
1Ol	98.1	15.0	52.4	99.0	5.99	46.6	98.1	19.8	112	105	5.14	84.8
1Ol2/2Ol	81.6	19.9	-	128	0.73	-	94.6	32.3	-	127	10.7	-
1St	99.0	34.5	50.5	121	20.6	45.6	135	43.2	112	123	4.22	82.6
Di-MCPDEs												
Ln-Ln	1.0	97.3	146	0.11	44.7	197	0.81	93.1	130	87.9	1.52	94.8
Li-Li	6.50	91.6	-	3.63	54.3	-	314	34.3	-	104	8.67	-
2Li-Li	8.20	26.9	-	5.41	123	-	12.1	13.7	-	105	9.67	-
Pa-Li	137	26.9	-	1520	82.9	-	520	87.1	-	93.4	9.97	-
Ol-Li	126.	23.3	-	0.15	200	-	0.95	132	-	105	5.04	-
Pa-Pa	109	17.5	-	114	36.8	-	94.4	22.2	3.53	100	4.52	105
2Pa-Pa	138	11.6	-	78.9	116	-	63.1	35.7	-	104	1.92	-
Pa-Ol	95.1	20.2	-	43.1	103	-	153	10.1	-	89.0	3.62	-
2Pa-Ol	65.6	1.30	-	68.2	16.3	-	80.8	3.45	-	88.1	1.14	-
Ol-Ol	55.2	3.77	4.30	73.8	28.2	0.40%	58.8	30.6	3.26	91.8	9.20	113
Pa-St	11.9	12.6	-	9.28	10.4	-	184	20.1	-	98.5	3.34	-
Ol-St	3.00	40.1	-	7.74	70.5	-	71.7	47.8	0.14	101	3.34	112

[a] 50 ng/mL was spiked into the edible oil. Reco.: recovery; RSD: relative standard deviation.

Table S5. Spike recovery rate of GEs and MCPDEs using multi-SPE purification in high-fat-content food (infant formula, n = 6).

Muti-SPE	C18-Si-Si			Si-Si-C18			Si-HLB-Si			Si-Si-HLB			Si-Si-HLB plus		
	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.	% reco.	%RSD	IS reco.
Glycidyl esters															
La-GE	65.2	1.33	56.6	79.3	1.35	41.3	80.1	1.35	107	88.1	1.47	68.7	97.6	0.60	103
My-GE	88.1	13.6	61.5	92.3	6.82	54.9	85.1	6.12	117	74.2	5.71	80.5	103	1.46	85.8
Ln-GE	72.6	7.33	74.5	90.8	20.2	52.4	81.7	8.47	110	78.4	4.48	74.9	91.5	1.43	89.9
Li-GE	95.1	4.94	71.7	93.5	1.76	63.0	80.6	2.89	112	79.2	9.53	78.0	106	1.30	89.4
Pa-GE	119	5.30	27.2	133	5.60	24.8	85.4	6.34	114	77.7	0.92	77.7	99.2	0.86	89.2
Ol-GE	87.9	3.53	39.2	96.5	5.53	36.5	89.0	8.50	107	82.5	5.57	73.3	103	0.81	88.3
St-GE	93.1	8.77	37.1	90.1	3.89	36.4	86.8	1.78	105	85.4	6.51	77.5	98.7	0.22	90.2
Mono-MCPDEs															
1La	143	12.3	-	99.1	10.1	48.3	118	8.99	-	78.5	3.69	-	87.3	6.42	-
1My	132	24.0	-	121	3.71	-	112	0.81	-	84.3	1.78	-	92.1	12.9	-
1Ln	160	13.2	-	149	4.12	-	107	11.7	-	86.5	8.17	-	98.4	2.55	-
1Li	156	5.92	-	136	19.8	-	108	5.93	-	81.5	0.41	-	85.8	15.8	-
1Li2	128	14.2	-	127	6.68	-	100	8.23	-	90.8	2.75	-	82.6	8.94	-
1Pa/2Pa	136	22.9	-	107	28.8	-	111	16.2	-	86.8	2.61	-	100	0.66	-
1Pa2	163	12.98	-	99.4	13.4	-	99.5	5.44	-	90.8	14.1	-	94.9	14.5	-
1Ol	109	22.4	-	94.5	22.3	-	102	4.46	-	87.4	1.36	-	90.6	0.61	-
1Ol2/2Ol	136	19.7	-	114	4.84	-	123	13.9	-	93.3	0.96	-	86.9	2.97	-
1St	151	21.5	52.4	101	27.1	75.9	104	5.80	109	106	5.25	91.8	117	7.36	108
Di-MCPDEs															
Ln-Ln	0.17	38.4	174	0.62	3.79		86.9	6.30	102	78.5	5.77	79.9	81.9	0.18	93.9
Li-Li	6.05	14.4	-	404	72.9		104	7.69	-	73.7	0.38	-	78.9	0.95	-
2Li-Li	0.66	0.00	-	9.00	34.8		109	32.1	-	58.8	19.3	-	87.1	3.40	-
Pa-Li	395	82.3	-	35.0	48.3	49.9	35.3	13.9	-	37.0	20.8	-	80.1	4.05	-
Ol-Li	0.00	0.00	-	0.05	85.8		62.8	1.81	-	66.2	4.50	-	120	0.31	-
Pa-Pa	140		-	83.1	6.58		88.4	6.57	97.3	70.4	9.12	102	92.4	0.55	100
2Pa-Pa	65.5	41.8	-	58.3	27.5		119	5.42	-	82.0	15.6	-	102	2.98	-
Pa-Ol	37.3	26.4	-	186	17.7		40..3	5.15	-	64.3	12.4	-	82.6	0.86	-
2Pa-Ol	76.6	3.00	-	28.6	16.7		76.8	17.1	-	69.0	20.6	-	89.6	0.10	-
Ol-Ol	60.5	6.70	0.25	61.5	68.1	0.48	22.3	10.9	10.4	45.4	39.0	32.6	74.3	3.14	103
Pa-St	6.55	14.6	-	224	5.80		361	19.2	-	164	5.63	-	107	4.05	-
Ol-St	10.0	9.22	-	2.42	84.7	0.03	39.5	5.26	7.34	49.6	19.9	20.2	91.9	0.12	84.5

