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Analysis of Processing Contaminants in Edible Oils. Part 1. Liquid Chromatography–Tandem Mass Spectrometry Method for the Direct Detection of 3-Monochloropropanediol Monoesters and Glycidyl Esters

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ABSTRACT: A new analytical method has been developed and validated for the detection of glycidyl esters (GEs) and 3-monochloropropanediol (3-MCPD) monoesters in edible oils. The target compounds represent two classes of potentially carcinogenic chemical contaminants formed during the processing of edible oils. Target analytes are separated from edible oil matrices using a two-step solid-phase extraction (SPE) procedure. The extracts are then analyzed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI). Chromatographic conditions that separate sn-1 and sn-2 monoesters of 3-MCPD have been developed for the first time. The method has been validated for GEs, sn-1 3-MCPD monoesters of lauric, myristic, linolenic, linoleic, oleic, and stearic acids, and sn-2 3-MCPD monoesters of oleic and palmitic acids in coconut, olive, and palm oils using an external calibration curve. The range of average recoveries and relative standard deviations (RSDs) across the three oil matrices at three spiking concentrations are 84–115% (3–16% RSD) for the GEs, 95–113% (1–10% RSD) for the sn-1 3-MCPD monoesters, and 76.8–103% (5.1–11.2% RSD) for the sn-2 3-MCPD monoesters, with limits of quantitation at or below 30 ng/g for the GEs, 60 ng/g for sn-1 3-MCPD monoesters, and 180 ng/g for sn-2 3-MCPD monoesters.

KEYWORDS: *3-monochloropropanediol, 3-MCPD, glycidol, glycidyl esters, LC–MS/MS, processing contaminants, edible oils*

INTRODUCTION

Many edible oils are industrially processed to improve consumer acceptance by removing components that can negatively impact appearance, taste, and shelf stability. However, it is possible for chemical changes to take place in the oil during the refining process. 3-Monochloropropanediol (3-MCPD) esters, 2-monochloropropanediol (2-MCPD) esters, and glycidyl esters (GEs) are contaminants that are not present in virgin unrefined oils but can be produced during processing, specifically during high-temperature deodorization.[1–3] While the mechanisms of their formation have not been conclusively elucidated,[4,5] there is evidence suggesting that 3-MCPD esters are formed from iron chloride and/or natural organochlorines present in native oils.[6] In addition, the fact that MCPD esters begin forming at 200 °C makes mitigation difficult because deodorizations are generally run at temperatures greater than 200 °C.[5]

Glycidol, 3-MCPD, and 2-MCPD present concerns for food safety. Free 3-MCPD has been shown to be carcinogenic in rats, with demonstrated effects on kidneys and reproductive systems.[7] It was classified by the European Scientific Committee on Food in 2001 as a non-genotoxic threshold carcinogen.[8] The Joint Food and Agriculture Organization/World Heath Organization Expert Committee on Food Additives (JECFA) recommended a maximum tolerable daily intake of 2 μg/kg of body weight per day.[9] Glycidol is a genotoxic carcinogen that is probably carcinogenic to humans.[10] It is a contaminant that should be kept at concentrations as low as is reasonably achievable in food.[11] There are toxicological concerns related to 2-MCPD, although its effects are different from those reported with 3-MCPD, and publications studying the toxicology of 2-MCPD are limited. One study did report that, at high doses in rats, the main health effects were found in striated muscles and the heart, as well as in the kidney and the liver.[12] Because of the limited studies, currently, there is not sufficient toxicological data to establish a maximum tolerable daily intake value for 2-MCPD.[12]

While the majority of the toxicological work on these contaminants has been in the free, unesterified forms, work has begun on the toxicological properties of the esters.[11–13] Recent *in vivo* toxicological work has demonstrated that free 3-MCPD is liberated from the diester form with 86% efficiency in rats.[14] Initial risk assessments conducted by the Federal Institute for Risk Assessment in Berlin, Germany, have concluded that, using a worst case scenario, infants who are fed only commercial infant formulas could potentially ingest significant amounts of glycidol and 3-MCPD amounts that would exceed the JECFA recommended maximum tolerable daily intake levels.[13] To date, there have been no published studies on the toxicological properties of the fatty acid esters of 2-MCPD.

The fact that these contaminants are present in processed edible oils, which are commonly consumed worldwide and used in the production of infant formula, highlights the need for the development of accurate analytical methodology. The esters of 3-MCPD and glycidol have been the subject of a great deal of analytical method

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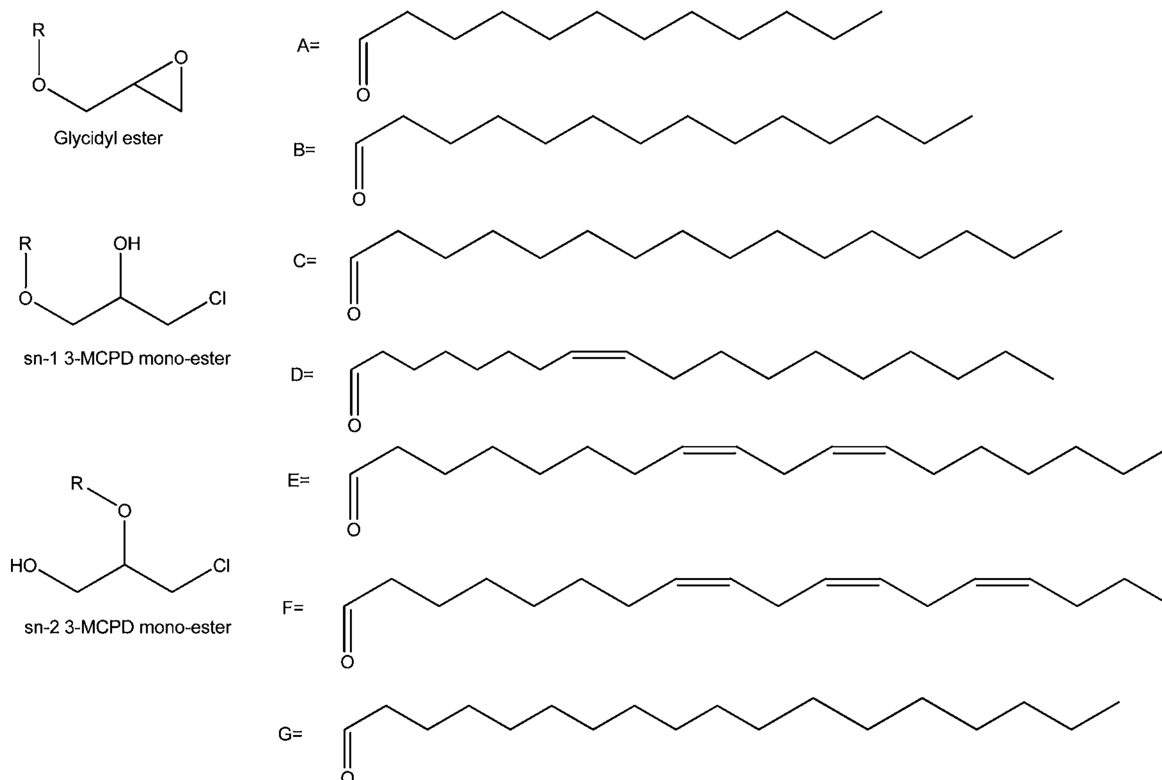

