Application note | 001225

Food analysis

Automated and high-throughput derivatization for FAMEs analysis in vegetable oils and animal fats

Authors

Silvia Tagliabue[1], Laura Della Bella[1], Giulia Riccardino[2], Chiara Guerrieri[2], Cavagnino Daniela[2], and Daniel Kutscher[3]

[1]Innovhub-SSOG, Milan, IT

[2]Thermo Fisher Scientific, Milan, IT

[3]Thermo Fisher Scientific, Bremen, DE

Keywords

Automated sample preparation, derivatization, TriPlus RSH SMART, fatty acids, edible oils, animal fats, fatty acid methyl-esters (FAMEs), gas chromatography, GC, flame ionization detection, FID

Goal

The aim of this study is to demonstrate the suitability of an automated sample preparation workflow for the derivatization of fatty acids in edible oils and animal fats using a Thermo Scientific™ TriPlus™ RSH SMART Robotic Sampler Handler autosampler.

Introduction

Fatty acids are carboxylic acids with medium and high chain length (C12-C28), typically found in fats and oils. They are characterized by different numbers of carbon atoms and double bonds. Therefore, they can be classified as saturated (no double bonds), monounsaturated (one double bond), polyunsaturated (two or more double bonds), and *trans* fatty acids (unsaturated fatty acids with at least one double bond in the trans configuration). Fatty acid composition is an important index of food quality as it provides key information on the nutritional value of fat-containing food. To assist consumers in maintaining healthy dietary practices, the U.S. Food and Drug Administration (FDA) implemented the Nutrition Labeling and Education Act in the 1990s, which states that total fat and saturated fat content must be listed in the nutrition label of conventional foods and dietary supplements.[1] This rule was later extended to trans fatty acids in 2003,[2] as the intake of both trans fatty acids as well as saturated fatty acids may increase the risk for coronary heart disease.

To support contract testing laboratories with analysis of fatty acids, the International Organization for Standardization (ISO) and the Association of Official Analytical Chemists (AOAC) have published several methods[3-5] describing standard procedures for the determination of total, saturated, and unsaturated fats using gas chromatography coupled to flame ionization detection (GC-FID), applicable to a variety of food commodities.

Sample preparation is one of the most critical steps in analytical workflows as it may affect the overall accuracy and reliability of the results. It usually requires multiple steps resulting in time-consuming and expensive procedures that are prone to errors and cross-contamination.

Automated sample preparation represents a viable solution to overcome these challenges, delivering more accurate data for greater confidence, increasing sample throughput, and improving analyst's safety by minimizing exposure to potentially harmful chemicals. The TriPlus RSH SMART autosampler provides advanced, built-in robotics that deliver exceptional precision and reproducibility, combined with an unprecedented flexibility to fully automate daily sample handling operations or customize more complex sample preparation workflows. The autosampler also provides an additional layer of reliability and confidence in the analytical results thanks to the automatic SMART syringe identification and usage tracking capabilities.[6]

In this study, the reliability of an automated workflow for derivatization of fatty acids from edible oils and animal fats was assessed. To improve the sample throughput, the TriPlus RSH SMART autosampler was operated in the unique Clone Mode configuration for sample injection. This configuration allows to serve two Thermo Scientific™ TRACE™ 1610 GC systems, each equipped with two Thermo Scientific™ iConnect™ split/splitless (iC-SSL) injectors and two Thermo Scientific™ iConnect™ flame ionization (iC-FID) detectors with one TriPlus RSH SMART autosampler; the autosampler operates as two independent autosamplers[7] so that the liquid injection of the derivatized samples can be performed into two GCs to double the productivity. Moreover, the full system is controlled by Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software in a seamless fashion.

Experimental

The analysis of fatty acid (FAs) is usually carried out by extracting the fat content from the food matrix using organic solvents, followed by acid or basic hydrolysis of the triglycerides, so that free fatty acids result. Those free acids are then converted into methyl-esters (FAMEs) by esterification with methanol to increase their volatility and hence make them amenable to GC analysis. As a general rule, a single trans-esterification step can be applied when the FAs content is ≤ 0.5% mass fraction, while for oils with FAs content ≥ 0.5%, the derivatization procedure involves two consecutive steps: an hydrolysis to produce free FAs and a methylation step where the free FAs are esterified with methanol in presence of an acid catalyst like boron trifluoride (BF_3) or sulfuric acid (H_2SO_4). The use of BF_3 offers the advantage of short derivatization times (approximately 2–3 minutes) compared to the use of other acids (2–3 hours),[8] although this reagent must be handled with care because of its toxicity.

The AOAC 996.01[3] and the ISO 12966-2[4] methods provide guidelines for the preparation of fatty acid methyl esters in food matrices. The derivation procedures described in these methods were automated with the TriPlus RSH SMART autosampler through a dedicated prep cycle. A prep cycle includes the sequence of steps sent to the autosampler for the execution of the workflow. Figure 1 shows the required configuration of the TriPlus RSH SMART. A detailed description of the autosampler configuration, including a complete list of suggested consumables, is reported in Appendix 1. The developed prep cycle can be applied also for the analysis of olive oil according to the Annex X of the EU Regulation 2015/1833.[9] The analyst is required to weigh each sample into a 10 mL headspace vial and place it into the autosampler together with an empty 2 mL vial where the fully derivatized sample is transferred prior to GC injection. The automatic tool change (ATC) station available on the autosampler allows for the automatic swap between a dedicated 10 mL syringe for reagents dispensing and a 10 µL syringe for sample injection into the analytical system. Two solvent stations, each with up to three 100 mL bottles, provide a reservoir for the addition of the required reagents.

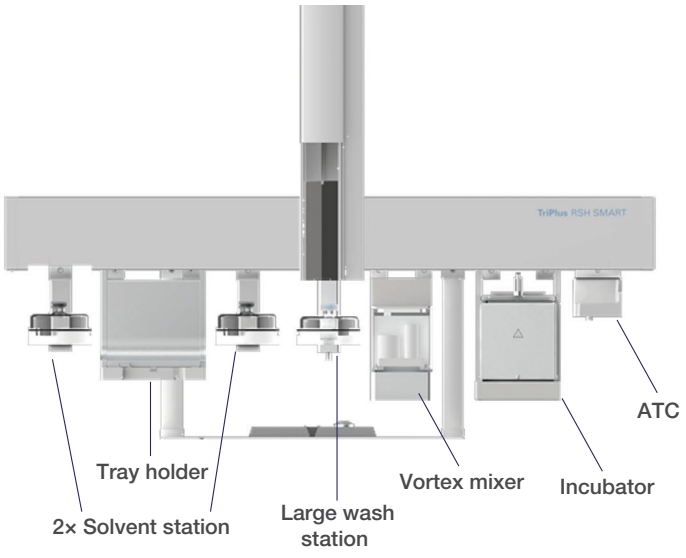


Figure 1. TriPlus RSH SMART autosampler configuration for automated esterification of fatty acids from edible oils and animal fats

The vortex mixer allows for efficient mixing, extraction, and derivatization of the fatty acids, while the incubator ensures uniform temperature when the incubation step is required. The incubator can host up to six samples to shorten the sample preparation time. Moreover, multiple solvents can be used for syringe washing, thus minimizing the risk of carryover and cross-contamination.

The use of the automated sample preparation approach reduces user exposure to harmful and toxic reagents such as BF_3 while permitting faster preparation of samples for increased throughput. The developed prep cycle allows for the preparation of a maximum number of four batches (total number of samples = 24) in one sequence with subsequent injection of the derivatized samples. Up to n = 48 samples can be prepared in 8 working hours following the ISO 12966-2 "rapid" method providing a single trans-esterification step. When an acid-catalyzed trans-methylation is needed according to the AOAC 996.01 method, n = 24 samples can be prepared in approximately 4.30 working hours. A schematic of the workflow showing the UE Reg. 2015/1833 for olive oil, the ISO 12966-2 "rapid" method for other oils (e.g., seed oils), and the AOAC 996.01 automated procedures is reported in Figure 2.

