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An Automated System for the analysis of fatty acid methyl esters (FAME) in edible oils

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Illinois Institute of Technology, Chicago, IL

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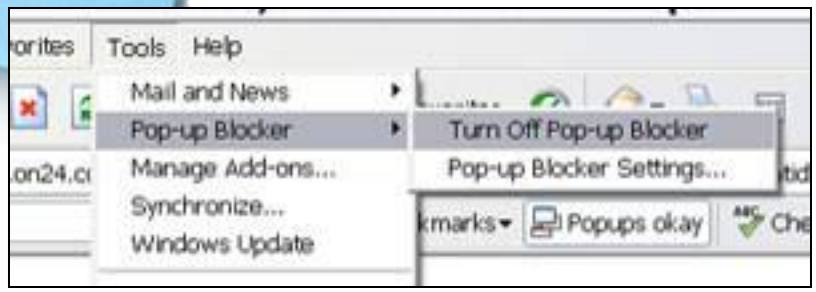

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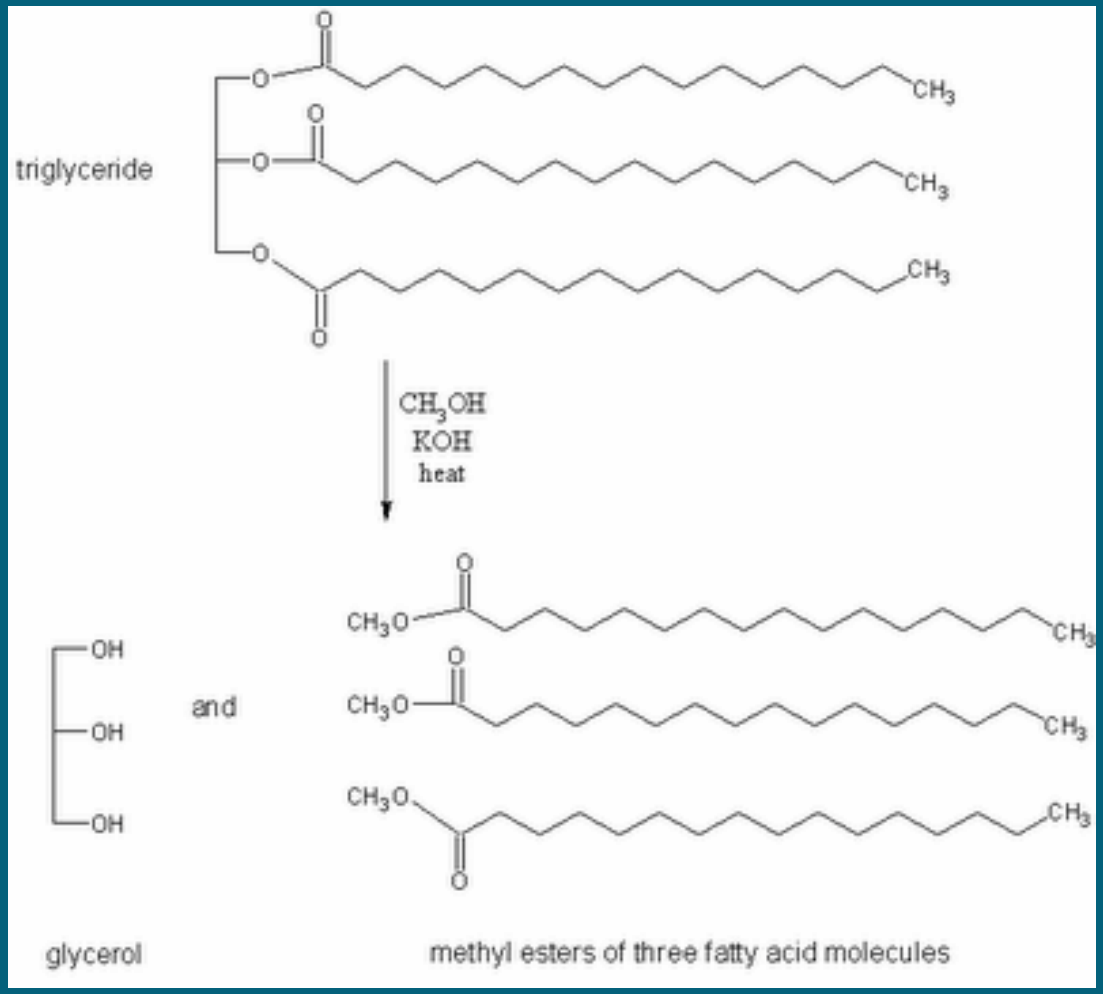
Outline

- GC/MS and GC/FID method
- WorkBench and manual sample preparation methodology
- Agilent 7696A Sample Prep WorkBench method validation
- Analysis of EVOO and adulterated EVOO
- Conclusion

Triglyceride

- 1 fat/3 fatty acid chains
- Glycerol Backbone
- Derivatization with Boron Trifluoride in methanol
- Product: 3 fatty acid methyl esters



Analysis

- AOAC 996.06
- AOCS Ce 1h-05
- Acid Hydrolysis of Sample Matrix (Mojonnier Fat Method)
- Esterification with methanol to form Fatty Acid Methyl Esters
- GC

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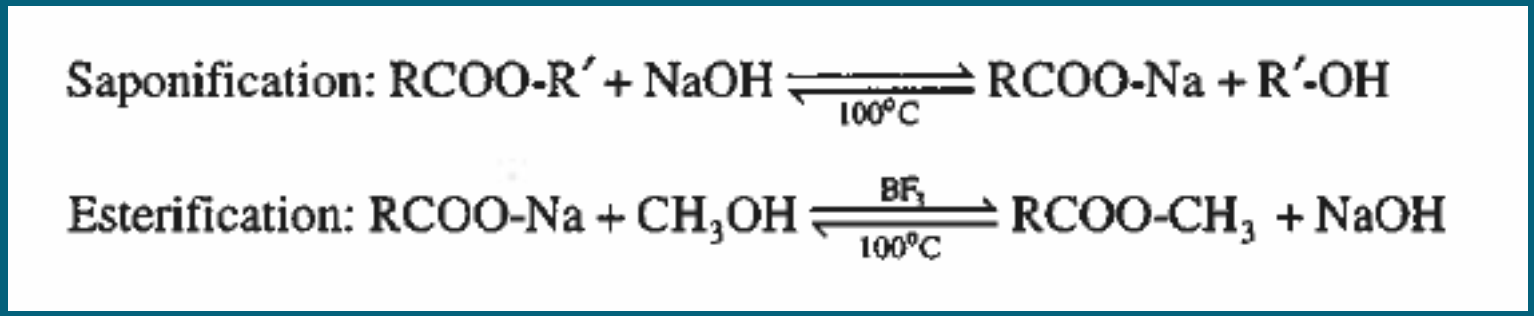
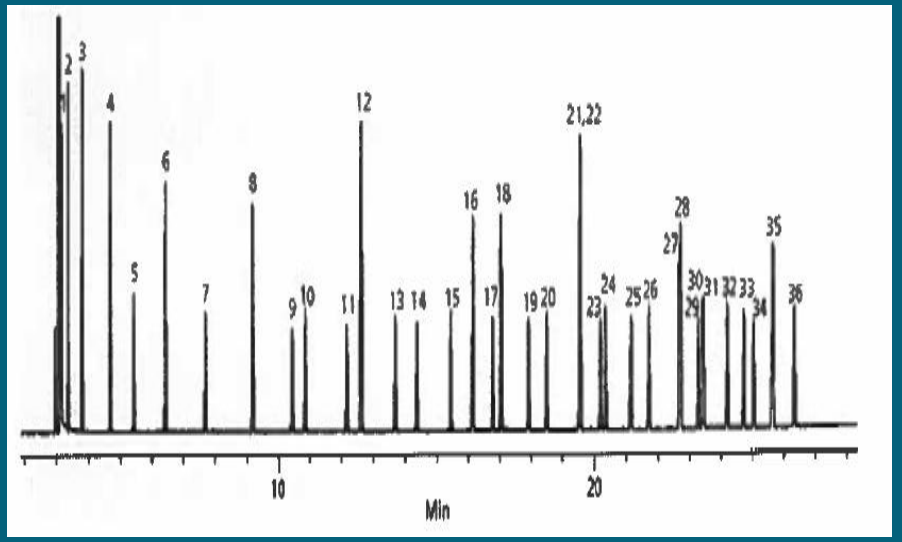
Esterification



GC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Instrumentation and Analytical Conditions for the GC/MS system

GC chromatograph	Agilent 6890A Series
Column:	HP-88, 60m x 250mm x 0.25 µm (p/n 112-8867)
GC Inlet:	250°C, Split ratio 100 : 1
Carrier gas:	Helium, constant flow mode, 1 mL/min
Oven temperature program:	140°C (5 min), 4°C/min to 240°C (0 min)
Injection volume:	1 µL
Mass selective detector:	5973 MSD
Transfer line:	280°C
Solvent delay:	4 min
Acquisition mode:	Scan (40 - 500 amu)
Liner:	Split liner, tapered, deactivated (p/n 5183-4711)

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Instrumentation and Analytical Conditions for the GC/FID system

GC chromatograph	Agilent 6890A Series
Column:	HP-88, 60m x 250mm x 0.25 µm (p/n 112-8867)
GC Inlet:	250°C, Split ratio 60:1
Carrier gas:	Helium, constant flow mode, 1.3 mL/min
Oven temperature program:	140°C (5 min), 4°C/min to 240°C (0 min)
Injection volume:	1 µL
Detector:	FID @300°C
Transfer line:	280°C
Liner:	Split liner, tapered, deactivated (p/n 5183-4711)

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Preparation Of Fatty Acid Methyl Esters with an Acid Catalyzed Reaction Manually

- 50 mg sample in 15 ml centrifuge tube
- Add 2 ml of 2N NaOH in methanol
- Heat 80ºC for 1 hour; Allow to cool
- Add 2 ml of 25% BF_3 in methanol
- Heat 80ºC for 1 hour; Allow to cool
- Add 5 ml of water and 5 ml of hexane
- Shake well
- Allow the phase to separate or centrifuge
- Transfer supernatant to GC autosampler vial

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Developing a method for WorkBench

- Calibration curve standards
- Validate the automated method
  - Convert manual AOAC method to WorkBench reduced volumes
  - Verify WorkBench method gives the same results as a manual method

Test samples with an acid catalyzed reaction:

- EVOO from Spain, Italy, Greece, and California
- Refined olive oils
- Seed oil (grapeseed, sunflower, corn, canola, and vegetable)

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Calibration Curve Standard Preparation on WorkBench

- GLC Reference STD 603 (from NU-CHEK PREP, INK.)
- ISTD - Methyl Undecanoate (from NU-CHEK PREP, INK.)
- Six level calibration curve (0.001- 0.1mg/ml, 0.040 - 40mg/ml – depending on the specific FAME concentration)
- Linear dilutions in 500µL of hexane
- Complete in 40 minutes
- Excellent linearity (Avg R^2=0.999)

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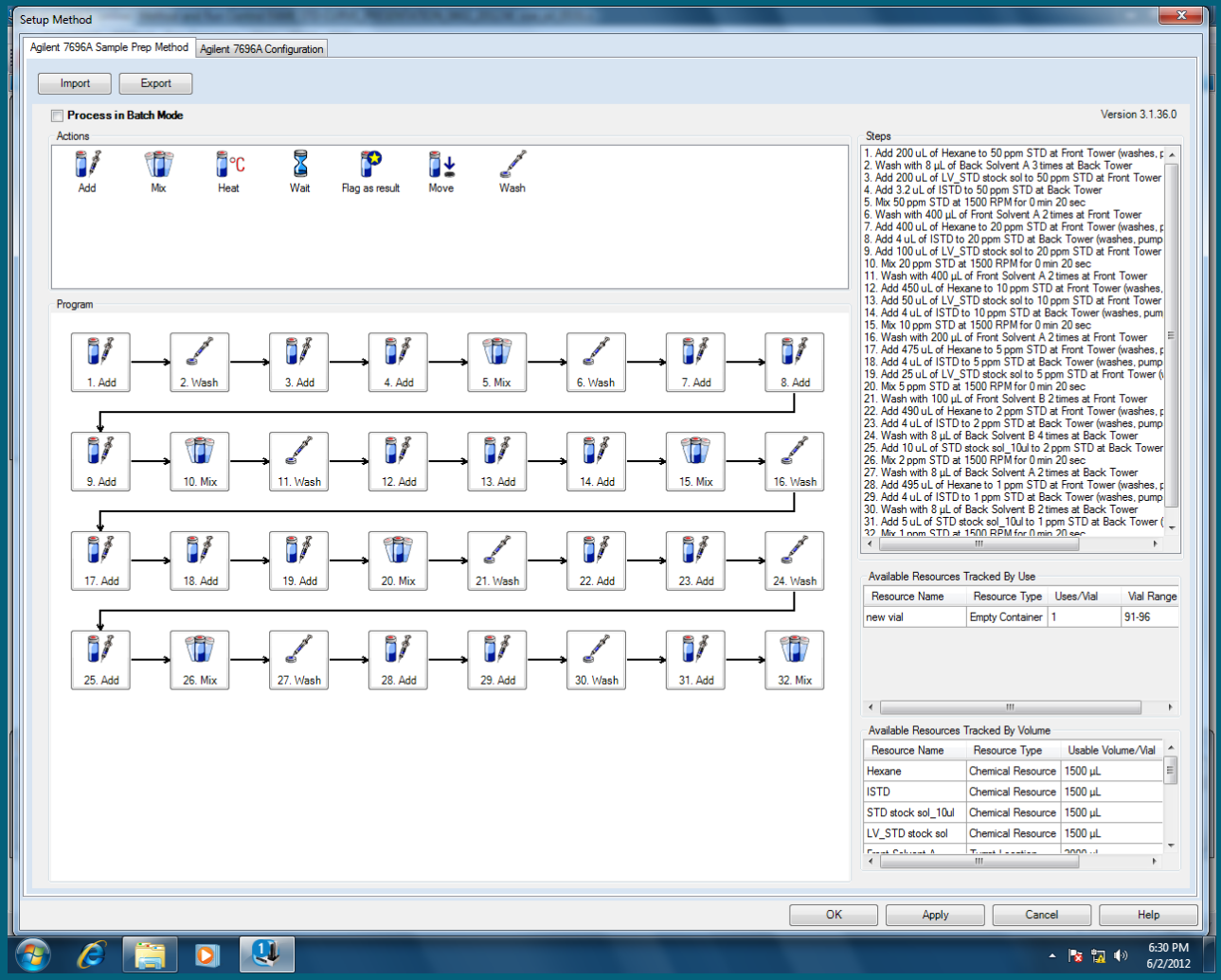
Work Bench

