APPLICATION NOTE

Determination of Fatty Acid Methyl Esters in olive oil using GC-FID

AN174v2; SCION Instruments

Introduction

The determination of Fatty Acid Methyl Esters (FAME) is a commonly performed analysis, to determine the quality of extra virgin olive oil (EVOO). Natural EVOO is made by pressing or centrifuging olives, without exposing the olives to any chemical processing. A way of producing cheaper olive oil is to mix them with cheaper oils, such as sunflower oil and refined olive oils. This mixing with refined olive oils results in a divergent fatty acid content in the end product.

The determination of fatty acids for quality control in EVOO's is performed by Gas Chromatography (GC). Therefore, the fatty acids need to be trans esterified into FAME because of the thermally instable volatile behaviour of the fatty acids. This results in a more reliable analysis. In the transesterification process free and bonded fatty acids react with an alcohol in the presence of a catalyst, forming a mixture of FAME compounds and an alcohol.[1] The composition of FAME compounds in EVOO can say a lot about the quality and origin of the olive oil, which makes this analysis very valuable for quality control.

This application will study the quantification and qualification of FAME within EVOO's, by GC with a Flame Ionization Detector (FID).

This application can be performed on either the SCION Instruments 8300 GC & 8500 GC platform with the SCION 8400PRO Autosampler, shown in Figure 1. A SCION-FAME column is used for obtaining the best separation of the cis and trans-isomers of most FAME's.

Figure 1 SCION Instruments 8300 & 8500-GC equipped with the 8400PRO Autosampler.

Table 1 details the GC parameters used throughout this analysis.

Table 1: Instrumentation operating conditions GC

GC Part	Settings
Injector	Temperature 220°C Split ratio 10:1
Injection Volume	2.0 µL
Liner	SCION 1177 4 mm S/SL taper Focus
Column	SCION-FAME 100m x 0.25mm x 0.2µm
Carrier Gas	Helium 1.5mL/min
Oven Program	100°C (hold 4.0 min), 3°C/min to 240°C (hold 6.33 min)
Detector	Flame Ionization Detector Temperature 250°C Air: 300 mL/min Hydrogen: 30 mL/min Make up (N_2): 25 mL/min
Run Time	57 min
Software	Compass CDS

Experimental

For this application a FAME standard was purchased for the qualification and quantification of unknown samples.

The FAME standard contained 37 FAME compounds with a concentration range from 150 – 600 µg/mL.

The compounds present in the FAME standard are shown in Table 2, along with their concentrations, and displayed in elution order for this application.

The SCION-FAME column provides a perfect resolution between the cis and trans FAME isomers that are present in the FAME-mix, seen in the chromatogram in Figure 2. Therefore, all 37 FAME components were successfully identified according to the retention time match observed from analysing the FAME standard with known concentrations.

Table 2: Components and concentrations of the FAME-standard.

#	Component	µg/mL	#	Component	µg/mL
1	Methyl Butyrate	399.6	20	Methyl Linoleate	198.0
2	Methyl Hexanoate	399.6	21	Methyl Arachidate	399.7
3	Methyl Octanoate	399.8	22	Methyl Gamma Linolenate	197.6
4	Methyl Decanoate	399.8	23	Methyl cis-11-Eicosenoate	199.2
5	Methyl Undecanoate	199.8	24	Methyl Linolenate	199.4
6	Methyl Laurate	399.7	25	Methyl Heneicosanoate	199.2
7	Methyl Tridecanoate	198.2	26	Cis-11,14-Eicosadienoic Acid Methyl Ester	199.8
8	Methyl Myristate	399.7	27	Methyl Behenate	399.7
9	Methyl Myristoleate	199.8	28	Cis-8,11,14-Eicosatrienoic Acid Methyl Ester	163.0
10	Methyl Pentadecanoate	199.6	29	Methyl Erucate	199.9
11	Cis-10-Pentadecenoic Acid Methyl Ester	198.1	30	Cis-11,14,17,Eicosatrienoic Acid Methyl Ester	150.0
12	Methyl Palmitate	599.6	31	Methyl Arachidonate	199.8
13	Methyl Palmitoleate	199.8	32	Methyl Tricosanoate	179.0
14	Methyl Heptadecanoate	159	33	Cis-1316-Docosadienoic Acid Methyl Ester	199.7
15	Cis-10-Heptadecenoic Acid Methyl Ester	199.9	34	Methyl Tetracosanoate	399.6
16	Methyl Stearate	399.6	35	Methyl Cis-5,8,11,14,17-Eicosapentaenoate	150.0
17	Methyl Elaidate	199.8	36	Methyl Nervonate	196.8
18	Methyl Oleate	399.7	37	Cis-4,7,10,13,16,19-Docosahexaenoate	154.0
19	Methyl Linolelaidate	199.8			

