OVID Meeting

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Direct determination of MCPD esters and glycidyl esters by LCMS

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Why Develop an LCMS method?

- The DGF method does not work adequately.
  - -ISTD recovery is acceptable ONLY if NaCl is used in the wash
  - -Loss of virtually all ISTD if Na_2SO_4 is used in the wash
  - -Conversion to the brominated form if NaBr is used in the wash.

- The DGF method claims the method analyzes the total of MCPD plus glycidol, but MCPD and glycidol have not been shown to be the only precursors that can produce MCPD under the reaction conditions.

- The difference between the two treatments in the DGF method has been implied as a measure of glycidol, but there is no evidence glycidol is the only substance which contributes to this difference in results.

- LCMS provides direct analysis of MCPD mono-esters and di-esters and glycidyl esters without chemical modifications that can give incorrect results.
- LCMS provides very specific correct identification of analytes.
- LCMS allows use of isotopic internal standards for very accurate analysis.

Effect of Salts on the DGF method

Destruction of 3-MCPD in the absence of chloride

	3-MCPD-d5 Area		3-MCPD Area	
	Sodium chloride Preparation	Sodium Sulfate Preparation	Sodium chloride Preparation	Sodium Sulfate Preparation
Palm Stearine	2,743,461	ND	1,316,656	142,956
RBD Soy	2,873,546	356,407	296,788	304,444
Palm/Soy	3,042,333	ND	1,248,188	276,105
RBD Soy	2,915,739	ND	1,313,061	ND
RB Palm Olein	1,764,198	ND	264,339	ND
RBD Palm Olein	1,418,326	71,307	2,882,576	ND
Palm Kernel Stearine	1,385,577	ND	118,680	ND
Hydro Palm Kernel Olein	1,316,460	ND	103,383	25,000
ISTD Blank	1,704,685	268,083	ND	ND
Check Standard	1,304,732	143,892	1,052,039	90,996
Crude Palm Oil	2,481,477	ND	3,809,021	208,429

ND = Not Detected

ADM

Effect of Salts on the DGF method
Conversion of MCPD to MCBD in the presence of bromide

	3-MCPD-d5	3-MBPD-d5	3-MCPD	3-MBPD
Soy/Palm #1 **NaCl**	653,696	ND	ND	ND
Soy/Palm #1 **NaBr**	ND	613,122	ND	ND
Soy/Palm #2 **NaCl**	597,555	ND	1,450,981	ND
Soy/Palm #2 **NaBr**	ND	586,702	ND	1,306,095
Soy/Palm #3 **NaCl**	630,800	ND	1,590,794	ND
Soy/Palm #3 **NaBr**	ND	588,550	ND	1,307,056
Soy/Palm #4 **NaCl**	684,877	ND	1,367,516	ND
Soy/Palm #4 **NaBr**	ND	617,797	ND	1,066,853
RBD Palm #1 **NaCl**	663,686	ND	330,123	ND
RBD Palm #1 **NaBr**	ND	616,085	ND	326,312
RBD Soy **NaCl**	662,067	ND	373,531	ND
RBD Soy **NaBr**	ND	619,409	ND	341,145

TOF-LCMS Method for MCPD esters and glycidyl esters

- MCPD esters do not readily ionize to MH+ ions by either ESI or APCI
- Current approach used previously for diglycerides [1]
- Low concentration of sodium acetate in the mobile phase allows ESI ionization and detection of sodiated ions [MNa+]
- High resolution TOF-MS for detection allowed elimination of interferences for certain MCPD esters.
    - Eliminated by setting m/z resolution to 50 ppm (±0.016 da)

[1] Hannah L. Callender, Jeffrey S. Forrester, Pavlina Ivanova, Anita Preininger, Stephen Milne, and H. Alex Brown**,** "Quantification of Diacylglycerol Species from Cellular Extracts by Electrospray Ionization Mass Spectrometry Using a Linear Regression Algorithm" **Anal. Chem. 2007, 79, 263-272**

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LC-TOFMS

•Detects both mono- and di-esters of MCPD
•Limit of quantitation for total MCPD esters ~500 µg/kg
(~100 µg/kg MCPD equivalent concentration)

•Method also optimized for detection of glycidyl esters
•Limit of quantitation for total glycidyl esters ~100 µg/kg
(~20 µg/kg glycidol equivalent concentration)

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Standards

- 3-MCPD monopalmitate, 3-MCPD monostearate, 3-MCPD dipalmitate, were purchased from Toronto Research Chemicals, Inc (North York, ON, Canada).
- Glycidyl stearate was purchased from TCI America (Portland, OR, USA).
- Glycidyl linolenate, glycidyl linoleate, glycidyl oleate and glycidyl palmitate were purchased from Wako Pure Chemical Industries (Tokyo, Japan).
- Glycidyl-31d-palmitate synthesized enzymatically as internal standard for glycidyl esters
- 3-MCPD mono- and diesters of oleic, linoleic and mixed fatty acids, and deuterated internal standard (dioleyl-d5-MCPD), and glycidyl esters of palmitic, oleic and mixed fatty acids were synthesized enzymatically.