Calibration Curve Standard Preparation on WorkBench method



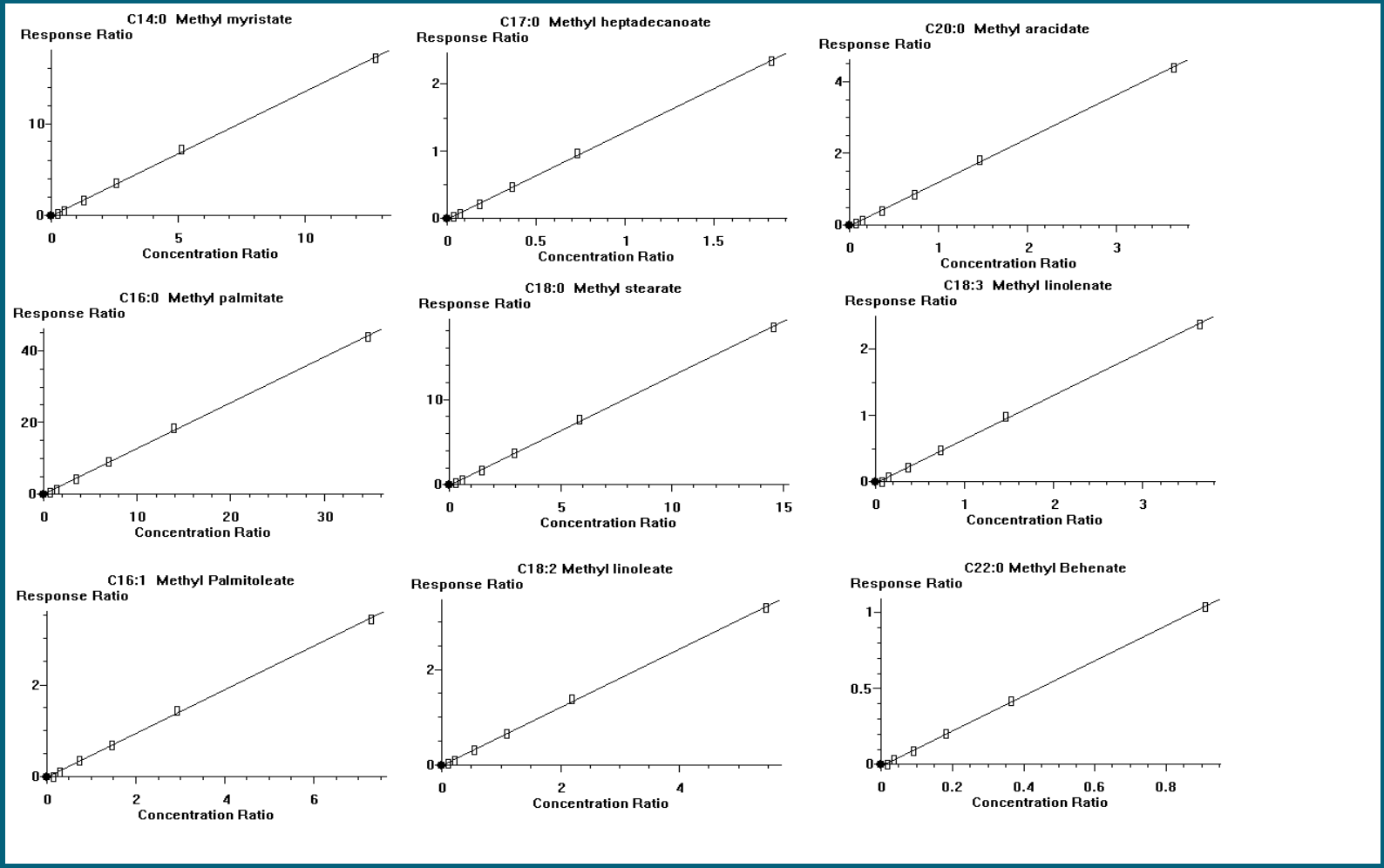
Instrument Calibration Data for FAME standards prepared with the Agilent 7696A Sample Prep WorkBench

#	FAME name	Chain	R^2
1	Methyl Myristate	C14:0	0.9994
2	Methyl Palmitate	C16:0	0.9992
3	Methyl Palmitoleate	C16:1	0.9991
4	Methyl Heptadecanoate	C17:0	0.9997
5	Methyl Stearate	C18:0	0.9996
6	Methyl Oleate	C18:1	1.0000
7	Methyl Vaccenate	C18:1	0.9987
8	Methyl Linoleate	C18:2	0.9994
9	Methyl Arachidate	C20:0	0.9997
10	Methyl Linolenate	C18:3	0.9994
11	Methyl 11-Eicosenoate	C20:1	0.9989
12	Methyl Behenate	C22:0	0.9997
13	Methyl Lignocerate	C24:0	0.9999

Instrument Calibration Data for FAME standards prepared manually

#	FAME name	Chain	R^2
1	Methyl Myristate	C14:0	0.9919
2	Methyl Palmitate	C16:0	0.9901
3	Methyl Palmitoleate	C16:1	0.9943
4	Methyl Heptadecanoate	C17:0	0.9989
5	Methyl Stearate	C18:0	0.9906
6	Methyl Oleate	C18:1	0.9912
7	Methyl Vaccenate	C18:1	0.9961
8	Methyl Linoleate	C18:2	0.9957
9	Methyl Arachidate	C20:0	0.9969
10	Methyl Linolenate	C18:3	0.9970
11	Methyl 11-Eicosenoate	C20:1	0.9976
12	Methyl Behenate	C22:0	0.9997
13	Methyl Lignocerate	C24:0	0.9989

Calibration curves for FAME standards prepared with the Agilent 7696A Sample Prep WorkBench



Edible oil Sample Preparation on the WorkBench

- Add 10 µL of oil sample
- Add 3.3 µL of internal standard
- Add 120 µL of 2N NaOH in methanol
- Mix 20 s at 1500 rpm
- Heat at 70ºC for 5min, cool 5 min
- Add 240 µL of 12.5% BF_3 in methanol
- Mix 20 s at 1500rpm
- Heat at 70ºC for 5 min, cool 5 min
- Add 300 µL of water
- Add 300 µL of hexane
- Mix 20 s at 1500 rpm
- Transfer top layer (of 300 µL) to a new GC vial

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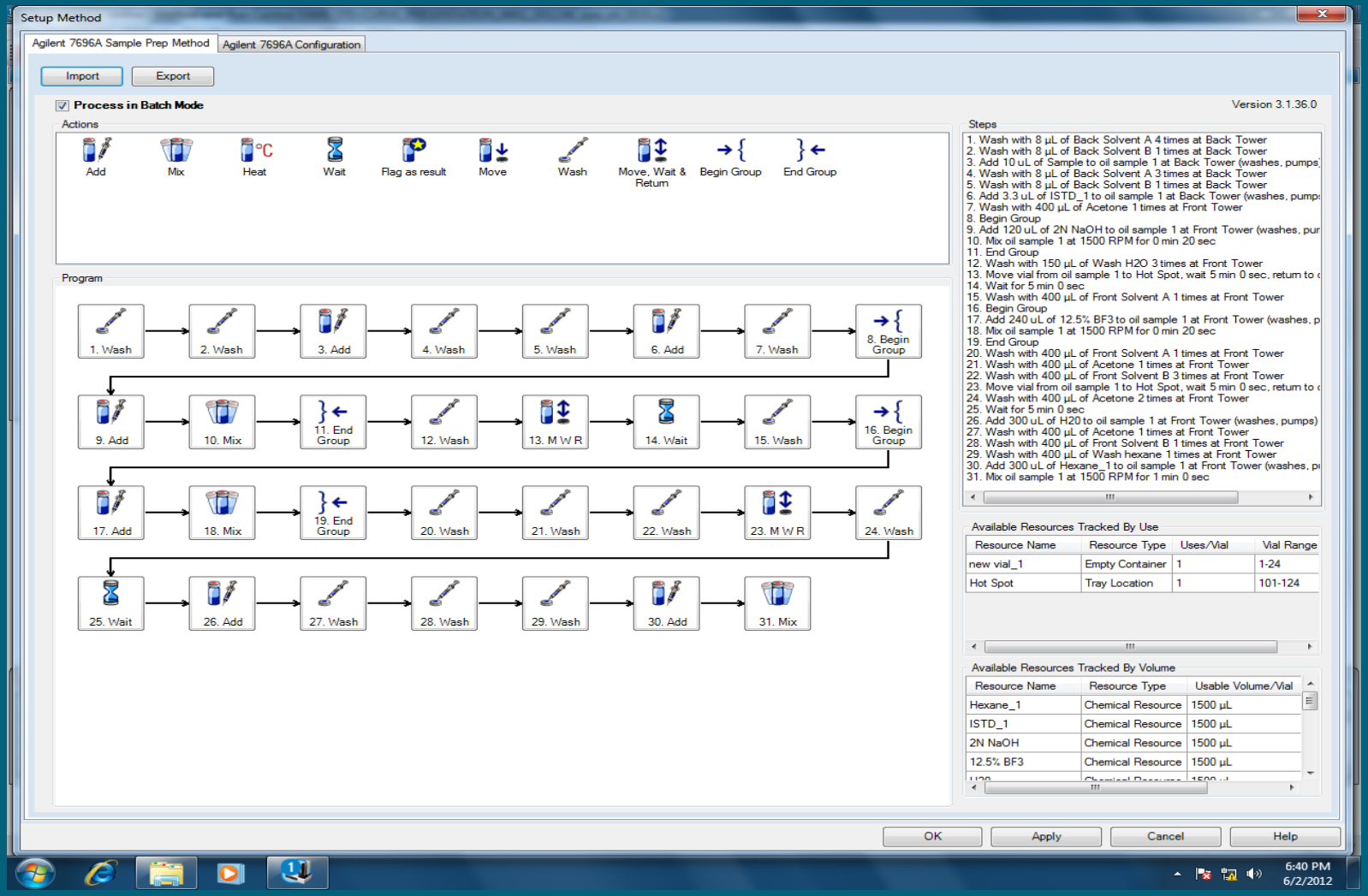
Edible oil Sample Preparation on the WorkBench



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WorkBench Method Validation

- Three edible oils (refined olive oil, Spanish EVOO, and California EVOO) were prepared manually and by WorkBench and analyzed on different days
- %RSD values for 6 samples were very similar to the manual sample preparation %RSD
- Generally recoveries were greater in the WorkBench prepared samples
- WorkBench made samples are as good as manually prepared samples
- WorkBench used 17 times less solvents and reagents

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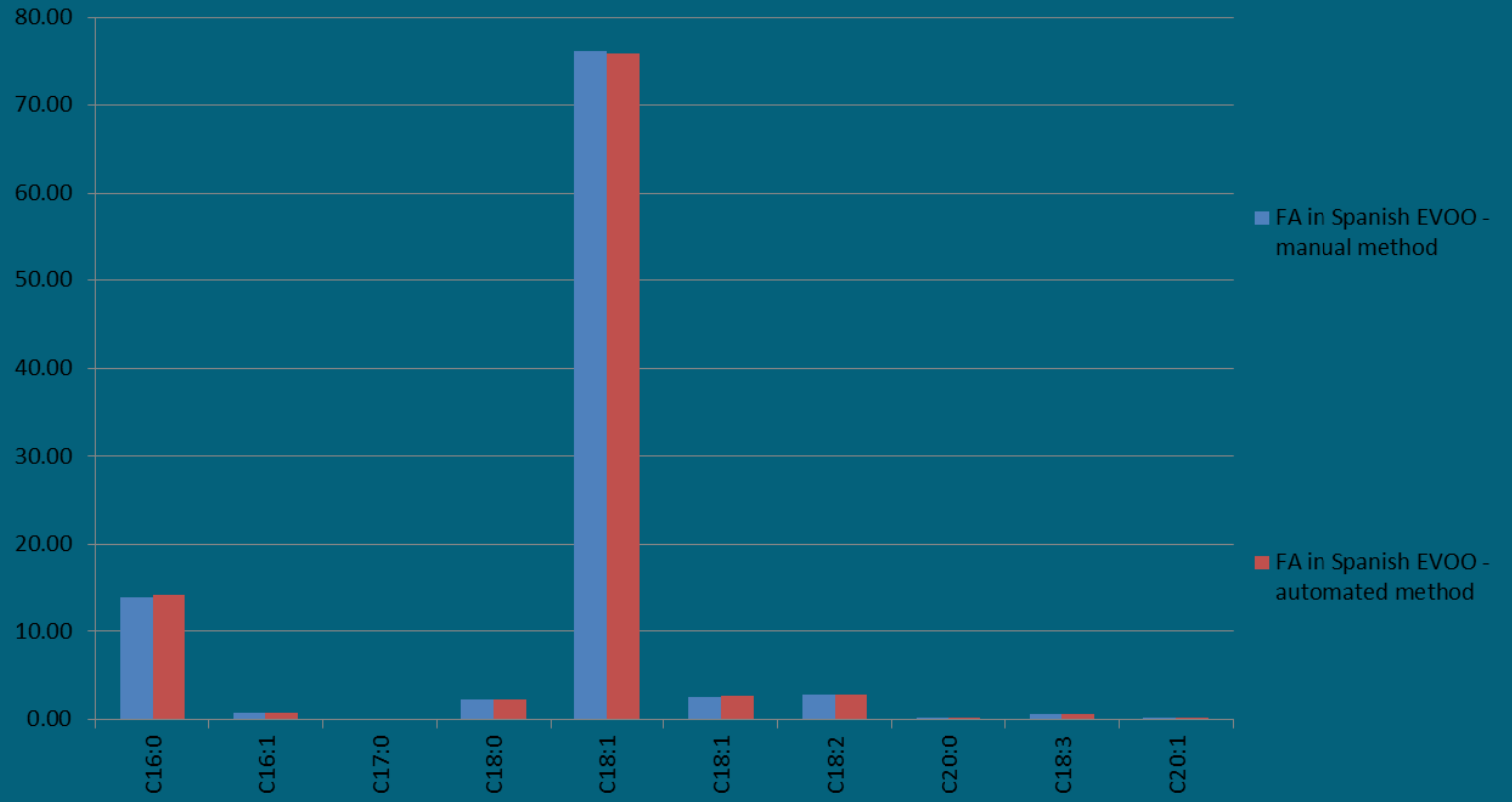
Free Fatty Acid results comparison using manual and automated sample preparation method