Chromatographic separation of the fatty acids was achieved on a Thermo Scientific™ TRACE™ TR-FAME column (10 m × 0.10 mm × 0.20 µm, P/N 260M096P). The high operating temperature (up to 260 °C) for this high polarity phase (70% cyanopropyl polysilphenylene-siloxane) ensured extremely low bleed, making this column ideal for FAME analysis. Hydrogen was used as carrier gas as recommended in the ISO 12966-4 method,[5] providing efficient chromatographic separation combined with short GC run times. The optional Thermo Scientific™ HeSaver-H_2Safer™ for iConnect SSL can be used to limit the total flow rate of the carrier gas and remove any safety risk associated with the use of hydrogen as a carrier gas, including the need to install the H_2 sensor into the GC oven.[10]

Additional GC-FID and autosampler parameters are detailed in Appendix 2.

Figure 2. Workflow showing the automated sample preparation according to the EU Regulation 2015/1833 (Annex X), the ISO 12966-2 "rapid" method, and the AOAC 996.01 procedures for fatty acid esterification

Data acquisition, processing, and reporting

The instrument control is fully integrated in the Thermo Scientific Chromeleon 7.3 CDS ensuring a streamlined automated workflow from sample preparation to sequence set up, sample injection, data acquisition and reporting, with minimal user intervention. Moreover, with the ever-evolving compliance requirements for data integrity and data security, Chromeleon CDS provides a secure platform for analytical laboratories to comply with modern regulatory guidelines including FDA 21 CFR Part 11 and European Commission (EU) Annex 11.

Standard preparation

Food industry FAME mix (37 components, 30 mg/mL total in methylene chloride, P/N 35077) was purchased from Restek and diluted 1:10 with hexane (Fisher Scientific, P/N M1007952500) before injection (injection volume = 0.5 µL) into the chromatographic system and used to assess peak retention times (RT_s), asymmetry (As), and chromatographic resolution (R_s).

Sample preparation

Several edible oil samples and one animal fat sample were provided by Innovhub-SSOG (Milan, Italy) and used to assess the consistency and reliability of the derivatization workflow. Inter- and intra-day extraction repeatability, precision, and quantitative performance were assessed to test the compliance of the developed workflow for everyday use in industrial and contract testing laboratories. Samples (0.01 g) were weighed into 10 mL HS vials and set into the autosampler. Sample derivatization was automatically performed according to the schematics reported in Figure 2.

Results and discussion

Chromatography

One of the main challenges associated with FAME analysis lies in the ability to achieve adequate chromatographic resolution as most of these analytes show similar chemical properties. The analysis of FAMEs is generally carried out using highly polar stationary phase (e.g., cyanopropyl or biscyanopropyl) and long capillary columns (usually 100 m) to provide sufficient chromatographic resolution of these analytes. However, the main disadvantage with such columns is that the analysis times are very long, thus representing a bottleneck to high throughput. The TRACE TR-FAME capillary columns have been designed to provide efficient separation with shorter lengths, reducing the run times and improving sample throughput. The TRACE TR-FAME capillary column used in this study had a length of only 10 m but ensured the chromatographic resolution of the target analytes in < 10 minutes, including the critical pairs C21:0, C20:2 and C20:3, C22:1, for which the calculated resolution (R_s) was ≥ 1.0 (USP formula applied).[9] Moreover, Gaussian peak shape was achieved for the analytes of interest with average calculated asymmetry factor (A_s) of 1.0. An example of chromatographic separation obtained for a FAME standard mix, diluted 1:10 with hexane, is reported in Figure 3.

Intra- and inter-day extraction repeatability

Intra-day extraction repeatability was assessed by preparing n = 4 batches of coconut oil samples (total number of samples = 24) applying the ISO 12966-2 “rapid method”. Inter-day extraction repeatability was assessed by derivatizing one batch of coconut samples (n = 6) per day over six working days to monitor the extraction performance. The consistency of the results was evaluated by monitoring both the absolute and the relative peak area repeatability across the analyzed batches of samples. The TriPlus RSH SMART autosampler ensured reliable analyte extraction and derivatization with overall intra- and inter-day absolute peak area RSD < 9% and relative peak area RSD < 3.3% over the testing period. Moreover, the engineered design of the TRACE TR-FAME column ensured stable chromatographic performance with average RT deviation and A_s of 0.005 min and 1.0, respectively, across the testing period, as reported in Figure 4.

Analysis of vegetable oils and animal fats

Several high-quality edible oils and one animal fat sample were derivatized by using the automated prep cycle, and results obtained were compared with the quality ranges established by the UNI regulation,[11] the FAO-WHO Codex Alimentarius,[12] and the UE Regulation 2019/1604[13] (for olive oil composition). A standard mix was injected at the beginning and end of the sequence to monitor instrument performance. The suitability of the developed prep cycle for the analysis of edible oils and animal fats was confirmed by the agreement between the obtained results and the quality ranges established in the aforementioned documents, confirming the high quality of the analyzed products. Examples of chromatograms showing the fatty acid methyl esters composition of some analyzed samples are reported in Figure 5. Appendix 3 shows the chromatograms and the quantitative analysis for the rest of the analyzed edible oils.

1=C4:0 Methyl butyrate
2=C6:0 Methyl caproate
3=C8:0 Methyl octanoate
4=C10:0 Methyl decanoate
5=C11:0 Methyl undecanoate
6=C12:0 Methyl dodecanoate
7=C13:0 Methyl tridecanoate
8=C14:0 Methyl myristate
9=C14:1 (*cis*-9) Methyl myristoleate
10=C15:0 Methyl pentadecanoate
11=C15:1 (*cis*-10) Methyl pentadecenoate
12=C16:0 Methyl palmitate
13=C16:1 (*cis*-9) Methyl palmitoleate
14=C17:0 Methyl heptadecanoate
15=C17:1 (*cis*-10) Methyl heptadecenoate
16=C18:0 Methyl stearate
17=C18:1 (*trans*-9) Methyl Octadecenoate
18=C18:1 (*cis*-9) Methyl oleate,
19=C18:2 (all-*trans*-9,12) Methyl linolelaidate
20=C18:2 (all-*cis*-9,12) Methyl linoleate
21=C18:3 (all-*cis*-6,9,12) Methyl linolenate
22=C18:3 (all-*cis*-9,12,15) Methyl linolenate
23=C20:0 Methyl arachidate
24=C20:1 (*cis*-11) Methyl eicosenoate
25=C20:2 (all-*cis*-11,14) Methyl eicosadienoate
26=C21:0 Methyl heneicosanoate
27=C20:3 (all-*cis*-8,11,14) Methyl eicosatrienoate
28=C20:4 (all-*cis*-5, 8, 11,14) Methyl arachidonate
29=C20:3 (all-*cis*-11,14,17) Methyl eicosatrienoate
30=C22:0 Methyl behenate
31=C22:1 (*cis*-13) Methyl erucate
32=C20:5 (all-*cis*-5,8,11,14,17) Methyl eicosapentaenoate
33=C22:2 (all-*cis*-13,16) Methyl docosadienoate
34=C23:0 Methyl tricosanoate
35=C24:0 Methyl lignocerate
36=C24:1 (*cis*-15) Methyl nervonate
37=C22:6 (all-*cis*-4,7,10,13,16,19) Methyl docosahexaenoate

