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Analysis of Processing Contaminants in Edible Oils. Part 2. Liquid Chromatography–Tandem Mass Spectrometry Method for the Direct Detection of 3-Monochloropropanediol and 2-Monochloropropanediol Diesters

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ABSTRACT: A method was developed and validated for the detection of fatty acid diesters of 2-monochloropropanediol (2-MCPD) and 3-monochloropropanediol (3-MCPD) in edible oils. These analytes are potentially carcinogenic chemical contaminants formed during edible oil processing. After separation from oil matrices using a two-step solid-phase extraction (SPE) procedure, the target compounds are quantitated using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI). The first chromatographic conditions have been developed that separate intact diesters of 2-MCPD and 3-MCPD, allowing for their individual quantitation. The method has been validated for 28 3-MCPD diesters of lauric, myristic, palmitic, linolenic, linoleic, oleic, and stearic acids in coconut, olive, and palm oils, as well as 3 2-MCPD diesters, 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 88–118% (2–16% RSD) with maximum limits of quantitation of 30 ng/g (ppb).

KEYWORDS: *3-monochloropropanediol, 3-MCPD, 2-MCPD, LC–MS/MS, processing contaminants, edible oils*

INTRODUCTION

Many edible oils undergo industrial processing to improve appearance, taste, and stability. However, chemical changes in the oil can occur during refining, producing fatty acid esters of 2-monochloropropanediol (2-MCPD) and 3-monochloropropanediol (3-MCPD).[1−6] Free 3-MCPD had carcinogenic effects on the kidneys and reproductive systems of rats during *in vivo* studies;[7] it was classified as a non-genotoxic threshold carcinogen by the European Scientific Committee on Food,[8] and the Joint Food and Agriculture Organization/World Heath Organization Expert Committee on Food Additives (JECFA) recommends a maximum tolerable daily intake (TDI) of 2 μg/kg of body weight per day.[9] Toxicological work has begun on the properties of the 3-MCPD fatty acid esters that occur in processed edible oils.[10−14] Free 3-MCPD is liberated from the diester form with 86% efficiency in rats,[13] and a study conducted by the Federal Institute for Risk Assessment in Berlin, Germany, has concluded that, using a worst case scenario, infants who are fed only commercial infant formulas could potentially ingest amounts of 3-MCPD that would exceed the JECFA recommended maximum TDI.[11] The toxicological concerns related to 2-MCPD are different from those for 3-MCPD because the main health effects of 2-MCPD were found in striated muscles and the heart, as well as in the kidney and the liver.[10] Currently, there is not sufficient toxicological data to establish a maximum TDI value for free 2-MCPD, and to date, there have been no published studies on the toxicological properties of 2-MCPD fatty acid esters.

MCPD esters have been the subject of a great deal of analytical method development. Initially, methodology was exclusively indirect, requiring hydrolysis of the fatty acid esters followed by derivatization prior to gas chromatography–mass spectrometry (GC–MS) analysis.[15−17] However, the original Deutsche Gesellschaft für Fettwissenschaft (DGF; German Society for Fat Science) 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 methodology, direct methods have been developed for 3-MCPD esters.[18−23] Indirect methodology has also improved, and there are now indirect methods that appear to provide accurate quantitative information for these esters in oils.[24,25] However, the possibility for compound loss or artifact formation with indirect methodology highlights the need for occurrence data to be collected for native ester-bound contaminants using direct methods.

Current direct methods for MCPD diesters are either not sufficiently rugged or reproducible,[18,19,21] do not reach desired limits of detection (LODs),[20] require complex sample preparation,[22] or do not provide accurate quantitative results.[23] In addition, none of the published direct methods for 3-MCPD esters provides chromatographic separation between isomeric 3-MCPD and 2-MCPD esters, which are indistinguishable by conventional analytical techniques. Indirect methods, which can distinguish between free 2-MCPD and 3-MCPD after hydrolysis because the isomers formed by derivatization can be separated by GC, have detected 2-MCPD esters in processed edible oils, although always at concentrations below 3-MCPD esters. The liquid chromatography–mass

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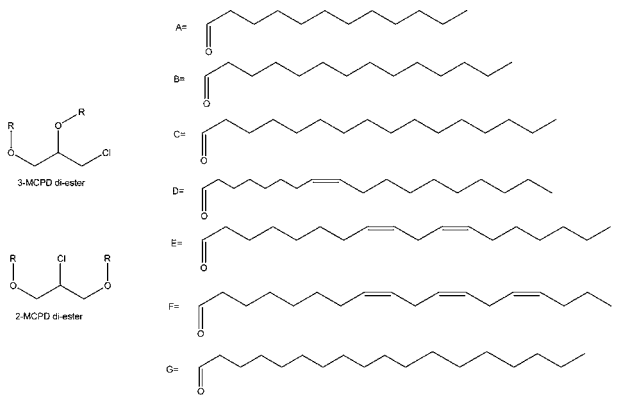
spectrometry (LC−MS) response for 2-MCPD diesters is 30% less than for the corresponding 3-MCPD diesters, leading to inaccurate quantitative results given that the two isomers elute as a single chromatographic peak and are quantified using a single analytical standard.[21] All published direct methodology to date for 3-MCPD diesters produce inaccurate quantitative results by attempting to quantify a single chromatographic peak containing isomeric 2-MCPD and 3-MCPD esters. In addition, given the difference in toxicological properties of these two classes of contaminants, it is essential that they are quantified separately. Even if it was possible to accurately quantify the combined concentrations of 2-MCPD and 3-MCPD esters without chromatographic separation, for useful occurrence data, these contaminants must be analyzed individually.