RT (min)	Chain	Name	Conversion Factor (Fa)	FA (mg/ml) in Spanish EVOO (#S6_L1)		FA (mg/ml) in California EVOO (#CA1_L1)		FA (mg/ml) in Olive oil (# O3_L1)	
				Manual n = 6	WorkBench n = 6	Manual n = 6	WorkBench n = 6	Manual n = 6	WorkBench n = 6
14.985	C16:0	Methyl Palmitate	0.9482	109.36	109.52	103.35	104.78	82.34	97.35
		STDEV		3.53	3.54	2.94	4.40	0.71	2.13
		%RSD		3.23	3.23	2.84	4.20	0.87	2.19
15.994	C16:1	Methyl Palmitoleate	0.9478	5.53	5.53	3.95	4.74	2.84	3.79
		STDEV		0.39	0.39	0.39	0.00	0.00	0.00
		%RSD		7.00	7.00	9.80	0.00	0.00	0.00
18.333	C18:0	Methyl Stearate	0.9531	18.11	17.31	17.31	17.63	22.87	28.12
		STDEV		0.60	0.72	0.72	0.80	0.00	0.52
		%RSD		3.33	4.14	4.14	4.52	0.00	1.86
19.182	C18:1	Methyl Oleate	0.9528	592.64	584.45	568.82	563.26	600.58	671.72
		STDEV		15.60	19.22	14.09	26.65	4.46	9.00
		%RSD		2.63	3.29	2.48	4.73	0.74	1.34
19.192	C18:1	Methyl Vaccenate	0.9528	20.17	20.17	17.94	18.26	17.31	19.06
		STDEV		0.72	0.72	0.39	0.39	0.39	0.00
		%RSD		3.56	3.56	2.17	2.13	2.25	0.00
19.826	C18:2	Methyl Linoleate	0.9525	21.91	22.07	45.56	45.56	43.34	49.37
		STDEV		0.60	0.72	1.11	1.53	0.52	1.27
		%RSD		2.75	3.25	2.44	3.35	1.20	2.56
21.467	C20:0	Methyl Arachidate	0.9571	1.91	1.91	1.91	2.87	1.91	2.87
		STDEV		0.00	0.00	0.00	0.00	0.00	0.00
		%RSD		0.00	0.00	0.00	0.00	0.00	0.00
21.964	C18:3	Methyl Linolenate	0.9521	4.92	4.92	4.13	4.44	3.81	4.13
		STDEV		0.39	0.39	0.49	0.49	0.00	0.49
		%RSD		7.90	7.90	11.92	11.07	0.00	11.92
22.273	C20:1	Methyl 11-Eicosenoate	0.9569	1.91	1.91	1.91	2.87	1.91	1.91
		STDEV		0.00	0.00	0.00	0.00	0.00	0.00
		%RSD		0.00	0.00	0.00	0.00	0.00	0.00

Comparison of Free Fatty Acids using manual and automated sample preparation methods in Spanish EVOO



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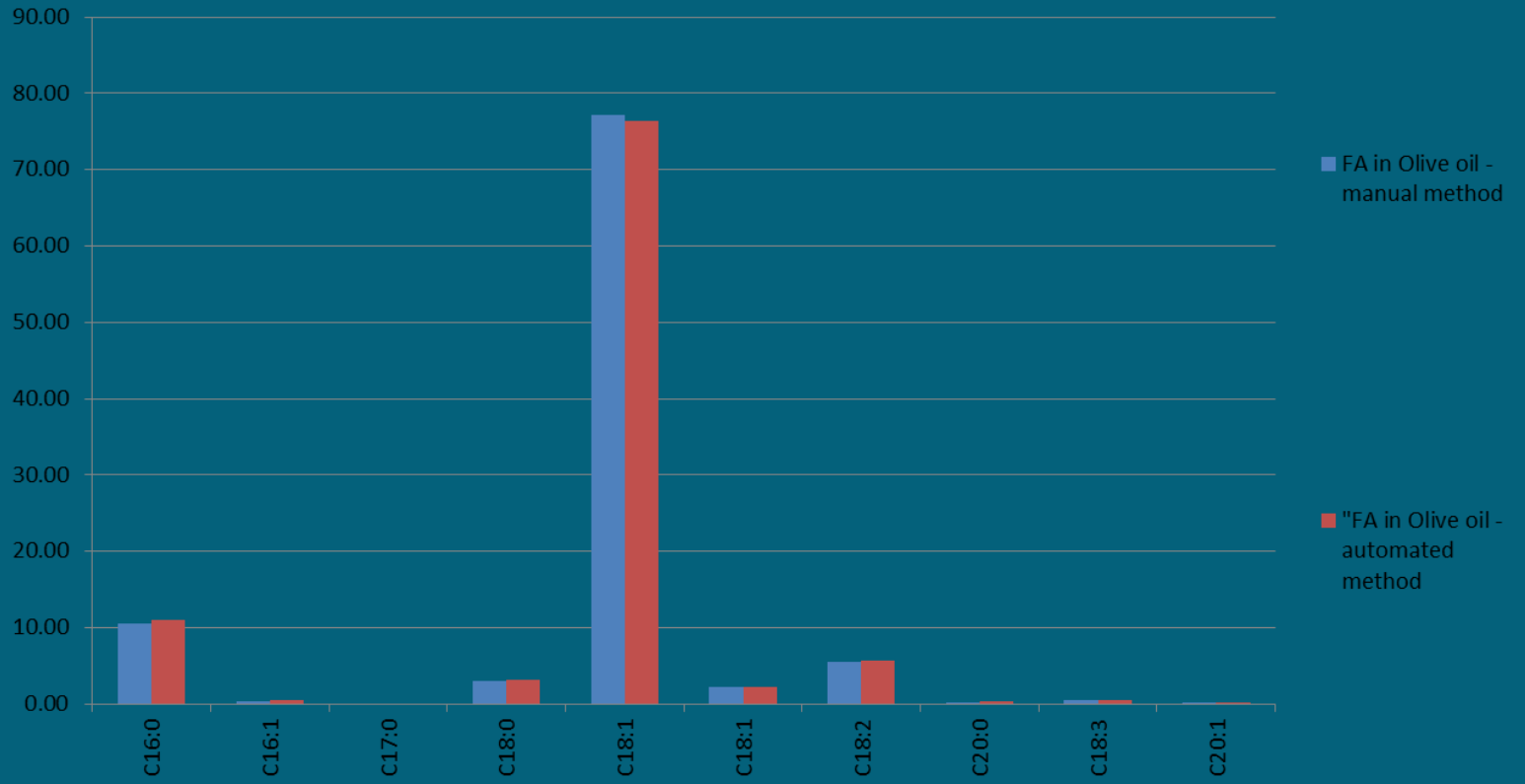
Comparison of Free Fatty Acids using manual and automated sample preparation methods in Olive Oil



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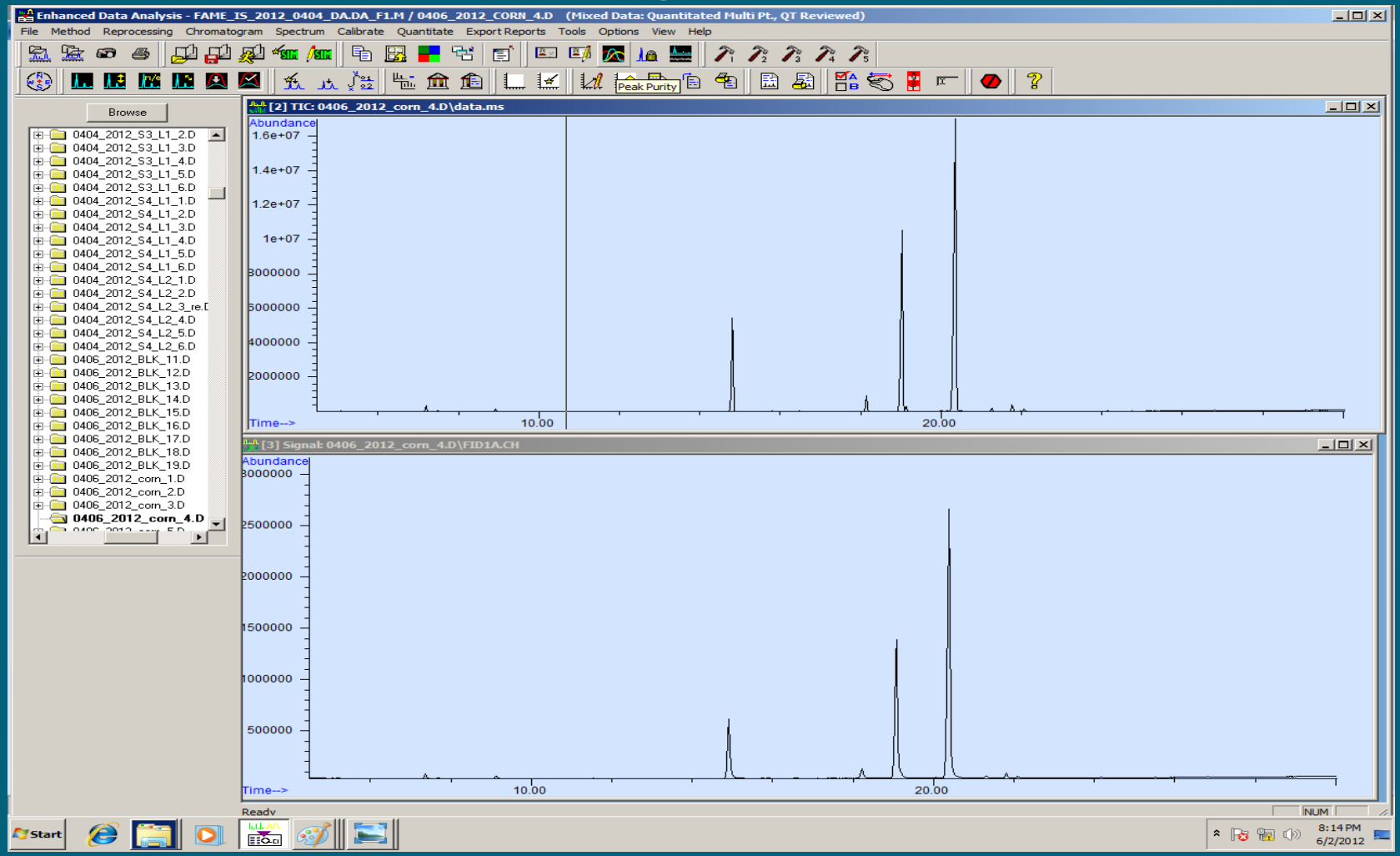
GC/MS and GC/FID chromatogram comparison



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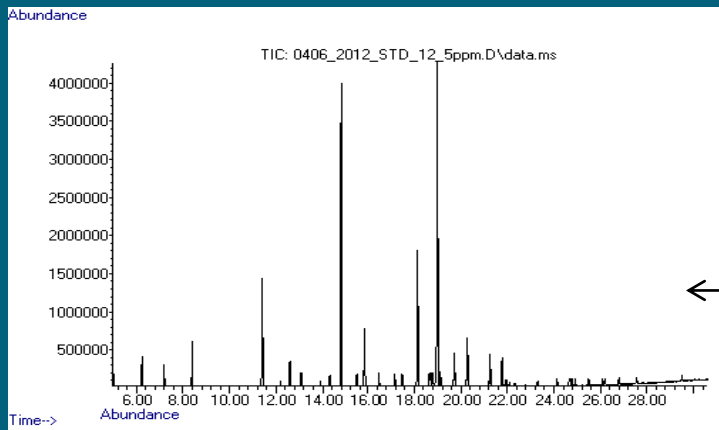
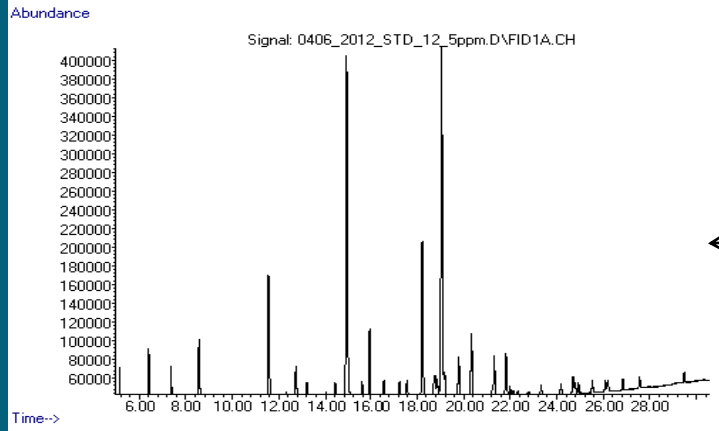
FAME standard chromatograms