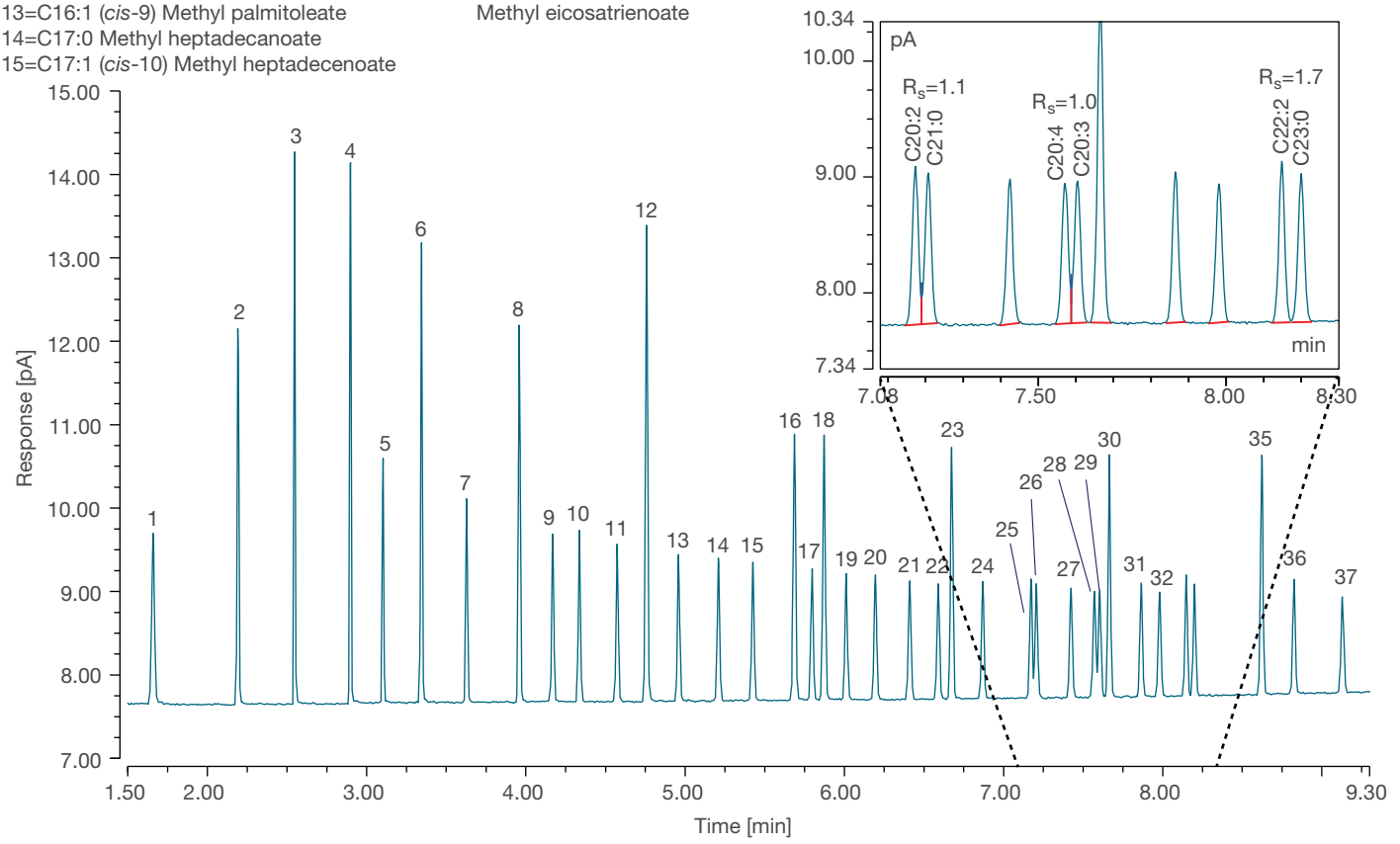
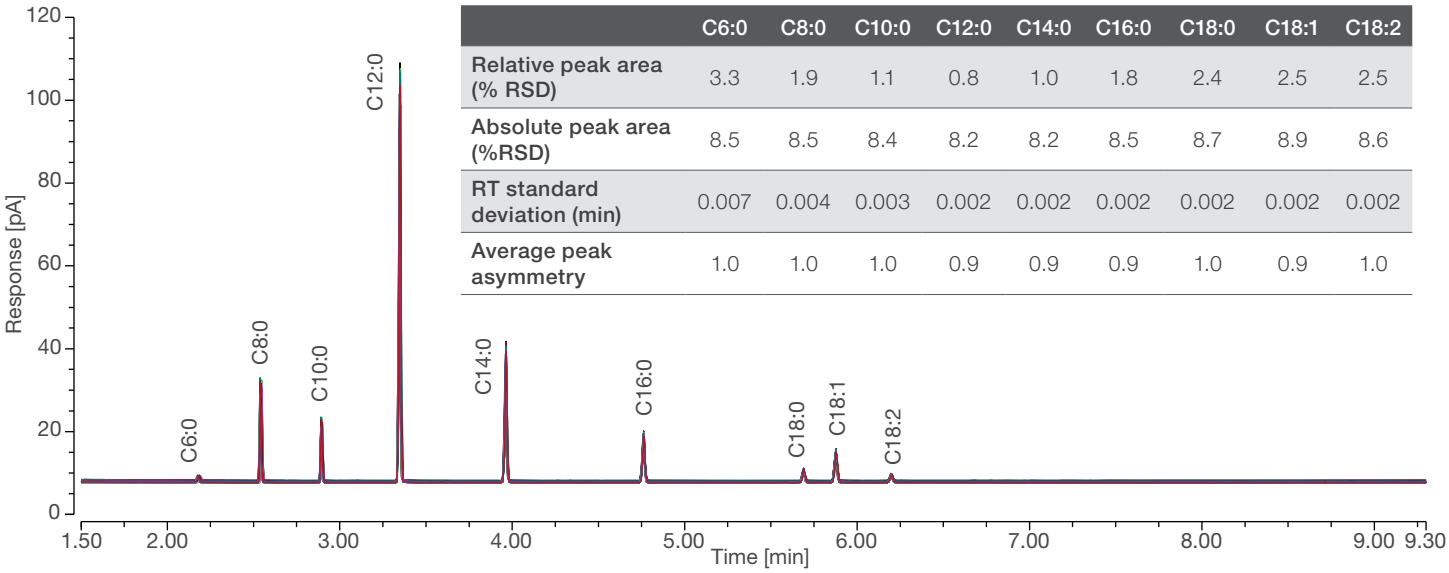
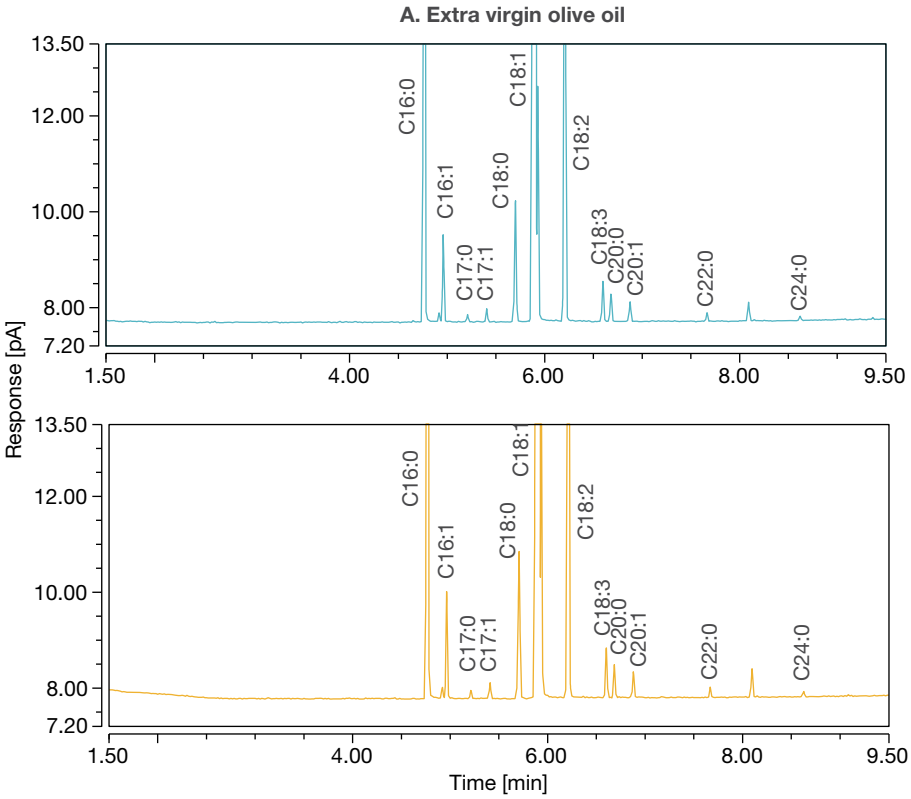


