APPLICATION NOTE

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

AN168v1; 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 stable volatile behaviour of these compounds. 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 much 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 Mass Spectrometry(MS).

This application can be performed on either the SCION Instruments 8300 GC & 8500 GC platform with 8700 Single Quad Mass Spectrometer (SQMS) and 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 and 8700 SQMS and 8400PRO Autosampler.

Table 1 details the GC and SQMS method parameters used throughout this analysis.

Table 1: Instrumentation operating conditions GC and SQMS

GC Part	Settings
Injector	220°C Split ratio 10:1
Injection Volume	1.0 µL
Column	SCION-FAME 100m x 0.25mm x 0.2µm
Carrier Gas	Helium 1.2mL/min
Oven Program	100°C (hold 4.0 min), 3°C/min to 240°C (hold 9.33 min)
Run Time	58 min
Software	MSWS
MS Part	**Settings**
MS transfer line temp	200°C
Ion source **temp**	250°C
MS mode	Electron Ionization
Delay collection time	9.00min
Scan mode	SIM mode

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 – 400µg/mL.

The components present in the FAME standard are shown in Table 2.

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 3. Therefore all 37 FAME components were successfully identified with NIST library search tool built into the MS Work Station (MSWS) software. The method was easily converted from Full scan into a SIM method, which increases the sensitivity of the method.

For example, compound #1 Methyl Butyrate is identified with the NIST library. When this component is added to the component list assigned to the method, the Quantifier (74 m/z) and Qualifier ions (43 and 71 m/z) are also inserted into the method. The retention time match and match with the known mass spec makes it very easy to identify unknown samples.

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

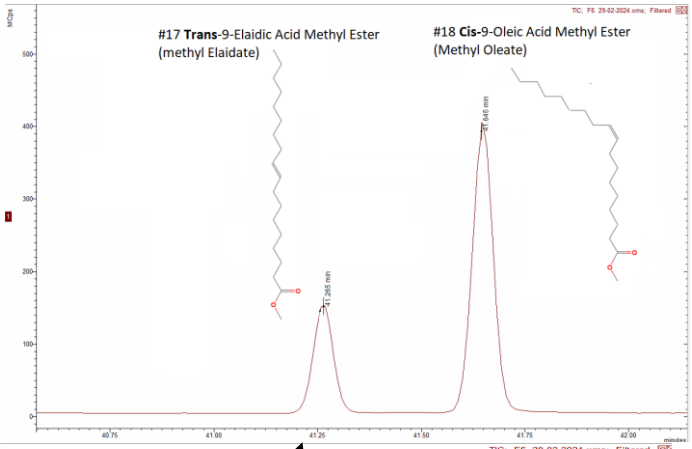
#	Component	µg/mL	#	Component	µg/mL
1	Methyl Butyrate	399.6	20	Methyl Linoleate	198.0
2	Methyl Hexanoate	399.6	21	Methyl Arachidate	399.7
3	Methyl Octanoate	399.8	22	Methyl Gamma Linolenate	197.6
4	Methyl Decanoate	399.8	23	Methyl cis-11-Eicosenoate	199.2
5	Methyl Undecanoate	199.8	24	Methyl Linolenate	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 0.20µg/mL up to 50µg/mL. Deuterated Methyl Heptadecanoate (50µg/mL) is used as an internal standard (IS) and added to all standards and samples. An IS working solution 1000µg/ml is prepared by dissolving 10mg Deuterated Methyl Heptadecanoate into n-heptane, in a 10mL volumetric flask.

The quality control sample (QC) is prepared[2] by dissolving 50µL extra virgin olive oil in n-Heptane in a 25mL volumetric flask. 0.1mL of the diluted olive oil is transferred into a test tube. To this tube 100µL of the IS work solution is added plus 1900µL n-heptane, followed by shaking. Then, 200µL of 2M KOH dissolved in methanol is added to perform the esterification process, by shaking vigorously for 30seconds. 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.