MSD



FID

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% FA in Spanish EVOO and adulterated EVOO with corn oil

Chain	Name	S1_L1-1	S2_L1	S3_L1	S4_L1	S4_L2	S6_L1	S5_L1	S5_L1 spk w/10% corn oil	S5_L1 spk w/25% corn oil	S5_L1 spk w/50% corn oil	Allowable FA Ranges % for EVOO
C14:0	Methyl Myristate	0.11	0.11	0.11	0.11	0.12	0.00	0.00	0.00	0.00	0.00	
C16:0	Methyl Palmitate	13.85	12.47	10.02	11.73	11.48	14.05	18.85	18.91	18.05	16.67	7.5-20
C16:1	Methyl Palmitoleate	1.15	1.07	0.57	0.76	0.69	0.71	1.45	1.29	1.03	0.66	0.3-3.5
C17:0	Methyl Heptadecanoate	0.11	0.11	0.11	0.11	0.12	0.00	0.13	0.13	0.13	0.13	
C18:0	Methyl Stearate	3.03	3.16	3.38	3.20	3.64	2.33	1.50	1.68	1.67	1.60	0.5-5
C18:1	Methyl Oleate	70.31	74.48	75.82	73.27	73.04	76.14	60.40	56.84	52.53	45.74	55.0-83.0
C18:1	Methyl Vaccenate	2.57	2.61	1.82	2.13	1.99	2.59	3.81	3.45	2.99	2.19	
C18:2	Methyl Linoleate	7.28	4.39	6.44	6.93	7.01	2.81	12.59	16.44	22.27	31.54	3.5-21.0
C20:0	Methyl Arachidate	0.36	0.33	0.34	0.36	0.40	0.25	0.26	0.25	0.26	0.27	<0.6
C18:3	Methyl Linolenate	0.79	0.77	0.82	0.82	0.92	0.63	0.51	0.51	0.54	0.66	<1.0
C20:1	Methyl 11-Eicosenoate	0.32	0.29	0.34	0.34	0.36	0.25	0.26	0.25	0.26	0.27	<0.4
C22:0	Methyl Behenate	0.11	0.11	0.11	0.12	0.12	0.12	0.13	0.13	0.13	0.13	
C24:0	Methyl Lignocerate	0.00	0.11	0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.13	
		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

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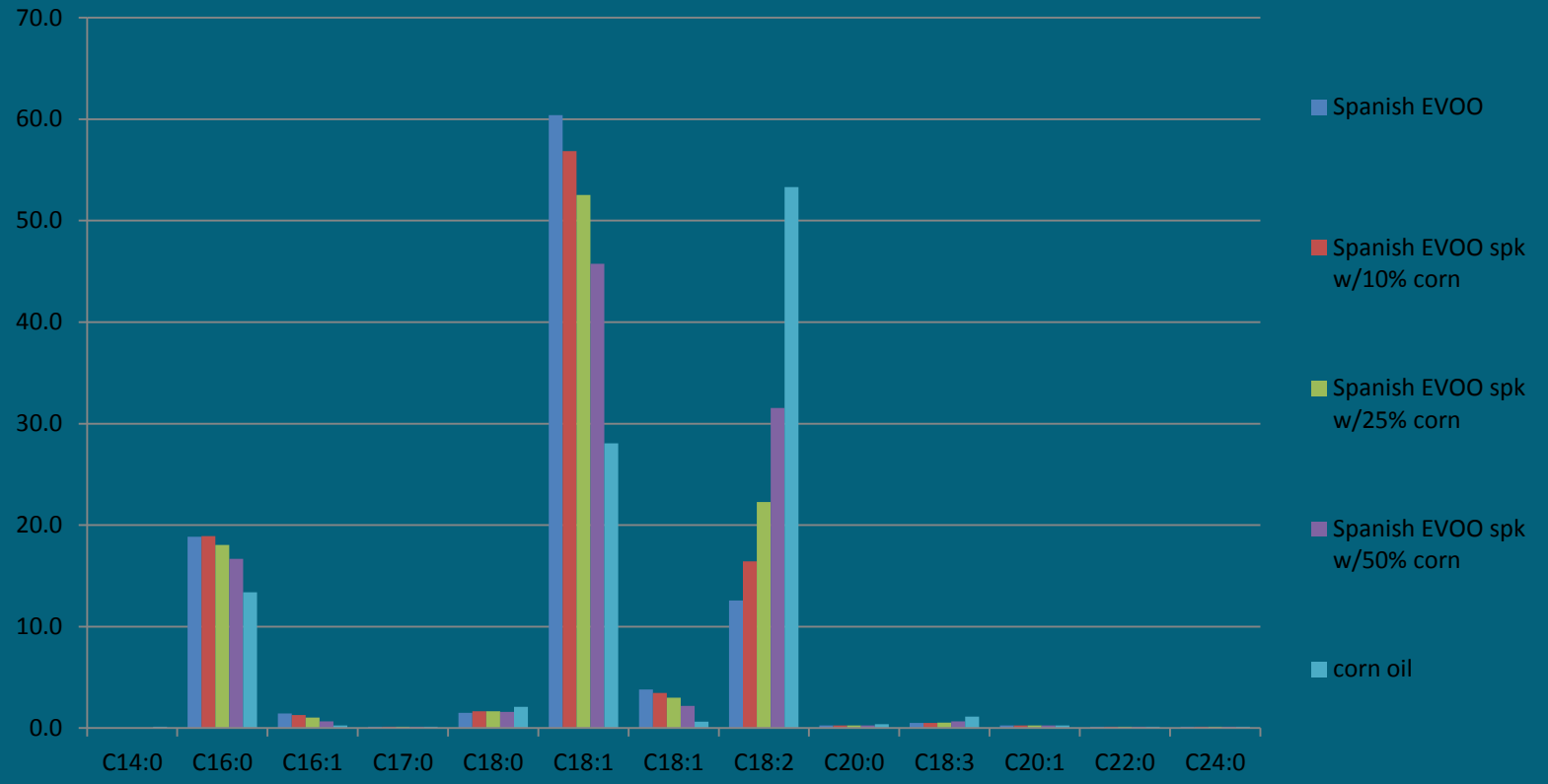
Fatty Acid distribution of spiked Spanish EVOO with corn oil



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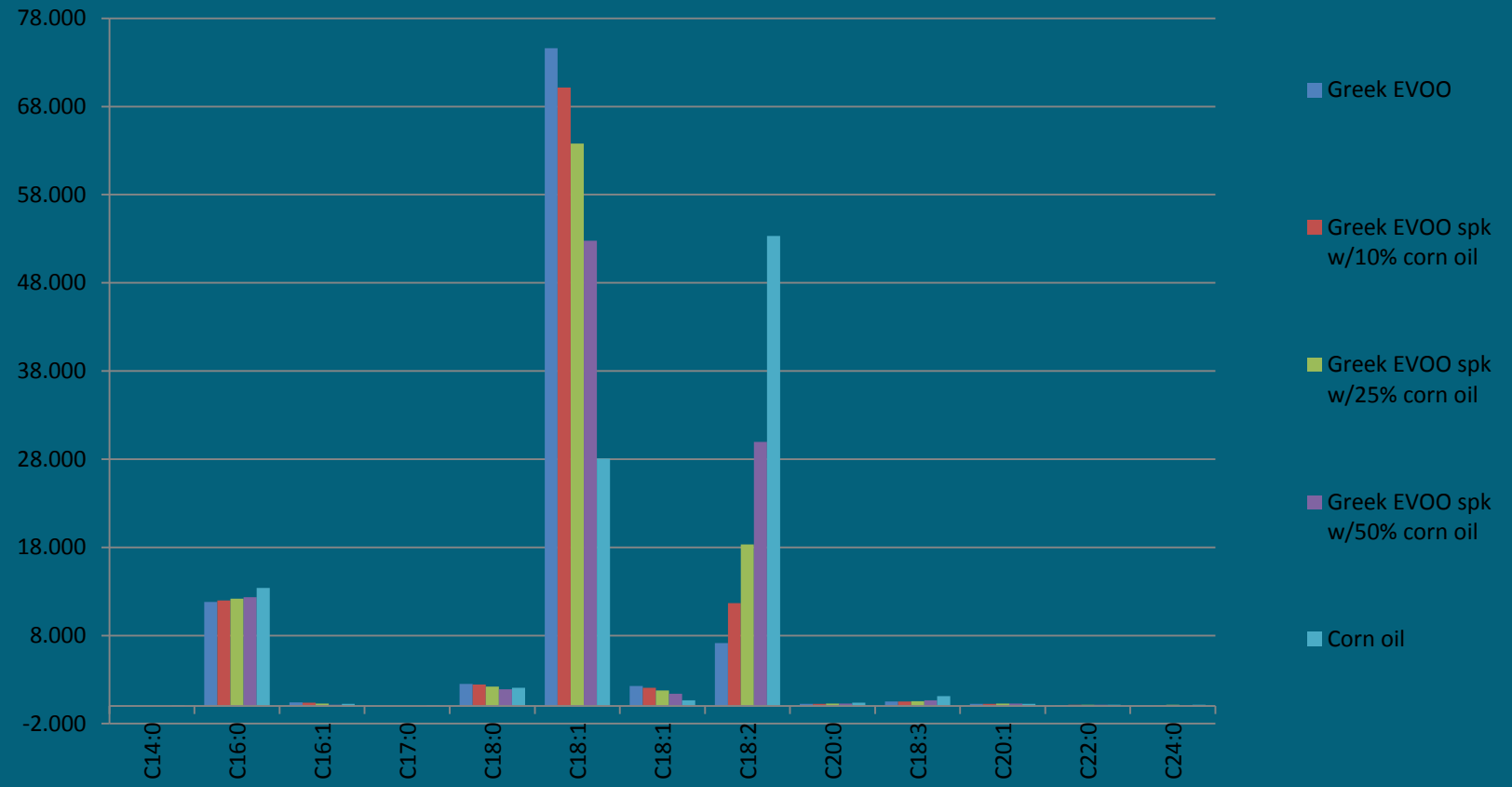
Fatty Acid distribution of spiked Greek EVOO with corn oil



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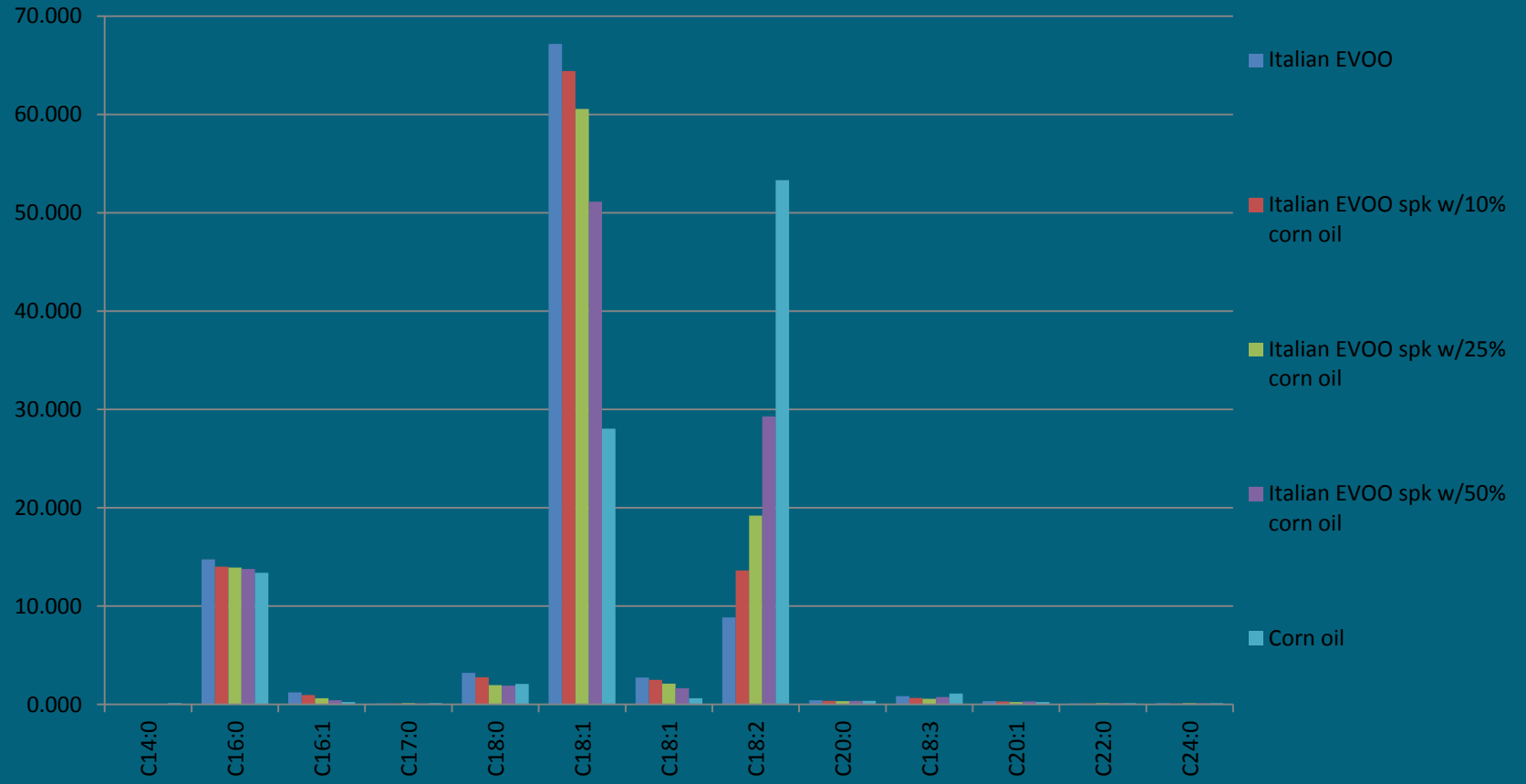
Fatty Acid distribution of spiked Italian EVOO with corn oil