Sample preparation

A calibration set from the FAME standards (diluted with n-Heptane) prepared from 0.20 µg/mL up to 50 µg/mL. Dodecane (50 µg/mL) is used as an internal standard (IS) and added to all standards and samples. An IS working solution 1000 µg/ml is prepared by dissolving 20 µL dodecane into n-Heptane, in a 15 mL volumetric flask.

To prepare the EVOO sample, 0.1 g of the olive oil is transferred into a test tube. To this tube 250 µL of the IS work solution is added plus 4750 µL n-Heptane, followed by shaking. Then 200 µL of 2M KOH dissolved in methanol is added to perform the esterification process, by shaking vigorously for 30 seconds. The solution is left to stratify until the upper layer of the solution becomes clear. The upper layer is transferred into a GC sample vial and is ready for injection.[2]

Blank injections of n-Heptane are performed in between samples to determine root-mean-square (RMS) Noise, and to ensure that the system is not contaminated after sample injections.

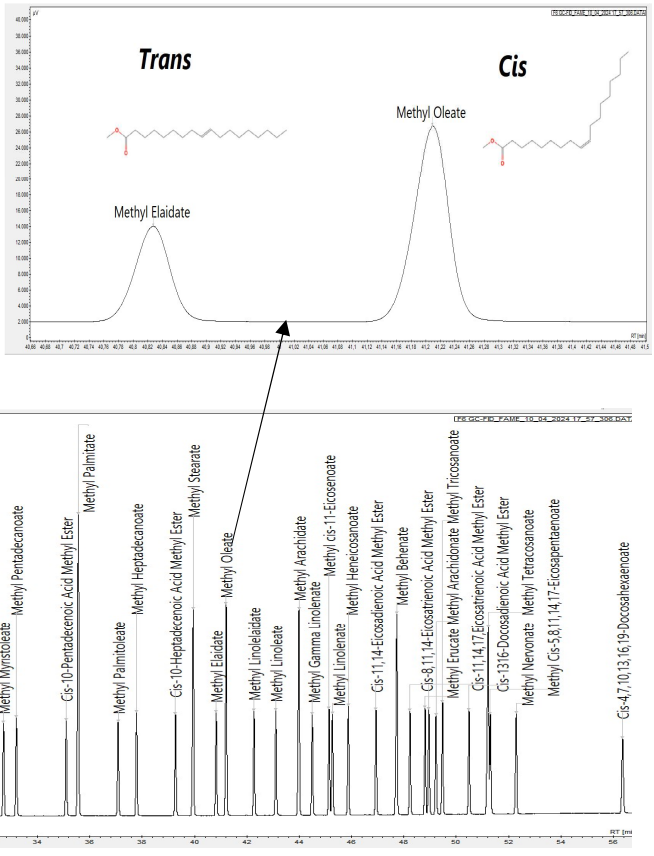


Figure 2 Example chromatogram of the FAME-standard, close up for resolution between isomers Methyl Elaidate(*Trans*) & Methyl Oleate(*Cis*)

Results

Due to the number of compounds present in this standard and method, not all compounds will be mentioned in the Results section.