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LC and TOF-MS conditions

- **Methanol-Sodium Acetate -** Methanol containing 0.26 mM sodium acetate was prepared by adding 21.3 mg sodium acetate to 1 liter methanol.
- **HPLC Mobile Phase A** - 90% methanol 10% acetonitrile with 0.026 mM sodium acetate. Mix 100 mL Methanol-Sodium Acetate, 800 mL methanol and 100 mL acetonitrile.
- **HPLC Mobile Phase B** - 80% methylene chloride 10% methanol 10% acetonitrile with 0.026 mM sodium acetate. Mix 100 mL Methanol-Sodium Acetate, 800 mL methylene chloride and 100 mL acetonitrile.
- **ISTD solution**: 2030 µg/mL dioleyl-d5-MCPD and 180 µg/mL glycidyl 31d-palmitate in HPLC Mobile B
- **Sample Preparation:**

  -In a 12 x 32 HPLC vial, accurately weigh 1 drop (~20-25 mg) oil
  -Add 975 µL ISTD Solution.

LC and TOF-MS conditions

- **HPLC Column**: Phenomenex Luna 3 µm C18 ,100Å 50 x 3.0 mm.
- **Flow Rate** - 0.25 mL / min.

Gradient Programming	
Run Time	% B
0 min.	0
5 min.	0
15 min.	65
16 min.	100
20 min.	100

- **TOF-MS –**
- Agilent 6210 TOF-MS upgraded to 4 GHz
  - ESI at 3.5 kV
  - Gas Temp. 300°C
  - Drying Gas 5 L/min.
  - Nebulizer Pressure 50 psi
  - Mass Range 300 to 700 m/z
- Polarity Positive, Instrument Mode 4 GHz, Data Storage - Centroid
- Monoheptadecanoin and Dinonadecanoin as Mass Reference Ions

