Determination of Mineral Oil Aromatic Hydrocarbons (MOAH) in vegetable oils and fats - Reverse Phase High-Performance Liquid Chromatography Fluorimetric (RP-HPLC-FLUO) method

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Mineral oil hydrocarbons (MOH) or mineral oil products are constituted by hydrocarbons containing from 10 to about 50 carbon atoms. Due to their extensive use, mineral oils could come into contact with food. The source of contamination is difficult to be predicted and can occur at any stage of food production. The first successful method was designed for the MOSH fraction detection by the Grob lab [1]. EN-ISO-16995 [7] is today referred to a method of choice for detecting MOSH and MOAH mineral oils in routine analysis. It has some critical points concerning mainly the preparation of the sample for analysis: automated purification procedures may be necessary to avoid or minimise the risk of interferences and the need to dedicate a specific system to this determination, with the consequent cost to be incurred [3]. This work suggests a simple and easily applicable method to carry out a pre-assessment of the MOAH content in vegetable oils and fat samples using an instrumentation commonly present in analytical laboratories (RP-HPLC-FLUO) and a simple sample preparation. The article reports the validation parameters: linearity, limit of quantification, recovery, and repeatability. To test the accuracy of the method, the laboratory has joined several international correlation circuits in the period 2020-2022. The method was applied mainly to vegetable fats and oils refined or crude and may be used also for animal fats.

Keywords: Mineral Oil Aromatics Hydrocarbons, MOAH, RP-HPLC-FLUO, Vegetable oils and fats.

1. INTRODUCTION

1.1 CHEMISTRY, SOURCE OF CONTAMINATION AND ANALYSIS HISTORY

Mineral oil hydrocarbons (MOH) or mineral oil products are constituted by hydrocarbons containing 10 to about 50 carbon atoms and are alkylated more than 98% [2]. It is a fraction obtained from petroleum refining and can be destined for various uses: cosmetic products, release agents (bakery industries), packaging materials (wax paper) or others such as technical products (lubricant oils). MOH comprises complex mixtures, principally of straight and branched open-chain alkanes (paraffins), largely alkylated cycloalkanes (aliphatic or aromatics such as naphthene), collectively classified as mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH).

MOAH, in particular contain at least one aromatic ring. They are considered as total content and/or in different fractions for their different biological effects. Recently, particular attention has been paid to MOAH due to the biological effects toward human health. Compounds such as benzene hydrocarbons derivatives may be of potential health hazard. Among the possible risks there is also that of being carcinogenic. In fact, it has also been proven that MOAH with 3-7 ring cyclic aromatic moiety are a main concern for the genotoxic and carcinogenic potential effect [2;4]. Recent studies have shown that mutagenicity decreases

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with the increase in the length of the alkyl chain that can be in these compounds. The reason is to be found in the greater or lower possibility of binding to the CYP450 coenzyme responsible for initiating oxidative reactions with the intercalation of activated metabolites in DNA [4]. The associated cellular damage could cause mutations, malformations, tumour and cancer.
Due to their extensive use, mineral oils can come into contact also with food. The source of contamination is difficult to be predicted and can occur at any stage of food production. Among the most common sources there is environmental contamination (e.g., from particulate matter originating from incomplete diesel combustion), and farming practices (phytosanitary and fertilising treatments). A part of contamination could be attributed to the biosynthetic mechanism intrinsic to the plant), to the food transformation process (lubricant and release agents) and to migration from packaging materials (recycled paperboard and mineral oil-based inks). Good harvesting practices are suggested to minimise the risk [5;15].
Historically, the method for the analysis of mineral oil and their components, was a standardised method based on a gravimetrical determination of the dimethyl sulfoxide extract residue [6]. The method suitable for pure mineral oil was not able to detect contamination in trace. Mineral oil products could migrate into food due to the wide application in technical products including food contact materials (FCM). The first successful method was designed for detection of the MOSH fraction by the lab of Grob in [1]; later also the MOAH fraction was investigated with a LC-GC-FID system; using this procedure it was not possible to resolve mineral oils into single components because the system resolved 2 fractions: MOSH and MOAH. This method today refers to a method of choice for detecting mineral oils in routine analysis and is known as EN-ISO-16995 [7]. Recently a new analytical method has been pointed out in ISO TC34 SC 11 Committee Standard to reach lower LOQ (Limit of Quantification) [11]. This new international standard, according to the results obtained, has been proven suitable for MOSH mass concentrations above 3 mg/kg and MOAH mass concentrations above 2 mg/kg. For this, an alternative method for the epoxidation of the MOAH fraction (with performic acid) and partially modified processing steps are improved. GCxGC-TOF-FID technique could be used for confirmation and characterisation of contaminants [8;10;13;14]. The revision of the UNI EN 16995:2017 standard is expected in 2024 (Draft ISO-FprEN 17517)[11].