Figure 3. Chromatographic separation of investigated FAMEs in a solvent standard diluted 1:10 with hexane



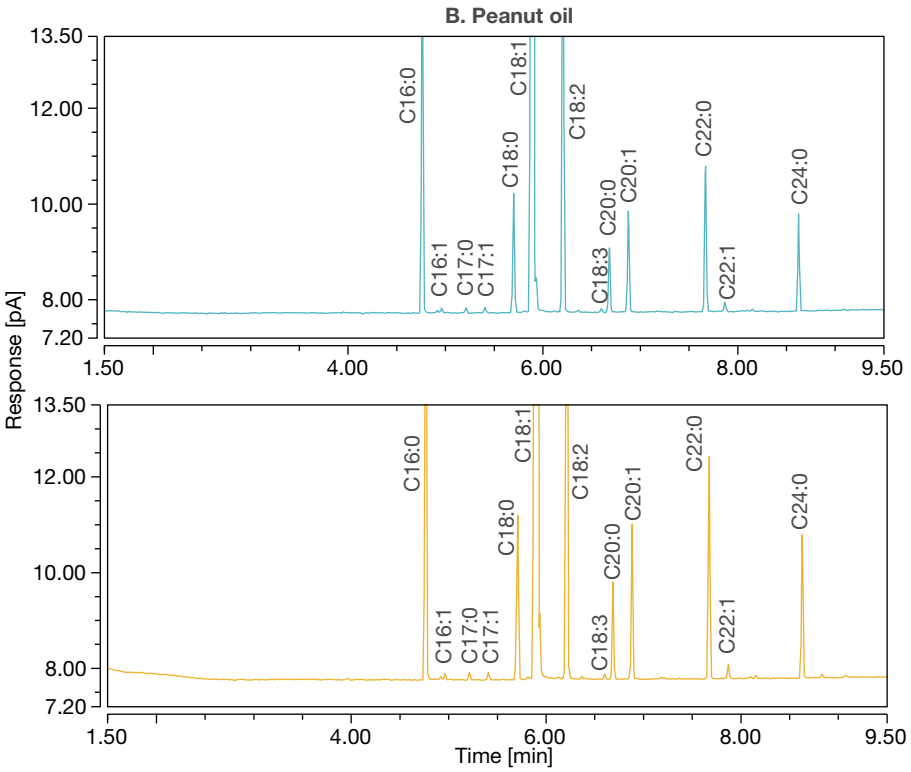
	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
Relative peak area (% RSD)	3.3	1.9	1.1	0.8	1.0	1.8	2.4	2.5	2.5
Absolute peak area (%RSD)	8.5	8.5	8.4	8.2	8.2	8.5	8.7	8.9	8.6
RT standard deviation (min)	0.007	0.004	0.003	0.002	0.002	0.002	0.002	0.002	0.002
Average peak asymmetry	1.0	1.0	1.0	0.9	0.9	0.9	1.0	0.9	1.0

Figure 4. Overlaid chromatograms showing intra- and inter-day extraction repeatability over six working days. Reliable analyte derivatization and extraction was obtained with overall absolute peak area RSD < 9% and relative peak area RSD < 3.3% over the testing period.



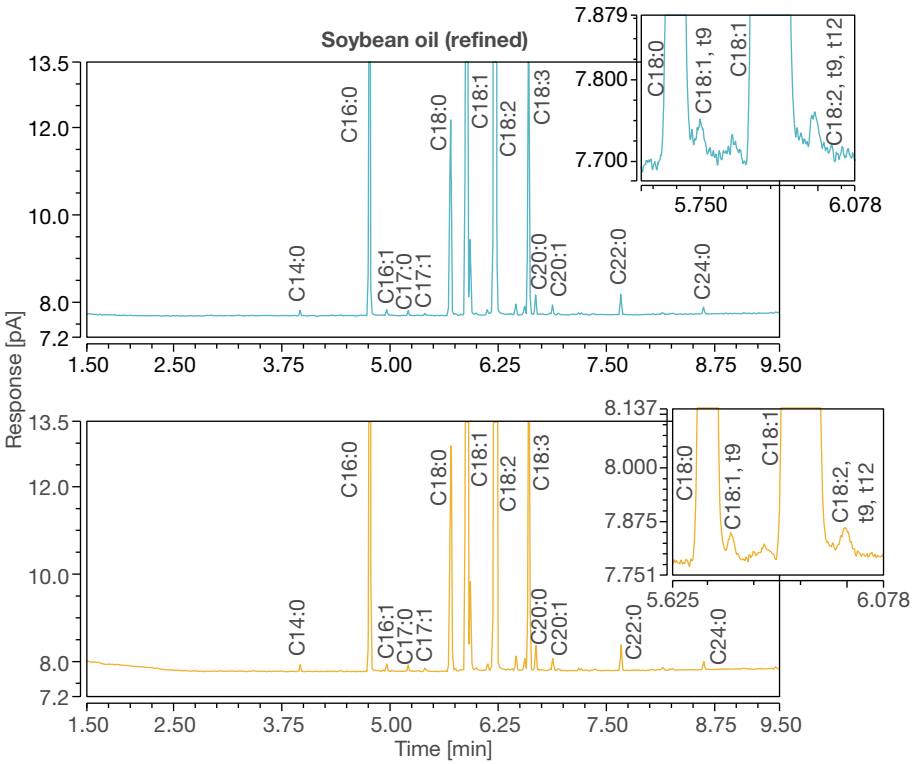
Fatty acid	Relative area %		Ref. UE 2019/1604
	UE Reg. 2015/1833 method	AOAC 996.01	
C14:0	n.d.	n.d.	max. 0.03
C16:0	14.0	14.2	7.5–20.0
C16:1	1.2	1.2	0.3–3.5
C17:0	0.1	0.1	max. 0.4
C17:1	0.2	0.2	max. 0.6
C18:0	2.0	2.0	0.5–5.0
C18:1	68.0	67.9	55.0–83.0
C18:2	9.6	9.6	2.5–21.0
C18:3	0.6	0.6	max. 1.0
C20:0	0.4	0.4	max. 0.6
C20:1	0.3	0.3	max. 0.5
C22:0	0.1	0.1	max. 0.2
C24:0	0.1	0.1	max. 0.2

Figure 5A. Chromatogram showing the fatty acid composition of extra virgin olive oil. Derivatization method: light blue chromatogram = AOAC 996.01 method, yellow chromatogram = UE Reg. 2015/1833 method.