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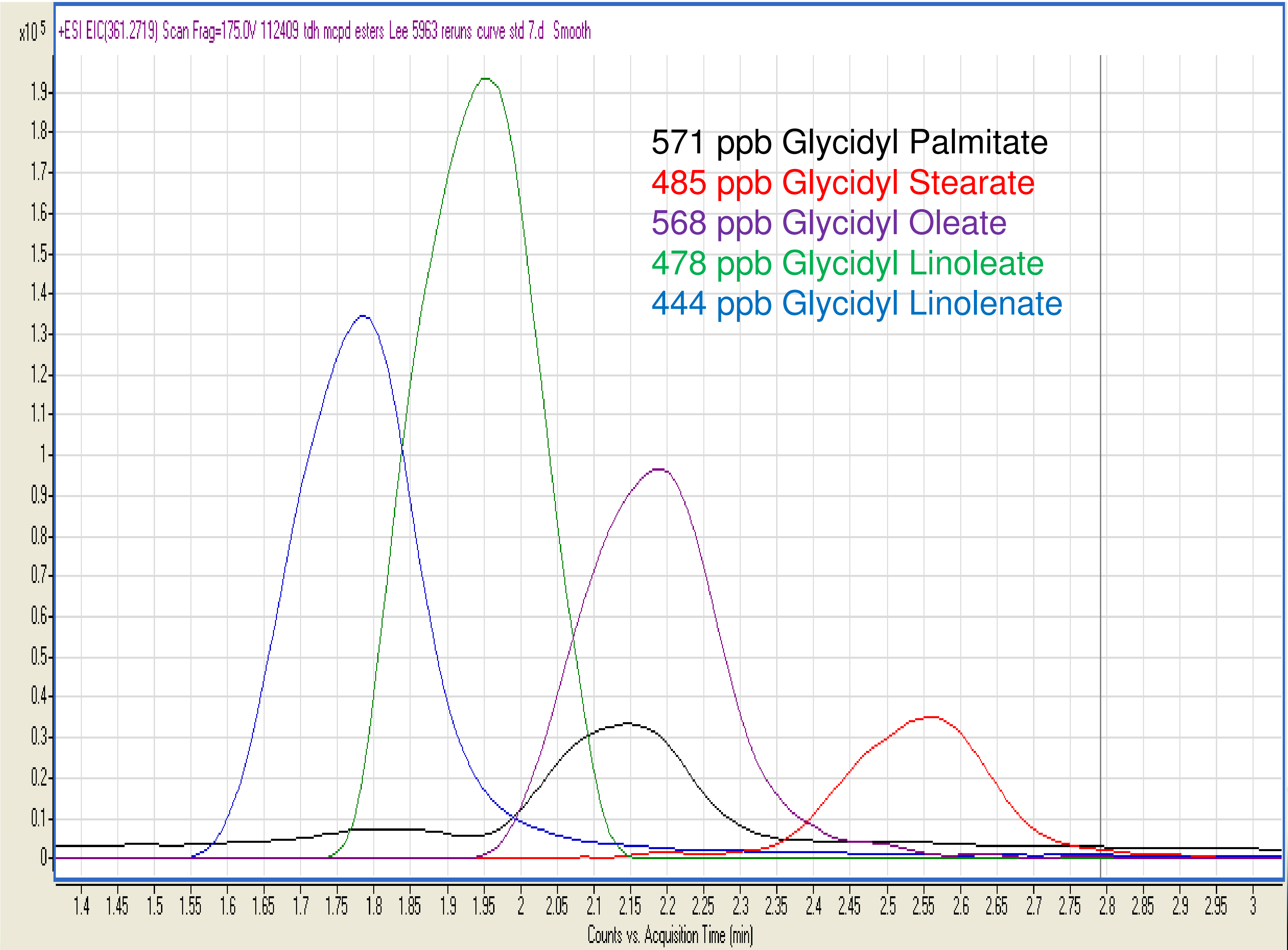
Compound	Formula	m/z [M+Na(+)]
Palmitic Acid Glycidol Ester	C19H36O3	335.25622
Stearic Acid Glycidol Ester	C21H40O3	363.28752
Oleic Acid Glycidol Ester	C21H38O3	361.27187
Linoleic Acid Glycidol Ester	C21H36O3	359.25622
Linolenic Acid Glycidol Ester	C21H34O3	357.24057
d31-Palmitic Acid Glycidol Ester Internal Standard	C19H5D31O3	366.45025
Palmitic Acid MCPD monoester	C19H37ClO3	371.23289
Stearic Acid MCPD monoester	C21H41ClO3	399.26419
Oleic Acid MCPD monoester	C21H39ClO3	397.24854
Linoleic Acid MCPD monoester	C21H37ClO3	395.23289
Linolenic Acid MCPD monoester	C21H35ClO3	393.21724
Palmitic Acid-Oleic Acid-MCPD diester	C37H69ClO4	635.47821
di-Palmitic Acid MCPD Diester	C35H67ClO4	609.46256
di-Oleic Acid MCPD diester	C39H71ClO4	661.49386
Palmitic Acid-Linoleic Acid MCPD diester	C37H67ClO4	633.46256
Oleic Acid-Linoleic Acid MCPD diester	C39H69ClO4	659.47821
Palmitic Acid-Stearic Acid MCPD diester	C37H71ClO4	637.49386
Oleic Acid-Stearic Acid MCPD Diester	C39H73ClO4	663.50951
di-Linoleic Acid MCPD diester	C39H67ClO4	657.46256
Linoleic Acid-Stearic Acid MCPD diester	C39H71ClO4	661.49386
di-Stearic Acid MCPD diester	C39H75ClO4	665.52516
di-Linolenic Acid MCPD diester	C39H63ClO4	653.43126
Oleic Acid-Linolenic Acid MCPD diester	C39H67ClO4	657.46256
Linoleic Acid-Linolenic Acid MCPD diester	C39H65ClO4	655.44626
Palmitic Acid-Linolenic Acid MCPD diester	C37H65ClO4	631.44691
Stearic Acid-Linolenic Acid MCPD diester	C39H69ClO4	659.47821
d5-MCPD Di-Oleic Acid Ester Internal Standard	C39H66D5ClO4	666.52524

