Determination of Fatty Acid Methyl Esters (FAMEs) in Olive Oil using Automated Sample Preparation

Application Note

Food Testing

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Abstract

There are different ways to analyze fatty acids (FAs) in oil. This application note shows how to analyze them after a base-catalyzed reaction and the advantages of preparing the samples with the Agilent 7696A Sample Prep WorkBench.

Introduction

The analysis of FAs is very common in olive oil industry and is usually done by gas chromatography. Due to their polar nature and their high boiling points, they generally show poor peak shapes and bad reproducibility. To avoid these problems, most methods use derivatization reactions to convert FAs to fatty acid methyl esters (FAMEs), which are easier to separate and exhibit better peak shapes.

There are a large number of derivatization reactions. One of the most common is the base-catalyzed reaction, which uses hexane and potassium hydroxide (KOH) in methanol. This method is quick, simple, and provides good results although it does not work on free fatty acids.

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Experimental

Materials

The materials used were, n-heptane, (hexane could also have been used), methanol (GC grade), and potassium hydroxide from Baker. A solution of KOH 2N was prepared by adding 11.2 g of KOH in 100 mL of methanol.

Heptane and water were used as wash solvents in the 7696A Sample Prep WorkBench. The syringe that extracts KOH solution had to be washed with both solvents, first with water to wash away the potassium hydroxide, and then with heptane. The syringe that extracts the heptane was washed with heptane alone.

Instrumentation

The usual method to analyze fatty acids in olive oil by basic derivatization uses 100 mg of sample, 10 mL of heptane and 100 µL of potassium hydroxide in a 20-mL tube. In this study, the utility of the WorkBench was tested. Therefore, all the quantities had to be divided by 10, because this instrument works with 2-mL vials.

This base-catalyzed reaction happens in a single step within a few minutes.

The WorkBench was used to automatically prepare all the samples injected into the GC/MS system.

The method used is as follows:

The software provides a Resource Manager showing where all the vials and reagents are allocated (see Figure 1).

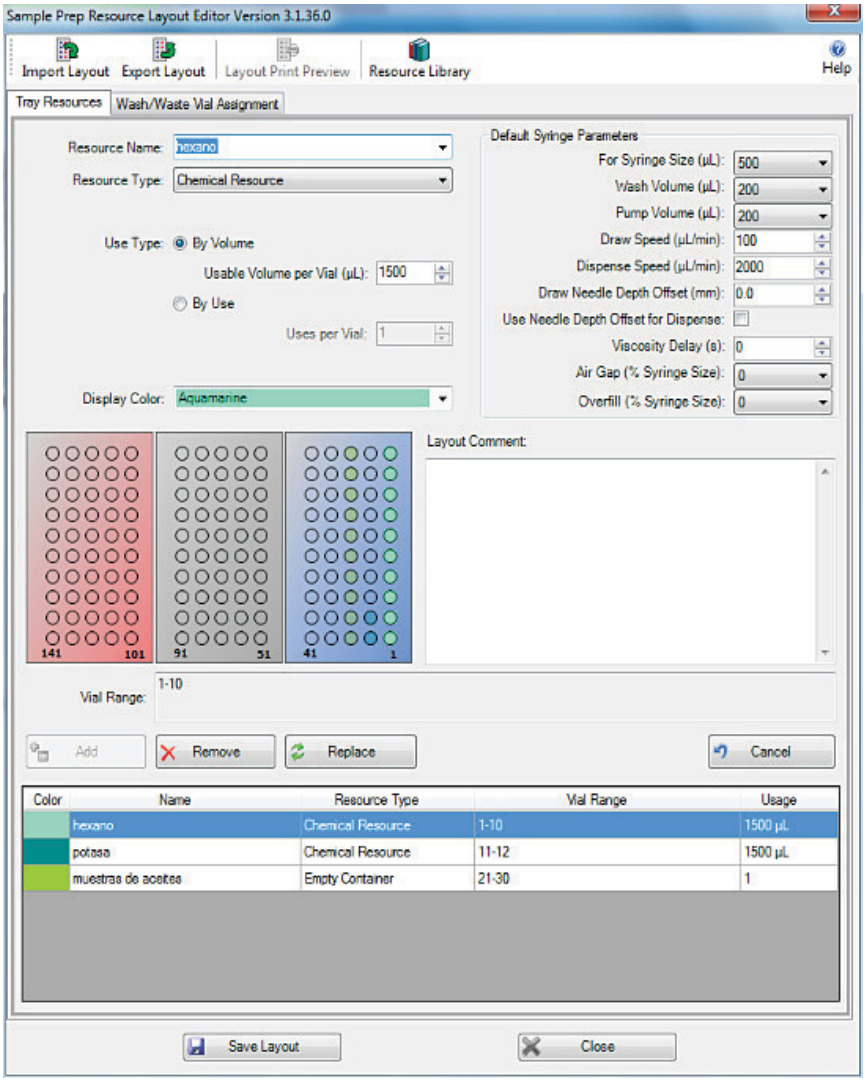

Figure 1. Resource layout.