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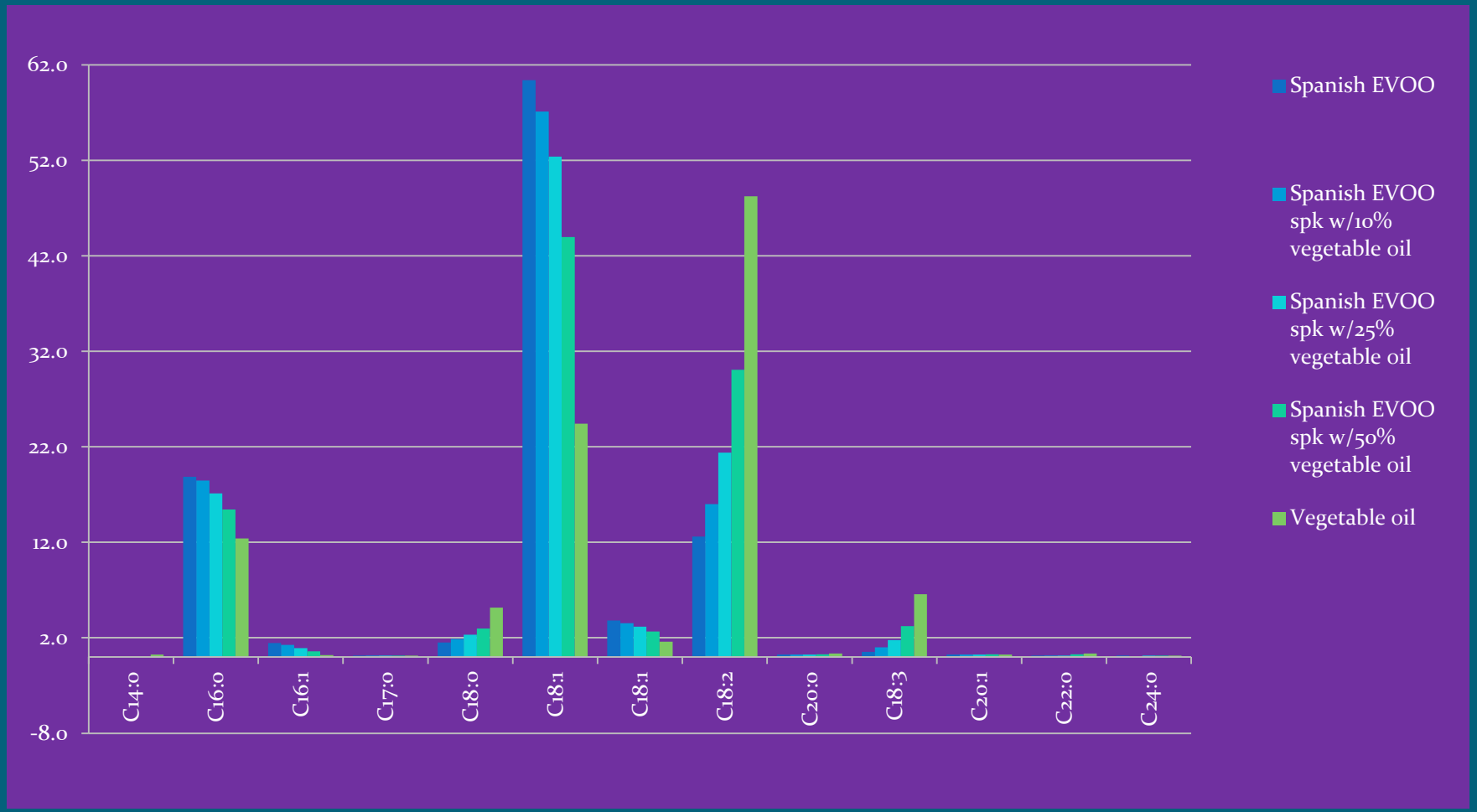
Fatty Acid distribution of spiked Spanish EVOO with vegetable oil



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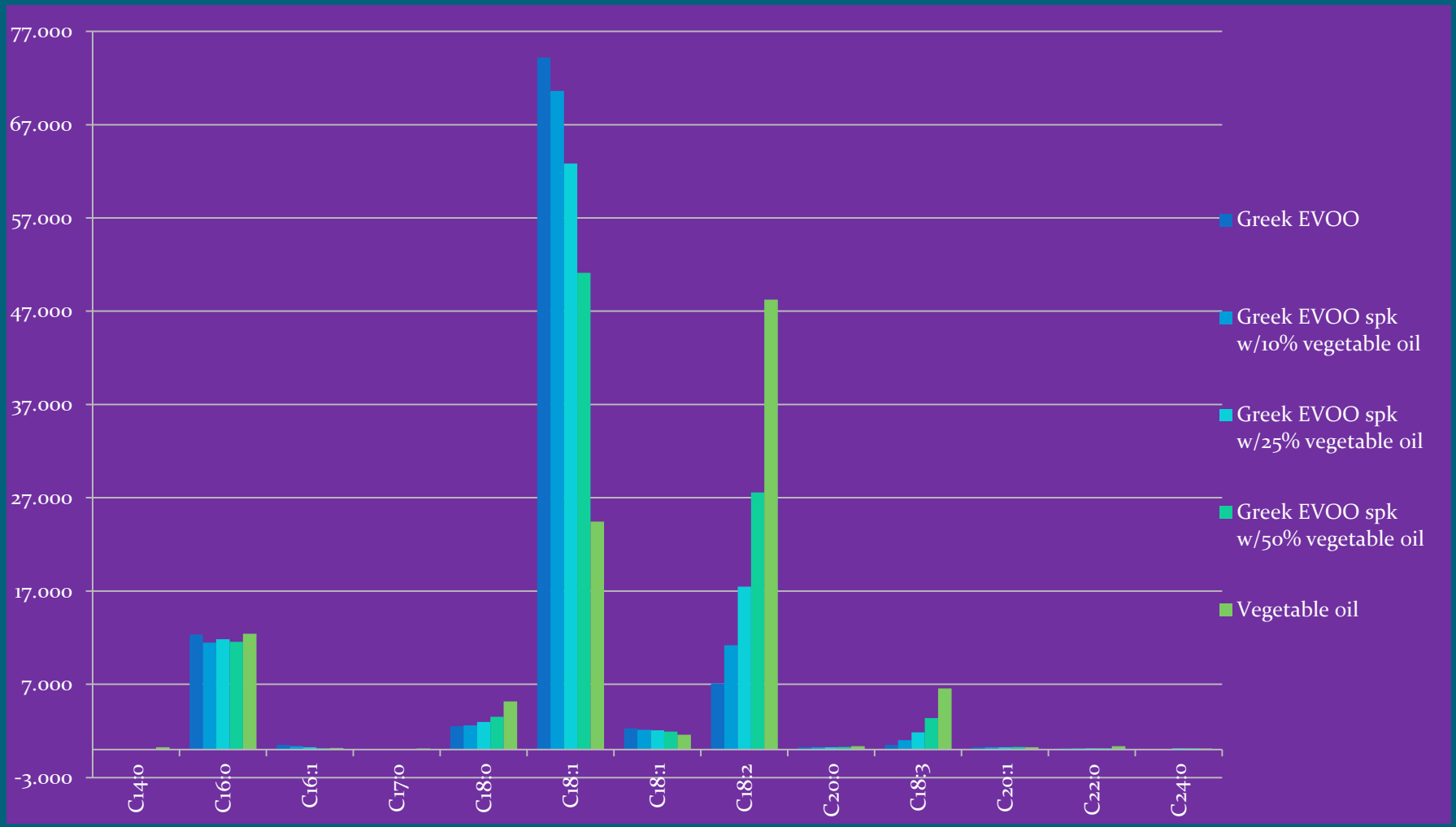
Fatty Acid distribution of spiked Greek EVOO with vegetable oil



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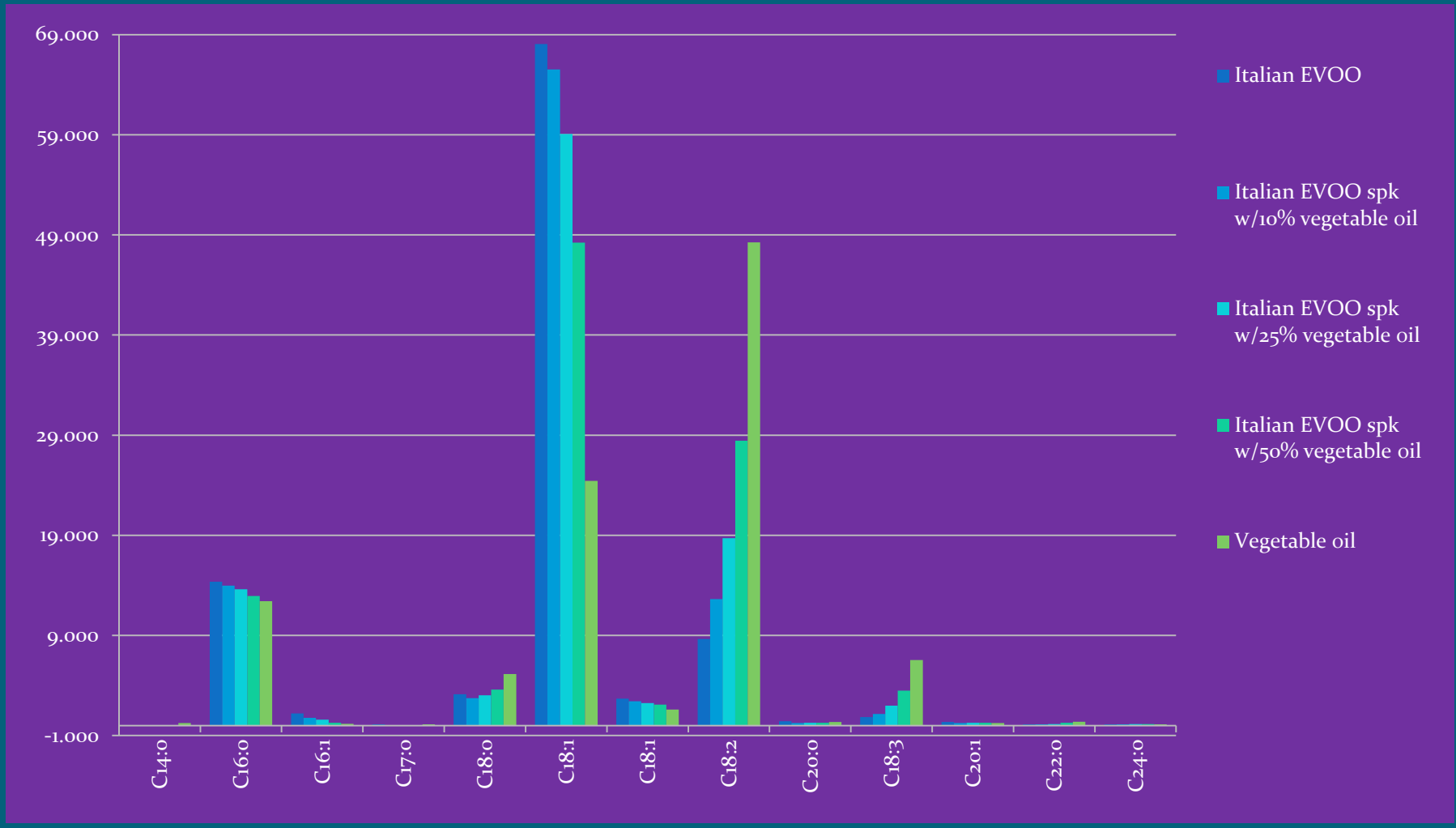
Fatty Acid distribution of spiked Italian EVOO with vegetable oil