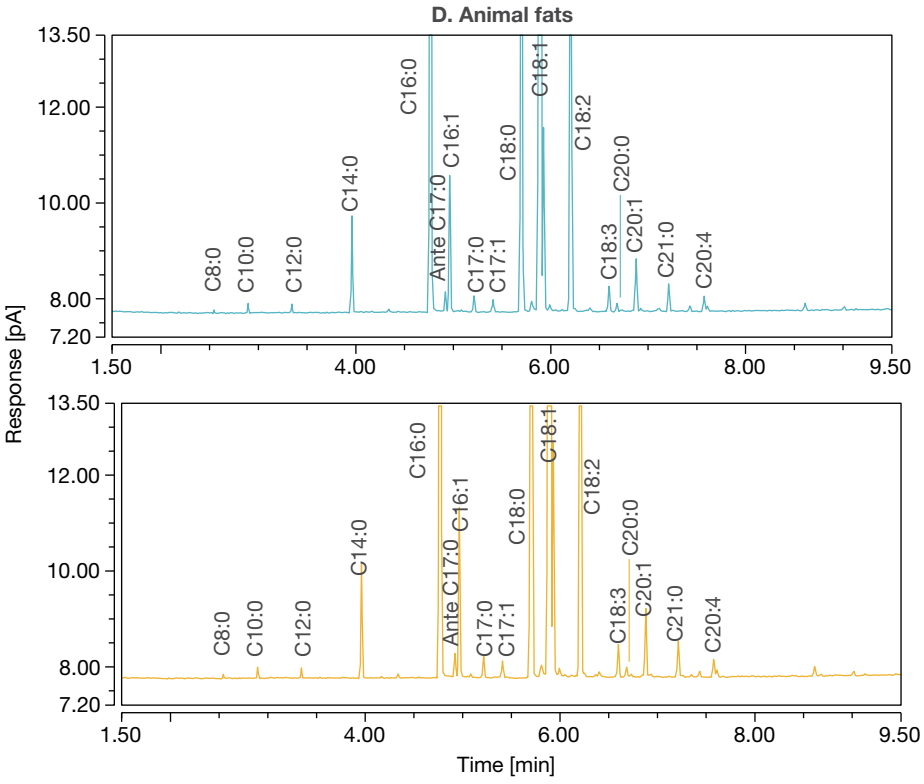
Fatty acid	Relative area %		Ref. UNI 22062
	ISO 12966-2 rapid method	AOAC 996.01	
C12:0	n.d.	n.d.	max. 0.03
C14:0	n.d.	14.2	7.5–20.0
C16:0	6.6	1.2	0.3–3.5
C16:1	0.1	0.1	max. 0.4
C17:0	0.1	0.2	max. 0.6
C17:1	0.1	2.0	0.5–5.0
C18:0	2.3	67.9	55.0–83.0
C18:1	73.4	9.6	2.5–21.0
C18:2	8.6	0.6	max. 1.0
C18:3	0.1	0.4	max. 0.6
C20:0	1.1	0.3	max. 0.5
C20:1	1.9	0.1	max. 0.2
C22:0	2.7	0.1	max. 0.2
C22:1	0.2	0.2	max. 0.3
C24:0	1.7	1.7	0.5–2.5

Figure 5B. Chromatogram showing the fatty acid composition of peanut oil. Derivatization method: light blue chromatogram = AOAC 996.01 method, yellow chromatogram = ISO 12966-2 “rapid” method.



Fatty acid	Relative area %		Ref. UNI 22067
	ISO 12966-2 rapid method	AOAC 996.01	
C12:0	0.0	0.0	max. 0.1
C14:0	0.1	0.1	max. 0.2
C16:0	10.5	10.6	8.0-13.5
C16:1	0.1	0.1	max. 0.2
C17:0	0.1	0.1	max. 0.1
C17:1	0.1	0.1	max. 0.1
C18:0	4.2	4.2	2.0-5.4
C18:1	25.8	25.8	17.0-30.0
C18:2	50.3	50.3	48.0-59.0
C18:3	6.0	6.0	4.5-11.0
C20:0	0.3	0.4	max. 0.6
C20:1	0.2	0.2	max. 0.5
C20:2	0.0	0.0	max. 0.1
C22:0	0.4	0.4	max. 0.7
C22:1	0.0	0.0	max 0.1
C24:0	0.1	0.1	max 0.5

Figure 5C. Chromatograms showing the fatty acid composition of soybean oil (refined). Derivatization method: light blue chromatogram = AOAC 996.01 method, yellow chromatogram = ISO 12966-2 "rapid" method.



Fatty acid	Relative area %		Ref. Codex Alimentarius
	ISO 12966-2 rapid method	AOAC 996.01	
C6:0	0.2	0.2	< 0.5 total
C8:0			
C10:0			
C12:0			
C14:0	1.3	1.4	1.0-2.5
C14:1	n.d.	n.d.	< 2.0
C15:0	0.1	n.d.	< 2.0
C16:0	24.5	24.9	20.0-30.0
C16:1	2.3	2.4	2.0-4.0
C16:2	n.d.	n.d.	< 0.1
Ante C17:0	0.3	0.3	< 0.1
C17:0	0.3	0.3	< 1.0
C17:1	0.2	0.2	< 1.0
C18:0	12.6	12.1	8.0-22.0
C18:1	41.9	41.5	35.0-55.0
C18:2	9.9	9.9	4.0-12.0
C18:3	0.6	0.7	< 1.5
C20:0	0.2	0.2	< 1.0
C20:1	1.0	1.0	< 1.5
C20:2	n.d.	n.d.	< 1.0
C20:4	0.3	0.3	< 1.0
C22:0	n.d	n.d	< 0.1
C22:1	n.d	n.d	< 0.5

Figure 5D. Chromatogram showing the fatty acid composition of animal fats. Derivatization method: light blue chromatogram = AOAC 996.01 method, yellow chromatogram = ISO 12966-2 "rapid" method.

Benefits of the automated derivatization workflow

The automated derivatization workflow allows for fully unattended operations, giving back to the user time for more valuable activities like data interpretation. The possibility to run samples overnight or over the weekend allows for an overall increase of sample throughput.

The autosampler configuration used for this work allows to prepare up to 48 samples in 8 working hours by applying the ISO 12966-2 “rapid” method, offering the possibility of preparing an additional 24 samples overnight, followed by liquid injection of both the sample batches prepared during the day and the ones prepared during the overnight hours.

The possibility to serve two GCs with a single autosampler, allows injection in four channels, significantly reducing the overall analysis time.

Conclusions

The results of these experiments demonstrate that the automated sample preparation capability of the TriPlus RSH SMART autosampler provides an ideal solution for laboratories looking to improve productivity and deliver confident results.

- The flexibility of the developed prep cycle allows for automated preparation of fatty acids methyl esters according to the ISO 12966-2, the UE Reg. 2015/1833, and the AOAC 996.01 methods.
- Up to n = 48 samples can be prepared in 8 working hours (ISO 12966-2 “rapid” method) while n = 24 samples can be prepared in approximately 4.30 working hours (AOAC 996.01 method) with subsequent injection of the derivatized samples, reducing the risk of errors, cross-contaminations and improving analysts’ safety by limiting exposure to toxic chemicals.
- The engineered TR-FAME column combined with the use of hydrogen as carrier gas ensures chromatographic separation of the target analytes in < 10 minutes with calculated $R_s \geq 1.0$, including the critical pairs C21:0, C20:2 and C20:3, C22:1.
- Reproducible preparation of extracts was demonstrated over a week period with overall absolute and relative peak area %RSD < 9 and 3.3, respectively, average RT deviations and A_s of 0.005 and 1.0, respectively, across the derivatized samples.
- The suitability of the automated procedure was confirmed by derivatizing several high-quality edible oils and one animal fat sample by using the ISO 12966-2 “rapid” method, the UE Reg. 2015/1833 method, and the AOAC 996.01 method. The obtained results were in agreement with the quality ranges established by the UNI regulation, the FAO-WHO Codex Alimentarius, and the UE Reg. 2019/1604, thus confirming the high quality of the products.

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Appendix 1. TriPlus RSH autosampler configuration for automated preparation of FAMEs and list of suggested consumables

Part number	TriPlus RSH SMART configuration
1R77010-2003	TriPlus RSH SMART Advanced for Liquid Injections, regular rail*
1R77010-1019	Automatic Tool Change Station (ATC) station. Stores and changes automatically up to three syringe tools. Up to two ATC stations can be configured on each TriPlus RSH SMART Advanced autosampler
1R77010-1031	Solvent station 3 × 100 mL solvent bottles. Bottles with seal and caps are included.
1R77010-1030	Large wash station for 2 × 100 mL solvent bottles and one waste position. Bottles with seal and caps are included.
1R77010-1033	Vortexer module for the intensive mixing of one vial at a time (suitable for 2 mL, 10 mL, or 20 mL vial)
1R77010-1032	Incubator/Agitator module Incubates and agitates up to 6 × 10 mL or 20 mL vials
1R77010-1068	10 mL Vial adapters for the incubation oven This kit contains 6 adapters to place 10 mL vials inside the incubation oven.
1R77010-1021	Tray holder for VT15, VT54, VT70, and R60 vial trays Tray holder able to house up to 3 different sample trays among VT15, VT54, VT70. Alternatively, the tray holder can accommodate one aluminum sample tray R60. Sample trays are not included.
1R77010-1023	VT54 Vial tray for 2 mL vials (sample tray for 54 vials of 2 mL)
1R77010-1022	VT15 Vial Tray for 10/20 mL vials. Sample tray for 15 vials of 10–20 mL. Vials are not included
1R77010-1007	Liquid syringe tool for syringes of 0.5, 1.0, 5, 10, 25, 50 or 100 µL with a 57 mm needle length
1R77010-1011	Liquid syringe tool for 10 mL syringe volume for a 10,000 µL syringe with a 57 mm needle length
1R77010-1182	Three-seat plastic holder for TriPlus RSH autosampler Optional replacement of the standard metal holder for large wash station or solvent station. Made in high density poly-ethylene (HDPE), it is suggested for workflows using aggressive media like BF_3, acids, and bases