Figure 2 shows the method used to prepare the samples.

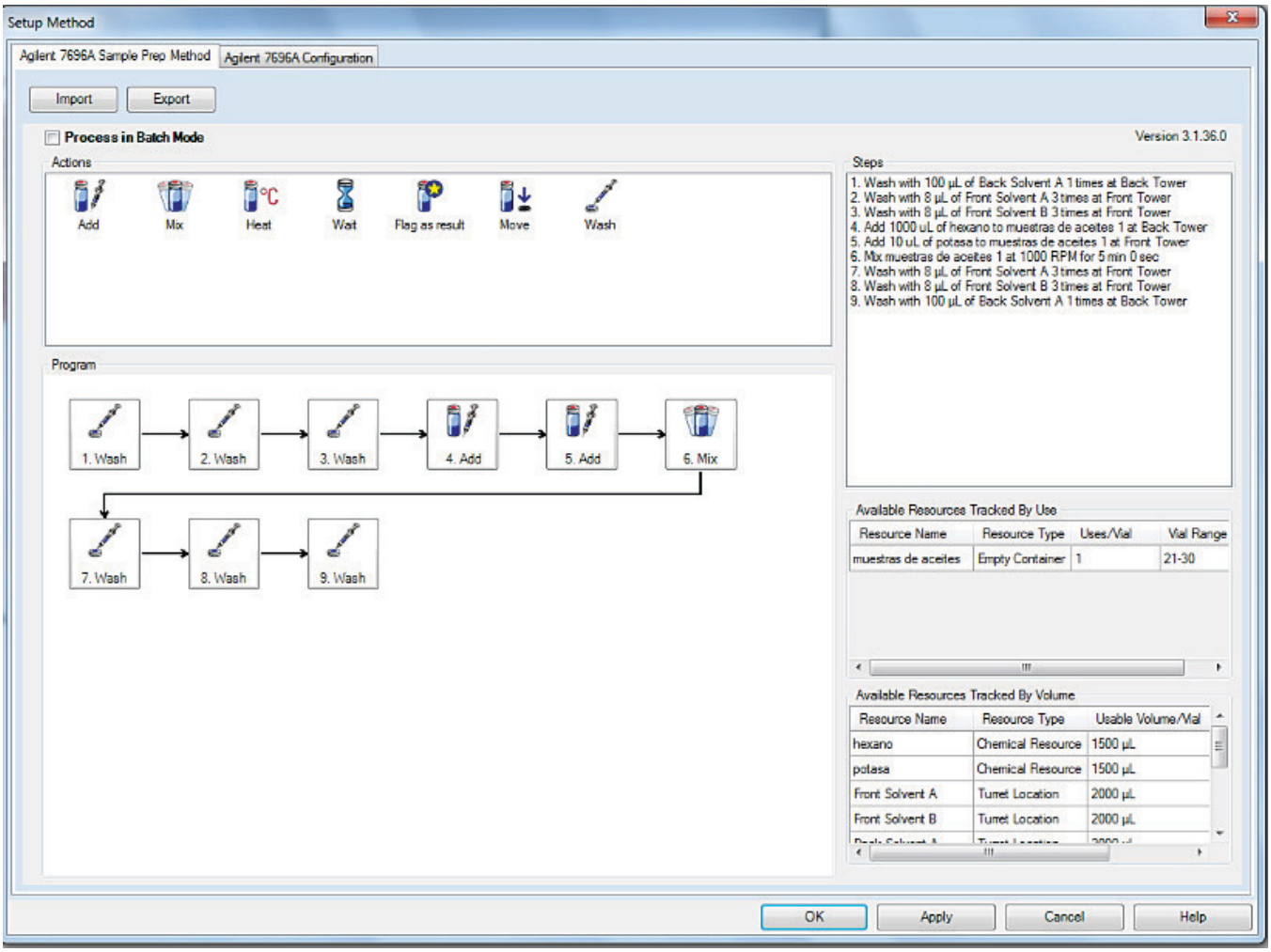

Figure 2. Agilent 7696A Sample Prep method.

In one of the trays, we set three rows of 2-mL vials, one with vials containing heptane, one containing vials with KOH, and the last row containing vials with one drop, about 10 µL, of olive oil (the weight must be noted). The SamplePrep WorkBench uses two syringes to add the necessary amount of each reagent: 1 mL of heptane and 10 µL of KOH. After both additions, the vial was agitated for 10 minutes.

