Analysis of Fatty Acid Methyl Esters in Edible Oils

APPLICATION NOTE - AN172

Introduction

In 2018 FDA stated that "Supportive but not conclusive scientific evidence suggests that daily consumption of about 1½ tablespoons (20 grams) of oils containing high levels of oleic acid, may reduce the risk of coronary heart disease. To achieve this possible benefit, oleic acid-containing oils should replace fats and oils higher in saturated fat and not increase the total number of calories you eat in a day."

Health benefits and high intrinsic value associated with extra virgin olive oil (EVOO) have, as the other side of a coin, a growing incidence of products adulteration along all the supply chain. This fraudulent attitude has become a major international financial and regulatory issue.

Most countries refer to the International Olive Council Standards, an international organization involved in the development of physical and chemical tests for olive oils and olive-pomace oils aimed at differentiating between each grade and at checking product authenticity.

Trade standards to be applied to olive oils and olive pomace oils that are object of international trade or food transactions are collected in COI/T.15/NC No3/ Rev.12 – June 2018

As Virgin olive oils are oils which are obtained from the fruit of the olive tree (Olea europea L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantations, centrifugation and filtration. Extra Virgin Olive Oil (EVOO) is virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.80 grams per 100 grams and the other physic-chemical and organoleptic characteristics of which correspond to those fixed for this category in this standard.

The International Olive Council has produced a list of the allowable levels for each of the fatty acids to be acceptable as extra virgin olive oil.

The determination of fatty acids for the evaluation of several purity criteria is one of the analyses included in the official regulations for the control of olive and olive pomace oils.

According to the international regulations, the analysis of fatty acids is performed by GC-FID. The determination of fatty acids require the preparation of the fatty acid methyl esters (FAME) for the subsequent analysis by gas chromatography with good precision and reproducibility.

In the present work FAMEs are analysed by DANI Master GC. The analysis has been performed on a standard mixture and then on commercially available oils (extra virgin olive oil, virgin olive oil, seeds oil). An evaluation of possible oil adulteration has been conducted on the obtained results comparing the amounts of selected compounds to the guidelines provided by the official methods.

EXPERIMENTAL

Sample

A standard mixture of 37-component FAMEs was purchased from Sigma-Aldrich. The standard mixture containing C4-C24 FAMEs in the 200-400ng/µL concentration range.

In order to perform a GC analysis of oils, non-volatile analytes have to be transesterified into thermally stable, volatile Fatty Acid Methyl Esters. At this regard, samples of extra virgin olive oil (EVO oil), virgin oil, corn oil and adulterated EVO oil were analyzed as methyl esters after suitable derivatization reaction. A 250mg of oil sample was weighed in a 20mL vial, 5mL of hexane and 0.25mL of 2N methanolic potassium hydroxide solution were added. The solution obtained was shaken vigorously for 1 minute and then left to separate until the formation of the two phases was obtained. The supernatant was then taken and injected into the GC.

System Configuration and Control

To perform the analyses, a DANI Master GC – Gas Chromatograph equipped with a PTV Injector and FID detector was used. Samples were introduced by means of Master AS – Automatic Liquid Sampler – Single Injection Mode. Master AS offered the complete automation of the sample introduction operations providing increased repeatability, sample throughput and, consequently, productivity. Instrumental conditions are summarized in *Table 1* on the right.

Master GC	
Column	DN-10 50m - 0.25mm - 0.20µm
Oven Temp. Program	60°C hold 6 minutes, 2°C/min to 240°C (2min)
PTV Injector	40°C, 999°C/min to 260°C (3min)
Split Ratio	1:80
Carrier Gas Flow (He)	1.2 mL in constant flow
FID Temp.	250°C
Injected Volume	0.8 µL

Table 1: Master GC Analytical Conditions

RESULTS AND DISCUSSION

The cyanopropyl polysiloxane stationary phase was used to improve the separation of cis-trans FAMEs and also this kind of column is able to resolve the typical coelutions of some components of 37 FAMEs MIX, for example C18:3n6 and C20:0; C20:3n6 and C22:0; C20:3n3, C20:4n6 and C22:1n9.

Figure 1 shows the separation of the 37-component FAMEs MIX using a DN-10 column ad PTV-GC-FID.