All of the deficiencies of current direct methods are addressed in the liquid chromatography−tandem mass spectrometry (LC−MS/MS) method described herein. The rugged, reproducible method provides baseline chromatographic resolution of the 3-MCPD diesters from three commercially available 2-MCPD diester standards, enabling separate identification and accurate quantitation of individual esters. The 3-MCPD diesters 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 50 ng/g (ppb). A LC−MS/MS method for the analysis of glycidyl esters and 3-MCPD monoesters is described in part 1 (10.1021/jf4005803) of this series.

MATERIALS AND METHODS

Reagents and Materials. Deuterated internal standards 1,2-dimyristoyl-3-chloropropanediol-d_5 (My-My-d_5), 1,2-dioleoyl-3-chloropropanediol-d_5 (Ol-Ol-d_5, CAS Registry No. 1246933-00-0), 1,2-dilinolenoyl-3-chloropropanediol-d_5 (Ln-Ln-d_5), 1,2-dilinoleoyl-3-chloropropanediol-d_5 (Li-Li-d_5), 1-oleoyl-2-linolenoyl-3-chloropropanediol-d_5 (Ol-Ln-d_5), linoleoyl-linolenoyl-3-chloropropanediol-d_5 (Li-Ln-d_5), palmitoyl-stearoyl-3-chloropropanediol-d_5 (Pa-St-d_5), and 1,2-distearoyl-3-chloropropanediol-d_5 (St-St-d_5, CAS Registry No. 1246818-85-8) were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). 3-MCPD diester standards were generously donated by the Archer Daniels Midland Company (Decatur, IL). Standards of 2-MCPD esters 1,3-distearoyl-2-chloropropandiol (2St-St, CAS Registry No. 26787-56-4), 1,3-dilinoleoyl-2-chloropropanediol (2Li-Li), and 1-linoleoyl-3-linolenoyl-2-chloropropanediol (2Li-Ln) were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Liquid-chromatographic-grade acetonitrile (ACN), isopropanol (IPA), methanol (MeOH), water (H_2O), dichloromethane, *n*-hexane (hexane), ethyl acetate (EtOAc), methyl *tert*-butyl ether (MTBE), and diethyl ether (Et_2O) were from Burdick and Jackson. Formic and ammonium formate were 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 (1000 mg of Si, 6 mL) and C18 SPE cartridges (1000 mg of C18, 6 mL) were purchased from Supelco (Sigma-Aldrich, St. Louis, MO). Disposable 15 mL glass tubes and polytetrafluoroethylene (PTFE) SPE inserts 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 high-performance liquid chromatography (HPLC) vials with preslit 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 (St. Louis, MO).

Standard Solutions. The 3-MCPD diester stock solution was prepared by weighing the appropriate amount of the 3-MCPD diester



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

Figure 1. Structures of MCPD diesters.

mixed standard [containing 4.0% lauroyl-myristoyl-3-chloropropanediol (La-My), lauroyl-linoleoyl-3-chloropropanediol (La-Li), lauroyl-oleoyl-3-chloropropanediol (La-Ol), lauroyl-palmitoyl-3-chloropropanediol (La-Pa), lauroyl-linolenoyl-3-chloropropanediol (La-Ln), myristoyl-palmitoyl-3-chloropropanediol (My-Pa), lauroyl-stearoyl-3-chloropropanediol (La-St), myristoyl-oleoyl-3-chloropropanediol (My-Ol), myristoyl-linoleoyl-3-chloropropanediol (My-Li), myristoyl-linolenoyl-3-chloropropanediol (My-Ln), myristoyl-stearoyl-3-chloropropanediol (My-St), palmitoyl-oleoyl-3-chloropropanediol (Pa-Ol), stearoyl-linoleoyl-3-chloropropanediol (St-Li), palmitoyl-linoleoyl-3-chloropropanediol (Pa-Li), oleoyl-linoleoyl-3-chloropropanediol (Ol-Li), stearoyl-linolenoyl-3-chloropropanediol (St-Ln), oleoyl-stearoyl-3-chloropropanediol (Ol-St), oleoyl-linolenoyl-3-chloropropanediol (Ol-Ln), linoleoyl-linolenoyl-3-chloropropanediol (Li-Ln), palmitoyl-linolenoyl-3-chloropropanediol (Pa-Ln), palmitoyl-stearoyl-3-chloropropanediol (Pa-St), 2.0% 1,2-dilauroyl-3-chloropropanediol (La-La), 1,2-dimyristoyl-3-chloropropanediol (My-My), 1,2-dipalmitoyl-3-chloropropanediol (Pa-Pa, CAS Registry No. 51930-97-3), 1,2-dilinolenoyl-3-chloropropanediol (Ln-Ln), 1,2-dilinoleoyl-3-chloropropanediol (Li−Li, CAS Registry No. 74875-96-0), 1,2-dioleoyl-3-chloropropanediol (Ol-Ol, CAS Registry No. 69161-73-5), and 1,2-distearoyl-3-chloropropanediol (St-St, CAS Registry No. 72468-92-9)] into a tared 25 mL volumetric flask and bringing to volume with IPA, producing the 3-MCPD diester stock solution containing 40 μg mL^{-1} (ppm) of each analyte (see Figure 1). Individual stock solutions for the

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Table 1. AB Sciex 5500 QTRAP MS/MS Conditions