1.2 LEGISLATION ASPECTS

Currently there is no law in force in the European Union that regulates the presence of MOAH in food products, but in some Member State there are national directives to prevent this type of contamination. In food products only national benchmark levels have been set: they are not safety levels but indicate that need to further investigation, to conduct a correct risk analysis. In 2021 German food industry established benchmark levels [9] in different food categories: for vegetable oils (excluding those of tropical origin) the MOAH level should be lower than LOQ (Limit of Quantification) of 2 mg/kg for each C-fraction (according to JRC guideline) [10]. The JRC technical report points out that analytical method used must be sufficiently sensible to ensure that food is not contaminated with potentially carcinogenic MOAH. The use of the most advanced methods of laboratory analysis is suggested; the on-line LC-GC-FID method and GC x GC-TOF-FID are recognised as reference methods, but any other detention technique is acceptable only if it provides equivalent results to the LC-GC-FID [7]. During 2023, the JRC updated the Technical Report introducing substantial changes: the quantification of MOH will no longer be carried out for the subfractions of MOSH and MOAH, it has also modified the performance requirements [13]. In 2022 the Member States of standing committee on plants, animals, food, and feed of European Commission agreed to withdraw or if necessary to recall products from the market when total concentration of MOAH is above the maximum LOQ of 2 mg/kg for fats and oils [19]. A recent update of the risk assessment of mineral oil hydrocarbons (MOH) in food has been published by EFSA in 2023. It suggests to pay attention for MOAH with 3-or more aromatic rings due to their human toxicity [20].

1.3 OFFICIAL METHOD EN ISO 16995

The official reference method is: EN ISO 16995 [7] Foodstuffs. “Vegetable oils and foodstuff on basis of vegetable oils. Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis”. It is an international standard method for the determination of saturated and aromatic hydrocarbons from 10 to 50 carbonic atoms in vegetable fats and oil and foodstuffs, interlaboratory validated with online LC-GC-FID. According to the results of interlaboratory studies, the method was deemed suitable for MOSH and MOAH in vegetable oils for a concentration above 10 mg/kg [7].
Although the LC-GC-FID method is nowadays used in several laboratories it has some critical points concerning mainly the preparation of the sample for analysis: automated purification procedures may be necessary to avoid or minimise the risk of interferences and the need to dedicate a specific system to this determination, with the consequent cost to be incurred.

2. AIM OF THE WORK

The purpose of this work is to suggest a simple and easily applicable method to carry out a pre-assessment of the MOAH content in vegetable oil and fat samples using instrumentation commonly present in analytical laboratories (RP-HPLC-FLUO) and a simple

sample preparation. This work also reports the validation results [17;18].
Vegetable oils and fats are dissolved in an appropriate volume of isopropyl alcohol or tetrahydrofuran, filtered and analysed with RP-HPLC-FLUO system. The proposed method is highly specific.
Fluorescence is the light emission by a molecule that has absorbed light or magnetic radiation.
This technique has already been used successfully in the analysis of polycyclic aromatic compounds; due to its high sensitivity, quickness, selectivity, and relative low cost we think it could also be applied to the MOAH analysis. This technique is also very sensitive due to the direct measurement of the emitted light intensity, has low background noise and is slightly affected by interferences species [12;16].
Because MOAH compounds contain at least one aromatic ring they are well suited for fluorescence technique because they have a molecular resonance spectrum due to their aromatic and polyaromatic structure.
The analysis is carried out on two acquisition channels set at 254 nm in excitation, 280 nm in emission for the determination of mono-aromatic compounds and 254 nm in excitation, 430 nm in emission for the determination of polyaromatic compounds. The amount of MOAH is calculated with the use of two external standards (one for each specific acquisition channel).