*Or equivalent TriPlus RSH base liquid configuration, in case of an existing instrument

Suggested consumables	Part number
10 µL Fixed needle SMART syringe 57 mm needle length, 26S gauge, cone needle type	365D0291-SM
10,000 µL Fixed needle gas-tight SMART syringe 57 mm needle length, 19S Gauge	365N2721-SM
Thermo Scientific™ SureSTART™ 10 mL glass screw top headspace vials, level 2 high-throughput applications	6ASV10-1
Thermo Scientific™ SureSTART™ 18 mm precision screw caps, level 3 high performance applications	6PMSC18-ST2
Thermo Scientific™ SureSTART™ 2 mL glass screw top vials, level 2 high-throughput applications	6ASV9-2P
Thermo Scientific™ SureSTART™ 9 mm screw pre-slit caps, level 3 high performance applications	6PSC9ST101X
TRACE TR-FAME GC column, 10 m × 0.10 mm × 0.20 µm	260M096P
Thermo Scientific™ LinerGOLD™ precision split/splitless liner with quartz wool	453A1255-UI

For more details about orders and quotations, please contact your Thermo Fisher sales representatives.

Appendix 2. TRACE 1610 GC parameters

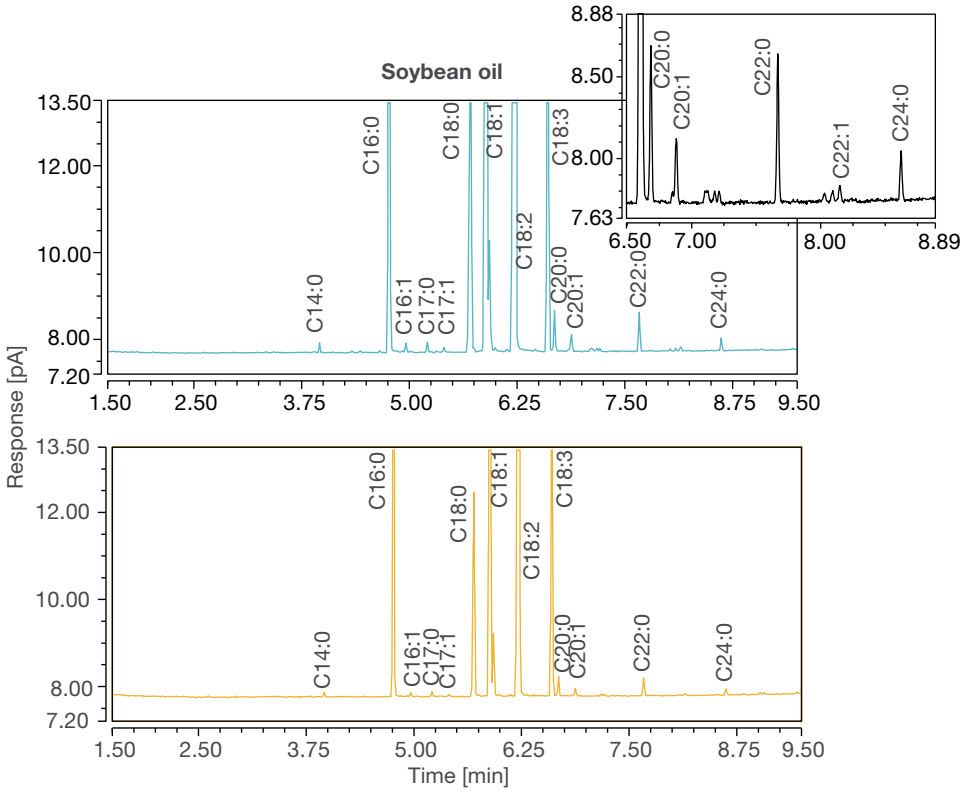
TRACE 1610 GC parameters

iC-SSL parameters	
Injection temperature (°C)	230
Liner	Precision split/splitless liner with quartz wool (P/N 453A1255-UI)
Inlet module and mode	SSL, split
Split flow (mL/min)	60
Split ratio	300:1
Septum purge flow (mL/min)	5, constant
Carrier gas, flow (mL/min)	H_2, 0.20

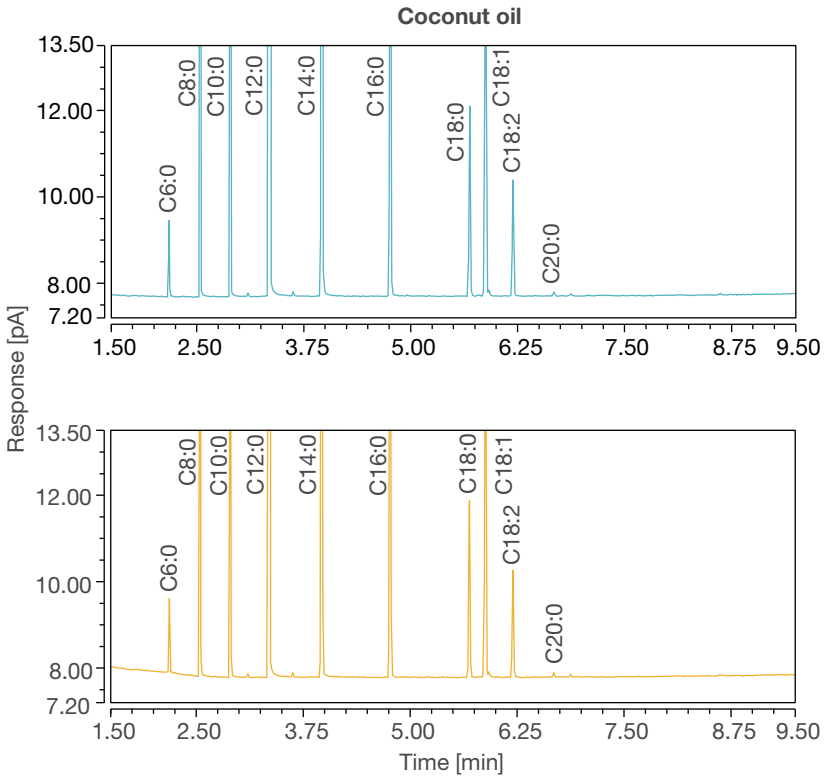
Oven temperature program	
Temperature (°C)	40
Hold time (min)	1
Rate (°C/min):	80
Temperature 2 (°C)	150
Rate (°C/min)	11
Temperature 3 (°C)	240
Hold time (min)	1
GC run time (min)	11.557

FID	
Temperature (°C)	280
Air flow (mL/min)	350
H_2 flow (mL/min)	35
N_2 flow (mL/min)	40
Aquisition rate (Hz)	25
Analytical column	
TRACE TR-FAME	10 m, 0.10 mm, 0.20 µm (P/N 260M096P)