ADM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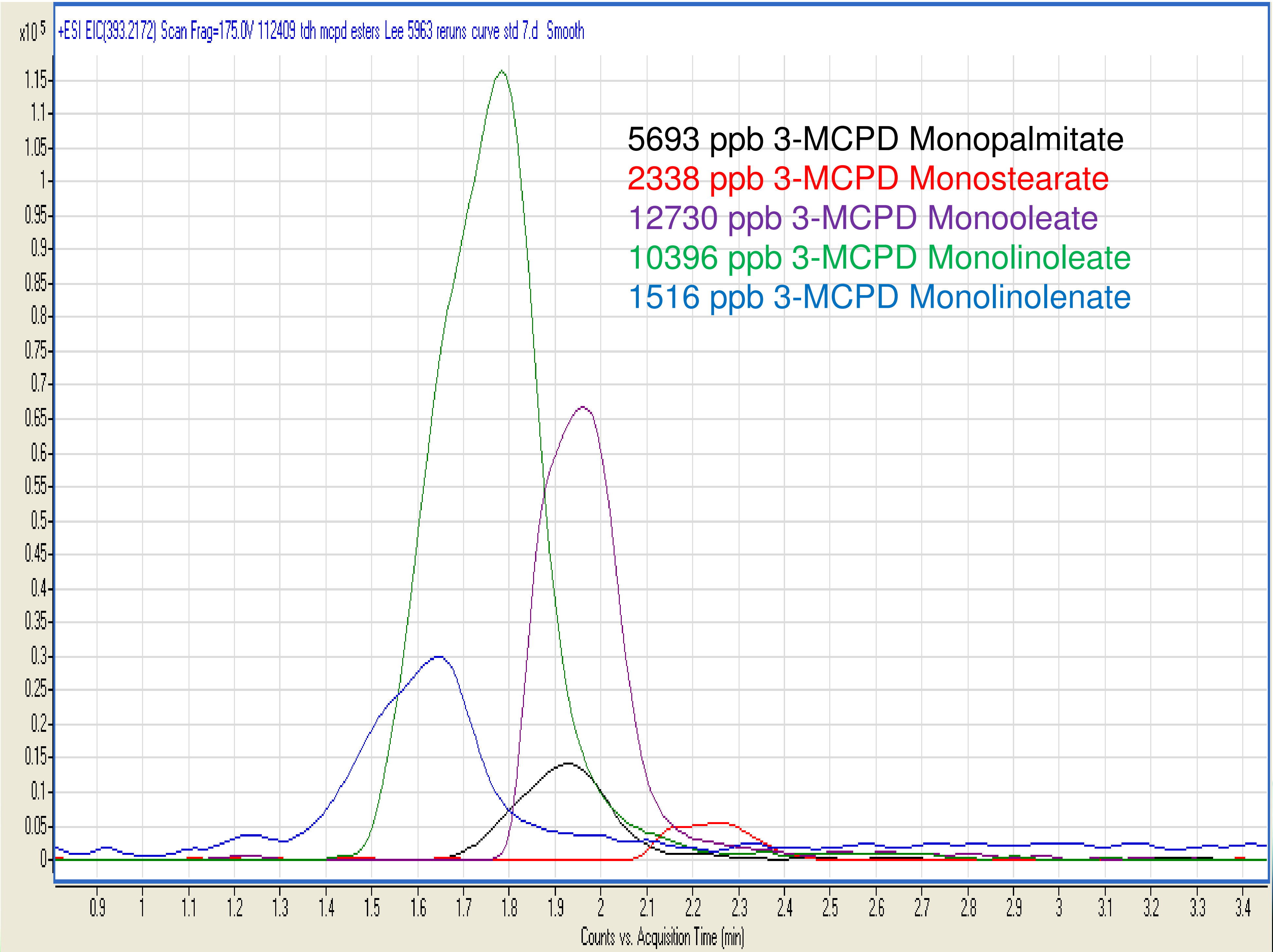
571 ppb Glycidyl Palmitate
485 ppb Glycidyl Stearate
568 ppb Glycidyl Oleate
478 ppb Glycidyl Linoleate
444 ppb Glycidyl Linolenate
Counts vs. Acquisition Time (min)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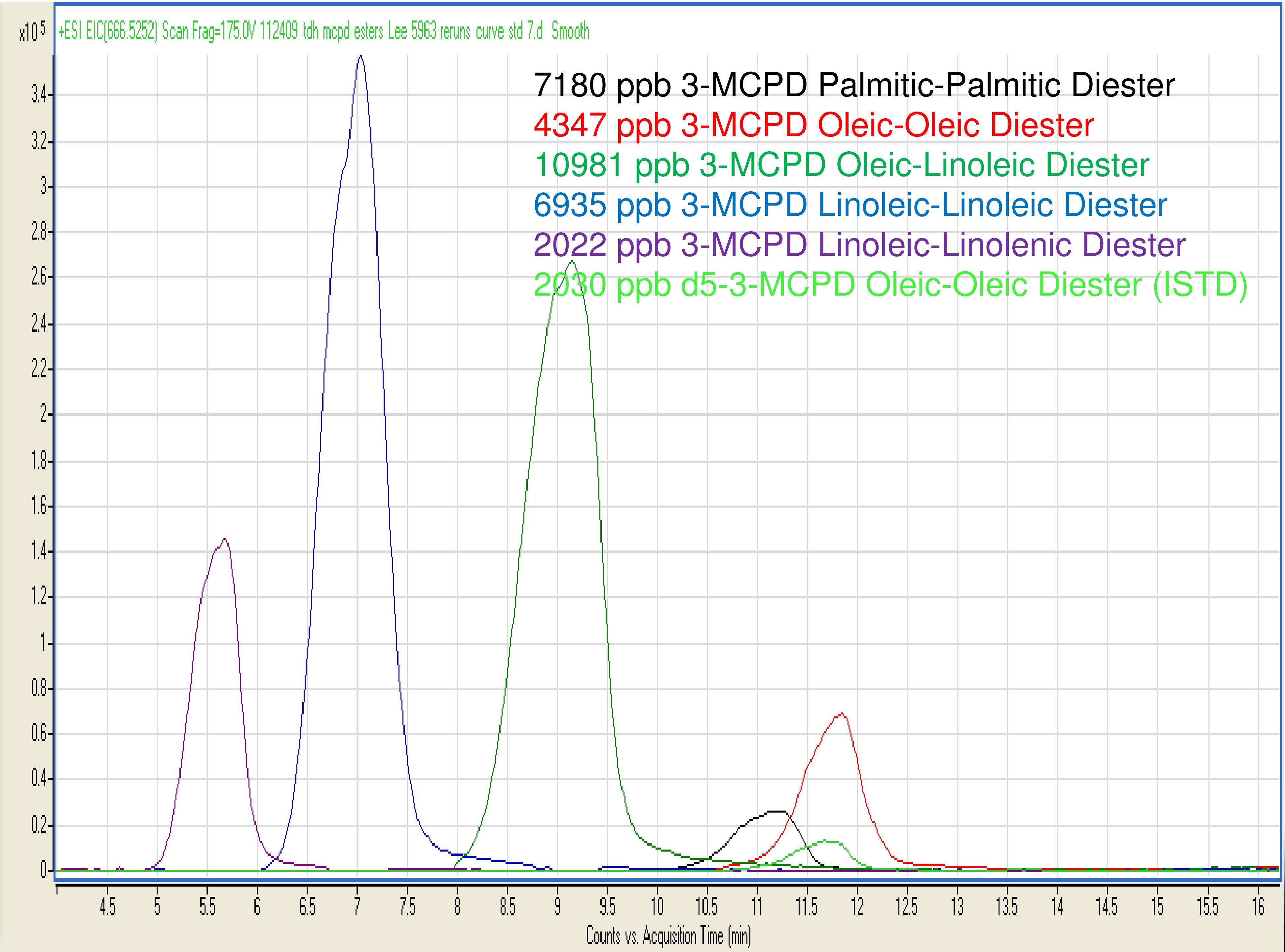
5693 ppb 3-MCPD Monopalmitate
2338 ppb 3-MCPD Monostearate
12730 ppb 3-MCPD Monooleate