Glycidyl Esters		
Compound Name	Abbreviation	R =
Glycidyl Laurate	La-GE	A
Glycidyl Myristate	My-GE	B
Glycidyl Palmitate	Pa-GE	C
Glycidyl Oleate	Ol-GE	D
Glycidyl Linoleate	Li-GE	E
Glycidyl Linolenate	Ln-GE	F
Glycidyl Stearate	St-GE	G
sn-1 3-MCPD Mono-esters		
1-Lauroyl-3-chloropropanediol	1-La	A
1-Myristoyl-3-chloropropanediol	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
sn-2 3-MCPD mono-esters		
2-Palmitoyl-3-chloropropanediol	2-Pa	C
2-Oleoyl-3-chloropropanediol	2-Ol	D

Figure 1. Structures of analytes included in the method.

development. Initial methodology was exclusively indirect analysis, requiring the hydrolysis of the esters, followed by derivatization and analysis by gas chromatography−mass spectrometry (GC−MS).[15−17] These methods were integral in bringing attention to the presence of these contaminants in fats and oils. However, the original DGF method[17] using sodium methoxide for hydrolysis was later shown to be inaccurate,[18] raising questions about the reliability of the hydrolysis techniques used in indirect methodology.

In response to the lack of reliability of early indirect methodology, direct methods have been developed for GEs[18−23] and 3-MCPD esters,[18,23−27] but these methods have some disadvantages. The large number of analytical standards required for direct methods

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must be synthesized in-house or purchased commercially, some at significant costs. Direct methods require looking at each ester individually; therefore, the sum of the limits of detection (LODs) for each ester in a direct method produces higher glycidol and 3-MCPD detection limits than indirect methodology. However, direct methodology requires no hydrolysis of the esters because contaminants are analyzed intact as they occur in the oils. This removes the possibility of the creation or destruction of 3-MCPD or glycidol during the relatively harsh acidic or basic hydrolysis conditions in indirect methods. Under these conditions, there is also the possibility of interconversion between 2-MCPD and 3-MCPD. In the case of 3-MCPD monoesters, the sn-1 monoester of 3-MCPD may be more easily hydrolyzed *in vivo* than the sn-2 3-MCPD monoester (see Figure 1), leading to possible differences in their *in vivo* toxicological properties.[13]

The quality of indirect methodology has improved, and there are now several indirect methods that appear to provide accurate quantitative information for these contaminants.[28,29] The advantage of indirect methods is that the conversion of all fatty acid esters to free 3-MCPD, 2-MCPD, and glycidol enables low LODs with only a small library of simple analytical standards and internal standards.

Potential differences in toxicology between monoesters, as well as the possibility for compound conversion, loss, or artifact formation with indirect methodology, highlight the importance that occurrence data be collected for the native ester-bound contaminants using direct methodology. However, current direct methods are either not sufficiently rugged or reproducible,[18,23,25] do not use detection modes suitable for identity confirmation,[19] do not reach desired LODs,[20,24] require complex sample preparation,[21,26] or require prior knowledge of an approximate concentration of the contaminants in samples before analysis.[22] None of the current direct methods for 3-MCPD esters provides chromatographic separation between 2-MCPD and 3-MCPD diesters or between sn-1 and sn-2 3-MCPD monoesters. The liquid chromatography−mass spectrometry (LC−MS) response for sn-2 3-MCPD monoesters is 40% less than sn-1 monoesters, leading to inaccurate quantitative results when both are analyzed with a single standard.[25] In addition, given the possibility that 3-MCPD will be released more easily from sn-1 monoesters,[13] it is important to be able to analyze these two contaminants separately.

All of these issues are addressed in the liquid chromatography−tandem mass spectrometry (LC−MS/MS) method described herein. The GEs and 3-MCPD monoesters of lauric, myristic, palmitic, linolenic, linoleic, oleic, and stearic acids (see Figure 1) are included, and method performance has been validated in coconut, olive, and palm oil matrices at spiked concentrations as low as 100 ng/g (ppb). A LC−MS/MS method for the analysis of the diesters of 2-MCPD and 3-MCPD is described in part 2 (10.1021/jf400581g) of this series.

■ MATERIALS AND METHODS

Reagents and Materials. Deuterated internal standards glycidyl laurate-d_5 (La-GE-d_5), glycidyl myristate-d_5 (My-GE-d_5), glycidyl palmitate-d_5 (Pa-GE-d_5), glycidyl linolenate-d_5 (Ln-GE-d_5), glycidyl linoleate-d_5 (Li-GE-d_5), glycidyl oleate-d_5 (Ol-GE-d_5), glycidyl stearate-d_5 (St-GE-d_5), 1-oleoyl-3-chloropropanediol-d_5 (1-Ol-d_5), and 1-palmitoyl-3-chloropropanediol-d_5 (1-Pa-d_5) were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). MCPD monoester standards 1-lauroyl-3-chloropropanediol (1-La, CAS Registry No. 20542-96-5), 1-myristoyl-3-chloropropanediol (1-My, CAS Registry No. 30557-03-0), 1-palmitoyl-3-chloropropanediol (1-Pa, CAS Registry No. 30557-04-1), 1-linolenoyl-3-chloropropanediol (1-Ln, CAS Registry No. 74875-99-3), 1-linoleoyl-3-chloropropanediol (1-Li, CAS Registry No. 74875-98-2), 1-oleoyl-3-chloropropanediol (1-Ol, CAS Registry No. 10311-82-7), 1-stearoyl-3-chloropropanediol (1-St, CAS Registry No. 22094-20-8), 2-oleoyl-3-chloropropanediol (2-Ol, CAS Registry No. 915297-48-2), and 2-palmitoyl-3-chloropropanediol (2-Pa, CAS Registry No. 20618-92-2) were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). GE standards glycidyl laurate (La-GE, CAS Registry No. 1984-77-6), glycidyl myristate (My-GE, CAS Registry No. 7460-80-2), glycidyl palmitate (Pa-GE, CAS Registry No. 7501-44-2), glycidyl linolenate (Ln-GE, CAS Registry No. 51554-07-5), glycidyl linoleate (Li-GE, CAS Registry No. 243085-63-3), glycidyl oleate (Ol-GE, CAS Registry No. 5431-33-4), and glycidyl stearate (St-GE, CAS Registry No. 7460-84-6) were synthesized according to a literature procedure,[30] and their purity was confirmed by proton nuclear magnetic resonance (^{1}H NMR). Liquid-chromatographic-grade acetonitrile (ACN), isopropanol (IPA), methanol (MeOH), water (H_2O), *n*-hexane (hexane), ethyl acetate (EtOAc), and methyl *tert*-butyl ether (MTBE) were from Burdick and Jackson. Formic acid and ammonium formate were high-performance liquid chromatography (HPLC)-grade from Sigma-Aldrich (St. Louis, MO). A Visiprep solid-phase extraction (SPE) manifold and Branson 2510 ultrasonic cleaner were purchased from Sigma-Aldrich (St. Louis, MO). Silica SPE cartridges (500 mg of Si, 3 mL) and C18 SPE cartridges (1000 mg of C18, 6 mL) were purchased from Supelco. Disposable 15 mL glass tubes were purchased from Thermo Fisher Scientific (Pittsburgh, PA). A DB-3 Dri-Block heater and sample concentrator were purchased from Bibby Scientific (Burlington, NJ). Clear-glass HPLC vials with preslit polytetrafluoroethylene (PTFE) caps were purchased from National Scientific (Rockwood, TN). Extra virgin coconut and extra virgin olive oils were purchased from a local organic grocery store. Organic palm oil was purchased from Sigma-Aldrich.