The results for the following FAME compounds found in the EVOO sample are shown: Methyl Palmitate (#12), Methyl Palmitoleate (#13), Methyl Stearate (#16), Methyl Oleate (#18), Methyl Linoleate (#20), Methyl Arachidate (#21), Methyl cis-11-Eicosenoate (#23) and Methyl Linolenate (#24). If necessary, the results from the other FAME components are accessible by request.

The calibration curves for the FAME standards were prepared from 0.2 µg/mL up to 50 µg/mL.

The precision of the instrument and method was obtained by ten consecutive injections of FAME standard #4 (range 10-25 µg/mL).

The system precision of the selected FAME compounds can be found in Table 3, along with the linearity results (R^2) obtained by the calibration curves.

Table 3: Summary of Results – Linearity and repeatability

No.	Rt (min)	Component	R^2	Repeatability (%RSD)
12	35.52	Methyl Palmitate	0.9934	0.50
13	37.07	Methyl Palmitoleate	0.9945	0.45
16	39.92	Methyl Stearate	0.9928	0.71
18	41.21	Methyl Oleate	0.9931	0.63
20	43.10	Methyl Linoleate	0.9935	0.56
21	43.95	Methyl Arachidate	0.9925	0.71
23	45.12	Methyl cis-11-Eicosenoate	0.9980	0.76
24	45.26	Methyl Linolenate	0.9934	0.67

For all FAME components an R^2 of 0.99 or higher was achieved. This is an excellent result, with many regulations requiring an R^2 value of only ≥0.98. Repeatability results show that for most FAME components the relative standard deviations (RSD%) are below 1%. For only 2 FAME components RSD ≤1.2%. This is a good precision for the method, since most acceptance criteria for method validation are requiring an RSD ≤2%.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to equations 1 and 2:

$$1)\ LOD = \frac{(3.3 * RMS\ Noise) * Concentration}{Peak\ height}$$

$$2)\ LOQ = \frac{(10 * RMS\ Noise) * Concentration}{Peak\ height}$$

Where the RMS Noise (in µV) is generated from a blank injection and calculated by CompassCDS, by root-mean-square (RMS) of the baseline over a selected time window. The concentration of the compounds is from the lowest calibration standard (800x dilution of FAME standard, in µg/mL) with corresponding peak heights (in µV).

The calculated LOD's were found to be ≤0.34 µg/mL, these are shown in Table 4. This table also displays that the LOQ's are ≤1 µg/mL. If lower LOD's and LOQ's are required it is recommended to perform this application with the SCION GC-SQMS configuration.

Identified FAME components found in the sample are shown in Table 4. According to regulations an EVOO FAME content (summation of total FAMES) should be ≤75 mg/kg.[3] If compensating for the dilution in n-Heptane the Σ FAME content is ≤51.3 µg/mL. In the olive oil sample, 8 FAME compounds are found, the concentration of each compound is calculated from the calibration curves equations. The total Σ FAME-content from all identified compounds is 34.51 µg/mL, and therefore within the specification mentioned in EU regulations.

Table 4: Summary of Results – LOD, LOQ and amount found in extra virgin olive oil sample.

No.	Component	LOD (µg/mL)	LOQ (µg/mL)	Amount Found in Sample (µg/mL)
12	Methyl Palmitate	0.11	0.34	10.70
13	Methyl Palmitoleate	0.10	0.31	1.06
16	Methyl Stearate	0.09	0.28	4.89
18	Methyl Oleate	0.10	0.31	8.84
20	Methyl Linoleate	0.09	0.09	6.60
21	Methyl Arachidate	0.09	0.28	1.36
23	Methyl cis-11-Eicosenoate	0.09	0.28	0.69
24	Methyl Linolenate	0.09	0.26	0.37
			Σ total FAME	34.51