Once the vial was mixed, the upper level was injected in a GC, equipped with a split/splitless inlet at 250 °C, and connected to a MSD. The column used was a HP88 (60 m × 250 µm, 0.2 µm), with a column flow rate of 1 mL/min. A temperature program of 175 °C for 5 minutes and 5 °C/min to 250 °C was used to achieve separation of the fatty acids. The inlet was set to Split mode with a split ratio of 100:1. All the analysis were performed in both SIM and SCAN modes.

Results and Discussion

To evaluate the reproducibility and accuracy of the chromatograms obtained using the WorkBench, 10 vials prepared with the WorkBench were injected on the GC/MS. Table 1 shows the results.

This application note compares the results of the four main compounds of the olive oil. The peak shape in the chromatograms is shown in Figure 3, and the area of the four peaks evaluated is shown in Table 2.

First, 10 vials were weighed after adding a drop of oil into them. Table 1 shows the values obtained.

Table 1. Weight of the 10 Samples Evaluated

Vial	Oil weight/mg
1	12.9
2	13.4
3	14.8
4	14.5
5	14.2
6	14.7
7	13.2
8	14.9
9	13.8
10	13.6

These vials were placed in the WorkBench tray to be automatically filled with the programmed amounts of each reagent.

Once the vials were ready, they were injected in the GC/MS under the conditions described above. Figure 3 shows the results.

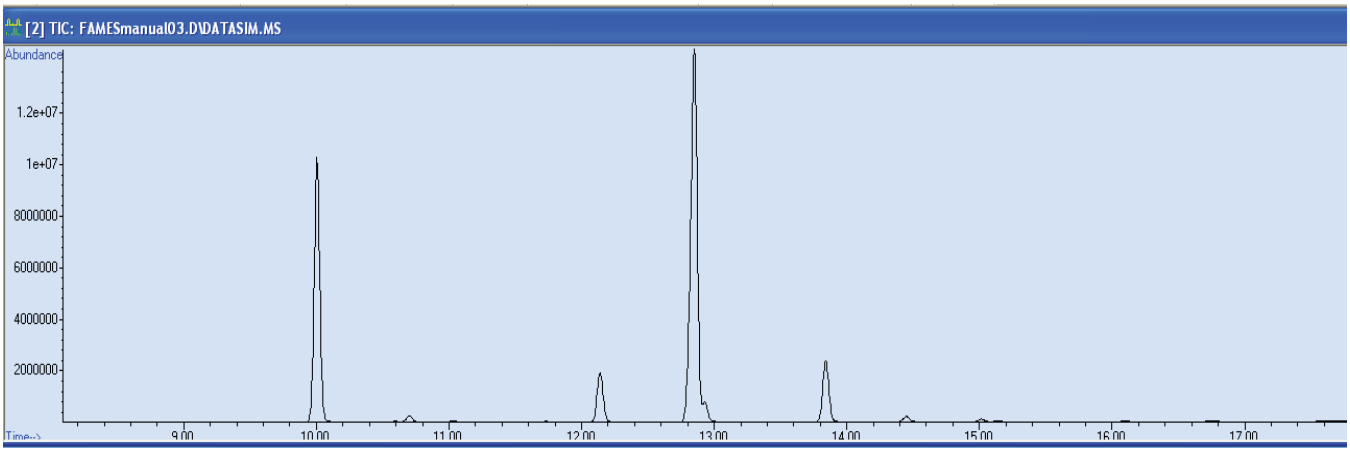

Figure 3. Chromatogram in SIM mode.

Table 2. Area of the Four Main Compounds of the Olive Oil

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	317343837.0	63331226.0	569320584.0	80584679.0
2	373510457.0	74825501.0	660064790.0	94609910.0
3	389137859.0	74174710.0	683431450.0	98106712.0
4	350160186.0	69553324.0	621849766.0	88281829.0
5	350311578.0	69513586.0	622622625.0	88233984.0
6	363692227.0	71973045.0	643859326.0	91639831.0
7	298792007.0	58778562.0	534781631.0	74997383.0
8	376569059.0	74878674.0	666439996.0	95109185.0
9	352698458.0	68424565.0	654254324.0	82569566.0
10	351745852.0	70145747.0	602155656.0	86951448.0
Average	**350409359.2**	**69188856.3**	**622601967.1**	**87561952.4**
Relative standard deviation	**27119463.9**	**5161865.2**	**46358289.5**	**7182432.9**
%RSD	**7.7**	**7.5**	**7.4**	**8.2**

Including the quantity of oil weight in each vial, the area or each compound per milligram is shown in Table 3.