Q1 mass (Da)	Q3 mass (Da)	RT	ID	internal standard	DP	EP	CE	CXP
492.5	275.2	14.9	La-La.1	My-My-d_5.1	85	4	23	16
494.5	277.2	14.9	La-La.2	My-My-d_5.2	85	4	24	20
570.5	517.4	17.4	La-Ln.1	Li-Ln-d_5.1	85	4	20	24
570.5	261.2	17.4	La-Ln.2	Li-Ln-d_5.2	85	4	23	12
570.5	275.2	17.4	La-Ln.3	Li-Ln-d_5.2	85	4	23	12
520.5	467.5	18.3	La-My.1	My-My-d_5.3	85	4	17	10
520.5	275.2	18.3	La-My.2	My-My-d_5.1	85	4	23	16
520.5	303.2	18.3	La-My.3	My-My-d_5.1	85	4	23	16
572.5	519.5	19.6	La−Li.1	My-My-d_5.3	85	4	18	6
572.5	275.2	19.6	La-Li.2	My-My-d_5.1	85	4	23	16
572.5	355.3	19.6	La-Li.3	My-My-d_5.1	85	4	23	8
653.5	358.3	20.1	Ln-Ln-d_5.1		85	4	28	8
653.5	261.2	20.1	Ln-Ln-d_5.2		85	4	27	12
648.5	353.2	20.2	Ln-Ln.1	Ln-Ln-d_5.1	85	4	28	8
648.5	261.2	20.2	Ln-Ln.2	Ln-Ln-d_5.2	85	4	27	12
598.5	545.5	21.1	My-Ln.1	My-My-d_5.3	85	4	20	6
598.5	303.2	21.1	My-Ln.2	My-My-d_5.1	85	4	27	4
598.5	353.2	21.1	My-Ln.3	My-My-d_5.1	85	4	24	20
553.5	308.2	21.9	My-My-d_5.1		85	4	23	8
555.5	310.2	21.9	My-My-d_5.2		85	4	23	8
553.5	500.5	21.9	My-My-d_5.3		85	4	17	10
548.5	303.2	22	My-My.1	My-My-d_5.1	85	4	23	8
550.5	305.2	22	My-My.2	My-My-d_5.2	85	4	23	8
548.5	275.2	22.1	La-Pa.1	My-My-d_5.1	85	4	24	16
548.5	331.2	22.1	La-Pa.2	My-My-d_5.1	85	4	23	8
548.5	495.5	22.1	LaPa.MyMy	My-My-d_5.3	85	4	17	10
574.5	521.5	22.4	La-Ol.1	My-My-d_5.3	85	4	18	24
574.5	275.2	22.4	La-Ol.2	My-My-d_5.1	85	4	23	16
574.5	357.3	22.4	La-Ol.3	My-My-d_5.1	85	4	23	8
655.5	602.5	22.4	Li-Ln-d_5.1		85	4	19	7
655.5	360.3	22.4	Li-Ln-d_5.2		85	4	30	8
655.5	358.2	22.4	Li-Ln-d_5.3		85	4	30	8
650.5	597.5	22.5	Li-Ln.1	Li-Ln-d_5.1	85	4	19	7
650.5	355.3	22.5	Li-Ln.2	Li-Ln-d_5.2	85	4	30	8
650.5	353.2	22.5	Li-Ln.3	Li-Ln-d_5.3	85	4	30	8
600.5	547.5	23.5	My-Li.1	My-My-d_5.3	85	4	20	6
600.5	303.2	23.5	My-Li.2	My-My-d_5.1	85	4	23	8
600.5	355.2	23.5	My-Li.3	My-My-d_5.1	85	4	23	8
657.5	360.2	24.8	Li-Li-d_5.1		85	4	30	8
657.5	263.2	24.8	Li-Li-d_5.2		85	4	27	16
659.5	362.2	24.8	Li-Li-d_5.3		85	4	30	8
652.5	355.2	24.9	Li-Li.1	Li-Li-d_5.1	85	4	30	8
652.5	263.2	24.9	Li-Li.2	Li-Li-d_5.2	85	4	27	16
654.5	357.2	24.9	Li-Li.3	Li-Li-d_5.3	85	4	30	8
626.5	573.5	25	Pa-Ln.1	St-St-d_5.1	85	4	21	7
626.5	331.2	25	Pa-Ln.2	St-St-d_5.2	85	4	29	8
626.5	353.2	25	Pa-Ln.3	St-St-d_5.3	85	4	29	8
657.5	604.5	25.2	Ol-Ln-d_5.1		85	4	20	7
657.5	358.2	25.2	Ol-Ln-d_5.2		85	4	30	8
657.5	362.2	25.2	Ol-Ln-d_5.3		85	4	27	16
652.5	599.5	25.4	Ol-Ln.1	Ol-Ln-d_5.1	105	4	23	14
652.5	353.2	25.4	Ol-Ln.2	Ol-Ln-d_5.2	85	4	30	8
652.5	357.2	25.4	Ol-Ln.3	Ol-Ln-d_5.3	85	4	30	8
576.5	331.2	25.9	My-Pa.1	My-My-d_5.1	85	4	23	8
576.5	303.2	25.9	My-Pa.2	My-My-d_5.1	85	4	23	8
576.5	523.5	26	MyPa.LaSt	My-My-d_5.3	85	4	17	24
576.5	275.2	26	La-St.1	My-My-d_5.1	85	4	25	12
576.5	359.2	26	La-St.2	My-My-d_5.1	85	4	23	8
602.5	549.5	26.2	My-Ol.1	My-My-d_5.3	85	4	17	6
602.5	303.2	26.2	My-Ol.2	My-My-d_5.1	85	4	24	8
602.5	357.2	26.2	My-Ol.3	My-My-d_5.1	85	4	24	8