Standard Solutions. Individual stock solutions of approximately 500 μg mL^{-1} (ppm) of each standard were prepared by weighing the appropriate amount of each reference standard (corrected for composition and purity) into separate tared 25 mL volumetric flasks (5 mL volumetric flasks for d_5 internal standards) and brought to volume with IPA. The spiking solution and the standard stock solution [containing La-GE, My-GE, Pa-GE, Ln-GE, Li-GE, Ol-GE, St-GE, 1-La, 1-My, 1-Pa, 1-Ln, 1-Li, 1-Ol, 1-St, 2-Ol, and 2-Pa at 10 μg mL^{-1} (ppm)] were prepared by pipetting the appropriate volume of each individual stock solution into separate 25 mL volumetric flasks. The internal standard spiking solution and the internal standard stock solution [containing La-GE-d_5, My-GE-d_5, Pa-GE-d_5, Ln-GE-d_5, Li-GE-d_5, Ol-GE-d_5, St-GE-d_5, 1-Pa-d_5, and 1-Ol-d_5 at 5 μg mL^{-1} (ppm)] were prepared by pipetting the appropriate volume of each individual stock solution into separate 10 mL volumetric flasks. One of the flasks was brought to volume with 20% EtOAc/MTBE, generating the internal standard spiking solution. The other flask was brought to volume with IPA, generating the internal standard stock solution. All individual stock solutions were stored at −20 °C and were stable for at least 1 year. The spiking and standard stock solutions were stored at 4 °C and were stable for at least 4 months.

Sample Preparation. A 1 g portion of oil was weighed in a tared 5 mL volumetric flask. A 250 μL aliquot of internal standard spiking solution and an appropriate volume of spiking solution were added to the flask, and the volume was brought to slightly below the 5 mL mark with 20% EtOAc/MTBE. The flasks were sonicated for 2 min to dissolve the entire sample, after which the solution was brought to the 5 mL mark to generate the sample solution.

A 1000 mg/6 mL C18 SPE cartridge was preconditioned with 6 mL of ACN without allowing the cartridge to dry. A 200 μL portion of the sample solution was added to the cartridge, and 14 mL of ACN was added and collected in a 15 mL glass tube. The elution rate was maintained at 1 drop per second using vacuum, as needed, and the cartridge was not allowed to dry until all 14 mL of ACN had been added. The ACN solution was dried at 70 °C under a stream of nitrogen.

A 500 mg/3 mL Si cartridge was preconditioned with 3 mL of 20% EtOAc/hexane without allowing the cartridge to dry. A 2 mL portion of 20% EtOAc/hexane was added to the glass tube containing dried residue from the previous SPE cleanup, sonicated for 15 s, added to the Si SPE cartridge, and collected in a new 15 mL glass tube. Another 2 mL portion of 20% EtOAc/hexane was added to the glass tube, vortex-mixed for 15 s, and added to the Si SPE cartridge; this was repeated 2 more times,

Table 1. AB Sciex 5500 QTRAP MS/MS Conditions[a]