3. EXPERIMENTAL

3.1 EQUIPMENT AND SUPPLIES

Standard Marker solutions for qualitative analysis

Naphthalene (Marker C10) $C_{10}H_8$ CAS Number 91-20-3 was purchased from Sigma-Aldrich (Merck Life Science srl Milano - Italy).
The stock standard solution was prepared at 0,5 mg/ml weighting accurately, to the nearest 0,1 mg, about 5 mg of naphthalene in a graduated 10 ml class A flask and filling to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v. The solution was diluted with acetonitrile/isopropyl alcohol solution 1/4 v/v to obtain a final concentration of 0,5 ug/ml.
Pyrene (Marker C16) $C_{16}H_{10}$ CAS Number 129-00-0 was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy).
The stock standard solution was prepared weighting accurately, to the nearest 0,1 mg, about 4 mg of pyrene in a graduated 10 ml class A flask and filling to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v. This solution was diluted up to a final concentration of 6 ng/ml.
Coronene (Marker C24) $C_{24}H_{12}$ CAS Number 191-07-1 was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy).
A coronene stock standard solution (concentration of 10 ng/µl) was diluted until a concentration of 2 ng/ml with acetonitrile/isopropyl alcohol solution 1/4 v/v.

Standard calibration solutions for quantitative analysis

4-Ethyl-Toluene standard (CAS 99 620-14-4) was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy). This standard was used for the determination of mono-arylated-aromatic compounds at 254 nm channel in excitation and at 280 nm in emission (Channel 1).
A stock standard solution was prepared weighing, to the nearest 0,1 mg, about 10 mg of 4-Ethyl-Toluene in a graduated 10 ml class A flask and filled to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v.
The standard working solutions for calibration curve were prepared in acetonitrile/isopropyl alcohol solution 1/4 v/v at different concentrations of 4-Ethyl-Toluene in the range of 0,1-1,0 µg/ml. Two injections for each level were performed.
The Chiron AS Stock Standard solution is a mixture of 20 compounds (C10-C15) dissolved in isooctane at a concentration of 100-500 µg /ml each, was purchased from Chiron AS (Stiklestadvn. 1 N-7041 Trondheim Norway). List of Chiron compounds solution is: 1-Methylnaphtalene (CAS 90-12-0), 2-Methylnaphtalene (CAS 91-57-6), 1,3-Dimethylnaphtalene (CAS 575-41-7), 1,4-Dimethylnaphtalene (CAS 571-58-4), 1,5-Dimethylnaphtalene (CAS 571-61-9), 1,6-Dimethylnaphtalene (CAS 575-43-9), 1,7-Dimethylnaphtalene (CAS 575-37-1), 2,6-Dimethylnaphtalene (CAS 581-42-0), 2,7-Dimethylnaphtalene (CAS 582-16-1), Biphenyl (CAS 92-52-4), Phenanthrene (CAS 85-01-8), 1-Methylphenantrene (CAS 832-69-9), 2-Methylphenantrene (CAS 2531-84-2), 3-Methylphenantrene (CAS 832-71-3), 9-Methylphenantrene (CAS 883-20-5), Dibenzotiophene (CAS 132-65-0), 1-Methyldibenzotiophene (CAS 31317-07-4), 2-Methyldibenzotiophene (CAS 20928-02-3), 3-Methyldibenzotiophene (CAS 16587-52-3), 4-Methyldibenzotiophene (CAS 7372-88-5). The total concentration of all compounds of Chiron AS Stock Standard solution was about 7000 µg/ml. The standard working solutions for calibration curve were prepared in acetonitrile/isopropyl alcohol solution 1/4 v/v at the different concentrations in the range 0,07-7 µg /ml as sum of all compounds. Two injections for each level were performed.
All the solvents used were HPLC grade.
To avoid contamination due to glassware washing residues it is recommended, prior use, to perform at least two rinses with acetone and n-hexane for HPLC and drying in an oven at 200°C for two hours.