[a] 50 ng/mL was spiked into the edible oil. Reco.: recovery; RSD: relative standard deviation.

Table S6. Evaluation of the robustness of six factors during determination of HPLC-MS/MS.

Factor	Parameter	- 1 level	Central point	+ 1 level
O	Organic solvent concentration (%)	70	75	80
B	Buffer concentration (mM/%)	1.5 mM / 0.01%	2 mM / 0.05%	2.5 mM / 0.1%
F	Flow rate (mL/min)	0.15	0.2	0.25
E	Extraction time (min)	5	10	15
MO	Matrix effect (%)	Recovery of internal standard in 12 food samples categorized into three pretreatment methods (edible oils, low-fat content food, and high-fat content food)		
ML				
MH				

Table S7. Comparison of the effectiveness of direct analytical methods: separated method (USFDA) and multi-phase extraction and clean-up system (this work).

	Food matrices	Direct analytical method	
		The present study	US FDA method
Usability		Various processed foods Oils and fats, law fat content food, and high fat content food	Edible oils and Infant formulas
Experiment efficiency	Oils and fats	One validated procedure and one injection on LC-MS/MS	2 validated procedures and 2 injection on LC-MS/MS
	Infant formula		
Analysis time	LC-MS/MS	69 mins	47 + 49 = 96 mins
Pretreatment time	Oils and fats	Shorter	Longer
Experiment cost		Lower	Higher
Accuracy	Oils and fats	75~127%	72~118%
	Infant formula	77~113%	88.7~107.5%
Precision	Oils and fats	RPD%= 0~18%	RSD%= 1~17%
	Infant formula	RPD%= 1~19%	RSD%= 1~9.5%
LOQ (ng/g)	Oils and fats	· MCPD mono-esters= 4.37~16.48 · MCPD di-esters= 2.07~8.81 (Ol-Li=38.21)	· MCPD mono-esters= 60~180 · MCPD di-esters= 10~30
Reference		This study	(Leigh and MacMahon 2016, MacMahon, et al. 2013, MacMahon, et al. 2013)