Automated Sample Preparation for FAME Analysis in Edible Oils Using an Agilent 7696A Sample Prep WorkBench

Application Note

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Abstract

Automating fatty acid derivatization and preparation of calibration standards on an Agilent 7696A Sample Prep WorkBench for fatty acid methyl esters (FAME) analysis can provide comparable precision, better recoveries, and more accurate calibration compared to manual procedures. Hands-on time, reagent usage, and exposure to hazardous chemicals are all greatly reduced. This automated approach has been used to detect adulteration of olive oils with less expensive vegetable oils.

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Introduction

Nutritional science on the influence of dietary fat and specific fatty acids on human health is rapidly evolving and driving public and regulatory interest in understanding the fat content of foods. This is especially true of edible oils in general, and olive oil in particular.

The US Food and Drug Administration (FDA) has approved a qualified health claim that consumption of monounsaturated fat from olive oil may reduce the risk of coronary heart disease. Recent studies have attributed the anti-inflammatory benefits of olive oil primarily to the extra virgin olive oil (EVOO) obtained from the first cold pressing of olives. EVOO is rich in antioxidants and anti-inflammatory compounds.

Given the interest in EVOO, consumers want to be certain that the olive oil products they purchase are indeed EVOO that has not been adulterated with other oils of lower quality or nutritional value. Accurate compositional analyses are required to meet this need. Fatty acids from mono-, di-, and triglycerides are commonly analyzed as fatty acid methyl esters (FAMEs) after saponification and derivatization. Analysis is performed using gas chromatography with mass spectral or flame ionization detection (GC/MS/FID).

The derivatization reactions are often done manually, but this approach can be time-consuming and can expose the analyst to hazardous chemicals. This application note demonstrates the ability of the Agilent 7696A Sample Prep WorkBench to automate the derivatization process, and the preparation of calibration standards. Calibration curves generated from standards prepared by the Sample Prep WorkBench were more linear than those from manually-prepared standards. The automated methods used much less solvent and fewer consumables, required much less hands-on time by the analyst, and generated much less chemical waste. FAME analysis of samples prepared on the Sample Prep WorkBench was used to detect EVOO that had been adulterated with less expensive vegetable oils.

Experimental

Standards and reagents

GLC Reference Standard GLC-603 (FAME) and Methyl Undecanoate (ISTD) were obtained from Nu-Chek Prep, Inc. (Elysian, MN). Boron trifluoride in methanol (50 % w/v) and sodium hydroxide (reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO). Hexane (reagent grade) and methanol (HPLC grade) were obtained from Burdick and Jackson (Muskegan, MI).

Instruments

The derivatization reactions and calibration standard preparation were performed on an Agilent 7696A Sample Prep WorkBench. The sample analysis was performed on an Agilent 6890A GC equipped with an Agilent 7683A Autosampler and coupled to an Agilent 5973 Series GC/MSD or an Agilent Flame Ionization Detector (FID). The GC/MS/FID conditions are listed in Table 1, and the automated Sample Prep WorkBench derivatization steps are listed in Table 3.

Table 1. GC/MS/FID Run Conditions

GC	
Column	Agilent HP-88, 60 m × 0.25 mm, 0.25 µm (p/n 112-8867)
Injection volume	1 µL
Inlet	Split ratio 100:1
Inlet temperature	250 °C
Liner	Split liner, tapered, deactivated (p/n 5183-4711)
Carrier gas	Helium (He), constant flow mode, 1 mL/min
Oven temperature program	140 °C hold 5 minutes 4 °C/min to 240 °C Hold 0 minutes Total run time = 30 minutes
MS	
Transfer line temperature	280 °C
Solvent delay	4 minutes
Acquisition mode	Scan, 40–500 amu
FID	
Temperature	300 °C

Statistical analysis

Principal Component Analysis was performed using Agilent MassProfiler Professional Software.