4. SAMPLE PREPARATION

0,5 g of well-homogenized vegetable oil or fat was weighted in a 5 ml volumetric class A flask. The sample was dissolved into a volume of isopropyl alcohol and the sample solution was mixed with vortex for 30 seconds. When necessary, to facilitate dissolution, an ultrasonic bath was used for about 5 minutes at room temperature. An aliquot solution (1 ml) for HPLC

analysis was filtered through a 13 mm Nylon 0,45 µm membrane syringe filter directly into a 2 ml HPLC vial. Three independent replicates were conducted for all samples and the result was the average of all the determinations.
For samples with a MOAH concentration level over 5 mg/kg and additional dilution is need to obtain a fluorimetric response less than 10% for channel 1, and 20% for channel 2 (of the full scan).
For fat solid samples, after the addition of solvent, it was necessary to heat the solution for a complete dissolution. The sample was left in the fridge at + 4°C for 5 hours to bring down triacylglycerides. Then, the supernatant was filtered first and then injected.

5. HPLC ANALYSIS

The HPLC system used was a HPLC quaternary pump with a degassing system (Shimadzu LC-30AD pump) equipped, with a C18 Reverse Phase column. The following column was selected for the determination: Repro-Sil 80 ODS-2 (250 mm x 4,0 mm, 3 µm - dr. Maisch GmbH). The analysis was carried out at 40°C column compartment and autosampler. The Fluorescence detector (Shimadzu RF-20A XS) was set up for the simultaneous acquisition on two channels: at 254 nm in excitation, 280 nm in emission for channel 1 and 254 nm in excitation, 430 nm in emission for channel 2. An integration system was needed. Photodiode Array Detector (PDA-Shimadzu SPD-M20A) for spectra recording (from 200 nm to 600 nm) could be used coupled with fluorescence detection when the analyte amount allows its detection also in PDA (spectra qualitative analysis).

5.1 ANALYSIS CONDITION

The elution was achieved with a linear gradient for 50 minutes starting from 60% of (A) HPLC grade water and 40% (B) HPLC grade Acetonitrile to 2% of (A) and 98% of (B). This condition was then maintained for 40 minutes. The flow rate was 0.4 ml/min. The total acquisition time was 90 minutes.
In the HPLC-FLUO system 20 µl of each sample solutions were injected.
The first sample injected as part of a series of analysis must always be a “blank” constituted by sample dissolution solvent (isopropyl alcohol or tetrahydrofuran) filtered through a 13 mm Nylon 0,45 µm membrane syringe filter. Pay attention to interfering signals present in the chromatographic run at the same retention time of calibration standards in the C-range considered (C10-C50). The blank content must be considered to be subtracted to the sample content.

5.2 CALIBRATION SOLUTIONS

Starting from calibration solutions, two calibration curves were obtained: one for 4-Ethyl-Toluene and one for Chiron AS Stock Standard Solution and the relative linear correlation equation forcing through the axis origin was calculated (Y = peak area, X = concentration in ng injected, Y = m x X, the respective slopes corresponded to unitary response for 1 ng of 4-Ethyl-Toluene and 1 ng Chiron AS Solution).

5.3 EXPRESSION OF RESULTS

Data processing was performed with Lab Solution software (Version 5.73 Shimadzu Corporation).
Quantification was performed and expressed in mg/kg with 4-EthylToluene as external standard on channel 1 and Chiron AS external Standard solution on Channel 2.
The total content of MOAH (C10-C50) expressed in mg of MOAH /kg of sample was the sum of the two contents.