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Table 1. continued

Q1 mass (Da)	Q3 mass (Da)	RT	ID	internal standard	DP	EP	CE	CXP
628.5	575.5	27.2	Pa-Li.1	Ol-Ol-d_5.3	85	8	20	7
628.5	331.2	27.2	Pa-Li.2	Ol-Ol-d_5.1	85	4	25	16
628.5	263.2	27.2	Pa-Li.3	Ol-Ol-d_5.4	85	4	25	12
654.5	601.5	27.5	Ol-Li.1	Ol-Ol-d_5.3	85	4	19	12
654.5	357.3	27.5	Ol-Li.2	Ol-Ol-d_5.1	85	4	31	8
654.5	355.3	27.5	Ol-Li.3	Ol-Ol-d_5.1	85	4	28	8
654.5	601.51	28.5	St-Ln.1	Ol-Ol-d_5.3	85	4	20	7
654.5	359.3	28.5	St-Ln.2	Ol-Ol-d_5.1	85	4	30	8
654.5	353.2	28.5	St-Ln.3	Ol-Ol-d_5.1	85	4	31	8
604.5	359.3	29.4	My-St.1	Ol-Ol-d_5.1	85	4	27	8
604.5	303.2	29.4	My-St.2	Ol-Ol-d_5.1	85	4	25	8
604.5	551.5	29.4	MySt.PaPa	Ol-Ol-d_5.3	85	4	16	6
604.5	331.2	29.4	Pa-Pa.1	Ol-Ol-d_5.1	85	4	25	8
606.5	333.2	29.4	Pa-Pa.2	Ol-Ol-d_5.2	85	4	25	8
630.5	577.5	29.6	Pa-Ol.1	Ol-Ol-d_5.3	105	4	22	12
630.5	331.2	29.6	Pa-Ol.2	Ol-Ol-d_5.1	85	4	27	8
630.5	357.3	29.6	Pa-Ol.3	Ol-Ol-d_5.1	85	4	27	8
661.5	362.3	29.7	Ol-Ol-d_5.1		85	4	28	8
663.5	364.2	29.7	Ol-Ol-d_5.2		85	4	28	8
661.5	608.5	29.7	Ol-Ol-d_5.3		85	4	20	7
656.5	357.3	29.8	Ol-Ol.1	Ol-Ol-d_5.1	85	4	28	8
656.5	603.5	29.8	Ol-Ol.3	Ol-Ol-d_5.2	85	4	20	7
658.5	359.2	29.8	Ol-Ol.2	Ol-Ol-d_5.3	85	4	28	8
661.5	265.2	29.8	Ol-Ol-d_5.4		85	4	27	8
656.5	603.51	30.5	St-Li.1	Ol-Ol-d_5.3	85	4	21	14
656.5	359.3	30.5	St-Li.2	Ol-Ol-d_5.1	85	4	28	8
656.5	263.2	30.5	St-Li.3	Ol-Ol-d_5.4	85	4	28	16
637.5	584.5	32.3	Pa-St-d_5.1		85	10	15	4
637.5	364.3	32.3	Pa-St-d_5.2		85	10	21	16
637.5	336.2	32.3	Pa-St-d_5.3		85	10	21	16
632.51	579.5	32.4	Pa-St.1	Pa-St-d_5.1	85	10	15	24
632.51	359.2	32.4	Pa-St.2	Pa-St-d_5.2	85	10	21	16
632.51	331.2	32.4	Pa-St.3	Pa-St-d_5.3	85	10	21	16
658.5	605.5	32.6	Ol-St.1	Pa-St-d_5.1	125	10	23	7
658.5	359.3	32.6	Ol-St.2	Pa-St-d_5.2	85	4	26	8
658.5	357.3	32.6	Ol-St.3	Pa-St-d_5.3	85	4	26	8
665.6	364.3	35	St-St-d_5.1		85	4	26	8
667.6	366.3	35	St-St-d_5.2		85	4	28	8
665.6	267.2	35	St-St-d_5.3		85	4	28	14
660.6	359.3	35.1	St-St.1	St-St-d_5.1	85	4	26	8
662.6	361.3	35.1	St-St.2	St-St-d_5.2	85	4	28	8
660.6	267.2	35.1	St-St.3	St-St-d_5.3	85	4	28	14

deuterated internal standards and 2-MCPD diesters were prepared by weighing the appropriate amount of standard into tared 5 mL volumetric flasks and bringing to volume with IPA. The spiking solution and the standard stock solution containing 10 μg mL^{-1} (ppm) of each analyte were prepared by pipetting the appropriate volume of the 3-MCPD diester stock solution into two separate 10 mL volumetric flasks. One of the flasks was brought to volume with 20% EtOAc/MTBE, generating the spiking solution. The other flask was brought to volume with IPA, generating the standard stock solution. The internal standard spiking solution and the internal standard stock solution [containing My-My-d_5, Li-Ln-d_5, Ln-Ln-d_5, Li-Li-d_5, Ol-Ln-d_5, Pa-St-d_5, St-St-d_5, and Ol-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 6 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, 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 Silica SPE cartridge was preconditioned with 5 mL of MeOH, 5.5 mL of dichloromethane, and two 6 mL portions (12 mL total) of 2% Et_2O/hexane without allowing the cartridge to dry. A 100 μL portion of the sample solution was added to a 15 mL glass tube and dried under a stream of nitrogen. A 2 mL portion of 2% Et_2O/hexane was added to the glass tube containing the dried residue from the previous step, sonicated for 15 s, added to the Si SPE cartridge, and collected in a new 15 mL glass tube. Another 2 mL portion of 2% Et_2O/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, totaling