Q1 (m/z)	Q3 (m/z)	RT	compound ID	internal standard	DP	EP	CE	CXP
310.2	183.1	4.9, 10.1	1-La.1	none	65	4	16	8
312.2	183.1	4.9, 10.1	1-La.2	none	65	4	16	8
310.2	57.1	4.9, 10.1	1-La.3	none	65	4	30	7
338.2	211.2	7.1, 14.9	1-My.1	1-Pa-d_5.1	75	10	16	10
340.2	211.2	7.1, 14.9	1-My.2	1-Pa-d_5.2	75	10	16	10
338.2	57.1	7.1, 14.9	1-My.3	1-Pa-d_5.1	75	10	30	7
366.2	239.2	9.3, 22.1	1-Pa.1	1-Pa-d_5.1	65	10	16	12
368.2	239.2	9.3, 22.1	1-Pa.2	1-Pa-d_5.2	65	10	16	12
366.21	239.2	9.5, 22.8	2-Pa.1	none	65	10	16	12
368.21	239.2	9.5, 22.8	2-Pa.2	none	65	10	16	12
388.3	261.2	6.9, 14.9	1-Ln.1	1-Pa-d_5.1	65	4	16	7
388.3	243.2	6.9, 14.9	1-Ln.2	1-Pa-d_5.1	65	4	16	6
390.3	261.2	6.9, 14.9	1-Ln.3	1-Pa-d_5.2	65	4	16	7
390.3	263.2	8.0, 18.9	1-Li.1	none	65	4	16	14
390.3	245.2	8.0, 18.9	1-Li.2	none	65	4	16	14
392.3	263.2	8.0, 18.9	1-Li.3	none	65	4	16	14
392.2	265.2	9.7, 23.3	1-Ol.1	1-Ol-d_5.1	75	14	20	14
392.2	247.2	9.7, 23.3	1-Ol.2	1-Ol-d_5.2	75	14	20	12
394.2	265.2	9.7, 23.3	1-Ol.3	1-Ol-d_5.3	75	14	20	14
392.21	265.2	9.9, 24.1	2-Ol.1	1-Ol-d_5.1	75	14	20	14
392.21	247.2	9.9, 24.1	2-Ol.2	1-Ol-d_5.2	75	14	20	12
394.21	265.2	9.9, 24.1	2-Ol.3	1-Ol-d_5.3	75	14	20	14
394.2	267.2	12.2, 27.5	1-St.1	none	75	4	16	7
396.2	267.2	12.2, 27.5	1-St.2	none	75	4	16	7
274.2	183.2	5.4	La-GE.1	La-GE-d_5.1	55	8	18	6
274.2	85.1	5.4	La-GE.2	La-GE-d_5.2	55	8	24	16
274.2	71.1	5.4	La-GE.3	La-GE-d_5.3	55	8	27	12
302.2	211.2	7.7	My-GE.1	My-GE-d_5.1	60	4	20	6
302.2	57.1	7.7	My-GE.2	My-GE-d_5.2	60	4	30	16
302.2	71.1	7.7	My-GE.3	My-GE-d_5.3	60	4	27	4
330.3	239.2	10.3	Pa-GE.1	Pa-GE-d_5.1	85	4	18	12
330.3	85.1	10.3	Pa-GE.2	Pa-GE-d_5.2	85	4	24	16
330.3	57.1	10.3	Pa-GE.3	Pa-GE-d_5.3	85	4	37	8
352.3	261.2	7.5	Ln-GE.1	Ln-GE-d_5.1	75	4	20	8
352.3	109.1	7.5	Ln-GE.2	Ln-GE-d_5.2	75	4	26	6
352.3	55.1	7.5	Ln-GE.3	Ln-GE-d_5.3	75	4	37	8
354.3	263.2	8.8	Li-GE.1	Li-GE-d_5.1	75	8	18	16
354.3	97.1	8.8	Li-GE.2	Li-GE-d_5.2	75	8	26	16
354.3	57.1	8.8	Li-GE.3	Li-GE-d_5.3	75	8	39	4
356.3	265.2	10.6	Ol-GE.1	Ol-GE-d_5.1	75	10	20	16
356.3	85.1	10.6	Ol-GE.2	Ol-GE-d_5.2	75	10	31	12
356.3	57.1	10.6	Ol-GE.3	Ol-GE-d_5.3	75	10	37	8
358.3	267.2	13.6	St-GE.1	St-GE-d_5.1	65	8	20	14
358.3	57.1	13.6	St-GE.2	St-GE-d_5.2	65	8	40	8
358.3	85.1	13.6	St-GE.3	St-GE-d_5.3	65	8	29	4
279.2	183.2	5.4	La-GE-d_5.1		55	8	18	6
279.2	85.1	5.4	La-GE-d_5.2		55	8	24	16
279.2	71.1	5.4	La-GE-d_5.3		55	8	27	12
307.2	211.2	7.6	My-GE-d_5.1		60	4	20	6
307.2	57.1	7.6	My-GE-d_5.2		60	4	30	16
307.2	71.1	7.6	My-GE-d_5.3		60	4	27	4
335.3	239.2	10.1	Pa-GE-d_5.1		85	4	18	12
335.3	85.1	10.1	Pa-GE-d_5.2		85	4	24	16
335.3	57.1	10.1	Pa-GE-d_5.3		85	4	37	8
357.3	261.2	7.4	Ln-GE-d_5.1		75	4	20	8
357.3	109.2	7.4	Ln-GE-d_5.2		75	4	26	6
357.3	55.1	7.4	Ln-GE-d_5.3		75	4	37	8
359.3	263.2	8.7	Li-GE-d_5.1		75	8	18	16
359.3	97.1	8.7	Li-GE-d_5.2		75	8	26	16
359.3	57.1	8.7	Li-GE-d_5.3		75	8	39	4
361.3	265.2	10.5	Ol-GE-d_5.1		75	10	20	16

Table 1. continued

Q1 (m/z)	Q3 (m/z)	RT	compound ID	internal standard	DP	EP	CE	CXP
361.3	85.1	10.5	Ol-GE-d_5.2		75	10	31	12
361.3	57.1	10.5	Ol-GE-d_5.3		75	10	37	8
363.3	267.1	13.4	St-GE-d_5.1		65	8	20	14
363.3	57.1	13.4	St-GE-d_5.2		65	8	40	8
363.3	85.1	13.4	St-GE-d_5.3		65	8	29	4
371.2	239.2	9.3, 22.0	1-Pa-d_5.1		65	10	16	12
373.2	239.2	9.3, 22.0	1-Pa-d_5.2		65	10	16	12
397.2	265.2	9.7, 23.1	1-Ol-d_5.1		75	14	20	14
397.2	247.2	9.7, 23.1	1-Ol-d_5.2		75	14	20	12
399.2	265.2	9.7, 23.1	1-Ol-d_5.3		75	14	20	14

[a]For the 3-MCPD monoesters, the first retention time is for the screening method and the second retention time is for the quantitative method.

totaling 8 mL. A 1 mL portion of 20% EtOAC/hexane was added directly to the Si SPE cartridge, resulting in a total of 9 mL of elution solvent. The elution rate was maintained at 1 drop per second using vacuum, and the cartridge was not allowed to dry until all 9 mL of 20% EtOAc/hexane had been added. The solution was dried at 55 °C under a stream of nitrogen, reconstituted in 1 mL of IPA, vortex-mixed for 30 s, and transferred to a HPLC vial for analysis.

LC−MS/MS Analysis. A Prominence UFLC XR liquid chromatography system (Shimadzu, Columbia, MD) with a Pursuit XRs C18 column, 2.0 × 150 mm, with 3.0 μm particles (Agilent, Santa Clara, CA), maintained at 30 °C, was used for the HPLC separation. Quantification of GEs and screening for 3-MCPD monoesters were performed as follows: an initial flow rate of 200 μL/min of 100% mobile phase A (2 mM ammonium formate/0.05% formic acid in 92:8 MeOH/H_2O) for the first 3 min (min), followed by stepping to 85% mobile phase A/15% mobile phase B (2 mM ammonium formate/0.05% formic acid in 98:2 IPA/H_2O) at 3.1 min, a linear ramp to 75% mobile phase A at 10.1 min, holding at 75% mobile phase A until 14.2 min, stepping to 0% mobile phase A and 250 μL/min at 14.5 min, holding at 0% mobile phase A until 20.5 min, returning to 100% mobile phase A at 20.75 min, holding at 100% mobile phase A at 250 μL/min until 26.75 min, returning to the initial flow rate of 200 μL/min at 27 min, and stopping the controller. The integrated Valco valve was directed to waste at 0.0 min, to the mass spectrometer at 4.0 min, and to waste at 14.2 min. The injection volume was 5 μL.

Alternate chromatographic conditions to separate and quantify sn-1 and sn-2 3-MCPD monoesters were developed using the same LC system and column described previously, with an initial flow rate of 200 μL/min of 100% mobile phase A (2 mM ammonium formate/0.05% formic acid in 75:25 MeOH/H_2O) for the first 2 min (min), followed by stepping to 70% mobile phase A/30% mobile phase B (2 mM ammonium formate/ 0.05% formic acid in 98:2 IPA/water) at 2.1 min, holding at 70% mobile phase A until 15 min, a linear ramp to 35% mobile phase A at 30.2 min, stepping to 0% mobile phase A and 250 μL/min at 30.5 min, holding at 0% mobile phase A until 40.5 min, returning to 100% mobile phase A at 40.75 min, holding at 100% mobile phase A at 250 μL/min until 46.75 min, returning to the initial flow rate of 200 μL/min at 47 min, and stopping the controller. The integrated Valco valve was directed to waste at 0.0 min, to the mass spectrometer at 9.0 min, and to waste at 30 min. The injection volume was 5 μL.