Blank injections of n-Heptane are performed in between samples, to ensure that the system is not contaminated after sample injections.



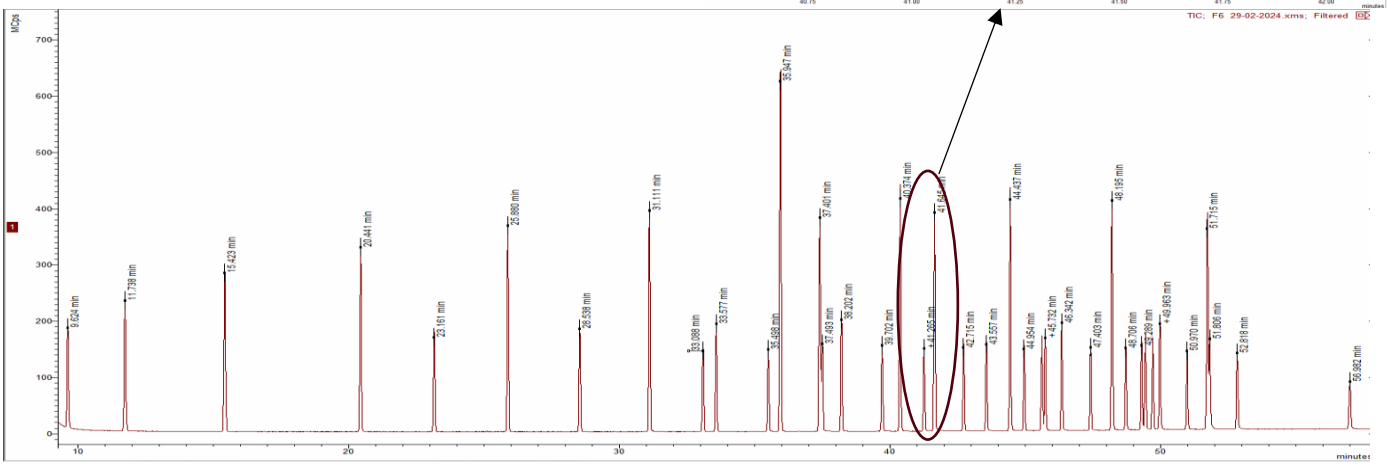


Figure 2 TIC chromatogram of the FAME-standard

Results

Because there are some many components present in this standard and method, not all components will be mentioned in the Results section.

If necessary, the full validation report is accessible by request. Selected compounds for showing results are FAME compounds that were found in the EVOO sample. Methyl Palmitate(#12), Methyl Palmitoleate(#13), Methyl Stearate (#16), Methyl Oleate(#18), Methyl Linoleate(#20), Methyl Arachidate (#21), and Methyl Linolenate (#24).

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

The precision of the method was obtained by seven consecutive injections of FAME standard #4. (range 10-25µg/ml)

The results of the 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	35.94	Methyl Palmitate	0.9935	0.34
13	37.48	Methyl Palmitoleate	0.9954	0.92
16	40.35	Methyl Stearate	0.9942	0.35
18	41.63	Methyl Oleate	0.9949	0.68
20	43.55	Methyl Linoleate	0.9931	0.41
21	44.40	Methyl Arachidate	0.9939	0.21
24	45.72	Methyl Linolenate	0.9937	0.56

For all FAME components an R^2 of 0.99 or higher was achieved, which 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%. 2 components are RSD ≤1,5.%. 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:

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

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

Where the noise (in kCps) is calculated from a blank injection, the concentration of the components is from calibration standard #1 (800x dilution of FAME standard, in µg/mL) with corresponding peak height (in kCps)

The calculated LOD's were found to be ≤0.04 µg/mL, these are shown in Table 4. These low LOD and LOQ's are easily achievable when measured in SIM-mode.