10396 ppb 3-MCPD Monolinoleate
1516 ppb 3-MCPD Monolinolenate
Counts vs. Acquisition Time (min)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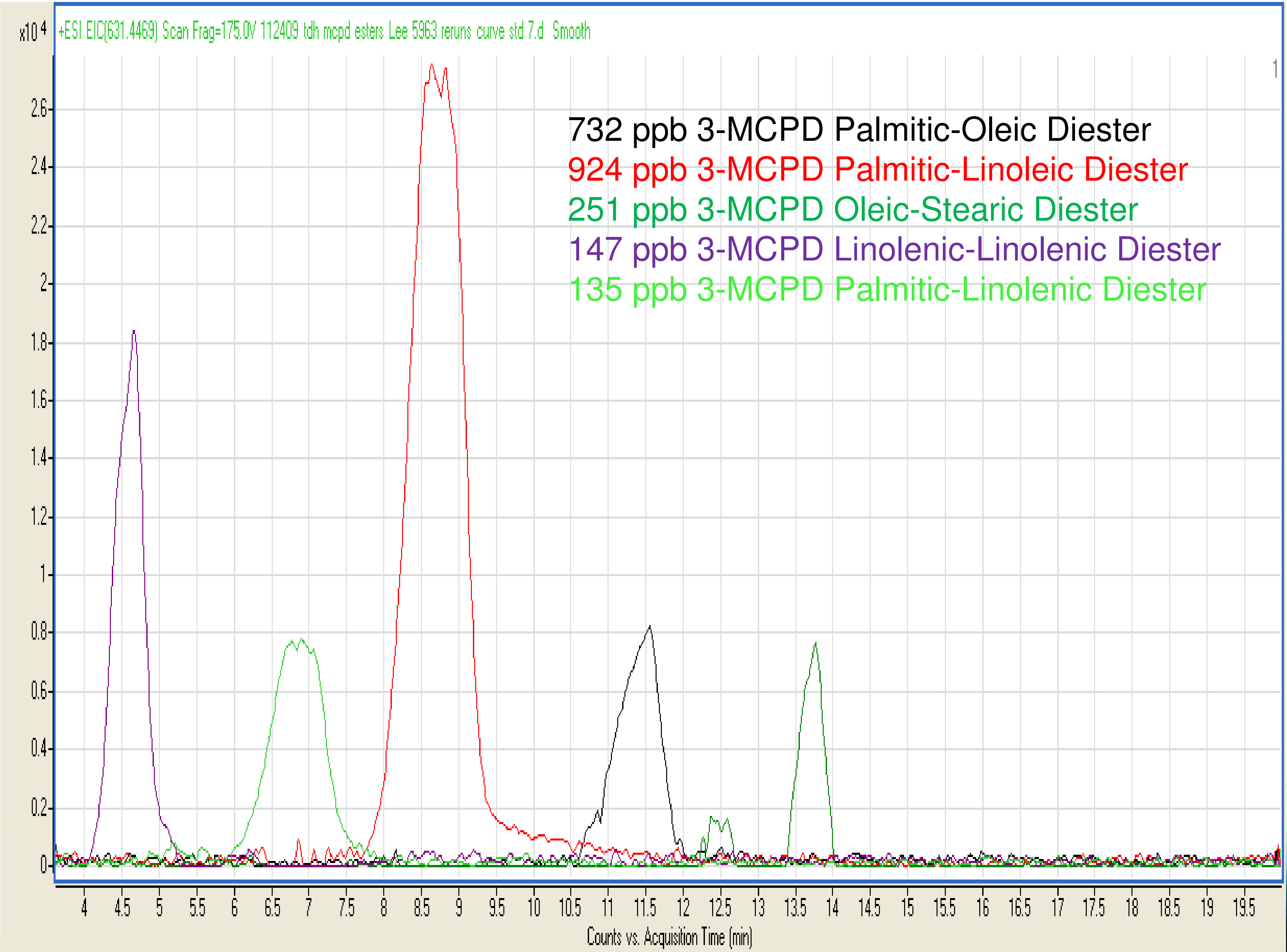
7180 ppb 3-MCPD Palmitic-Palmitic Diester
4347 ppb 3-MCPD Oleic-Oleic Diester
10981 ppb 3-MCPD Oleic-Linoleic Diester
6935 ppb 3-MCPD Linoleic-Linoleic Diester
2022 ppb 3-MCPD Linoleic-Linolenic Diester
2030 ppb d5-3-MCPD Oleic-Oleic Diester (ISTD)