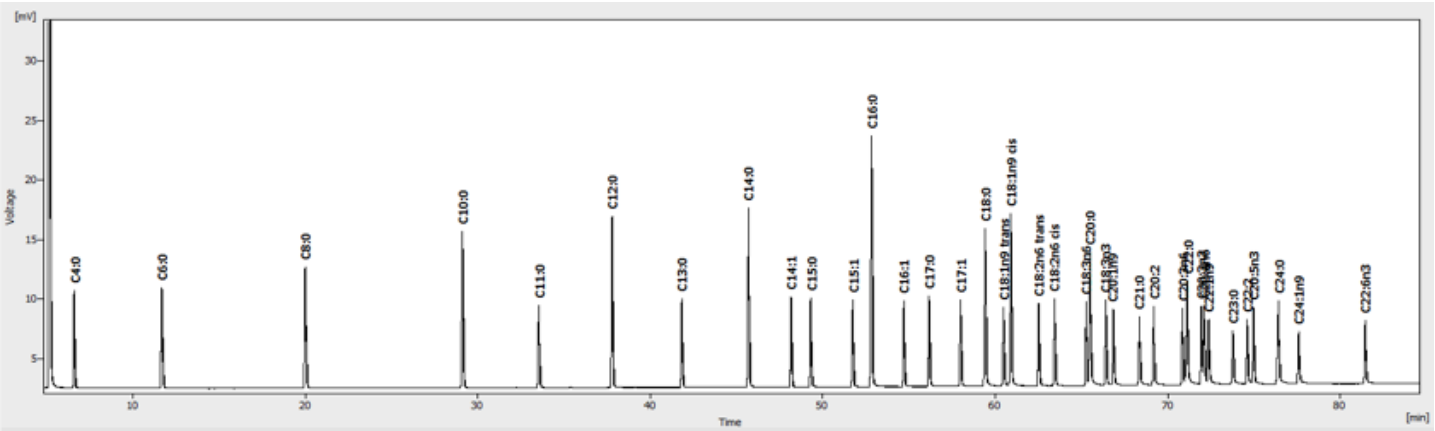

FIGURE 1: CHROMATOGRAM OF 37 FAMES MIX

37 FAMES MIX	
COMPOUND NAME	**ABBREVIATION**
Butyric Acid Methyl ester	C4:0
Caproic Acid Methyl ester	C6:0
Caprylic Acid Methyl ester	C8:0
Capric Acid Methyl ester	C10:0
Undecanoic Acid Methyl ester	C11:0
Lauric Acid Methyl ester	C12:0
Tridecanoic Acid Methyl ester	C13:0
Myristic Acid Methyl ester	C14:0
Myristoleic Acid Methyl ester	C14:1
Pentadecanoic Acid Methyl ester	C15:0
cis-10-Pentadecenoic Acid Methyl ester	C15:1
Palmitic Acid Methyl ester	C16:0
Palmitoleic Acid Methyl ester	C16:1
Heptadecanoic Acid Methyl ester	C17:0
cis-10-Heptadecanoic Acid Methyl ester	C17:1
Stearic Acid Methyl ester	C18:0
Elaidic Acid Methyl ester	C18:1n9 trans
Oleic Acid Methyl ester	C18:1n9 cis
Linolelaidic Acid Methyl ester	C18:2n6 trans
Linoleic Acid Methyl ester	C18:2n6 cis
γ-Linolenic Acid Methyl ester	C18:3n6
Arachidic Acid Methyl ester	C20:0
Linolenic Acid Methyl ester	C18:3n3
cis-11-Eicosenoic Acid Methyl ester	C20:1n9
Heneicosanoic Acid Methyl ester	C21:0
cis-11,14-Eicosadienoic Acid Methyl ester	C20:2
cis-8,11,14-Eicosatrienoic Acid Methyl ester	C20:3n6
Behenic Acid Methyl ester	C22:0
cis-11,14,17-Eicosatrienoic Acid Methyl ester	C20:3n3
Arachidonic Acid Methyl ester	C20:4n6
Erucic Acid Methyl ester	C22:1n9
Tricosanoic Acid Methyl ester	C23:0
cis-13,16-Docosadienoic Acid Methyl ester	C22:2
cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl ester	C20:5n3
Lignoceric Acid Methyl ester	C24:0
Nervonic Acid Methyl ester	C24:1n9
cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl ester	C22:6n3