Identified FAME components found in the sample are shown in table 4. According to regulations[3] an EVOO FAME content (summation of total FAMES) should be ≤75mg/kg. If compensated for the dilution in n-heptane the ΣFAME content this is ≤51,3µg/mL. In the olive oil sample 7 FAME compounds are identified in MSWS, the concentration of each component is calculated from the calibration curves equations. The total Σ FAME-content from all identified components is 39,96 µg/mL, and therefore within the specification mentioned in 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	0.0031	0.0094	15.05
13	Methyl Palmitoleate	0.0069	0.0210	1.10
16	Methyl Stearate	0.0036	0.0110	2.89
18	Methyl Oleate	0.0093	0.0283	9.18
20	Methyl Linoleate	0.0094	0.0284	8.18
21	Methyl Arachidate	0.0045	0.0135	2.09
24	Methyl Linolenate	0.0085	0.0257	1.46
			Σ total FAME	39.96

Figure 3 shows some example chromatograms from MSWS of the target peaks Methyl Oleate and Methyl Linoleate and their corresponding mass spectra, from the QC sample.

Also shown in Figure 3 is the NIST library reference mass spectra confirming the identity of each analyte.

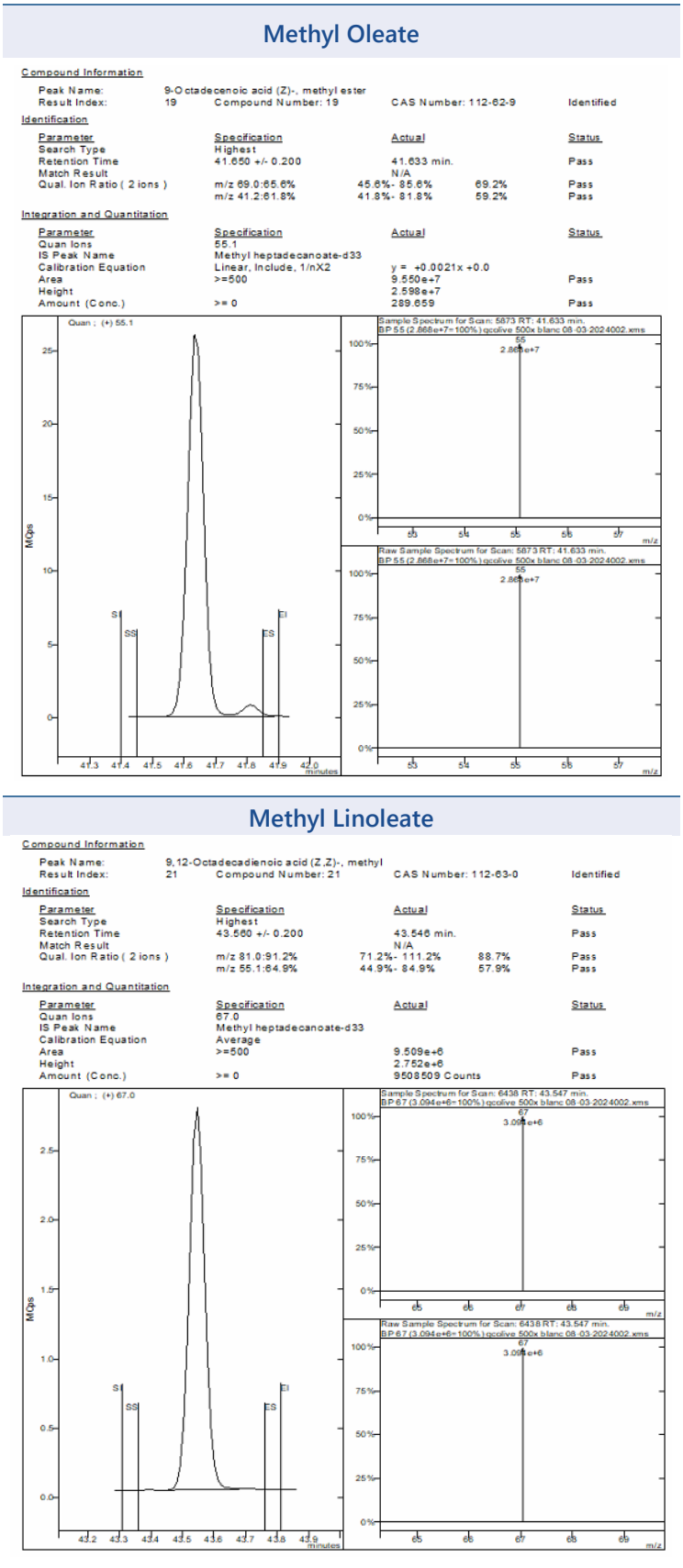


Figure 3: Example chromatograms for selected FAMES

Extra virgin olive oils are known for their health benefits, derived from a high content of Methyl oleate and a lower content Methyl Linoleate.

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	28.58	No
13	Methyl Palmitoleate	0.30 – 3.50	0.77	Yes
16	Methyl Stearate	0.50 – 5.00	5.64	Yes
18	Methyl Oleate	55.00 – 83.00	58.32	Yes
20	Methyl Linoleate	2.50 – 21.00	5.81	Yes
21	Methyl Arachidate	≤0.60	0.49	Yes
24	Methyl Linolenate	≤1.00	0.41	Yes

The composition range (%) of FAMEs mentioned in regulations[4] for EVOO are shown in table 5.

The composition of identified FAMES from the EVOO sample (% peak Areas) are compared with the set up specifications. Only 1 of the 7 FAME compounds is above spec (methyl palmitate). When a EVOO is suspected to be adulterated, this mostly results in a divergent Methyl Oleate or Methyl Linoleate contents, not for methyl palmitate.