5.4 SUB-FRACTION (C-FRACTION) DEFINITION

In the 2019 JRC Technical Report, sub-fractions of MOAH (so-called C-fractions) in the chromatograms are defined by the position of the elution signals of n-alkanes from the GC column. Each C-fraction starts at the retention time of the peak end of the first n-alkane of the range and stops at the retention time of the peak end of the second n-alkane of the range. Only the C-fraction ≥ n-C10 to ≤ n-C16 starts at the retention time of the peak start of n-C10 and stops at the retention time of the peak end of n-C16 [10].
They are:

Table I - C-fraction definition according to JRC Technical Report [10]

MOAH
MOAH ≥ n-C_{10} to ≤ n-C_{16}
MOAH > n-C_{16} to ≤ n-C_{25}
MOAH > n-C_{25} to ≤ n-C_{35}
MOAH > n-C_{35} to ≤ n-C_{50}

Also in HPLC elution it was possible to separate approximately some of those fractions but on the basis of the the retention times of the Naphthalene, Pyrene and Coronene markers. In fact, the retention time of those polyaromatic compounds, which are detectable in channel 2, were used to divide the different fractions range also on channel 1.
Each C-fraction starts at the retention time of the peak of the first polyaromatic reference compound and end at the retention time of the second polyaromatic reference compound, both in the first and second channel.
The HPLC C-fractions are not fully corresponding to JRC C-fractions.
Nowadays the JRC technical report 2023 [13] has eliminated the C-fraction in expression of results.

6. METHOD VALIDATION

The method was validated for linearity, detection limit, quantification limit (LOQ), recovery and repeatability; the method was compared with other methods participating in different collaborative studies between 2020-2022 for the accuracy evaluation (Table VIII).

6.1 CALIBRATION AND CORRELATION COEFFICIENT (LINEARITY)

Different calibration solutions for both types of external standards were prepared and analysed for the definition of the calibration curve and the relative slopes corresponding to the response factor of 1 ng di 4-Ethyl-Toluene on channel 1 and of 1 ng Chiron AS Solution on Channel 2. The correlation coefficient was ≥ 0,99 for both calibration standard curves. The slopes showed a repeatability CV% value of 7% for 4-Ethyl-Toluene and 11% for Chiron AS Solution obtained from statistical elaboration data from 7 different calibration curves. For both calibration standard curves the linearity residuals showed a value < 20% [17].

6.2 ACCURACY EVALUATION

To test the accuracy, the method was applied to several international proficiency tests in the period 2020-2022. Table III shows the results obtained after the statistical evaluation processing carried out by each of the organising committees.

6.3 HPLC-FLUO METHOD COMPARISON WITH EN 16995 METHOD

To test the possibility of using the HPLC-FLUO method as a screening method, some samples were compared to the results obtained by the LC-GC-FID method (EN 16995). Table IV shows the samples submitted to both analyses and the results obtained. The method was in agreement with the official method (LC-GC-FID), in some cases the content was above those quantified with the reference justifying proceeding, in this case, with a more accurate analysis.

Table II - C-fraction definition in HPLC FLUO method [10]

	MOAH
1	MOAH ≥n-C_{10} Naphthalene to < n-C_{16} Pyrene
2	MOAH ≥n-C_{16} Pyrene to < n-C_{24} Coronene
3	MOAH ≥n-C_{24} Coronene

6.4 RECOVERY

The determination of the recovery value was performed by analysing a refined sunflower oil sample suitably spiked with MOAH from mineral oil at six different concentration levels between 0,5 and 20,0 mg/kg.
Table V shows the results obtained for each level of concentration.

6.5 REPEATABILITY

To test the repeatability of the method, 10 independent replicates were performed on an olive oil sample obtained from a proficiency test with an assigned value of MOAH content. The data obtained were processed with a software program for the statistical analysis of the data provided by ARPAT (ARPA Tuscany region). On all the data obtained, the presence of a normal distribution was verified according to the Shapiro-Wilks test at the 95% confidence level. Subsequently single Dixon, single and pair Grubbs and Huber tests were performed, always with a 95% confidence level.

Table III - Accuracy evaluation through participation to proficiency tests

Year	**Sample**	**PT Commitee Organizative**	**Assigned Value mg/kg (C10-C50)**	**HPLC-PDA- FLUO Value mg/kg**	**Z score**
2020	Contaminated vegetable oil	Innovhub	13,3	8,1	2,47
2021	Vegetable oils blend	27th DGF *	3,91	3,61	-0,1
	Olive oils blend	27th DGF *	4,39	5,21	0,32
	Contaminated vegetable oil	Innovhub	21,6	22,2	0,83
	Contaminated olive oil	IOC **	40,9	51,92	1,08
	Certified diatermic oil	Certified Material	<1	0,9	-
2022	Olive oil	JRC ***	43,54±2,41	10,78	-2,85
	Olive oil	JRC ***	2,8±0,5	3,95	1,31
	Contaminated olive oil	Innovhub	5,5	5,7	0,30

(*) DGF = Deutsche Gesellschaft für Fettwissenschaft (German Society for Fat Science).
(**) IOC = International Olive Council.
(***) JRC = Join Research Centre (European Commission).
For one sample the Z score is not present as due to the low content and the organizer decided that it was not possible to proceed with the statistical processing.