Counts vs. Acquisition Time (min)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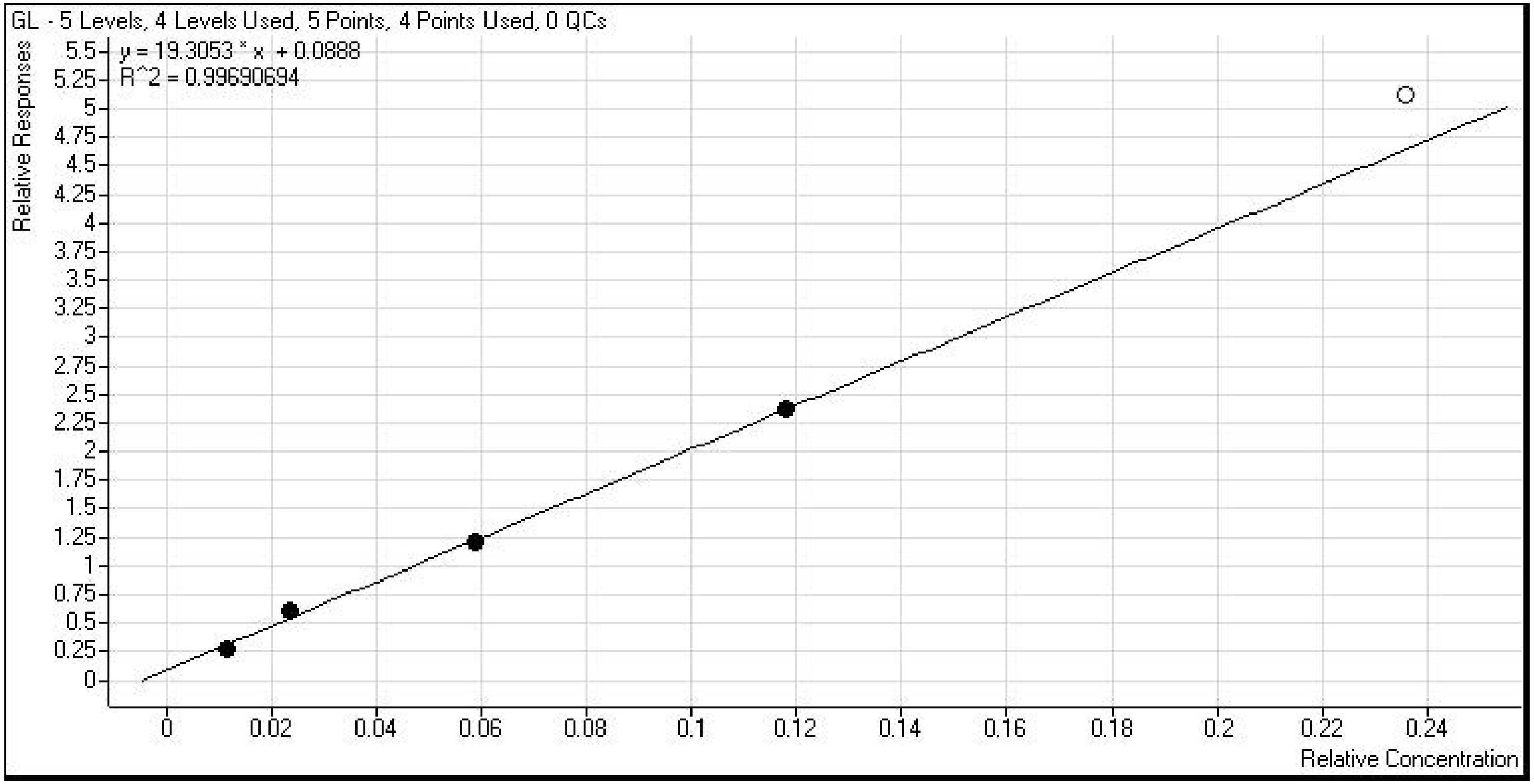
+ESI EIC(631.4469) Scan Frag=175.0V 112409 tdh mcpd esters Lee 5963 reruns curve std 7.d Smooth
732 ppb 3-MCPD Palmitic-Oleic Diester
924 ppb 3-MCPD Palmitic-Linoleic Diester
251 ppb 3-MCPD Oleic-Stearic Diester
147 ppb 3-MCPD Linolenic-Linolenic Diester
135 ppb 3-MCPD Palmitic-Linolenic Diester
Counts vs. Acquisition Time (min)