Figure 3 shows an example chromatogram generated in CompassCDS of the EVOO sample. The composition range (%) of FAMEs mentioned in regulations for EVOO are shown in Table 5.[4]

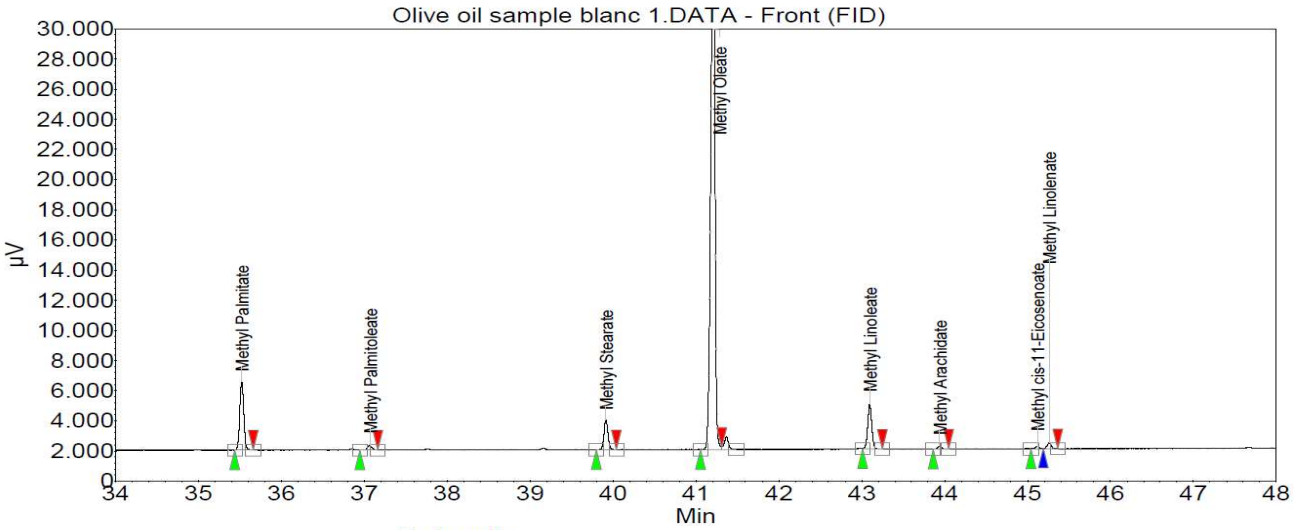
Table 5: Results FAME composition in EVOO sample compared to specifications mentioned in regulations[4]

No.	Component	Specification range %	Found in sample (%)	Passed spec?
12	Methyl Palmitate	7.50 – 20.00	9.34	Yes
13	Methyl Palmitoleate	0.30 – 3.50	0.67	Yes
16	Methyl Stearate	0.50 – 5.00	4.08	Yes
18	Methyl Oleate	55.00 – 83.00	77.99	Yes
20	Methyl Linoleate	2.50 – 21.00	6.3	Yes
21	Methyl Arachidate	≤0.60	0.45	Yes
23	Methyl cis-11-Eicosenoate	N.D.	0.31	N.D.
24	Methyl Linolenate	≤1.00	0.83	Yes

The composition of identified FAMES from the EVOO sample (% peak Areas Ratio) are compared with the set up specifications for a genuine extra virgin olive oil, and all compounds passed the criteria set out in the specifications. #23 Methyl cis-11-Eicosenoate wasn't mentioned in the regulations and is therefore marked as N.D.

Another confirmation that EVOO is not adulterated is to check for the presence of trans-isomers. Trans-isomers (for example #17 Methyl Elaidate) in double bonded fatty acids are not present in natural EVOO, but can be formed after heating processes. In this case the analysed EVOO does not contain any trans-FAME compounds which indicates that the sample has not been exposed to any heating processes.