Results and Discussion

Calibration linearity

A typical FAME chromatographic separation is shown in Figure 1. Six FAME calibration standards were prepared both manually and with the Sample Prep WorkBench, in the range of 0.001–0.1 mg/mL and 0.040–40 mg/mL, depending on the specific concentration of the FAME in the GLC-603 standard. All calibration curves were linear over the entire concentration range (Figure 2).

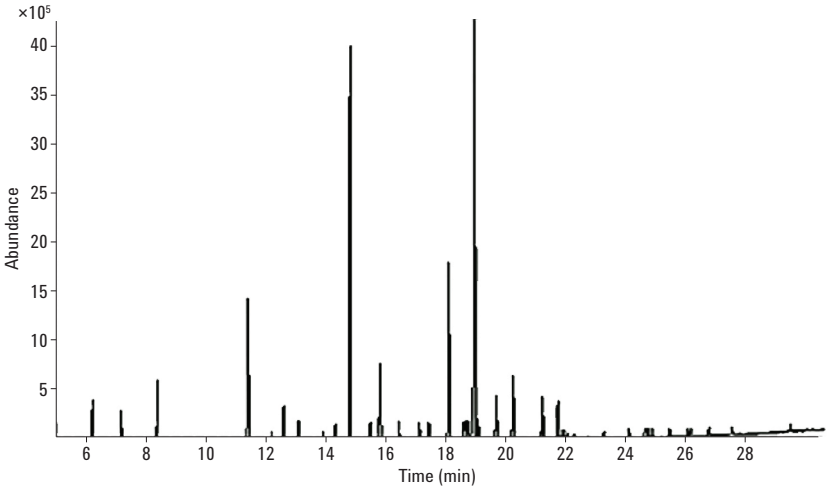

Figure 1. Representative chromatogram for analysis of the GLC Reference Standard GLC-603 using the Agilent 5973 Series GC/MSD.