Table 3. Area per mg of Oil

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	24600297.4	4909397.4	44133378.6	6246874.3
2	27873914.7	5583992.6	49258566.4	7060441.0
3	26293098.6	5011804.7	46177800.7	6628831.9
4	24155874.9	4796781.0	42886190.8	6088402.0
5	24669829.4	4895323.0	43846663.7	6213660.8
6	24740967.8	4896125.5	43799954.1	6234002.1
7	22635758.1	4452921.4	40513759.9	5681619.9
8	25273091.2	5025414.4	44727516.5	6383166.8
9	25557859.3	4958301.8	47409733.6	5983301.9
10	25863665.6	5157775.5	44276151.2	6393488.8
Average	**25097420.2**	**4954369.4**	**44585290.7**	**6272059.8**
Relative standard deviation	**1391411.9**	**284657.5**	**2430391.6**	**371694.7**
%RSD	**5.5**	**5.7**	**5.5**	**5.9**

Table 4 shows the area percentage of each FAME for the 10 samples prepared.

Table 4. Area Percentage of Each Peak of the Chromatogram

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	30.8	6.1	55.2	7.8
2	31.0	6.2	54.9	7.9
3	31.3	6.0	54.9	7.9
4	31.0	6.2	55.0	7.8
5	31.0	6.1	55.1	7.8
6	31.1	6.1	55.0	7.8
7	30.9	6.1	55.3	7.8
8	31.0	6.2	54.9	7.8
9	30.5	5.9	56.5	7.1
10	31.7	6.3	54.2	7.8
Average	**31.0**	**6.1**	**55.1**	**7.7**
Relative standard deviation	**0.3**	**0.1**	**0.6**	**0.2**
%RSD	**1.0**	**1.9**	**1.0**	**2.9**

In this experiment, both methods, the original (100 mg of oil) and the method adapted to the WorkBench, are compared. The results from the manual preparation methods are shown in Table 5 and Table 6.

Table 5. Area per mg of Oil Using the Quantities of the Original Method

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	2674181.8	529275.8	4610749.8	674892.3
2	2562129.3	505970.3	4442449.3	648040.5
3	2596966.1	511187.6	4504510.0	655770.4
4	2388663.8	466760.2	4168008.4	601931.7
5	2721157.8	535230.9	4722598.6	688465.5
6	2789232.0	549999.6	4813189.6	704034.8
7	2330855.0	453164.1	4057061.6	589335.4
8	2645696.1	528725.3	4579552.0	669544.2
9	2650632.8	520264.3	4600138.2	668931.5
10	2660736.3	520639.8	4632201.2	671882.6
Average	**2594658.8**	**510416.9**	**4501404.4**	**655410.2**
Relative standard deviation	**142531.1**	**30276.4**	**236121.4**	**36110.5**
%RSD	**5.5**	**5.9**	**5.2**	**5.5**

As seen, the %RSD are similar to the results using the WorkBench.

The same sample preparation used by the WorkBench was performed manually: one weighed drop of oil in a 2-mL vial, plus 1 mL of heptane and 10 µL of KOH in methanol using Agilent syringes, and shaken gently by the operator. The results of the analysis are shown in Table 6.

Table 6. Area per mg of Oil After Manual Sample Preparation using WorkBench Quantities

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	24414278.4	4280301.6	34483064.7	5405051.1
2	21953969.5	4385041.9	34340981.7	5496525.8
3	25176754.2	4987565.4	39311102.4	6258162.4
4	23806050.0	4723341.1	36249791.4	5917479.9
5	23413864.7	4659269.7	36103230.9	5862013.3
6	22388861.8	4441774.0	34988087.2	5625015.1
7	23345774.4	4655270.9	36540218.6	5654628.9
8	21758664.6	4326697.7	31010500.3	5465899.5
9	22268704.8	4448969.7	34833834.8	5598507.6
10	21726270.7	4324528.6	34099881.5	5188441.0
Average	**22970768.0**	**4513461.5**	**35078976.3**	**5633014.3**
Relative standard deviation	**1194355.0**	**226067.9**	**2129375.1**	**301856.8**
%RSD	**5.2**	**5.0**	**6.1**	**5.4**

As seen, the %RSD are similar to the results using the WorkBench.

Conclusions

The Agilent 7696A Sample Prep WorkBench is a very comfortable, fast, easy and reliable tool to automate some typical laboratory work such as sample preparation. The results detailed in this application note how the reproducibility of the analysis when performed with the WorkBench. The results obtained from the WorkBench preparation are very similar to those obtained with a manual preparation both with original resource quantities and WorkBench-scale quantities.

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