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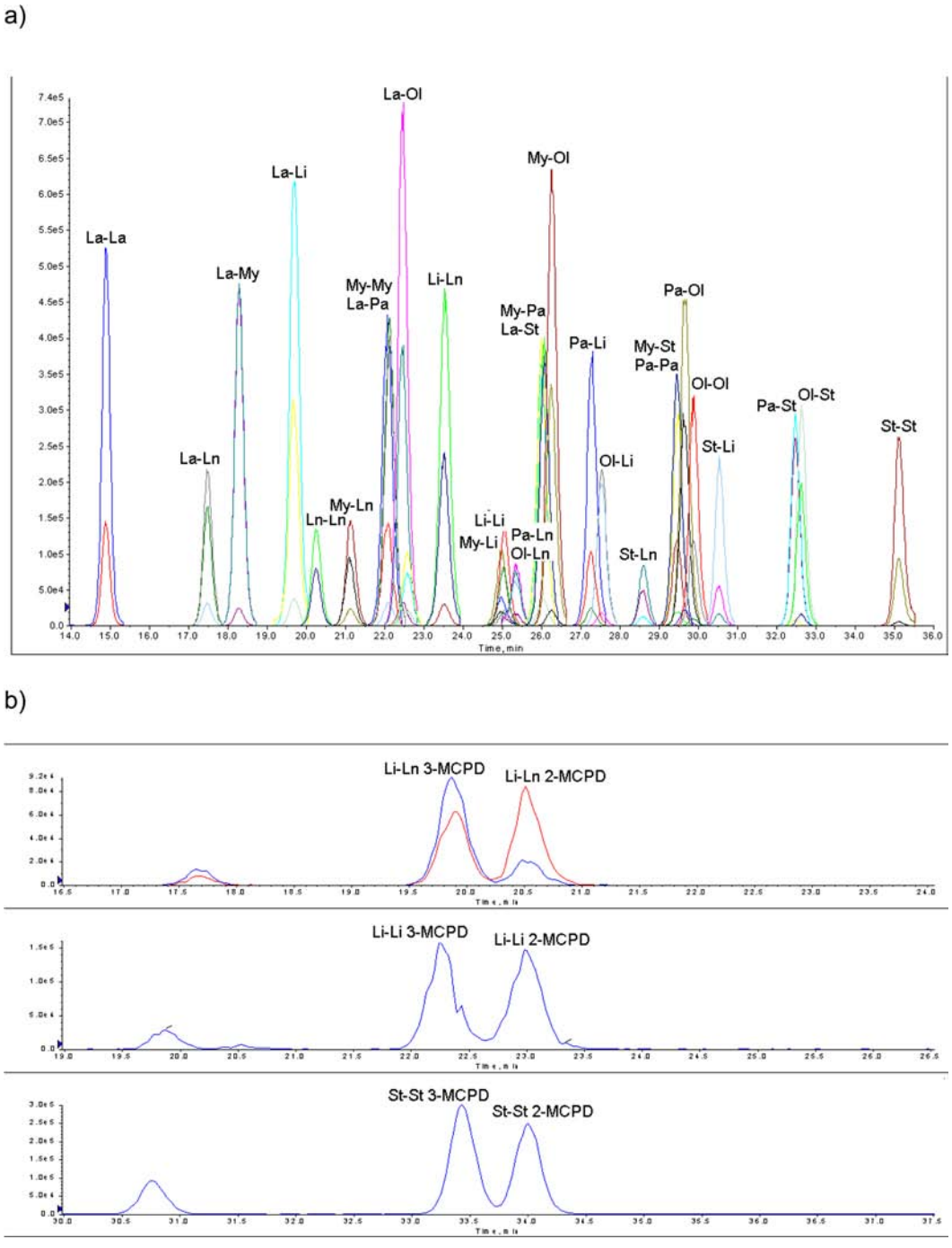

Figure 2. LC−MS/MS data for (a) library of 28 3-MCPD diesters and (b) separation of 2-MCPD and 3-MCPD diesters of Li-Ln, Li-Li, and St-St.

8 mL. A 5.5 mL portion of 2% Et_2O/hexane was added directly to the Si SPE cartridge, resulting in a total of 13.5 mL of elution solvent. The elution rate was maintained at 1 drop per second using vacuum, as needed, and the cartridge was not allowed to dry until all 13.5 mL of 2% Et_2O/hexane had been added. The solution was dried at 55 °C under a stream of nitrogen.

A 1000 mg/6 mL C18 cartridge was preconditioned with 6 mL of 40% EtOAc/ACN without allowing the cartridge to dry. A 2 mL portion of 40% EtOAc/ACN 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 5 mL glass tube. Another 2 mL portion of 40% EtOAc/ACN was added to the glass tube, vortex-mixed for 15 s, and added to the Si SPE cartridge; this was repeated 2 more times, totaling 8 mL. A 1 mL portion of 40% EtOAc/ACN was added directly to the C18 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 40% EtOAc/ACN had been added. The solution was dried at 70 °C under a stream of nitrogen, reconstituted in 0.500 mL of IPA, vortex-mixed for 30 s, and added to a HPLC vial for analysis.

Instrumental 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), was used for the HPLC separation, with 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 2 min, stepping to 70% mobile phase A/30% mobile phase B (2 mM ammonium formate/0.05% formic acid in 98:2 IPA/H_2O) at 2.1 min, a linear ramp to 50% mobile phase A at 20 min, a linear ramp to 17% mobile phase A at 36 min, stepping to 0% mobile phase A and 250 μL/min at 36.25

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min, holding at 0% mobile phase A until 42.5 min, returning to 100% mobile phase A with the increased flow rate of 250 μL/min at 42.75 min, holding these conditions until 48.75 min, returning to the initial flow rate of 200 μL/min at 49 min, and stopping the controller. The integrated Valco valve was directed to waste at 0.0 min, to the mass spectrometer at 13.0 min, and to waste at 36.25 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); see Table 1 for a summary of multiple reaction monitoring (MRM) transitions. 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 500 °C; gas 1 pressure was set at 60 au; and gas 2 pressure was set at 70 au. The DP, EP, CE, and CXP for the individual compound transitions as well as approximate analyte retention times (RTs) are shown in Table 1. The RTs for the target compounds were determined by analyzing a mixed standard under the conditions described above using standard 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 50 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 3-MCPD diester 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. A linear calibration curve with $1/X^2$ weighting provided a good fit for all 3-MCPD diesters. 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 3-MCPD esters is significantly impacted by the number of standards used in the analysis. As relative ester concentrations follow the fatty acid composition of the individual oils,[21] 3-MCPD diesters 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).