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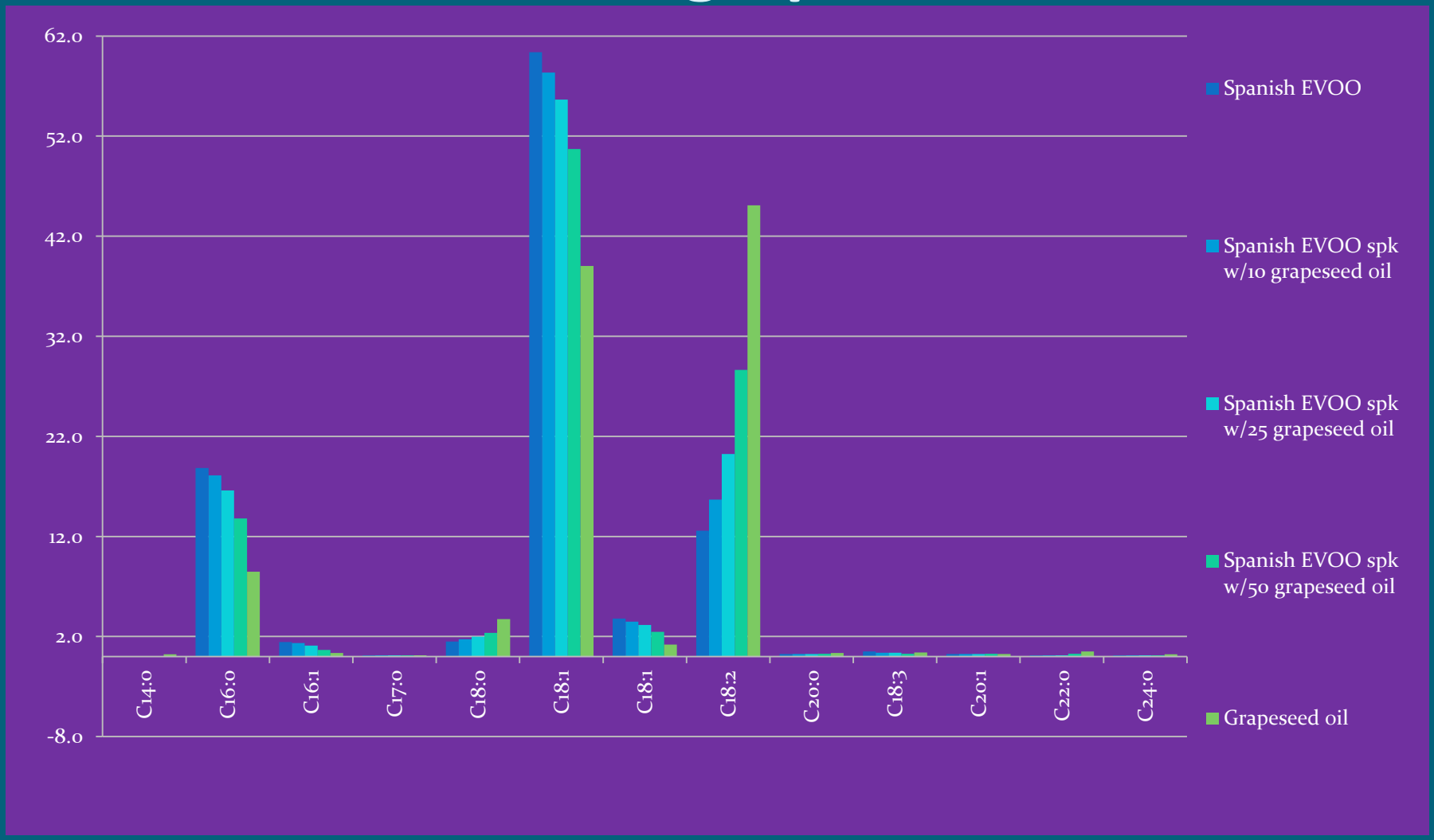
Fatty Acid distribution of spiked Spanish EVOO with grapeseed oil



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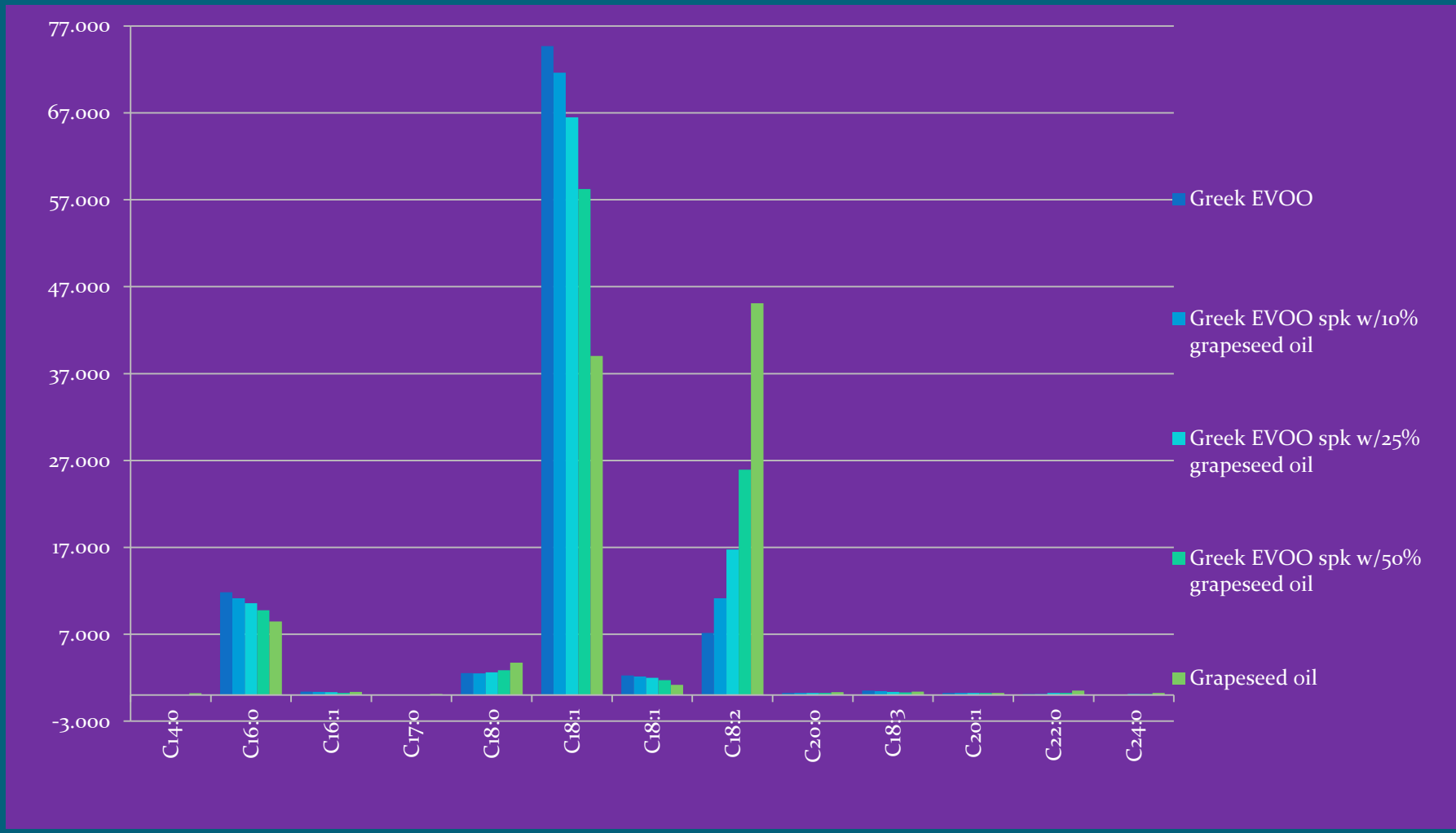
Fatty Acid distribution of spiked Greek EVOO with grapeseed oil



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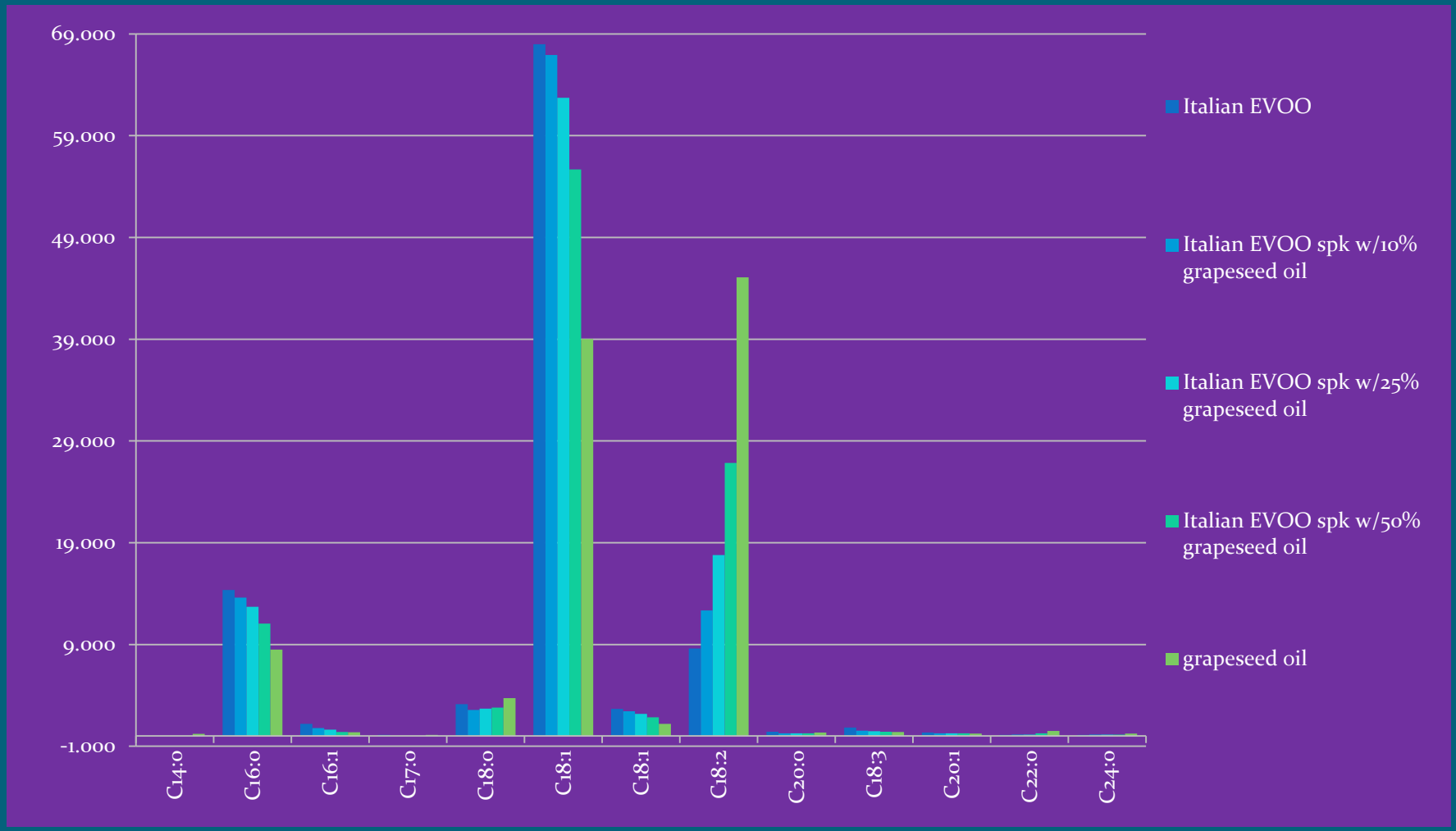
Fatty Acid distribution of spiked Italian EVOO with grapeseed oil



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Greek, Italian and Spanish EVOO adulterated with Grapeseed oil

		1	2	3	4	5
		Variance	Percent	Cumulative	Press Cal	
1	Factor1	276.8874	42.5981	42.5981	373.1126	
2	Factor2	210.1967	32.3380	74.9360	162.9158	
3	Factor3	121.0540	18.6237	93.5597	41.8618	
4	Factor4	14.9576	2.3012	95.8609	26.9042	
5	Factor5	8.9521	1.3772	97.2381	17.9521	
6	Factor6	7.6124	1.1711	98.4093	10.3398	
7	Factor7	4.3834	0.6744	99.0836	5.9564	
8	Factor8	2.2217	0.3418	99.4254	3.7347	
9	Factor9	1.7935	0.2759	99.7014	1.9412	
10	Factor10	1.1156	0.1716	99.8730	0.8256	
11						

- **PCA Factor Variance Analysis**

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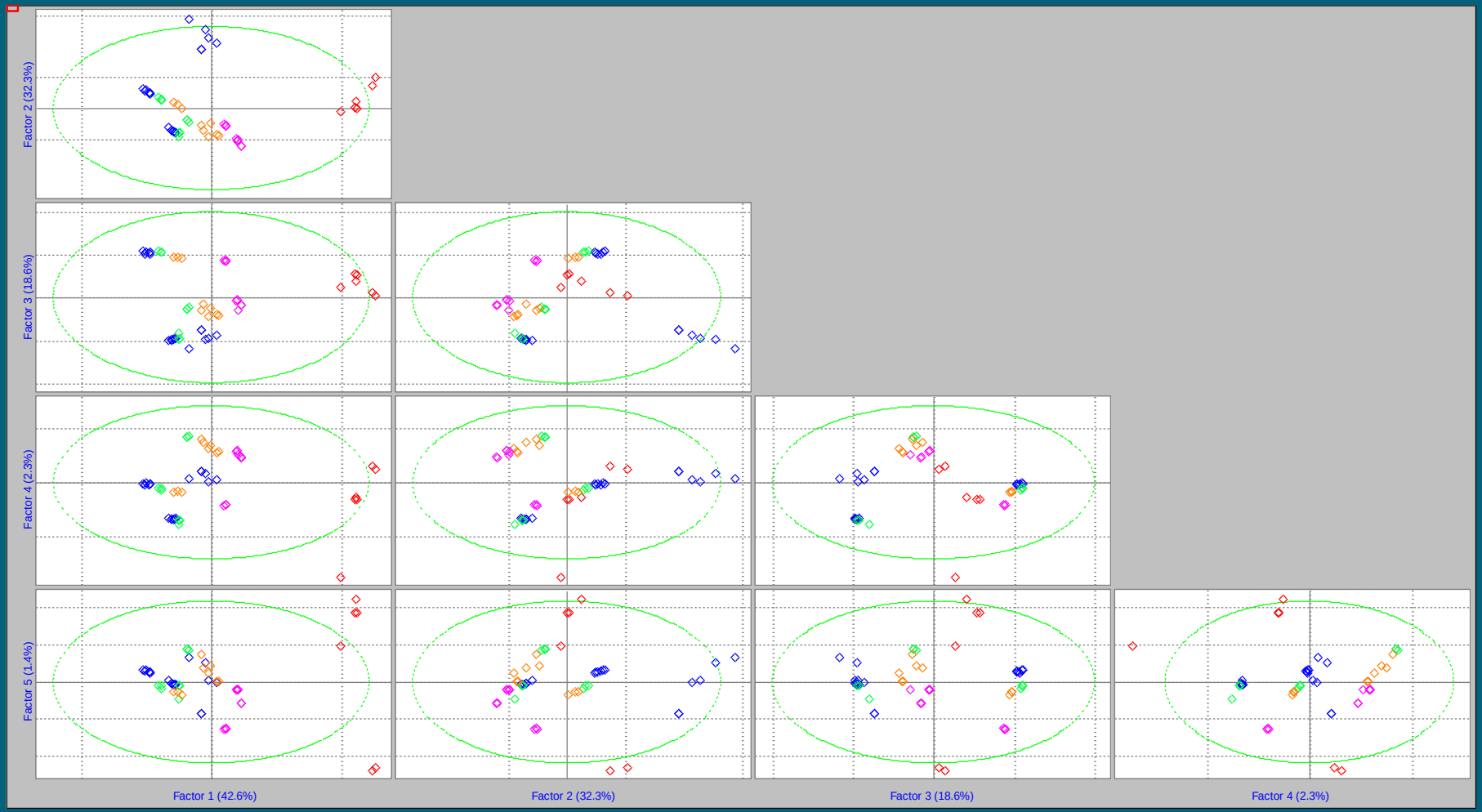
Italian, Spanish, and Greek EVOO spiked with grapeseed oil