With the total FAME content mentioned in Table 4, the FAME composition of EVOO sample which can be found in Table 5, and that there was no presence of trans-isomers in the sample it can be concluded that the analysed sample is a genuine EVOO, which has not been exposed to dilution with cheaper oils, or any heating or chemical processes.



Peak results :

Index	Name	Time [Min]	Height [µV]	Area % [%]	Res. USP	Area [µV.Sec]
1	Dodecane (IS)	9,71	36791,6	40,738	0,00	111167,8
2	Methyl Palmitate	35,52	4534,2	5,532	309,27	15095,7
3	Methyl Palmitoleate	37,07	306,1	0,401	17,56	1093,6
4	Methyl Stearate	39,91	1972,2	2,421	32,30	6605,8
5	Methyl Oleate	41,21	36669,9	46,245	14,58	126197,7
6	Methyl Linoleate	43,10	2968,7	3,699	21,37	10093,0
7	Methyl Arachidate	43,94	220,7	0,274	8,22	746,7
8	Methyl cis-11-Eicosenoate	45,12	152,0	0,191	13,20	522,5
9	Methyl Linolenate	45,26	394,5	0,500	1,75	1365,3
Total			84009,9	100,000		272888,0

Figure 3: Example chromatogram and peak results for EVOO sample.

Conclusion

The SCION 8500 GC platform equipped with a split/spitless injector, SCION FAME column and Flame Ionization Detector with 8400PRO sampler is a perfect solution for the determination of FAME in olive oil. Good system precision, good linearity results and low LOQ's and LOD's are easily achieved for this application.

The SCION-FAME column shows good resolution between cis and trans FAME isomers, which was an easy way to verify that there were no trans-FAME isomers present in an EVOO that could indicate heating in the production process.

The analysed EVOO sample is, according to multiple results obtained by this application, most likely a natural EVOO that has not been exposed to adultery in the production process.

This method is also applicable on the SCION Instruments 8300 or 8500 GC-platform with 8700 Single Quad Mass Spectrometer (SQ-MS). This technique can achieve lower LOD's and LOQ's by GCMS measuring in SIM-mode, compared to the GC-FID application. The use of the MSWS-software allows unknown FAME components to be easily identified with our NIST-Libraries. This application note 'Determination of FAME in olive oil using GC-SQMS' can be found our Application Library.

Order Information

Ordering Information for the 8300 GC	
Part	**Part Number**
8300 GC with S/SL inlet and FID (120V)	839001701
8300 GC with S/SL inlet and FID (230V)	839001702
8400 PRO Autosampler for 8300 GC and 8500 GC	840000001
Compass CDS Base License Software	BR502002

Suggested Consumables	
Part	**Part number**
15% Graphite/85% Vespel Ferrule 1/16" with 0.4 mm hole pk/10	41312148
BTO Septa 9 mm, pk/50	CR298713
10µL fixed needle syringe, 5 cm, 0.47 mm OD, 26 g conical needle	41312133
SCION-FAME column 100m x 0,25mm x 0,2 µm	SC37301
SCION 1177 4 mm S/SL taper Focus	41312102

References

1. INTERNATIONAL OLIVE COUNCIL; DETERMINATION OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY, https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-T.20-Doc.-No-33-Rev.-1-2017.pdf (accessed 23 Feb 24)

2. COMMISSION IMPLEMENTING REGULATION (EU) Characteristics of olive oil and olive-residue oil and on the relevant methods of analysis 2015, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R1833&rid=8 (accessed 14 Feb 24)

3. COMMISSION REGULATION (EU) Characteristics of olive oil and olive-residue oil and on the relevant methods of analysis 2011, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0061 (accessed 30 Apr 25)

4. INTERNATIONAL OLIVE COUNCIL: TRADE STANDARD APPLYING TO OLIVE OILS AND OLIVE POMACE OILS JUNE 2019 , https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-T.15-NC.-No-3-Rev.-13-2019-Eng.pdf (accessed 29 Mar 24)

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