A 5500 QTRAP with an electrospray ionization (ESI) source in positive-ion mode with Analyst 1.5.2 software was used to control LC and MS (AB Sciex, Foster City, CA). Source parameters, such as gas flows, ion spray voltage, and source temperature, were optimized for each analyte, as was collision energy (CE), declustering potential (DP), collision cell exit potential (CXP), and entrance potential (EP). Q1 and Q3 were set at unit resolution. The curtain gas was set at 20 arbitrary units (au); the collisionally activated dissociation (CAD) gas was set at medium; the ion spray voltage was 5500 V; the source temperature was 300 °C; gas 1 pressure was set at 80 au; and gas 2 was set at 80 au. The individual MS/MS transitions, approximate retention times (RTs), internal standards, DP, EP, CE, and CXP for each analyte are shown in Table 1, with the quantitation ions listed first, followed by one or two confirmatory ions for each analyte.

The isomeric MS/MS transitions for the sn-1 and sn-2 monoesters were distinguished from one another by increasing the parent mass of the sn-2 monoester by 0.01 Da; this was solely to enable the software to process the two isomers independently and did not impact the data quality on a unit resolution mass spectrometer. The retention times for the target compounds were determined by analyzing a mixed standard under the conditions described above using standard multiple reaction monitoring (MRM) mode (not scheduled MRM). The MS/MS data for all validation samples were collected in scheduled MRM mode, with unit resolution in Q1 and Q3, a 5 ms pause between mass ranges, a MRM detection window of 45 s, and a target scan time of 1 s. A representative LC−MS/MS chromatogram is shown in Figure 2.

Quantitation. To calculate recoveries for validation, an eight-point calibration curve with solutions at 2, 5, 10, 25, 50, 125, 250, and 400 ng/mL (ppb) of each GE and MCPD monoester standard [0.05−10.00 μg/g (ppm) on a sample basis] each with 50 ng/mL (ppb) of deuterated internal standard [1.25 μg/g (ppm) on a sample basis] were prepared in IPA and used for quantitation. The calibration curves were generated using the ratio of the MRM chromatographic peak area for each analyte to that of the corresponding internal standard. When appropriate internal standards were not available (1-La, 2-Pa, 1-Li, and 1-St), these analytes were quantified directly by their peak areas. A linear calibration curve with $1/X^2$ weighting provided a good fit for both the GEs and 3-MCPD monoesters. This fit was chosen to ensure proper weighting for lower concentration standards given the large linear range of the calibration curve. All curves had R^2 values of 0.990 or greater.

RESULTS AND DISCUSSION

Selection of Standards. The quality of the results for direct analyses of intact 3-MCPD esters and GEs is significantly impacted by the number of standards used in the analysis. As relative GE concentrations follow the fatty acid composition of the individual oils, GEs and sn-1 MCPD monoesters of lauric, myristic, palmitic, linolenic, linoleic, oleic, and stearic acids were selected to provide suitable analytical coverage for the contaminants in nearly all commonly consumed edible oils (see Figure 1).

Deuterated GEs of the seven common fatty acids included in the method were commercially available from multiple suppliers at the time of the analysis. Despite concerns related to stability and shifts in retention time of the deuterated GEs when compared to ^{13}C-labeled GEs,[21] no issues were encountered during method development and validation using deuterated GEs as internal standards. Additionally, the use of deuterated GEs avoided the need for costly custom synthesis of ^{13}C-labeled internal standards.

At the time of these analyses, there were only two commercially available labeled 3-MCPD monoester internal standards: 1-Ol-d_5 and 1-Pa-d_5. Method development demonstrated that 1-Pa-d_5 can act as a suitable internal standard for the monoester of palmitic acid as well as myristic and linolenic acids and 1-Ol-d_5 can act as an internal standard for both the sn-1 and