Table IV - Comparison between data obtained from LC-GC-FID method (EN 16995) and HPLC-FLUO method

Sample	Matrix	Total MOAH Content LC-GC-FID Method (EN 16995) mg/kg	Total MOAH Content HPLC-PDA-FLUO Method mg/kg
1	EXTRA VIRGIN OLIVE OIL	<2,0	0,9
2	EXTRA VIRGIN OLIVE OIL	2,7	2,2
3	EXTRA VIRGIN OLIVE OIL	<2,0	0,9
3	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
4	EXTRA VIRGIN OLIVE OIL	<2,0	0,2
5	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
6	EXTRA VIRGIN OLIVE OIL	2,0	0,5
7	EXTRA VIRGIN OLIVE OIL	<2,0	0,2
8	EXTRA VIRGIN OLIVE OIL	<2,0	0,1
9	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
10	OLIVE OIL	2,0	1,1
11	FRYING OIL	<2,0	0,2
12	GRAPESEED OIL	7,4	8,6
13	GRAPESEED OIL	8,0	15
14	GRAPESEED OIL	4,4	5,3
15	GRAPESEED OIL	<2,0	0,8
16	GRAPESEED OIL	<2,0	2,5
17	GRAPESEED RAW OIL	4,9	11,5
18	GRAPESEED RAW OIL	4,8	13,1
19	GRAPESEED RAW OIL	6,1	13,2
20	GRAPESEED RAW OIL	5,0	15,7
21	PEANUTS OIL	<2,0	0,2
22	PEANUTS OIL	4,4	2,1
24	CORN OIL	<2,0	1,6
25	CORN OIL	<2,0	2,0
26	CORN OIL	<2,0	2,1
27	CORN OIL	<2,0	1,0
28	CORN OIL	4,2	2,4
29	CORN OIL	<2,0	1,7
30	SUNFLOWER OIL	<2,0	0,2
31	SUNFLOWER OIL	<2,0	2,0
32	SUNFLOWER OIL	<2,0	1,9
33	SUNFLOWER OIL	<2,0	2,5
34	SUNFLOWER OIL	<2,0	2,0
35	SUNFLOWER OIL	<2,0	0,5
36	SUNFLOWER OIL	<2,0	1,2
37	SUNFLOWER OIL	<2,0	1,8
38	SUNFLOWER OIL	<2,0	1,9
39	SUNFLOWER OIL	<2,0	1,0
40	SUNFLOWER OIL	<2,0	0,2
41	SUNFLOWER OIL	<2,0	0,7
42	SUNFLOWER OIL	<2,0	1,8
43	SUNFLOWER OIL	<2,0	1,2
44	SUNFLOWER OIL	<2,0	0,6
45	SUNFLOWER OIL	<2,0	1,3
46	SUNFLOWER OIL	<2,0	2,1
47	SUNFLOWER OIL	<2,0	1,4
48	SUNFLOWER OIL	<2,0	0,1
49	SUNFLOWER OIL	<2,0	1,4
50	SUNFLOWER OIL	<2,0	1,3

Table V - Recovery data on six different concentration level

Sample	Theoretical MOAH Content (mg/kg)	MOAH Content HPLC-FLUO method (mg/kg)	Recovery (%)
1	0,5	0,5	106
2	1,0	1,7	106
3	2,0	1,7	83
4	5,0	4,4	88
5	10,0	8,9	86
6	20,0	17,5	88

Table VI - Sample repeatability data

N° of Replication	Total MOAH content mg/kg
1	5,9
2	5,6
3	5,6
4	5,4
5	5,5
6	5,6
7	5,5
8	5,6
9	5,4
10	5,5
Average	5,5
Standard Deviation	0,1
Repeatability	0,3
Variation Coefficient %	1,4
Expanded Uncertainty	0,3

Table VI reports the results obtained from the repeatability test and the related statistical indices.