A large number of deuterated 3-MCPD diester standards 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 RT of the deuterated standards when compared to ^{13}C-labeled standards, no issues were encountered during method development and validation using deuterated standards because internal standards avoided the need for costly custom synthesis of ^{13}C-labeled internal standards.

The analysis includes all of the diesters of the seven most common fatty acids in edible oils, a total of 28 analytes. While ideal results could certainly be achieved using 28 deuterated internal standards, this approach was avoided because of the cost of purchasing or custom synthesizing all of these standards. Method development demonstrated that the use of eight deuterated internal standards provides suitable coverage for all 28 target analytes. Two compounds, My-My-d_5 and Ol-Ol-d_5, were appropriate internal standards for 19 of the 28 analytes in the method. The recoveries of the remaining nine analytes were corrected through the use of the remaining six internal standards, with Pa-St-d_5, St-St-d_5, and Li-Ln-d_5 each accounting for two analytes and Ln-Ln-d_5, Ol-Ln-d_5, and Li-Li-d_5 as appropriate surrogates for the respective unlabeled compound.

Method Development. The dissolution of the oil in the 2% diethyl ether/hexane solution used in the Silica SPE cleanup step was attempted, but it did not fully dissolve palm or coconut oils used in the study. The use of 4:1 MTBE/EtOAc is derived from the official American Oil Chemists' Society (AOCS) glycidyl ester methodology[26] and was selected to match the solvent system used in the related method to analyze 3-MCPD monoesters and glycidyl esters,[26] enabling one sample preparation for both analyses.

Sample cleanup is more effective when the Si SPE is performed before the C18 SPE because 3-MCPD diesters are less polar than most components in the oil matrix, including most triglycerides and all di- and monoglycerides. This first step provides the majority of the sample cleanup because conditions were chosen where the MCPD diesters elute and the majority of the matrix remains on the Si SPE cartridge. The method was initially begun by equilibrating the Si SPE cartridge with the diethyl ether/hexane solution before loading the sample. However, the reproducibility performance of the method under these conditions was unacceptable, particularly when switching to different lots/manufacturers of Si SPE cartridges. It was discovered that when the Si SPE cartridges were exposed to air, even for 24 h, the retentive properties are impacted and the elution of MCPD diesters decreases significantly, lowering method recoveries. Because the likely explanation was that the silica cartridges, which are known to be hygroscopic, were absorbing moisture from the air, attempts were made to remove the moisture and properly equilibrate the cartridges prior to analysis. The application of methanol to the Si SPE cartridge to remove water, followed by dichloromethane to remove the methanol, and finally, 2 cartridge volumes of eluent to remove dichloromethane and equilibrate the cartridge for sample analysis enabled reproducible method performance from lot to lot and manufacturer to manufacturer. While the authors have not seen this phenomenon in Si SPE previously, it is likely due to the extremely low elutropic strength (∼0.01) that the 2% diethyl ether/hexane solution has on silica, making proper equilibration essential. The final analytes to elute from the Si SPE cartridge are the diester of linolenic acid and the diester of linolenic and linoleic acids. Internal standards are included for both of these analytes to ensure that they are completely captured by the method. The total volume of eluent used in the silica SPE is kept strictly at 13.5 mL because less than this does not fully elute the final two analytes, while an increase beyond this volume leads to significant ion suppression of Pa-Li, Ol-Li, and St-Li in coconut oil samples by an unidentified matrix interference. Heated drying at 55 °C under nitrogen in glass tubes does not produce any detectable loss of target analytes. The process takes approximately 30 min, and samples are promptly removed once the elution solvent is completely removed.

The subsequent C18 SPE cleanup removes any remaining triglycerides and waxes, which would be extremely nonpolar and could potentially contaminate the mass spectrometer. The 3-MCPD diester of stearic acid is the final analyte to completely elute from the C18 SPE cartridge under these conditions, and a labeled internal standard for St-St is included to ensure accurate

recovery. Heated drying at 70 °C under nitrogen in glass tubes takes approximately 45 min, and samples are promptly removed once the elution solvent is completely removed.

Because of the differences in toxicological properties and MS responses between 2-MCPD and 3-MCPD diesters, the development of chromatographic conditions, which provided separation between these isomers, was essential to the success of the method. While it is not possible to separate the analytes by SPE and given that 2-MCPD and 3-MCPD esters produce identical ions upon fragmentation, the separation has to take place by HPLC. The goal was to develop a method suitable for routine regulatory analysis so that the use of expensive specialty columns was avoided. At the time of analysis, the only commercially available 2-MCPD diester standards were 2Li-Ln, 2Li-Li, and 2 St-St (see Figure 1). While ideally more 2-MCPD diester standards will become available commercially, these three were suitable for method development because Li-Ln is among the earliest analytes to elute, Li-Li elutes near the majority of the analytes in the method, and St-St is the final analyte to elute on C18. Numerous column chemistries were attempted, most providing little or no chromatographic separation between 2-MCPD and 3-MCPD isomers. The first column to show the potential to provide acceptable chromatographic separation was the Agilent Pursuit XRs C18 column; its enhanced performance is due to a very high degree of carbon loading. Many different combinations of mobile phases, buffer systems, and gradient elutions were attempted before the final conditions that provide nearly baseline resolution were established. In all cases, the diester of 2-MCPD elutes after the corresponding 3-MCPD diester because of the more symmetrical 2-MCPD structure being slightly less polar than 3-MCPD and, therefore, better retained on the nonpolar C18 column (see Figure 2). The combination of SPE cleanup and HPLC conditions has been extremely rugged because a single HPLC column has been used through the entire method development process with no visible decrease in performance and without the need to vent and clean the vacuum portion of the mass spectrometer.