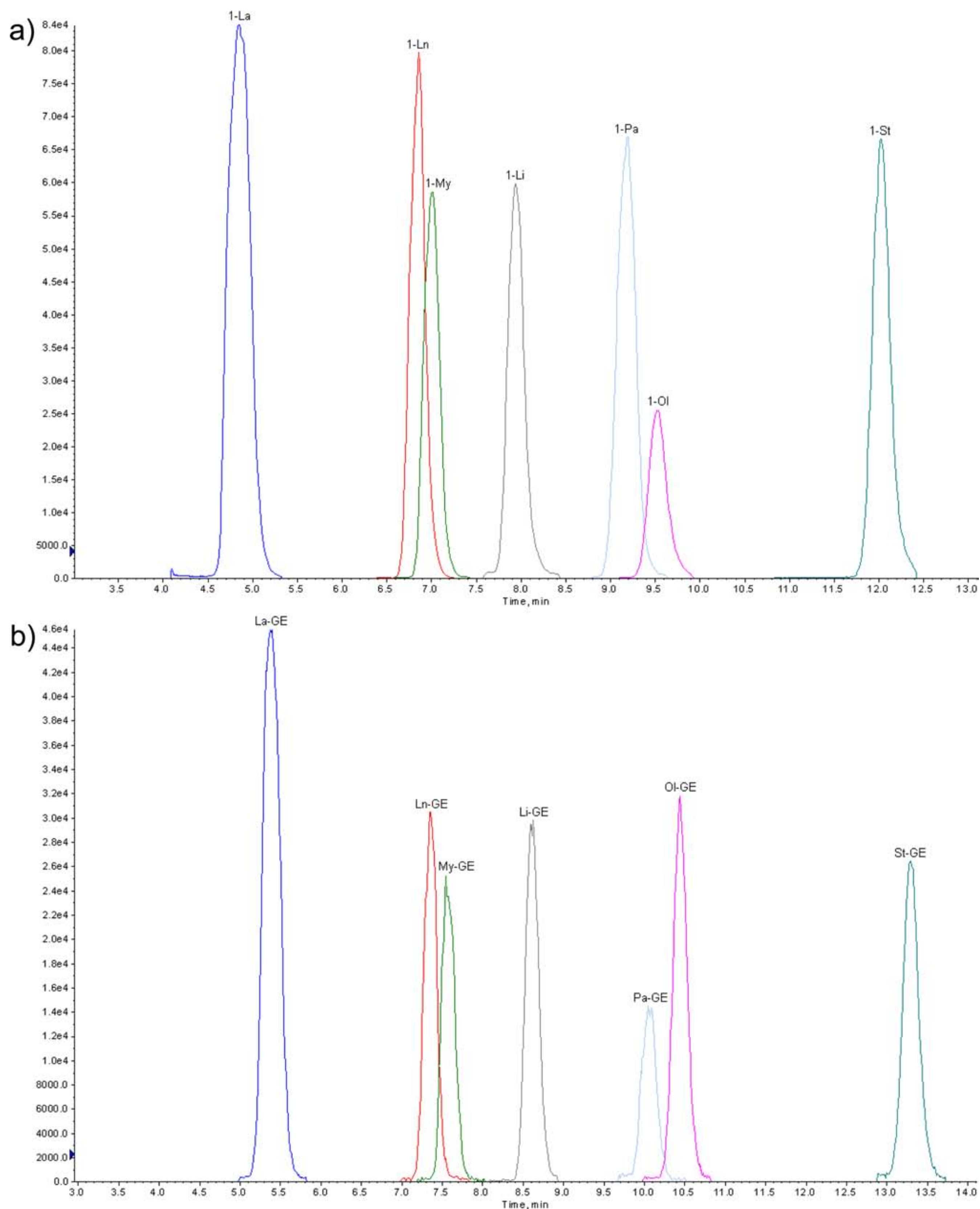

Figure 2. LC−MS/MS data for (a) 3-MCPD monoesters and (b) GEs using the rapid chromatographic separation system for a 1 ppm olive-oil-spiked sample (equivalent to 40 ng/mL on column).

sn-2 monoesters of oleic acid. The lack of internal standards for the remaining monoesters did not have a significantly negative effect on their method performances relative to those monoesters with internal standards, likely because of the lack of matrix suppression or enhancement in the regions where these analytes elute by HPLC.