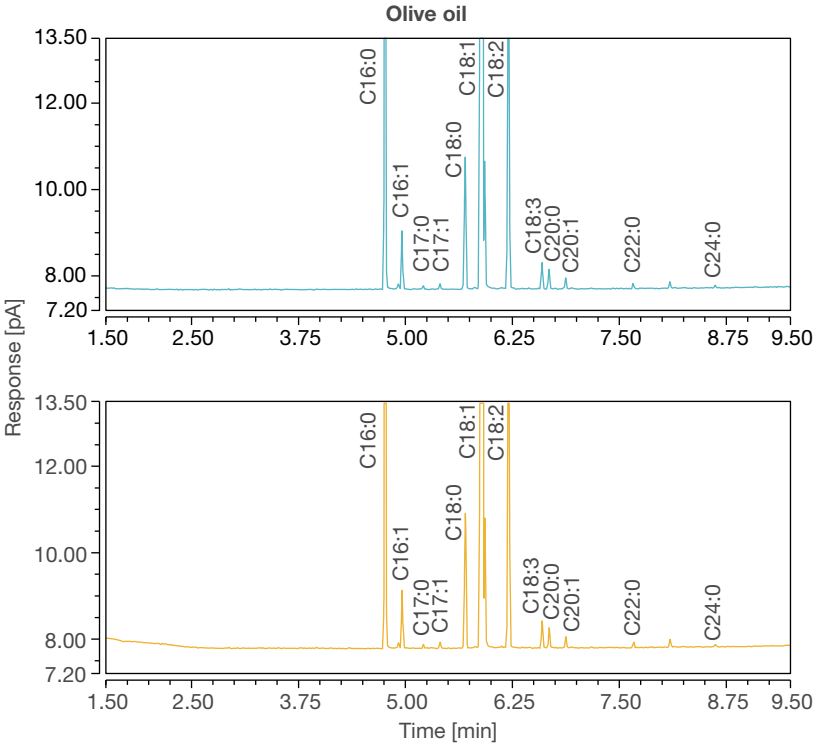
Appendix 3. FAMEs content in different seed oils



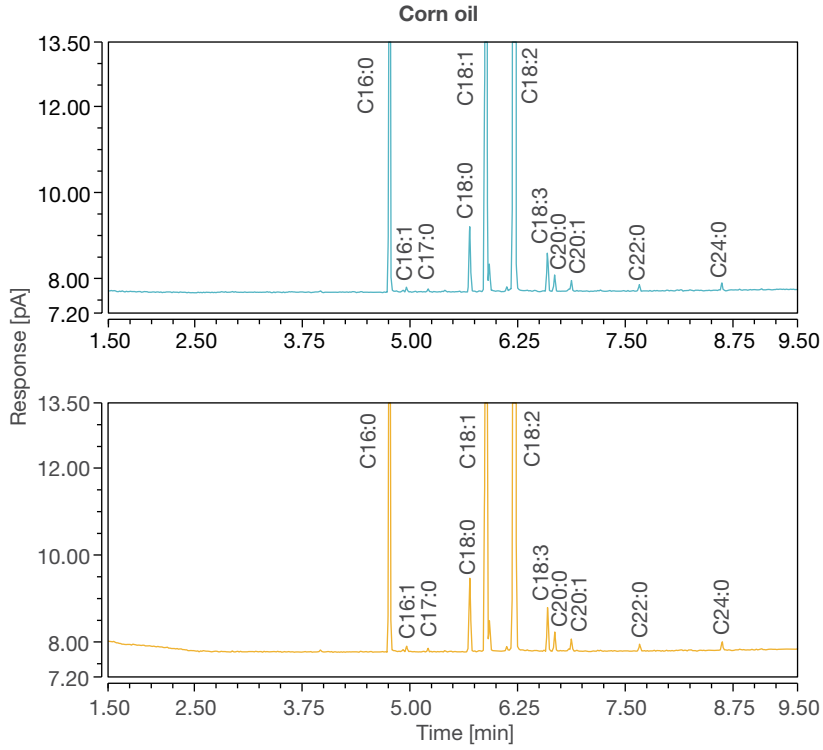
Fatty acid	Relative area %		Ref. UNI 22067
	ISO 12966-2 rapid method	AOAC 996.01	
C12:0	n.d	n.d	max. 0.1
C14:0	0.1	0.1	max. 0.2
C16:0	10.3	10.1	8.0–13.5
C16:1	0.1	0.1	max. 0.2
C17:0	0.1	0.1	max. 0.1
C17:1	n.d.	0.1	max. 0.1
C18:0	4.9	4.8	2.0–5.4
C18:1	23.2	22.9	17.0–30.0
C18:2	51.8	51.9	48.0–59.0
C18:3	7.0	7.1	4.5–11.0
C20:0	0.4	0.4	max. 0.6
C20:1	0.2	0.2	max. 0.5
C20:2	n.d.	n.d.	max. 0.1
C22:0	0.4	0.4	max. 0.7
C22:1	n.d.	n.d.	max. 0.1
C24:0	0.2	0.1	max. 0.5



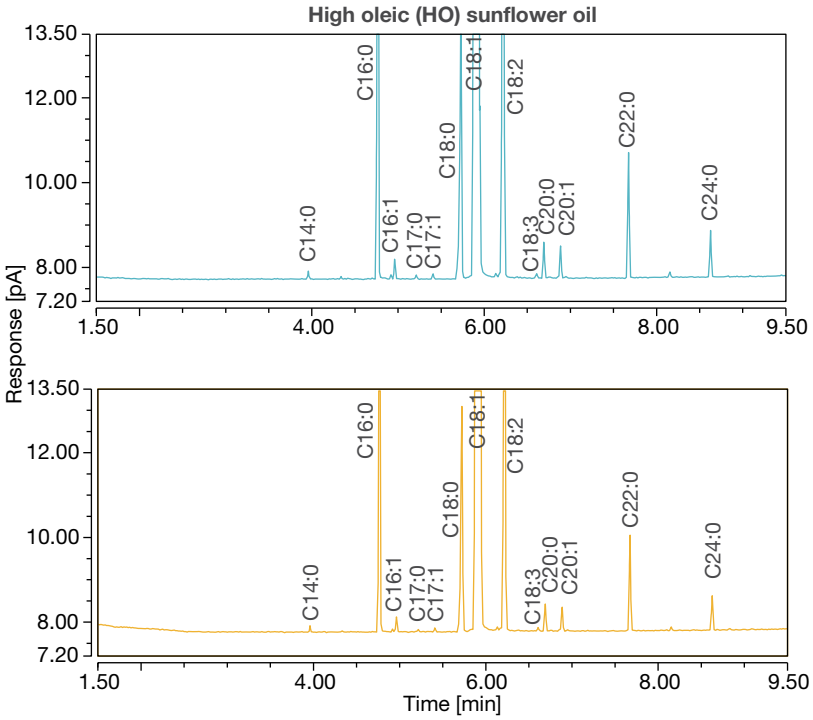
Fatty acid	Relative area %		Ref. Codex Alimentarius
	ISO 12966-2 rapid method	AOAC 996.01	
C6:0	0.7	0.6	max. 0.7
C8:0	8.8	8.7	4.6–10.0
C10:0	5.9	5.9	5.0–8.0
C12:0	48	48.4	45.1–53.2
C14:0	18.8	19.1	16.8–21.0
C16:0	7.7	7.6	7.5–10.2
C16:1	n.d.	n.d.	n.d.
C17:0	n.d.	n.d.	n.d.
C17:1	n.d.	n.d.	n.d.
C18:0	2.4	2.3	2.0–4.0
C18:1	5.8	5.6	5.0–10.0
C18:2	1.5	1.5	1.0–2.5
C18:3	n.d.	n.d.	max. 0.2
C20:0	n.d.	n.d.	max. 0.2
C20:1	n.d.	n.d.	max. 0.2
C20:2	n.d.	n.d.	n.d.
C22:0	n.d.	n.d.	n.d.
C22:1	n.d.	n.d.	n.d.
C22:2	n.d.	n.d.	n.d.
C24:0	n.d.	n.d.	n.d.
C24:1	n.d.	n.d.	n.d.