Table 2: 37-FAMEs MIX

Abbreviation	Retention Time RSD%	Area RSD%
C4:0	0.16	2.6
C6:0	0.12	2.34
C8:0	0,07	2,22
C10:0	0,04	2,57
C11:0	0,04	2,15
C12:0	0,03	1,37
C13:0	0,02	2,05
C14:0	0,02	1,02
C14:1	0,02	1,61
C15:0	0,02	2,02
C15:1	0,02	1,40
C16:0	0,02	2,71
C16:1	0,02	1,88
C17:0	0,01	2,48
C17:1	0,01	2,56
C18:0	0,02	2,82
C18:1n9 trans	0,02	2,38
C18:1n9 cis	0,01	2,39
C18:2n6 trans	0,01	2,43
C18:2n6 cis	0,01	2,09
C18:3n6	0,01	2,64
C20:0	0,01	2,01
C18:3n3	0,01	2,29
C20:1n9	0,01	1,37
C21:0	0,01	2,48
C20:2	0,01	1,53
C20:3n6	0,01	2,70
C22:0	0,01	2,52
C20:3n3	0,01	2,69
C20:4n6	0,01	2,57
C22:1n9	0,01	2,95
C23:0	0,01	1,72
C22:2	0,01	1,58
C20:5n3	0,01	1,80
C24:0	0,01	1,71
C24:1n9	0,01	1,97
C22:6n3	0,01	2,34

Table 3: RSD% of Retention Time and Area Obtained with 6 consecutive injections

The repeatability was evaluated on 6 consecutive injections of FAMEs standard solution. The repeatability could be a critical aspect of this analysis considering the evaporation of the solvent and sample and the run time of a little bit more than 80 minutes. The table 3 lists the RSD% of area and retention time for each peak.