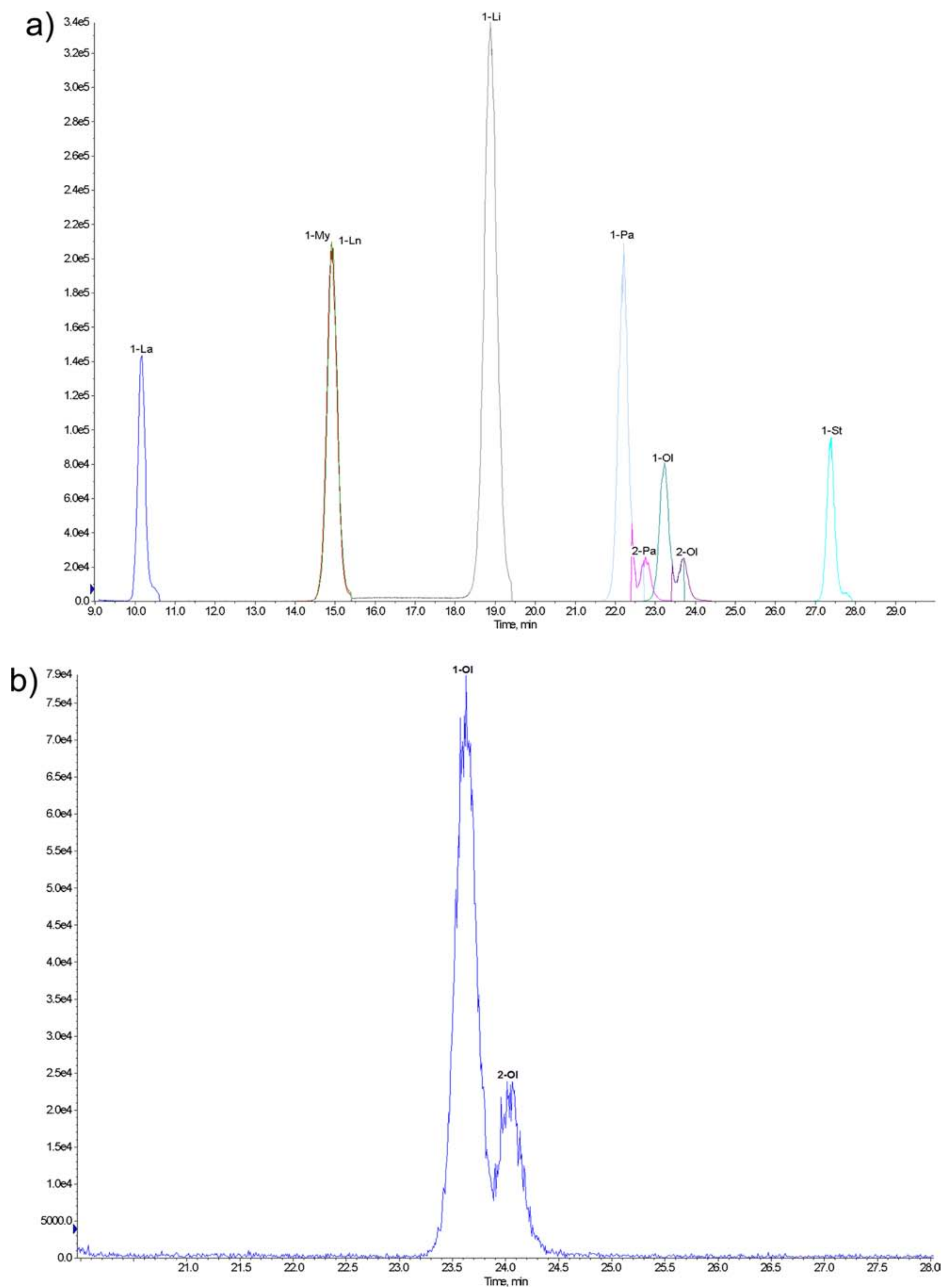

Figure 3. continued

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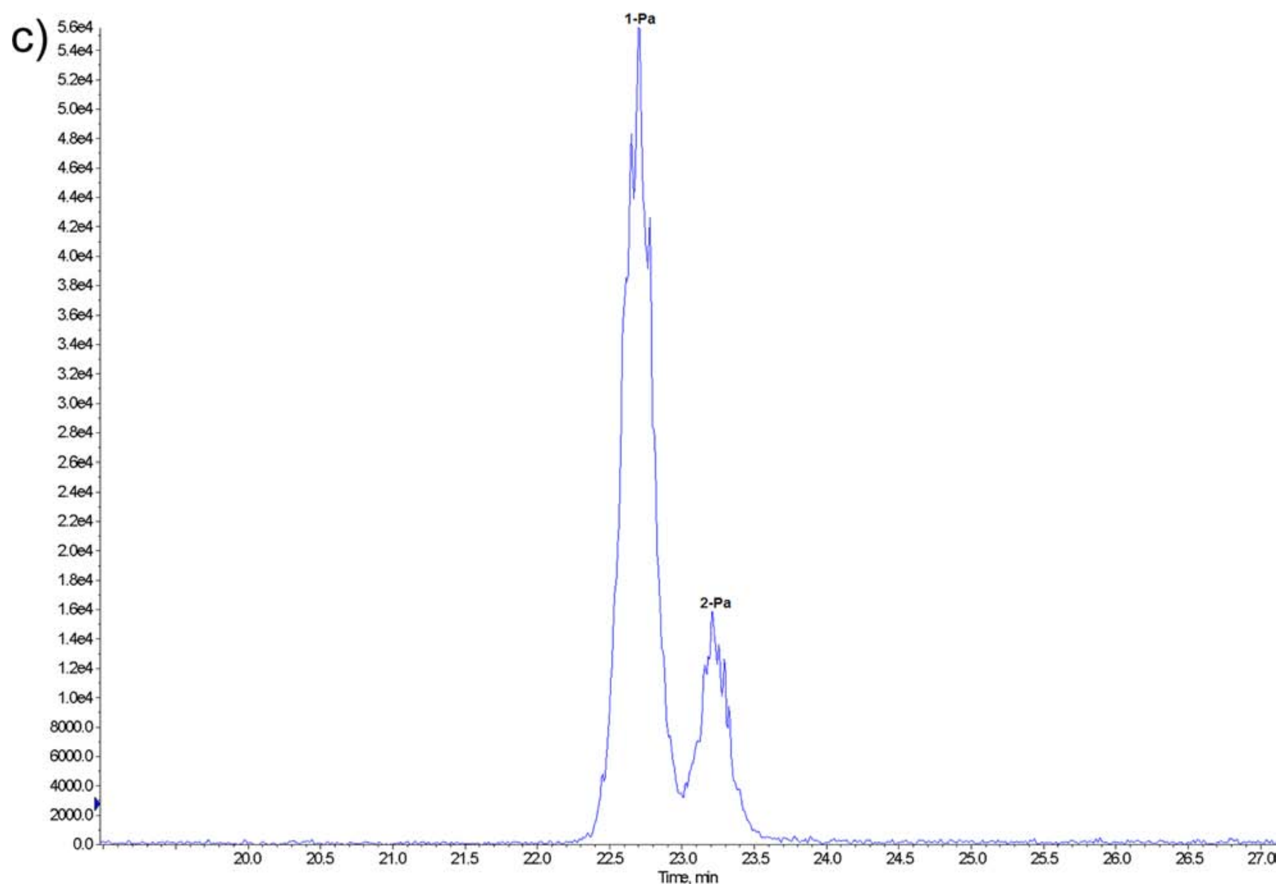

Figure 3. LC−MS/MS data using the alternate LC conditions for separating sn-1 and sn-2 3-MCPD monoesters for (a) 3-MCPD monoesters, (b) separation of 1-Ol and 2-Ol, and (c) separation of 1-Pa and 2-Pa.

Method Development. The dissolution of the oil in a solvent system, which more closely matched the polarity of the ACN that is used in the C18 SPE separation, was attempted; none provided suitable solubility. An attempt to disperse the oil in ACN to load the samples onto the SPE cartridge was also unsuccessful and led to low recoveries, particularly for fatty acid esters of stearic and palmitic acids. The use of 4:1 MTBE/EtOAc is derived from the official American Oil Chemists' Society (AOCS) GE methodology.[19] To minimize the amount of nonpolar solvent initially loaded onto the C18 SPE cartridge, the amount of solvent used to dissolve 1 g of oil was decreased from the 10 mL used in the AOCS official method to 5 mL. Both ACN and MeOH were evaluated to effect the separation of the MCPD monoesters and GEs from triglycerides by C18 SPE. ACN provided more effective removal of triglycerides, which could potentially contaminate the mass spectrometer. The final analyte to elute from the C18 SPE cartridge was the GE of stearic acid, which elutes completely with 14 mL of ACN. Heated drying at 70 °C under nitrogen in glass tubes did not produce any detectable loss of target analytes. The process took approximately 90 min, and samples were promptly removed once the elution solvent was completely removed. The following Si SPE cleanup removed mono- and diglycerols along with other polar matrix components. The 3-MCPD monoester of linolenic acid was the final analyte to completely elute from the silica SPE cartridge.

Initial attempts were made to use atmospheric pressure chemical ionization (APCI) for LC−MS/MS analysis. APCI is generally less sensitive to matrix effects than ESI, and the target analytes are all small and relatively nonpolar, which makes them suitable candidates for APCI. However, under APCI conditions, sn-1 3-MCPD monoesters reacted in the source to form the corresponding GEs. Initial concerns that the GEs were an impurity in the analytical standards were proven incorrect because the GEs formed from in-source fragmentation of the sn-1 3-MCPD monoesters were chromatographically resolved from the GE standard of the corresponding fatty acid. Therefore, ESI was used, and matrix effects were taken into account using available deuterated-labeled internal standards for all seven GEs as well as the 3-MCPD monoesters of palmitic and oleic acids.

It is possible that sn-1 monoesters are more easily hydrolyzed *in vivo* than sn-2 3-MCPD monoesters, impacting their respective toxicological properties.[13] In addition, the LC−MS response for the sn-2 3-MCPD monoesters is 40% less than the sn-1, leading to inaccurate quantitative results when they are quantified without chromatographic separation.[25] To ensure accurate quantitation, these isomeric analytes must be monitored separately. Given that several previously reported direct methods report no detectable concentrations of MCPD monoesters in deodorized oils[18,26] and to allow for analysis of samples using the related 3-MCPD diester analytical methodology[30] without changing mobile phases on the Shimadzu Prominence UFLC XR liquid chromatography system, the chromatographic conditions described in the LC−MS/MS Analysis section are initially applied in quantifying GEs and screening for the presence of 3-MCPD monoesters in all samples. In the event that an edible oil sample contains detectable concentrations of 3-MCPD monoesters using the screening approach, mobile phase A is replaced, the chromatographic system is purged, and the alternate LC conditions described in the LC−MS/MS Analysis section are applied

Table 2. Average Method Performance As Calculated by Use of an Eight-Point Calibration Curve with Deuterated Internal Standards for (a) GEs and (b) 3-MCPD Monoesters (n = 6 at Each Concentration, with Duplicate Spikes in Olive, Palm, and Coconut Oils)

(a)	percent recovery, percent RSD						
spike concentration (μg/g) (ppm)	La-GE	My-GE	Pa-GE	Ln-GE	Li-GE	Ol-GE	St-GE
0.1	113, 3.8	104, 7.4	107, 9.5	107, 3.1	103, 9.7	110, 4.6	108, 3.8
1.0	105, 2.6	101, 2.2	98.6, 2.4	107, 2.1	99.9, 3.3	103, 8.7	101, 2.7
8.0	101, 2.6	105, 1.3	95.4, 2.6	107, 3.6	98.6, 4.9	98.5, 7.9	98.0, 4.8

(b)	percent recovery, percent RSD								
spike concentration (μg/g) (ppm)	1-La	1-My	1-Pa	2-Pa	1-Ln	1-Li	1-Ol	2-Ol	1-St
0.1	108, 10.2	88.9, 8.1	91.9, 9.5	99.1, 9.8	76.0, 11.9	96.9, 9.0	107, 9.5	103, 11.2[a]	91.7, 7.5
1.0	104, 8.8	84.1, 8.4	86.2, 5.0	76.8, 12.7	77.7, 10.8	98.0, 5.9	98.8, 3.1	94.0, 9.0	88.8, 7.5
8.0	114, 9.7	103, 10.2	98.9, 10.6	84.5, 14.9	105, 10.0	100, 4.9	115, 5.8	108, 6.2	97.9, 6.4

[a]The spike concentration of 2-Ol = 0.2 μg/g (ppm).

to quantify the monoesters. The only sn-2 3-MCPD monoesters commercially available at the time of analysis were esters of oleic and palmitic acids; therefore, a chromatographic system that provided adequate separation between 1-Ol/2-Ol and 1-Pa/2-Pa was developed (see Figure 3). The remaining sn-2 monoesters will be purchased as they become commercially available; of particular interest is the sn-2 monoester of linoleic acid, because linoleic, oleic, and palmitic acids are the most commonly found fatty acids in edible oils.