Initial development of MS/MS experiments monitored the formation of cyclic acyloxonium ions formed by the loss of one of the two fatty acid esters from a given 3-MCPD diester. For unsymmetrical 3-MCPD diesters consisting of two different fatty acids, the two transitions resulting from the loss of each fatty acid were monitored; in symmetrical 3-MCPD diesters when the two fatty acids were identical to one another, this single transition was monitored, as well as the same transition for the Cl-37 isotope for confirmation. While monitoring these transitions provided accurate and reproducible recoveries in spiked samples, it became clear that these transitions were not appropriate for the quantitation of unsymmetrical 3-MCPD diesters in processed oil samples, because the two monitored transitions for a given analyte produced very different results.

While the use of 28 analytical standards does provide coverage for all of the possible diester combinations of lauric, myristic, palmitic, linolenic, linoleic, oleic, and stearic acids, it does not account for sn-1 and sn-2 isomerism possible in unsymmetrical 3-MCPD diesters. The analytical standard mixture provided by the Archer Daniels Midland Company contains unsymmetrical diesters with an approximately 50:50 mixture of the fatty acids at the sn-1 and sn-2 positions. However, this is not necessarily the composition found in edible oils, where unsaturated fatty acids preferentially populate the sn-2 position and saturated fatty acids are more common in the sn-1 position. The fatty acids in the sn-1 and sn-2 positions of a given 3-MCPD diester are not lost via MS/MS to form cyclic acyloxonium ions equally; the fatty acid in the sn-1 position is lost preferentially, generally 2.5−4 times more than the fatty acid in the sn-2 position.[23] As a result, unsymmetrical 3-MCPD diesters cannot be accurately quantified by their most sensitive transitions unless the ratio of isomers in the sample is identical in the analytical standard. Given that this is an impossible task because isomer ratios can vary between samples of the same oil and even more between different oils, a single MS/MS transition that responds equally to quantify both isomers needed to be developed. At this point, the use of a high-resolution accurate mass system was considered because it would avoid the need to produce any fragments. However, given the advantages in specificity of monitoring two or more MS/MS transitions and the increased quantitative accuracy on a triple quadrupole, efforts were dedicated to the development of an acceptable MS/MS approach for these analytes.

The MS/MS fragment produced by the loss of NH_4Cl, which was ignored during method development because of the low sensitivity of that transition relative to the loss of a fatty acid, produces the identical response for the two 3-MCPD isomers of a given diester because it is not impacted by sn-1/sn-2 isomerism. This was confirmed by comparing the Archer Daniels Midland Company standard, a 50:50 mixture of isomers, to commercially purchased single-isomer unsymmetrical diester standards. Pa-St, Ol-St, and Ol-Ln were selected to be representative of all diester standards because they consist of two saturated fatty acids, a saturated and an unsaturated fatty acid, and two unsaturated fatty acids, respectively. Several spiked and unknown samples were analyzed using separate calibration curves of these two types of standards; the responses for the analytical standards as well as the quantitative results produced by monitoring the loss of NH_4Cl were very similar using both curves, ensuring accurate quantitation of both isomers with this single transition. The larger intensity fragments resulting from the loss of the fatty acids while not suitable for quantitation of unsymmetrical diesters are monitored to provide structural confirmation for each analyte. However, the cyclic acyloxonium ion base peak is suitable for quantitation of 3-MCPD diesters consisting of the same fatty acid and should be acceptable for quantitation of 2-MCPD diesters given that these structures have chlorine in the sn-2 position and are not affected by the preference in fragmentation from the sn-1 position relative to the sn-2 position. In addition, the 2-MCPD diesters produce a higher response for the fatty acid acylium ion compared to the 3-MCPD isomer, providing another possible quantitation ion for 2-MCPD diesters.

It is not possible to individually monitor La-Pa, My-Pa, La-St, and My-St selectively using the loss of NH_4Cl because they coelute with different 3-MCPD diesters of the same mass, which result in two compounds coeluting and producing identical NH_4Cl fragment mass. However, given that they are made up of solely saturated fatty acids, which tend to populate the sn-1 and sn-2 positions at approximately equal concentrations, this is not a tremendous concern for quantitative accuracy. In addition, there are no commonly consumed edible oils that contain both of the fatty acids in any of these contaminants at more than 10% of their total fatty acid composition; therefore, they are unlikely to be found in most oils.

Method Performance. The goal was to develop a validated method that worked across all edible oils. With this in mind, the method was validated using three very different oil matrices to ensure reliable 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, four 1.0 ± 0.01 g portions of coconut, olive, and palm oils were spiked with 0, 10, 100, and 800 μL of spiking solution, respectively, generating oils that were blank, 0.1 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 26 of the 28 3-MCPD diesters in olive, coconut, and palm oils without the use of deuterated internal standards ranged from 76 to 137% [relative standard deviation (RSD) range of 2−23%], with only Ln-Ln, Li-Ln, and Li-Li showing average recoveries below 100%. The results for the remaining two analytes, St-St (average recovery of 159%, RSD of 25%) and Ol-Ln (average recovery of 297%, RSD of 64%), showed significant matrix enhancement and variability. However, strategically chosen deuterated internal standards (see Table 1) to adequately correct recoveries for the entire library of 3-MCPD diesters were available affordably through commercial channels; after their incorporation, the average recoveries (88−118%) and reproducibilities (RSD of 2−16%) for the method 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 ranged from 40 to 985 ng/g (ppb) and were subtracted out before recovery calculations, and none of the target analytes was detected in the coconut and olive oils used in the validation. The method performance of 3-MCPD diesters containing two different fatty acids was validated for all analytes at a low spiking concentration of 100 ppb in all three matrices, and recoveries for diesters containing two of the same fatty acid were validated with a low spike of 50 ppb. Several of the analytes (Pa-Pa, Pa-Ol, and Ol-Li) were present in the palm oil sample at higher concentrations than the low- and middle-level spikes, slightly impacting the reproducibility for these compounds. Recoveries of 3-MCPD diesters were not impacted by the addition of the corresponding 2-MCPD diesters, confirming that the method can separately quantify these two isomeric analytes. The method was validated in the same manner for the three 2-MCPD diesters using the corresponding deuterated 3-MCPD diester internal standard. The average recoveries and reproducibilities in the three matrices across the three spiking levels for 2Li-Li (101.5% recovery, 13% RSD), 2Li-Ln (86.8% recovery, 11.3% RSD), and 2St-St (90.2% recovery, 5.0% RSD) were comparable to those seen for isomeric 3-MCPD diesters.