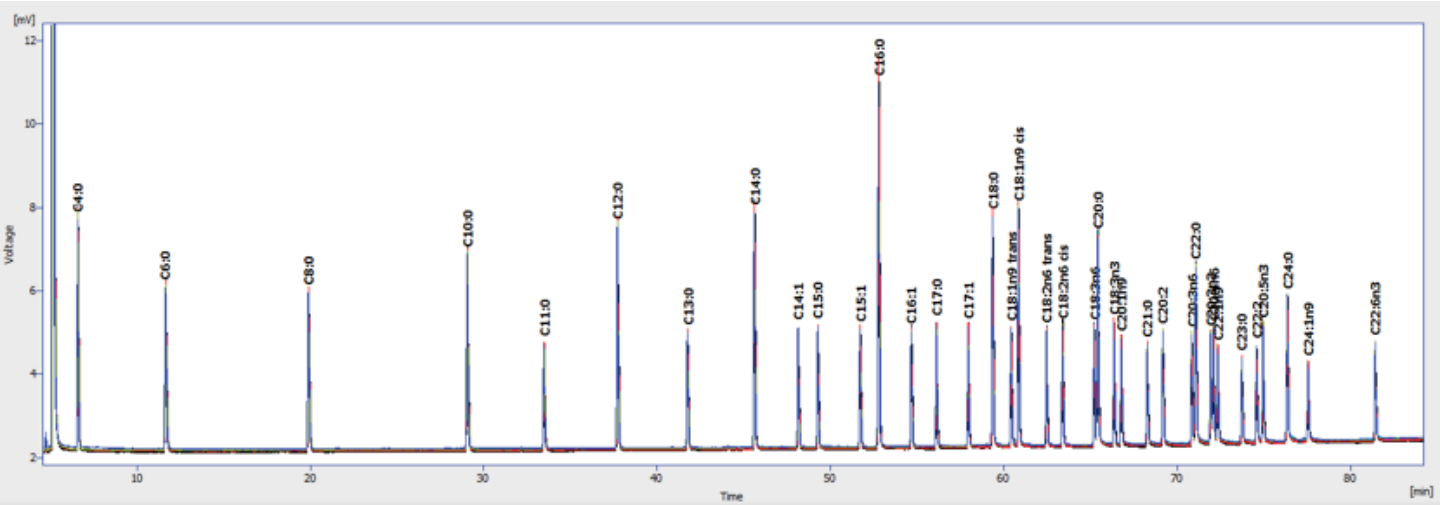

Figure2. Overlay of 6 chromatograms of 37-FAMEs MIX

Real oil samples were extracted, methylated and analyzed.
Figures 3,4,5 show the results obtained for Extra Virgin Olive Oil, Virgin Olive Oil, and Corn Oil.
Chromatograms show that both Extra Virgin Olive Oil and Virgin Olive Oil are very rich in oleic acid and poor in linoleic acid. On the other hand, the analysed seed oil shows an inverse relationship between these two compounds.
For the Extra Virgin Olive Oil, in fact, an acceptable range in area % for the oleic acid is between 55-83%, while linoleic acid is typically between 3.5-21%.
The area% of oleic acid in the analysed samples in the present work is 73.11% in Extra Virgin Olive Oil and 74.55% in the Virgin Olive Oil sample, while the linoleic acid was 7.62 % and 8.15% respectively. These values perfectly fall within the internationally accepted range.
As far as the seed oil is concerned, instead, the amount of the linoleic acid in the corn oil was 53,74%.
The area% of linoleic, oleic and palmitic acids in olive oil can provide some useful information about the possible geographic origin of the olives/oils.
Italian, Spanish and Greek olive oils are, in fact, high in oleic and low in palmitic acid, while African and Australian oils are , vice versa, rich in linoleic and lower in oleic acids.
Both the analysed olive oils here were Italian, and the chromatograms confirmed this pattern.

Another aspect that is important to take in consideration is that the majority of the fatty acids in the olive oils feature only a single double bond (the others are mostly showing single chemical bond). In other edible oils (i.e. seed oils) there is a higher number of fatty acids with double bonds. In nature, double bonds are in their cis-form. Under particular conditions, none of which happens naturally, double bonds can turn into their trans-form. This may happen, for example, upon heating or deodorizing processes.
Figure 5 shows a chromatogram of the analysis of corn oil. Since the final step of the production of corn oil involves deodorization by distillation at 232-260°C, it is possible to find trans-fatty acids. In fact, Trans-Palmitoleic Acid Methyl Ester and Trans-Linolelaidic Acid Methyl Ester are present in the chromatogram.