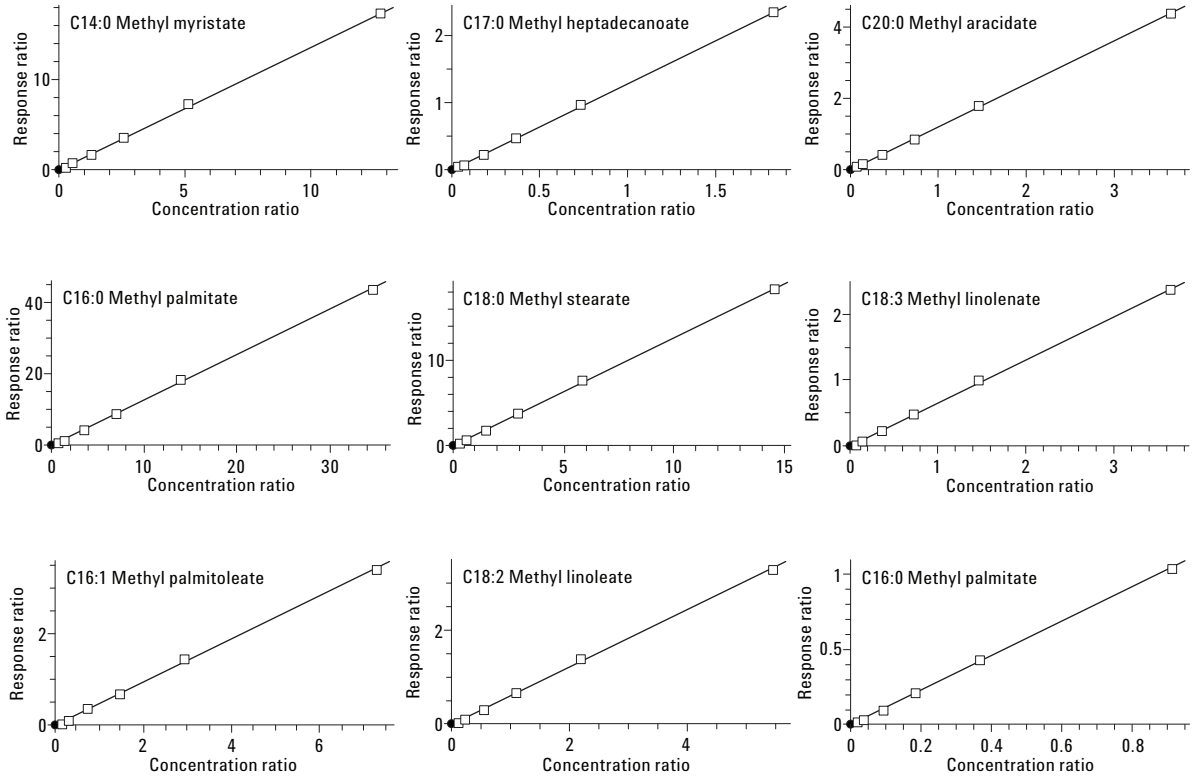

Figure 2. Representative calibration curves for FAME standards, using the Agilent 7696A Sample Prep WorkBench.

However, the correlation coefficients for the curves prepared using the Sample Prep WorkBench were uniformly higher, with an average correlation coefficient (R^2) for all of the fatty acids of 0.999, versus 0.995 for the manually prepared calibration curves (Table 2). The relative standard deviations (RSDs) for analyses done on six replicates were less than 5 % for both manual and automated preparation of calibration standards.

Table 2. Linearity of Calibration Curves

		Correlation coefficient (R^2)	
FAME	**Chain**	**Manual**	**Agilent 7696A Sample Prep WorkBench**
Methyl myristate	C14:0	0.9919	0.9994
Methyl palmitate	C16:0	0.9901	0.9992
Methyl palmitoleate	C16:1	0.9943	0.9991
Methyl heptadecanoate	C17:0	0.9989	0.9997
Methyl stearate	C18:0	0.9906	0.9996
Methyl oleate	C18:1	0.9912	1.0000
Methyl vaccenate	C18:1	0.9961	0.9987
Methyl linoleate	C18:2	0.9957	0.9994
Methyl arachidate	C20:0	0.9969	0.9997
Methyl linolenate	C18:3	0.9970	0.9994
Methyl 11-eicosenoate	C20:1	0.9976	0.9989
Methyl behenate	C22:0	0.9997	0.9997
Methyl lignocerate	C24:0	0.9989	0.9999

Automated derivatization

Table 3 provides a comparison of the manual derivatization procedure with the procedure automated on the Sample Prep WorkBench. While the automated procedure uses more discrete steps, it greatly reduces operator hands-on time, reagent and solvent usage, and waste disposal costs. The fatty acid compositions for Spanish EVOO were essentially identical, using either manual or automated derivatization (Figure 3), and recoveries were generally better with the automated method. The relative standard deviations (RSDs) were also very similar for the two methods, ranging from 0.00 to 8 % (Table 4).

Table 3. Automated and Manual Derivatization Steps

Step no.	Automated	Manual
1	Add 10 µL of diluted oil sample in hexane (oil:hexane/50:50).	Place 50 mg of sample in a 15-mL centrifuge tube.
2	Add 3.3 µL of internal standard.	Add 2 mL of 2 N NaOH in methanol.
3	Add 120 µL of 2 N NaOH in methanol.	Heat at 80 °C for 1 hour; allow to cool.
4	Mix for 20 seconds at 1,500 rpm.	Add 2 mL of 25 % BF3 in methanol.
5	Heat at 70 °C for 5 minutes, cool 5 minutes.	Heat at 80 °C for 1 hour; allow to cool.
6	Add 240 µL of 12.5 % boron trifluoride (BF3) in methanol.	Add 5 mL of water and 5 mL of hexane.
7	Mix for 20 seconds at 1,500 rpm.	Shake well.
8	Heat at 70 °C for 5 minutes, then cool 5 minutes.	Allow the phase to separate or centrifuge.
9	Add 300 µL of water.	Transfer supernatant to GC autosampler vial.
10	Add 300 µL of hexane.	
11	Mix for 20 seconds at 1,500 rpm.	
12	Transfer the top layer (of 300 µL) to a new GC vial.	