Typical Standard Curve

GL, ng/mL	4.79	11.96	23.93	47.85	119.63	239.25	478.5



Results for Commercial Refined Oils

- MCPD monoesters are never found in deodorized oils. This means that glycidol is not likely to be the precursor of MCPD esters in oils.

- MCPD diesters have been not found in commercial vegetable oils other than some samples of palm oil.

- The DGF test does not accurately predict the amount of MCPD esters in oil samples.

DGF Method compared to LCMS method

Sample ID	DGF method run at SGS	Total MCPD Mono esters mg/kg (DL=1)	Total MCPD Diesters mg/kg (DL=0.5)	MCPD Equiv. by LCMS mg/kg	Total Glycidyl Esters mg/kg (DL=0.1)	Glycidol Equiv. mg/kg
Sample A : RBD Palm oil	**7.74**	nd	6.1	**1.2**	18.6	5.0
Sample A after ADM Treatment Process	**2.85**	nd	nd	**0.0**	nd	**0.0**
Sample B, Commercial Oil Blend	**3.64**	nd	nd	**0.0**	9.9	2.7
Sample B after ADM Treatment Process	**1.83**	nd	nd	**0.0**	nd	**0.0**

DGF method predicts much higher MCPD concentrations than LCMS when MCPD esters are present.

DGF Method still gives positive results even when MCPD and glycidyl esters are not present.

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MCPD Esters can form from contact with hydrochloric acid

Sample: RBD Corn Oil with ~20 mg/kg added glycidyl stearate	GE (ppm)	3-MCPD diesters (ppm)
Control - no acid wash	24.4	nd
Acetic acid wash	23.1	nd
Hydrochloric acid wash	20.1	40.8
ADM Treatment Process	nd	nd

Conditions of acid treatment: 0.1 v/wt% acid, 2 min shear mix, 60 min stir mix, 70 C, water wash to neutral, deodorized after washing

MCPD Esters can form from hydrochloric acid treated adsorbents

Sample of RB Palm Oil with ~20 mg/kg added glycidyl stearate	Glycidyl Esters (mg/kg)	MCPD diesters (mg/kg)
Control (No treatment)	18.8	nd
10% Magnesol XL, 110 C, 30 min	35.1	nd
10% Silica gel, 110C, 30 min	16.9	nd
10% alumina, acidic *, 110 C, 30 min	21.4	73.2
10% acid washed carbon *, 110 C, 30 min	22.2	215.6

* manufacturers confirmed that HCl was used in the acid washing

Note that MCPD increased without a decrease in glycidyl esters. Glycidyl esters were not converted to MCPD