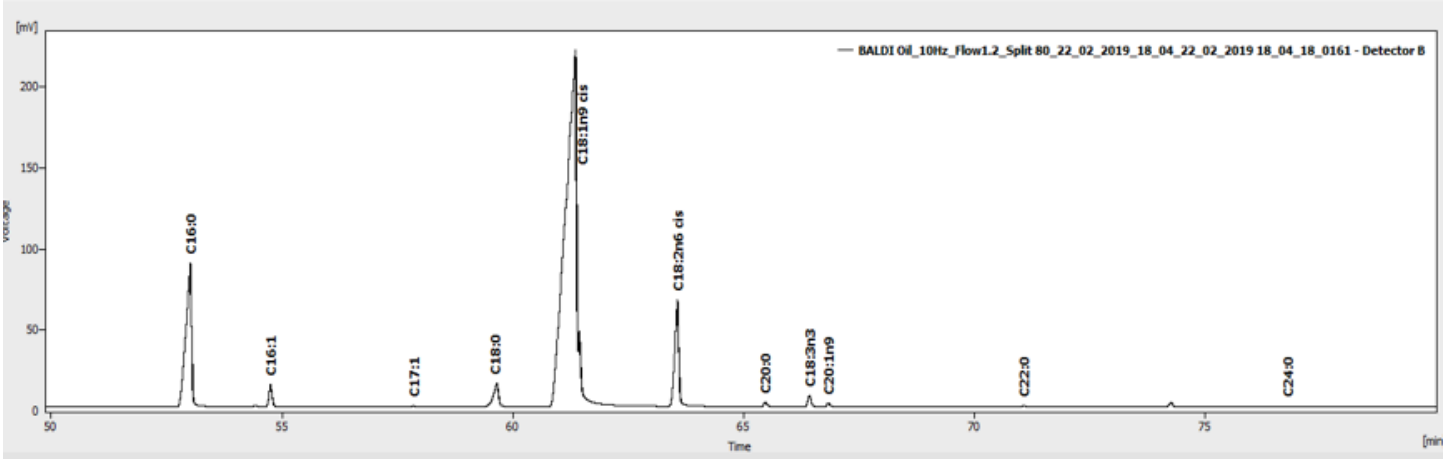

Figure 3. Chromatogram of EVOO oil

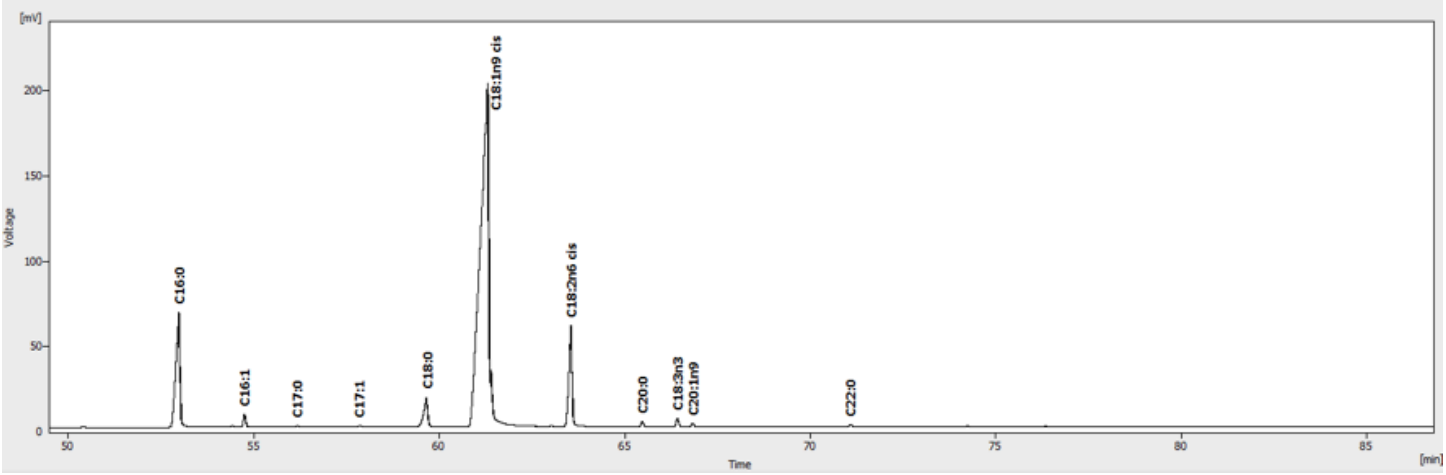

Figure 4. Chromatogram of Virgin olive oil

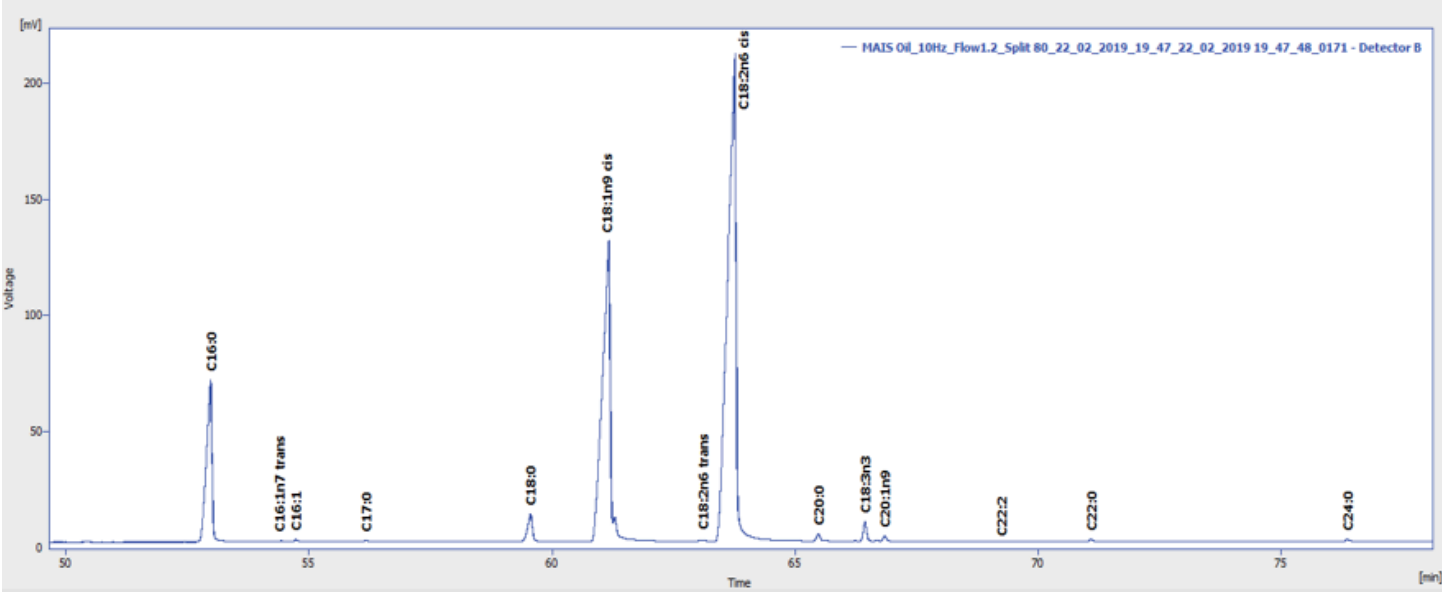

Figure 5. Chromatogram of corn oil

Adulterated Olive Oil

As previously stated, genuine Extra Virgin Olive Oil does not contain any trans-fatty acid. If found, like in the example shown in Figure 6, it is clear that the oil has been adulterated.

As a further confirmation, we can take in consideration the oleic / linoleic acid ratio, although this value can vary strongly according to many variables such as weather, temperatures etc. This ratio is not mentioned in the international regulations, however it is often used as a stability parameter. Ideally, a good Extra Virgin Olive Oil should show a oleic / linoleic ratio greater or equal to 7.