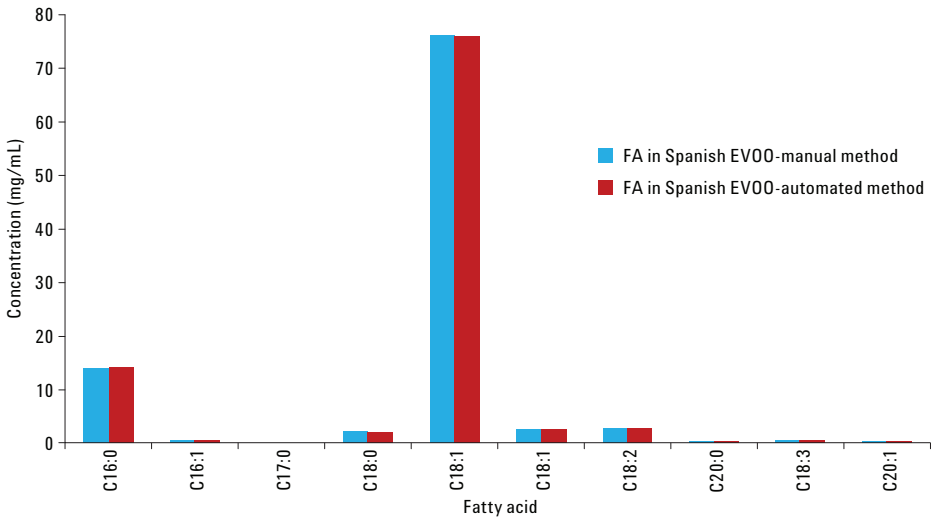

Figure 3. Comparison of fatty acid compositions determined using the manual and Agilent 7696A Sample Prep WorkBench automated derivatization methods.

Table 4. Determination of Fatty Acid Composition of Olive Oils using Manual and Automated FAME Derivatization (n = 6)

				Spanish EVOO		California EVOO	
FAME	Chain	RT (min)		Manual	Agilent 7696A Sample Prep WorkBench	Manual	Agilent 7696A Sample Prep WorkBench
Methyl palmitate	C16:0	14.985	mg/mL	109.36	109.52	103.35	104.78
			SD*	3.53	3.54	2.94	4.40
			%RSD	3.23	3.23	2.84	4.20
Methyl palmitoleate	C16:1	15.994	mg/mL	5.53	5.53	3.95	4.74
			SD*	0.39	0.39	0.39	0.00
			%RSD	7.00	7.00	9.80	0.00
Methyl stearate	C18:0	18.333	mg/mL	18.11	17.31	17.31	17.63
			SD*	0.60	0.72	0.72	0.80
			%RSD	3.33	4.14	4.14	4.52
Methyl oleate	C18:1	19.182	mg/mL	592.64	584.45	568.82	563.26
			SD*	15.60	19.22	14.09	26.65
			%RSD	2.63	3.29	2.48	4.73
Methyl vaccenate	C18:1	19.192	mg/mL	20.17	20.17	17.94	18.26
			SD*	0.72	0.72	0.39	0.39
			%RSD	3.56	3.56	2.17	2.13
Methyl linoleate	C18:2	19.826	mg/mL	21.91	22.07	45.56	45.56
			SD*	0.60	0.72	1.11	1.53
			%RSD	2.75	3.25	2.44	3.35
Methyl arachidate	C20:0	21.467	mg/mL	1.91	1.91	1.91	2.87
			SD*	0.00	0.00	0.00	0.00
			%RSD	0.00	0.00	0.00	0.00
Methyl linolenate	C18:3	21.964	mg/mL	4.92	4.92	4.13	4.44
			SD*	0.39	0.39	0.49	0.49
			%RSD	7.90	7.90	11.92	11.07
Methyl 11-eicosenoate	C20:1	22.273	mg/mL	1.91	1.91	1.91	2.87
			SD*	0.00	0.00	0.00	0.00
			%RSD	0.00	0.00	0.00	0.00

*SD = Standard deviation

Adulteration of olive oil

To determine if this method could be used to detect olive oil adulteration, several different vegetable oils were added to olive oil in various amounts. Samples of the pure vegetable and olive oils, as well as the mixtures, were derivatized on the Sample Prep WorkBench and analyzed by GC. Figure 4 illustrates one example, in which corn oil was added to Italian EVOO. A clear change in the fatty acid distribution is observed as corn oil is added, even at only 10 % v/v. A progressive decrease in the methyl oleate (C18:1) peak is accompanied by a steady increase in the methyl linoleate peak (C18:2), as the percentage of corn oil increases.