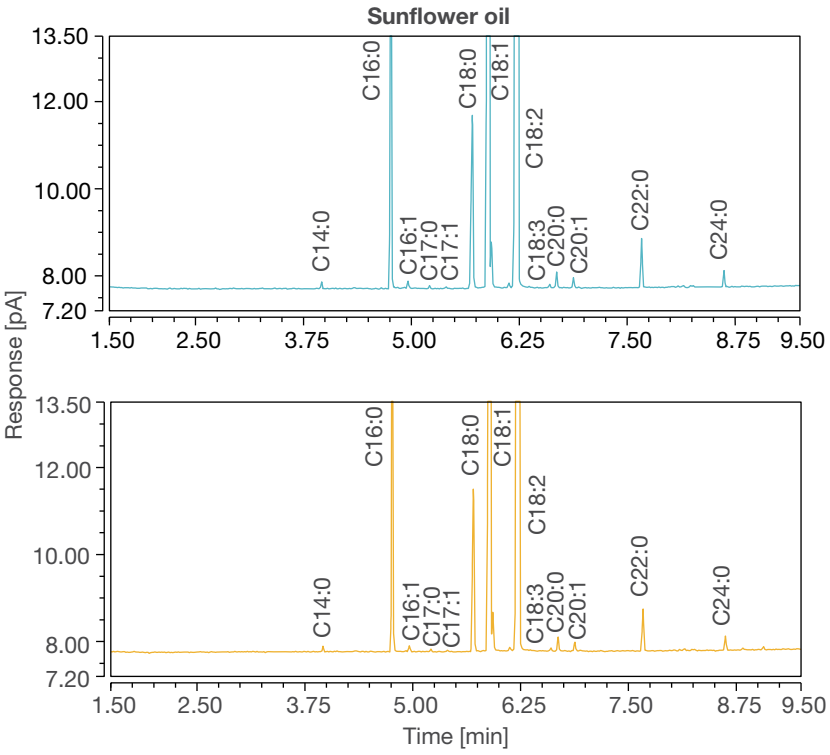
Fatty acid	Relative area %		Ref. UE 2019/1064
	ISO 12966-2 rapid method	AOAC 996.01	
C14:0	n.d.	n.d.	max. 0.03
C16:0	13.3	13.4	7.5–20.0
C16:1	1.1	1.1	0.3–3.5
C17:0	0.1	n.d.	max. 0.4
C17:1	0.1	0.1	max. 0.6
C18:0	3.1	3.1	0.5–5.0
C18:1	69.3	69.3	55.0–83.0
C18:2	9.2	9.3	2.5–21.0
C18:3	0.6	0.6	max. 1.0
C20:0	0.4	0.4	max. 0.6
C20:1	0.2	0.2	max. 0.5
C22:0	n.d.	n.d.	max. 0.2
C24:0	n.d.	n.d.	max. 0.2



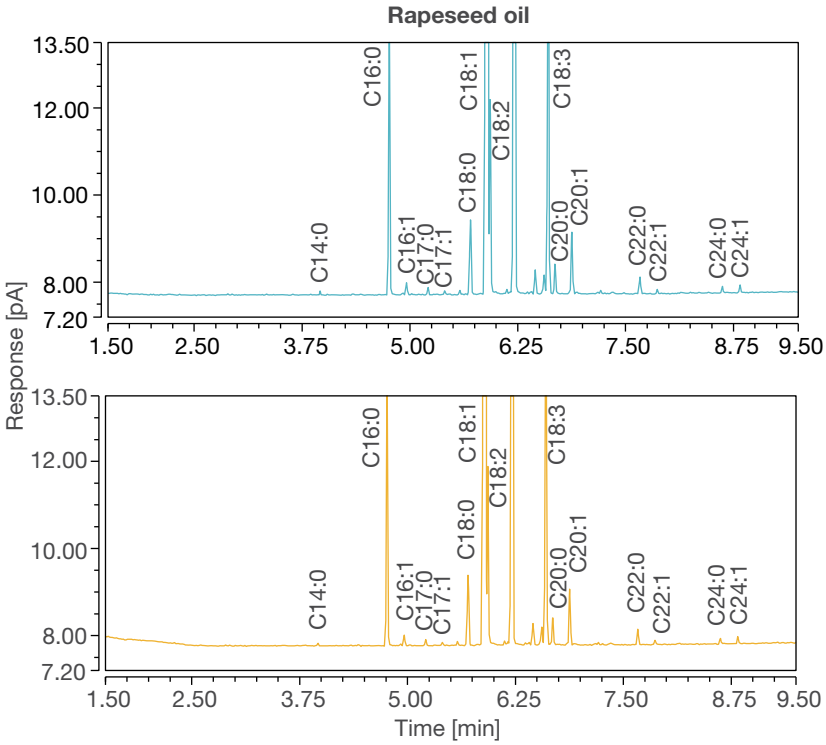
Fatty acid	Relative area %		Ref. UNI 22066
	ISO 12966-2 rapid method	AOAC 996.01	
C12:0	n.d.	n.d.	max. 0.3
C14:0	n.d.	n.d.	max. 0.3
C16:0	11.80	11.80	8.6–16.5
C16:1	0.10	0.10	max. 0.5
C17:0	0.10	0.10	max. 0.1
C17:1	n.d.	n.d.	max. 0.1
C18:0	1.70	1.70	max. 3.3
C18:1	29.00	28.90	20.0–42.2
C18:2	54.70	54.80	34.0–65.6
C18:3	0.90	0.90	max. 2.0
C20:0	0.40	0.40	0.3–1.0
C20:1	0.30	0.30	0.2–0.6
C20:2	n.d.	n.d.	max. 0.1
C22:0	0.10	0.20	max. 0.5
C22:1	n.d.	n.d.	max. 0.3
C24:0	0.20	0.20	max. 0.5



Fatty acid	Relative area %		Ref. UNI 22066
	ISO 12966-2 rapid method	AOAC 996.01	
C14:0	0.1	0.0	max. 0.1
C16:0	4.3	4.3	2.6-5.0
C16:1	0.1	0.1	max. 0.2
C17:0	n.d.	n.d.	max. 0.1
C17:1	n.d.	n.d.	max. 0.1
C18:0	2.7	2.7	2.0-6.2
C18:1	83.1	83.2	75.0-90.7
C18:2	6.7	6.7	2.1-17.0
C18:3	n.d.	n.d.	max. 0.3
C20:0	0.3	0.3	0.2-0.5
C20:1	0.3	0.3	0.1-0.5
C22:0	0.9	0.9	0.5-1.6
C22:1	n.d.	n.d.	max 0.3
C24:0	0.3	0.3	max 0.5



Fatty acid	Relative area %		Ref. UNI 22066
	ISO 12966-2 rapid method	AOAC 996.01	
C12:0	0.0	0.0	max. 0.1
C14:0	0.1	0.1	max. 0.2
C16:0	6.2	6.3	5.0–7.6
C16:1	0.1	0.1	max. 0.3
C17:0	0.0	0.0	max. 0.2
C17:1	0.0	0.0	max. 0.1
C18:0	3.3	3.2	2.4–6.5
C18:1	32.6	32.5	14.0–39.4
C18:2	55.6	55.6	48.3–74.0
C18:3	0.1	0.1	max. 0.3
C20:0	0.2	0.2	0.1–0.5
C20:1	0.2	0.1	max. 0.3
C22:0	0.7	0.7	0.3–1.5
C22:1	0.0	0.0	max 0.3
C24:0	0.2	0.2	max 0.5



Fatty acid	Relative area %		Ref. UNI 22066
	ISO 12966-2 rapid method	AOAC 996.01	
C14:0	0.1	0.1	max. 0.2
C16:0	4.6	4.7	2.5–7.0
C16:1	0.2	0.2	max. 0.6
C17:0	0.1	0.1	max. 0.3
C17:1	0.1	0.1	max. 0.3
C18:0	1.5	1.5	0.8–3.0
C18:1	59.7	59.6	51.0–70.0
C18:2	19.8	20.0	15.0–30.0
C18:3	7.6	8.0	5.0–14.0
C20:0	0.5	0.5	0.2–1.2
C20:1	1.0	1.0	0.1–4.3
C20:2	n.d.	n.d.	max. 0.1
C22:0	0.3	0.3	max 0.6
C22:1	0.1	0.1	max. 2.0
C22:2	0.0	0.0	max 0.1
C24:0	0.1	0.1	max. 0.3
C24:1	0.2	0.1	max. 0.4

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