The Extra Virgin Olive Oil used as a sample here, in fact, has a 9.14 ratio. In the adulterated oil, instead, this value is only 3,93.

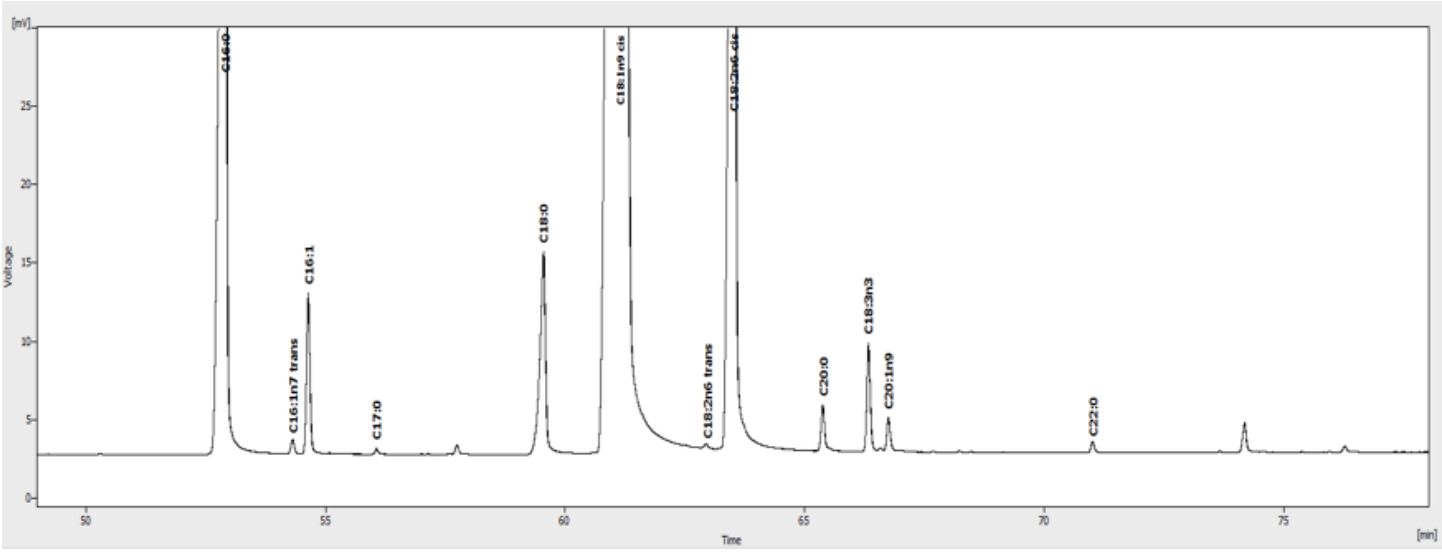

Figure 6. Chromatogram of Adulterated Olive Oil

CONCLUSIONS

The DANI Master GC Gas Chromatograph equipped a PTV injector and FID detector, along with the DANI Master AS Automatic Liquid Sampler, allowed the determination of Fatty Acid Methyl Esters with excellent accuracy and precision. The solution described in the present work has proven to be robust and reliable, as seen in the repeatability results.
Furthermore, it has been proved that with this solution it is possible to identify possible adulterations

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