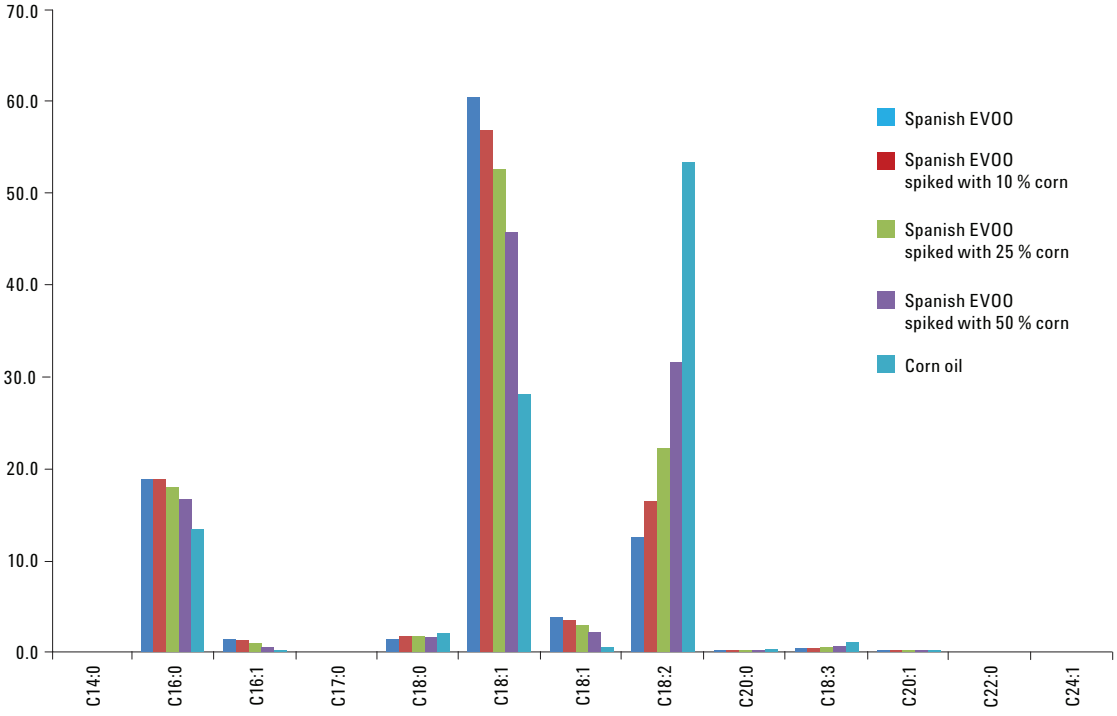

Figure 4. FAME analysis of Italian olive oil spiked with corn oil, using the Agilent 7696A Sample Prep WorkBench automated derivatization method.

To confirm that varying levels of adulteration could be differentiated, a Principal Component Analysis (PCA) was performed on the data using Agilent MassProfiler Professional Software. PCA is a frequently employed unsupervised multivariate analysis technique enabling data dimensionality reduction, while retaining the discriminating power in the data. It is performed through the transformation of measured variables into uncorrelated principal components, each being a linear combination of the original variables. It can be used as a quality control tool to provide an idea of how the data clusters, and to identify sample outliers. PCA of the entities that varied in amount between the Italian olive oil samples adulterated with various amounts of corn oil confirmed distinctive grouping of the data (Figure 5). As little as a 10 % addition of corn oil was easily discernable. Similar results were obtained for other olive oils adulterated with grapeseed oil and other vegetable oils (data not shown).