The LODs and limits of quantitation (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 30 ppb or below, with LODs of 10 ppb or below. The lower LOQs and LODs are generally for analytes that are being quantified by their cyclic acyloxonium ion base

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

	percent recovery, percent RSD										
spike concentration (μg/g) (ppm)	La-Ln	La-My	La-Li	My-Ln	La-Pa	La-Ol	Li-Ln	My-Li	Pa-Ln	Ol-Ln	My-Pa
0.1	109.8, 6.2	96.6, 6.5	97.1, 8.6	107.1, 5.6	109.8, 3.2	99.6, 10.1	96.7, 12.6	104.9, 5.5	106.9, 8.5	110, 10.4	117.6, 10.4
1.0	102.3, 12.4	91.0, 9.4	97.8, 6.7	98.3, 9.5	98.7, 8.9	97.8, 6.7	102.2, 14.2	101.5, 5.4	97.9, 11.3	102.6, 7.2	103.8, 7.3
8.0	101.8, 5.1	87.7, 5.2	105.7, 6.8	107.4, 7.8	97.4, 2.4	105.7, 6.8	103.2, 5.5	106.9, 6.1	89.7, 8.0	97.1, 7.0	106.7, 8.3
	percent recovery, percent RSD										
spike concentration (μg/g) (ppm)	La-St	My-Ol	Pa-Li	Ol-Li	St-Ln	My-St	Pa-Ol	St-Li	Pa-St	Ol-St	
0.1	114.2, 6.2	103.1, 6.3	108.1, 5.0	110.5, 10.4	99.1, 13.0	98.6, 9.5	99.8, 10.7	108.5, 5.7	110.9, 6.2	97.8, 5.5	
1.0	104.4, 8.4	101.5, 5.4	94.7, 10.0	90.8, 16.0	89.7, 14.7	98.9, 12.2	93.5, 11.6	94.7, 10.0	96.2, 9.2	89.8, 9.3	
8.0	104.2, 8.2	106.9, 6.1	107.6, 7.9	95.1, 2.9	99.6, 5.6	92.9, 1.8	93.8, 6.0	106.5, 7.9	95.3, 5.3	108.4, 4.9	
	percent recovery, percent RSD										
spike concentration (μg/g) (ppm)	La-La	Ln-Ln	My-My	Li-Li	Pa-Pa	Ol-Ol	St-St				
0.05	111.7, 5.0	101.5, 7.1	109.2, 4.3	101.4, 10.4	102.3, 11.4	111.5, 9.7	110.0, 6.9				
0.5	94.9, 9.4	96.2, 12.8	96.2, 8.5	96.5, 10.7	96.3, 11.7	98.9, 10.1	102.6, 7.2				
4.0	99.4, 4.0	108.9, 6.9	101.8, 2.4	96.9, 7.6	90.3, 4.0	97.5, 7.1	97.1, 7.0				

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Table 3. Method LODs and LOQs in ppb (ng/g) for 3-MCPD Diesters

	La-La	La-Ln	La-My	La-Li	Ln-Ln	My-Ln	My-My	La-Pa	La-Ol	Li-Ln	My-Li	Li-Li	Pa-Ln	Ol-Ln
LOD (ppb)	3	10	10	10	3	3	3	6	10	10	10	3	10	10
LOQ (ppb)	10	30	30	30	10	10	10	20	30	30	30	10	30	30
	My-Pa	La-St	My-Ol	Pa-Li	Ol-Li	St-Ln	My-St	Pa-Pa	Pa-Ol	Ol-Ol	St-Li	Pa-St	Ol-St	St-St
LOD (ppb)	6	6	10	6	10	10	6	3	6	3	6	6	6	3
LOQ (ppb)	20	20	30	20	30	30	20	10	20	10	20	20	20	10

peak. The LOD and LOQ, respectively, for 2Li-Ln (10 ppb, 30 ppb), 2Li-Li (10 ppb, 30 ppb), and 2St-St (10 ppb, 30 ppb) were higher for 2Li-Li and 2St-St than those for isomeric 3-MCPD diesters because of lower LC−MS/MS responses for the 2-MCPD diesters. Despite lower LC−MS/MS responses for 2Li-Ln compared to Li-Ln, the limits are identical for these two analytes. The MS/MS base peak produced by the loss of a fatty acid from the sn-1 position can be used for quantitation of 2Li-Ln because 2-MCPD diesters are symmetrical and are not affected by the sn-1/sn-2 isomerism present in 3-MCPD diesters.

Applicability in Sample Analysis. This is the first direct method that allows for the separate and accurate quantitation of isomeric 3-MCPD and 2-MCPD diesters. This is possible through the development of the first HPLC conditions that chromatographically resolve 2-MCPD and 3-MCPD esters. Once a suitable library of 2-MCPD analytical esters are synthesized in house or become commercially available, the method will be validated for their quantitation, as well. The method does not require 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 across all edible oils. The lack of the need for matrix-matched standards combined with a straightforward and rugged two-step SPE cleanup allows for rapid analysis of numerous samples in different matrices in a single analytical batch. Given their different relative responses by LC−MS/MS, the method described herein is currently the only approach that is capable of accurately quantifying intact 2-MCPD and 3-MCPD diester concentrations in edible oils. Along with methodology to quantify 3-MCPD monoesters and glycidyl esters (10.1021/jf4005803), 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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