No direct methods have analyzed for monoesters of 2-MCPD; therefore, it is unclear if they are even present in processed oils. However, reliable indirect approaches have found 2-MCPD esters in some deodorized oil samples, although always at concentrations well below 3-MCPD esters.[28,29] At the time of analysis, no monoesters of 2-MCPD were commercially available. Given their structural similarities and chromatographic behavior in previously published work, it is very likely that 2-MCPD monoesters and the sn-2 3-MCPD monoesters consisting of the same fatty acids would both coelute and give similar MS/MS responses using our current methodology.[25] Therefore, if the isomers cannot be separated chromatographically, it should be possible to accurately quantify the combined concentrations of these two esters separately from the corresponding sn-1 3-MCPD monoester concentration. As 2-MCPD esters become commercially available or are synthesized in house, this will be explored further.

Method Performance. The goal was to develop a validated method that is suitable to analyze a wide variety of edible oils. With this in mind, the method was validated using three very different oil matrices to ensure robust performance. Olive oil consists of largely unsaturated fatty acids; palm oil contains mostly saturated fatty acids; and coconut oil contains mostly shorter chain fatty acids. To generate validation data, five 1.0 ± 0.01 g portions of coconut, olive, and palm oils were spiked in duplicate with 0, 10, 20, 100, and 800 μL of spiking solution, respectively, generating oils that were blank, 0.1 μg/g (ppm), 0.2 μg/g (ppm), 1.0 μg/g (ppm), and 8.0 μg/g (ppm), respectively. Each oil sample was spiked with 250 μL of internal standard spiking solution, generating oils containing 1.25 μg/g (ppm) of each internal standard. Average recoveries for the seven GEs in olive, coconut, and palm oils without their respective deuterated internal standards ranged from 69 to 110% [relative standard deviation (RSD) range of 7−19%]. However, deuterated internal standards for each target analyte were available affordably through commercial channels; after their addition to the method average recoveries (95−113%) and reproducibilities (RSD of 1−10%), both improved greatly. The method recoveries and RSDs averaged for three spiking concentrations in the three matrices are depicted in Table 2; contaminants found in the organic palm oil sample blank ranged from 50 to 405 ng/g (ppb) and were subtracted out before recovery calculations, while coconut and olive oil samples did not contain any target analytes. The LOD and limit of quantitation (LOQ) were defined as a signal-to-noise ratio of 3 and 10, respectively, and were determined experimentally by analyzing spiked olive oil samples; the results are shown in Table 3. The performance of the GE method was compared by processing several spiked samples in various matrices and comparing the results to the AOCS official method.[19] The results were similar in the analyses; however, the current method offers significantly lower LOQs, increased specificity with the use of MS/MS as opposed to selected ion monitoring (SIM) in the AOCS methodology, and the ability to analyze for 3-MCPD monoesters.

Table 3. Method LODs and LOQs in ng/g (ppb) for (a) GEs and (b) 3-MCPD Monoesters, Determined in Spiked Olive Oil

(a)	La-GE	My-GE	Pa-GE	Ln-GE	Li-GE	Ol-GE	St-GE
LOD (ng/g)	6	6	20	6	6	6	10
LOQ (ng/g)	20	20	60	20	20	20	30

(b)	1-La	1-My	1-Pa	2-Pa	1-Ln	1-Li	1-Ol	2-Ol	1-St
LOD (ng/g)	20	10	10	20	10	10	20	60	10
LOQ (ng/g)	60	30	30	60	30	30	60	180	30

The average recoveries of the 3-MCPD monoesters in the absence of any internal standards across the three oil matrices ranged from 55 to 151% (5−22% RSD). Matrix enhancement is an issue for several of the 3-MCPD monoesters, particularly at lower concentrations. The recoveries for the analytes impacted by matrix enhancement (1-My, 1-Ln, and 1-Pa) were corrected using 1-Pa-d_5, which was a suitable internal standard for all three of these analytes; method validation results can be seen in Table 2. The performance for sn-2 monoesters showed more variability than the isomeric sn-1 monoesters; this was expected because of lower MS/MS responses relative to isomeric sn-1 monoesters and close chromatographic proximity to the larger sn-1 monoester MS/MS peak (see Figure 3). The low spiking concentration was increased to 200 ppb for 2-Ol, because 100 ppb was below the experimentally determined LOQ. The method performance of 2-Pa was suitable at the 100 ppb spiking concentration in all three matrices because this was above the LOQ.

The LODs and LOQs (see Table 3) were determined experimentally by analyzing spiked olive oil samples. When the

signal-to-noise ratio reached 3 and 10, the spiking concentration for that analyte was used to determine the LOD and LOQ, respectively. Olive oil was chosen as the representative oil for limit determination because it consists predominantly of 18 carbon unsaturated fatty acids as do most other commonly consumed edible oils, including soybean, canola, corn, sesame, sunflower, and peanut oils. All target analytes have LOQs of 60 ppb or below, with the exception of 2-Ol, which has a LOQ of 180 ppb.

Applicability in Sample Analysis. This method allows for reliable quantitation of GEs and 3-MCPD monoesters in oil samples without the need for matrix-matched standards, which is important given the difficulty of finding samples of most deodorized oils that do not contain any of the target analytes. It was rigorously validated using three very different edible oil matrices to ensure reliable method performance and reproducibility. The lack of matrix-matched standards combined with a straightforward two-step SPE cleanup allows for rapid analysis of numerous samples in different matrices in a single analytical sequence. This is the first published approach that provides chromatographic separation between sn-1 and sn-2 3-MCPD monoesters, allowing for the collection of accurate quantitative data for these two types of monoester. Given their different relative responses by LC−MS/MS, the method described herein is the only direct approach that is capable of accurately quantifying 3-MCPD monoester concentrations in edible oils. Along with methodology to quantify 2-MCPD and 3-MCPD diesters (10.1021/jf400581g), this technique is suitable for quantification of these toxicologically relevant processing contaminants in a manner suitable for the collection of occurrence data for risk assessment purposes.

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Notes

The authors declare no competing financial interest.

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