Another confirmation that be sure an EVOO is not adulterated is to check the presence of trans-isomers. Trans-isomers (for example Methyl Elaidate) in double bonded fatty acids are in nature not present in EVOO, but can be formed after heating processes. In this case the analyzed EVOO is not containing any trans-FAME compounds which could indicate that the sample is not exposed to any heating processes.

With the total FAME content mentioned in table 4, the composition of FAME components in table 5, and no presence of trans-isomers in the sample it can be assumed that the analyses sample is an genuine EVOO, that is most likely not exposed to dilution with cheaper oils, or any chemical processes.

Conclusion

The SCION 8500 GC platform equipped with a split/spitless injector, SCION FAME column and 8700 SQMS and 8400PRO sampler is a perfect solution for analyzing FAME in olive oil for qualitative and quantitative analysis. A good system precision, good linearity results and low LOQ's and LOD's are achieved for this application with the SCION Instruments GC-MS set up and MS-Work Station software.

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

The analyzed 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 Flame Ionisation Detector. (GC-FID)

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 23-02-2024)

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 14-02-2024)

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/LexUriServ/LexUriServ.do?uri=OJ:L:2011:023:0001:0014:en:PDF (accessed 18-02-2024)

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 29-03-2024)

Order Information

Ordering Information for the 8300 GC	
Part	**Part Number**
8300 GC with 8700-MS-SQ EI Select, with S/SL inlet (120V)	SCIONSQ83SEL311
8300 GC with 8700-MS-SQ EI Select, with S/SL inlet (230V)	SCIONSQ83SEL312
8400 PRO Autosampler for 8300 GC and 8500 GC	840000001
MS WorkStation Software	394195791
NIST 20 MS Library and Search Program for MSWS	4121057

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
78.5mm x 6.3mmOD x 4mmID; S/SL Recessed Gooseneck Qwool with o ring, pk/5	41312106

SCION offers other MS options such as the 8700 SQ Premium and 8900 TQ, as well as additional spectral libraries such as Designer Drugs and Wiley, please contact your local SCION sales representative to discuss your needs.

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