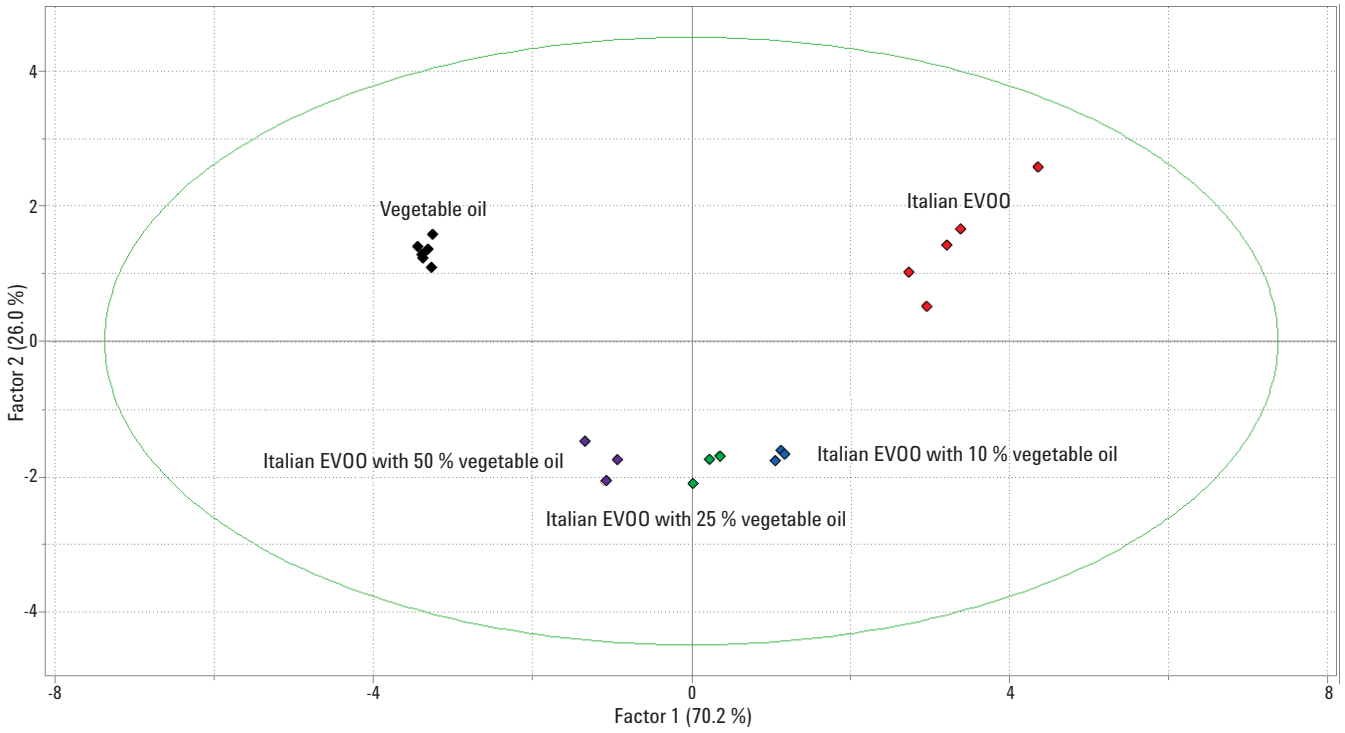

Figure 5. PCA analysis of olive oil adulterated with corn oil, illustrating the ability to differentiate them, even at 10 % adulteration.

Conclusion

Automated preparation of calibration standards on the Agilent 7696A Sample Prep WorkBench for FAME analysis results in calibration curves that are more linear than those prepared manually. The precision of six replicate analyses was comparable using the two methods. Hands-on operator time and exposure to hazardous chemicals are both greatly reduced, and solvent use is reduced 17-fold, accompanied by a reduction in consumables and generated waste. This method thus lends itself ideally to the determination of adulteration of olive oils, which can be readily determined using statistical analysis performed in Agilent MassProfiler Professional Software.

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