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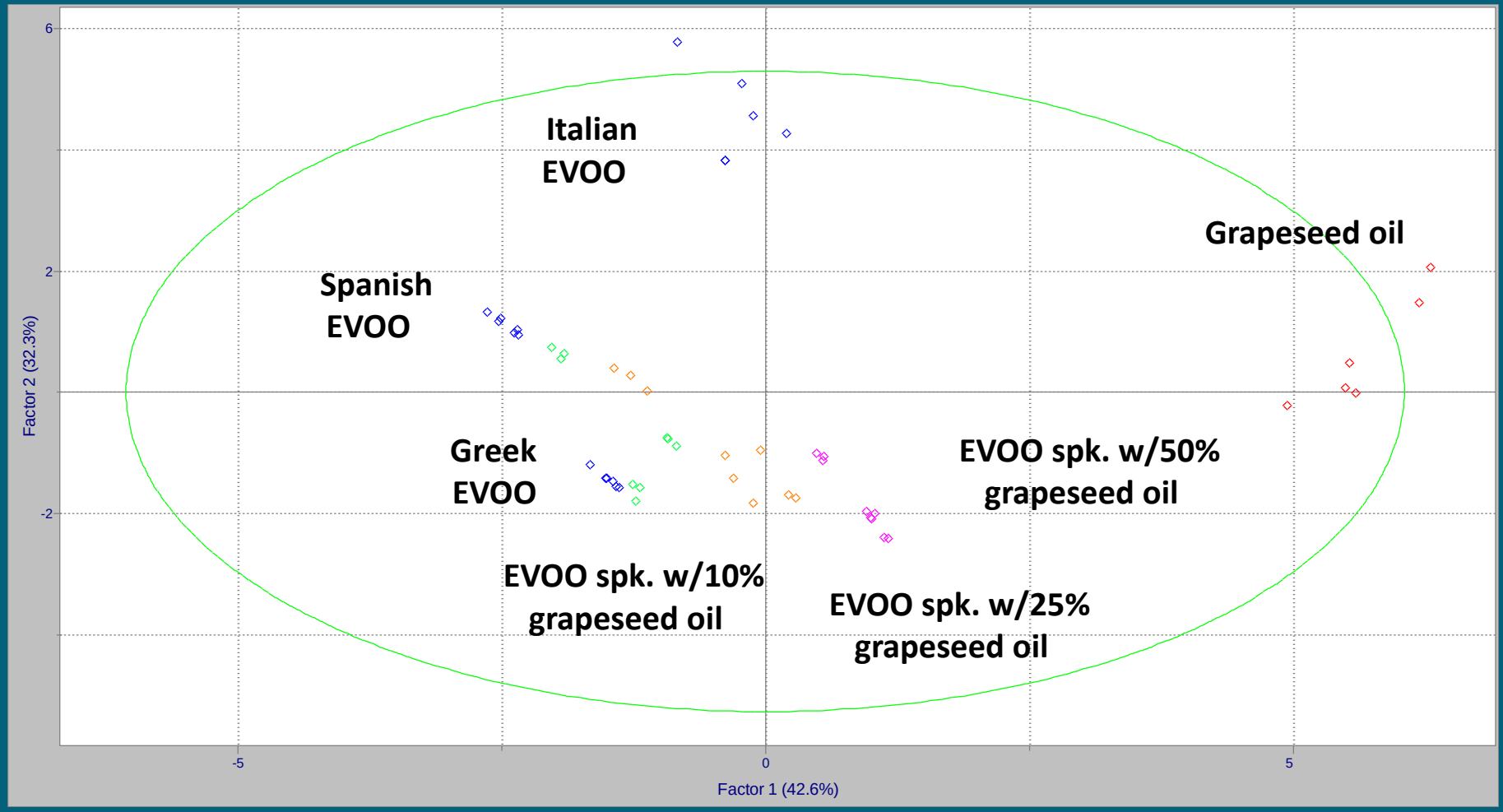
Italian, Spanish, and Greek EVOO spiked with grapeseed oil (cont.)



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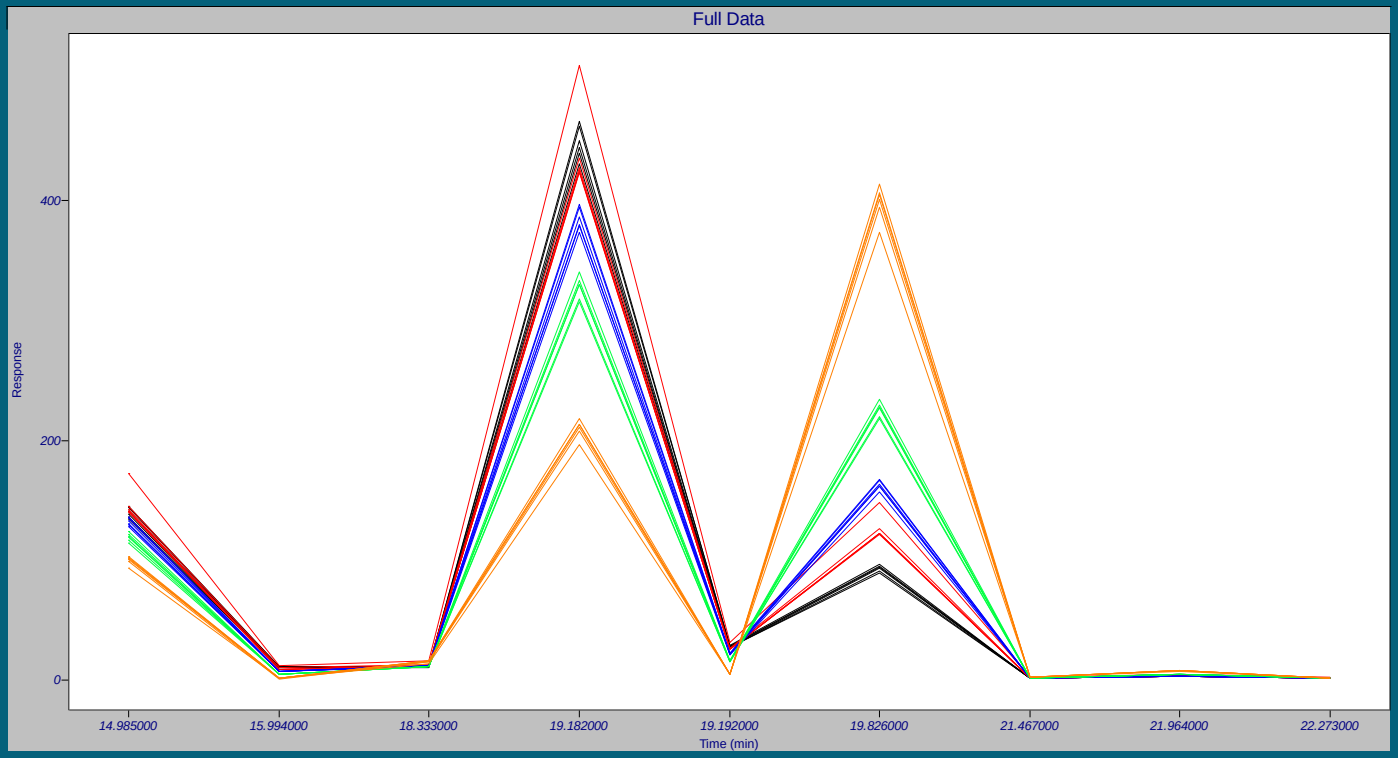
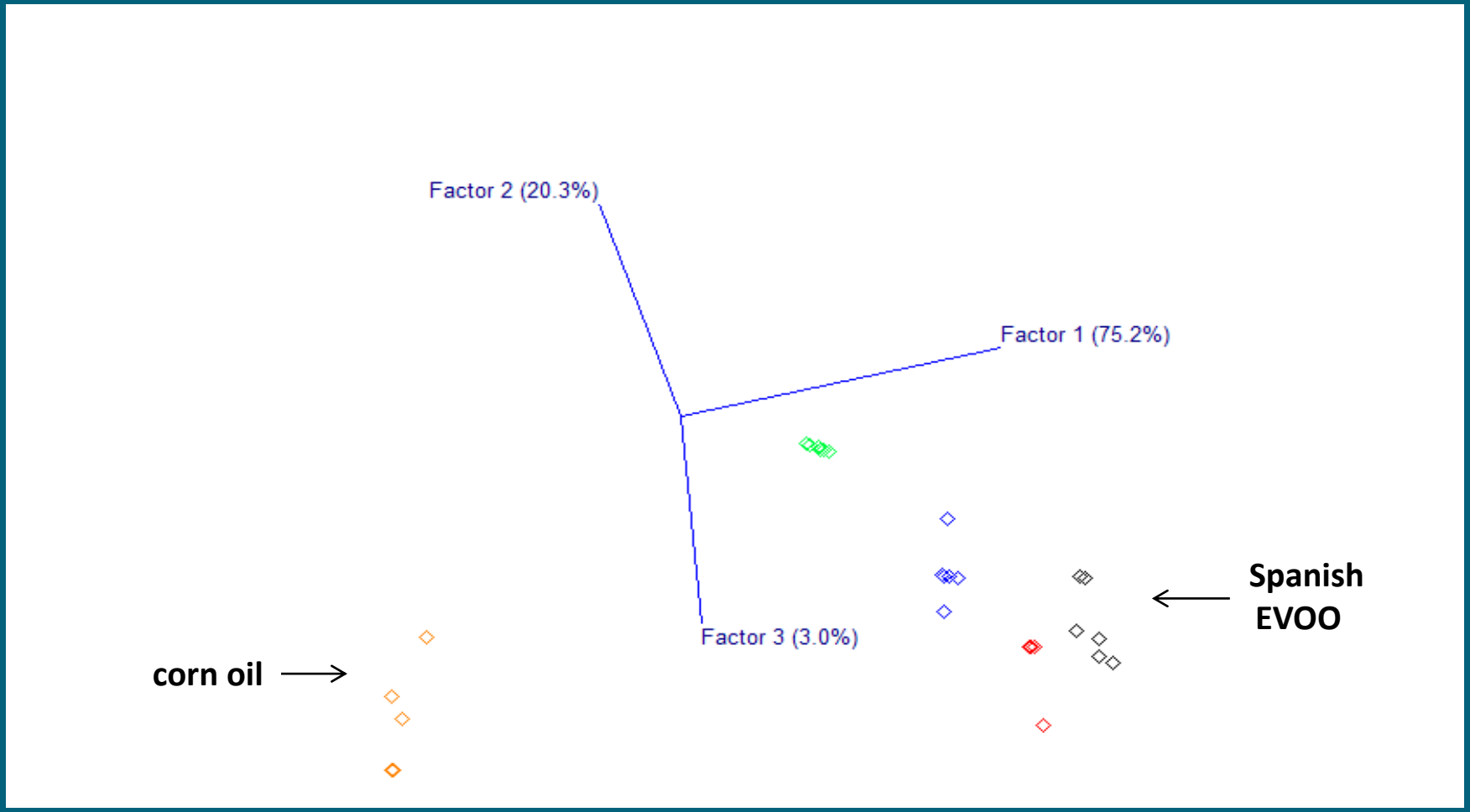
Spanish EVOO spiked with corn oil at 10%, 25%, and 50%