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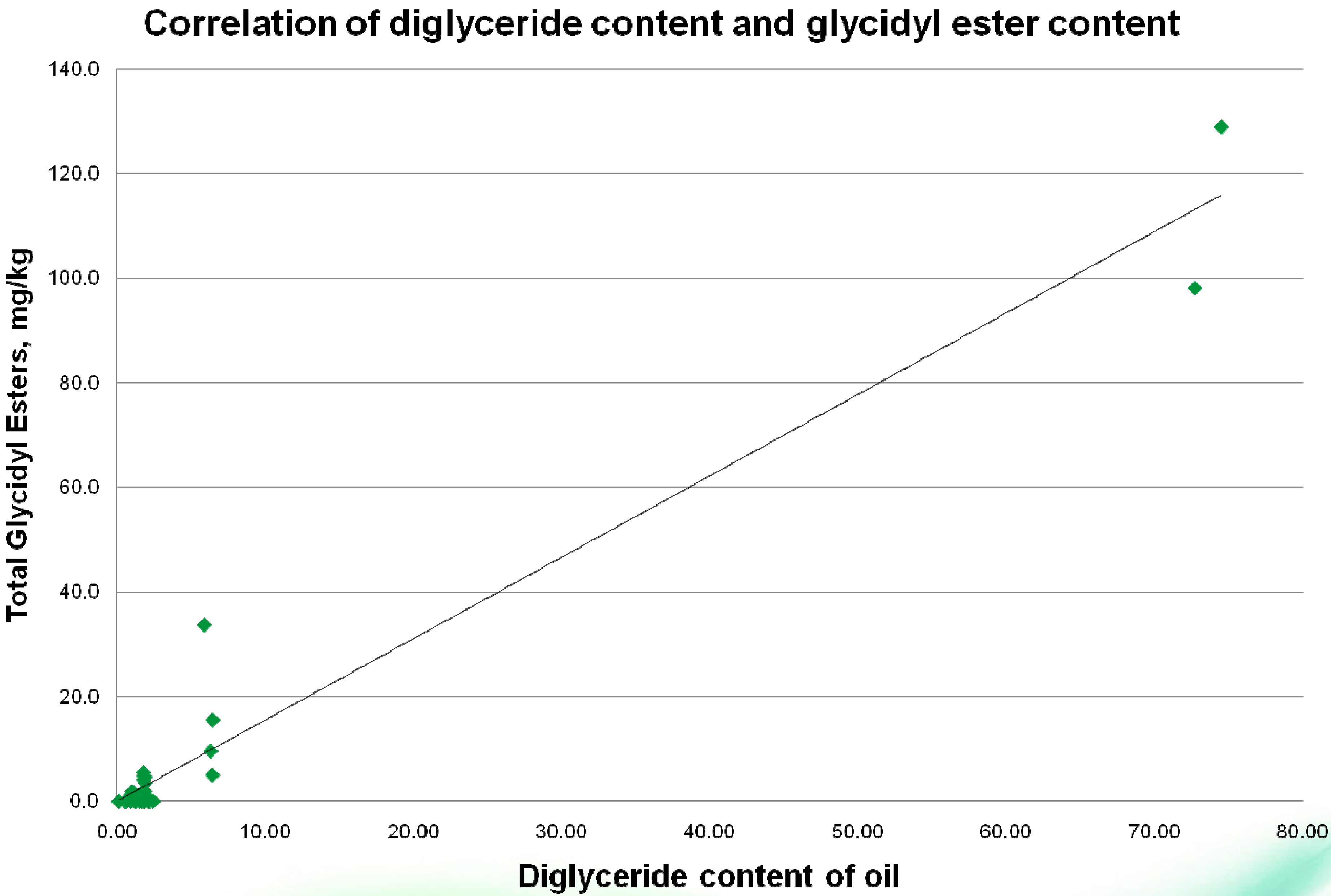
Glycidyl esters

•Glycidyl esters were found in significant quantities in some commercial vegetable oils.

•Diglyceride content seems to correlate somewhat with glycidyl ester content. High diglyceride content in oils predisposes them to formation of glycidyl esters

ADM


Correlation of diglyceride content and glycidyl ester content
Total Glycidyl Esters, mg/kg
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
Diglyceride content of oil

Conclusions

- The harsh chemistry of the DGF method creates incorrect results in the analysis of MCPD and glycidyl esters.
- Chemistry capable of transesterifying oils needs to be avoided in analysis of MCPD and glycidyl esters.
- Direct analysis by LCMS looks extremely promising.
- MCPD esters can form from contact with hydrochloric acid and from acid washed absorbents.
- Glycidyl esters are widely present in vegetable oils. Glycidyl esters seem to be a much more widespread issue than MCPD esters.
- Glycidyl esters and MCPD esters can be greatly reduced by processing.

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