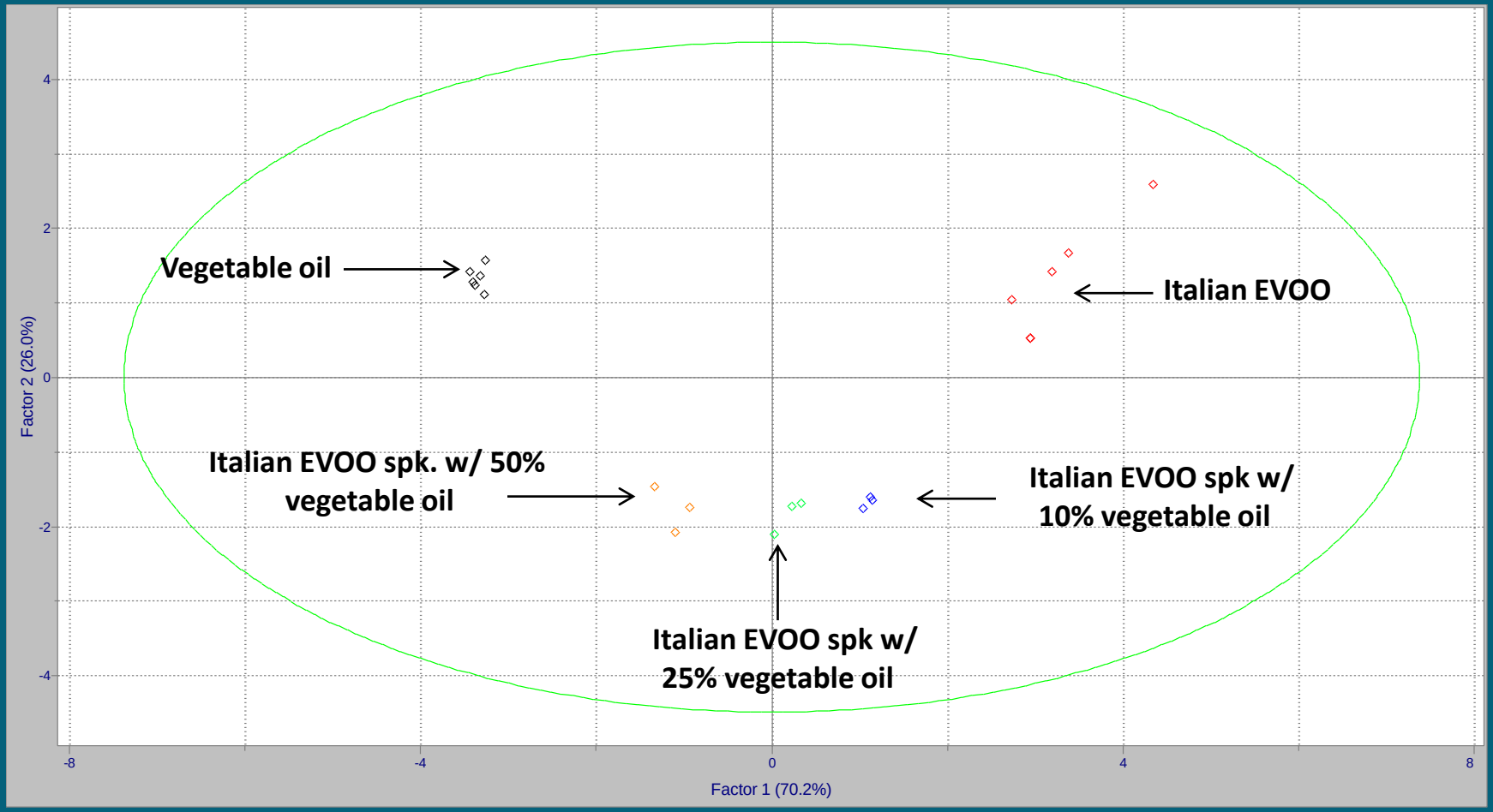


- Full data line plot view adulterated Spanish EVOO with corn oil

PCA Scores plot of Spanish EVOO adulterated with corn oil (10%, 25% and 50%)

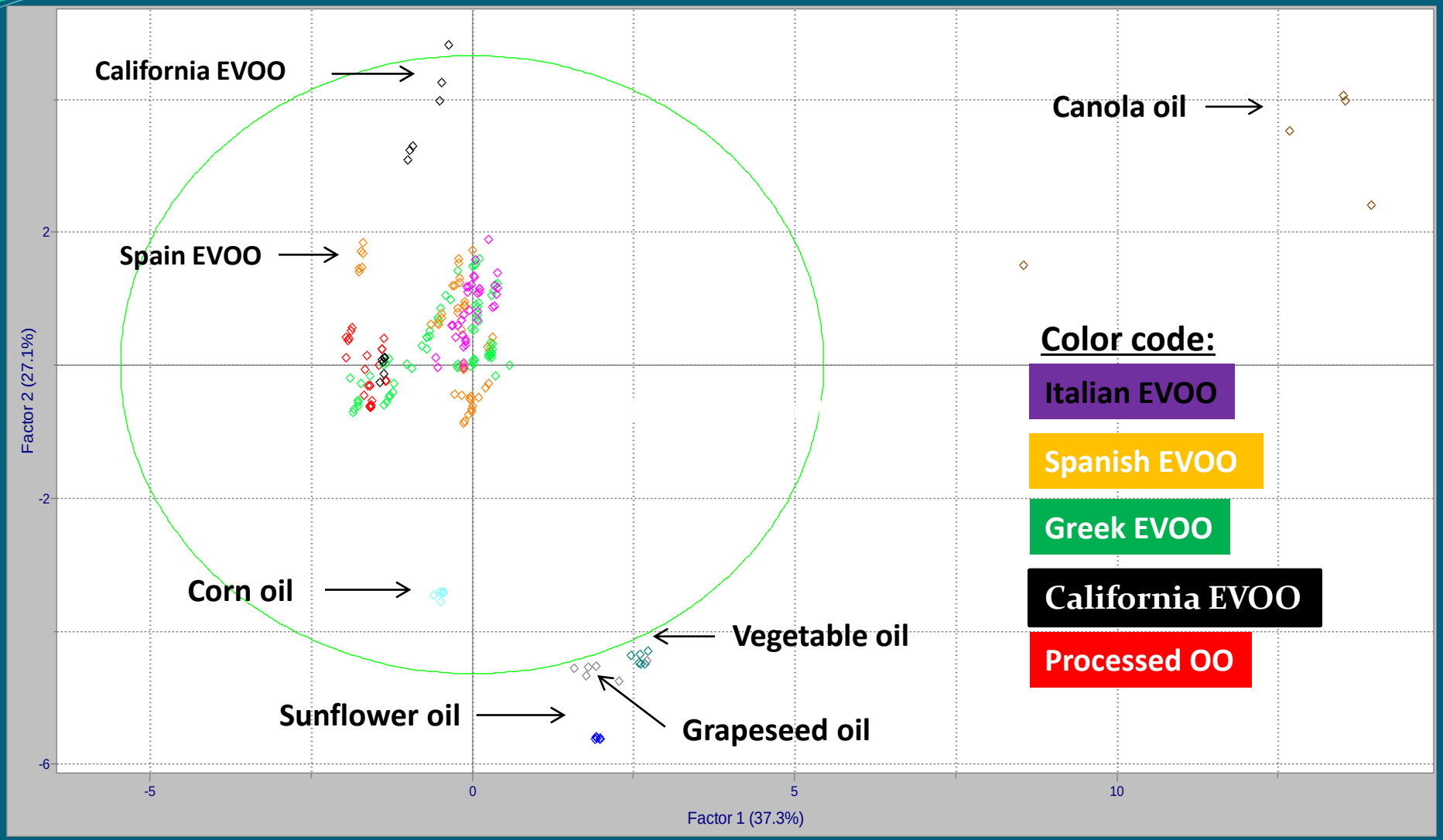


Italian EVOO spiked with vegetable oil



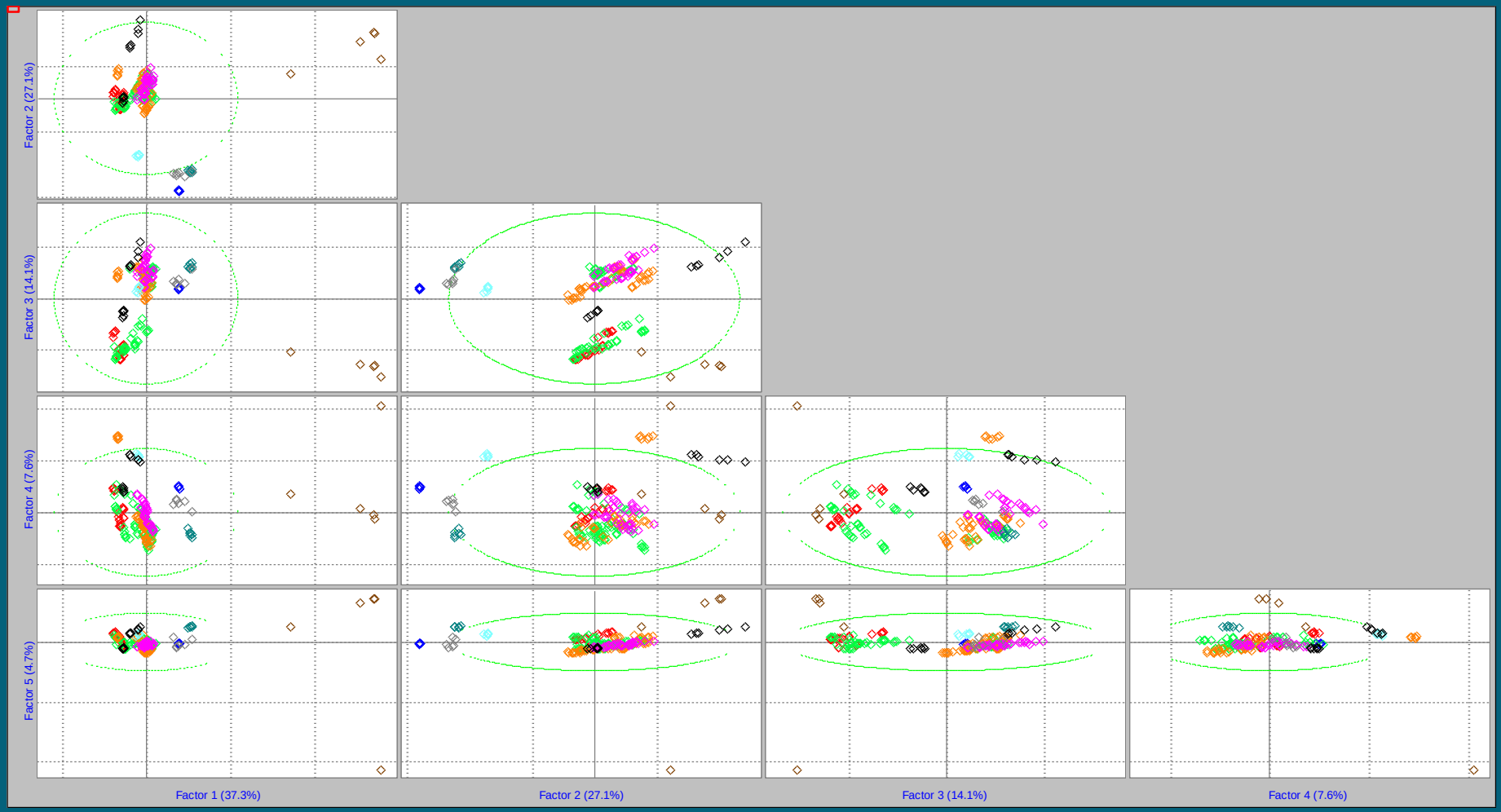
PCA Scores, all samples model, no cross validation

Edible oils distribution



- Factor 1 v Factor 2 Scores

Edible oils distribution (cont.)



- Scores Plots, factors 1 through 5

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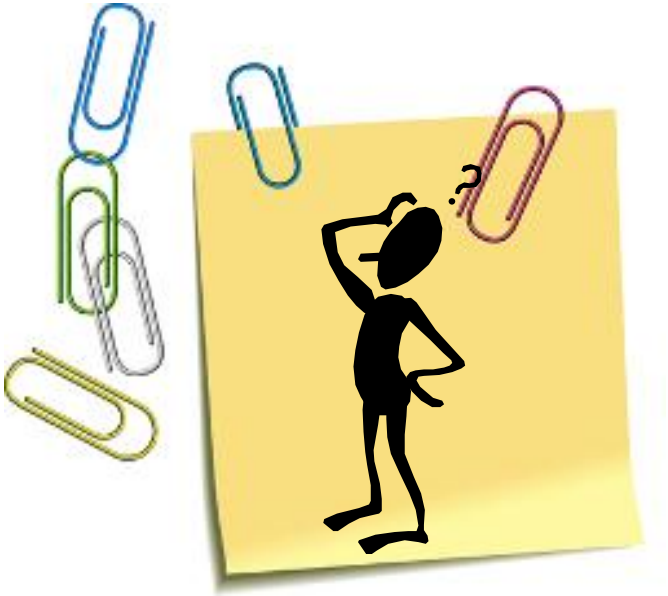
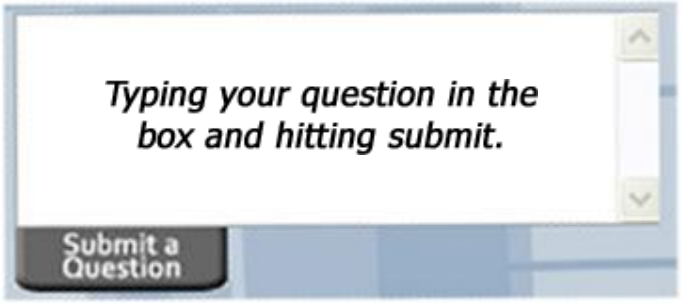
Conclusions

- The Agilent 7696A Sample Prep WorkBench, derivatization reactions were easily converted to automated method
- Samples prepared with WorkBench are reproducible compared to manual preparation
- Calibration standard preparation is fast and yields excellent results (r^2)
- WorkBench used 17 times less solvents and reagents
- This study demonstrates an efficient and economical GC/MS /FID method for determination of fatty acids in edible oils

Questions?



Please Submit your questions by:



Thank You!

We apologize for this brief interruption but we are experiencing technical difficulties and will resume shortly.