7. SAMPLES OF VEGETABLE OILS AND FATS ANALYSED

Several vegetable oil samples (109 samples) were analysed for screening purposes by our laboratory in the period 2020-2022. These were samples of olive oils, extra virgin olive oils, seed oils and vegetable oils and fats.
Before the analysis they were stored in the refrigerator at a temperature of +4°C, in glass bottles.
Table VII shows the list of analysed samples sorted by matrix and relative numerosity.

Table VII - Analysed sample numerosity, MOAH content (C_{10}-C_{50})

Sample	Number of sample analysed	Range of MOAH Content (C_{10}-C_{50}) mg/kg
Extra Virgin Olive oil	51	<0,1-6,8
Refined Olive oil	6	2,3-9,2
Lampante Olive oil	2	9,3-9,6
Refined Sunflower oil	17	0,2-20,1
Refined Almond oil	6	0,9-4,2
Refined/Raw Grapeseed oil	8	2,5-29.8
Refined Palm oil	2	9,3-24,2
Refined Coconut oil	2	0,6-8,2
Refined Sesame seed oil	2	0,6-6,1
Refined Lineseed oil	2	6,0-7,4
Pressed Hemp seed oil	2	7,5-15,3
Refined Corn seed oil	2	1,7-2,4
Refined Peanuts seed oil	2	0,2-2,1
Animal oil (chicken, salmon)	4	0,8-4,6
Anydrous milk	1	<0,1-0,4

8. COMPARISON OF PRECISION DATA BETWEEN JRC REQUEST

Table VIII shows values of LOQ, recovery and intermediate precision obtained with the HPLC-FLUO method in comparison with those suggested by JRC guidelines [10; 13]

9. CONCLUSIONS

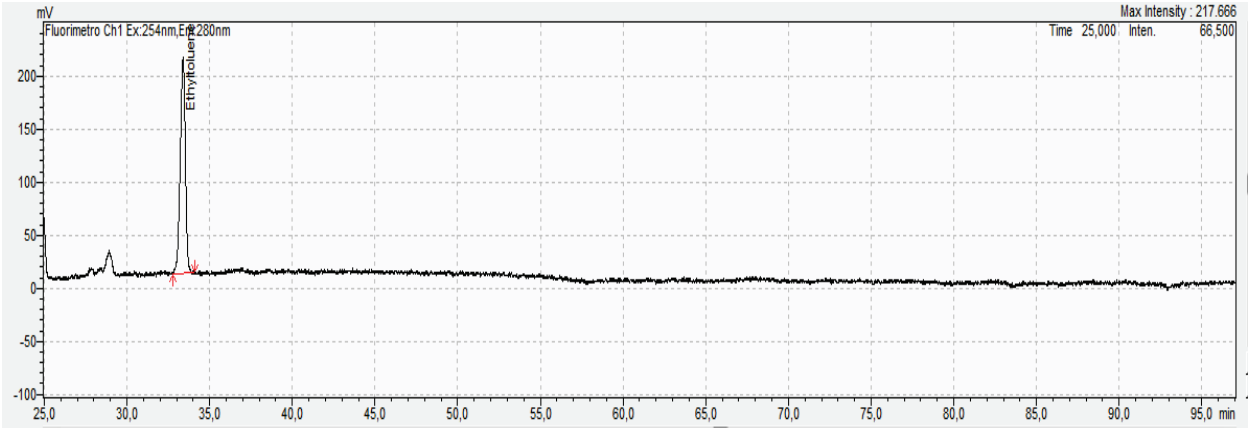
A method for the analysis of MOAH was developed using the HPLC-FLUORIMETRIC system. The method was fully validated for vegetable fats and oils and the LOQ was 0,5 mg/kg for both matrices. The validation of the method also showed suitable accuracy

Table VIII – Precision values obtained with the HPLC-FLUO method

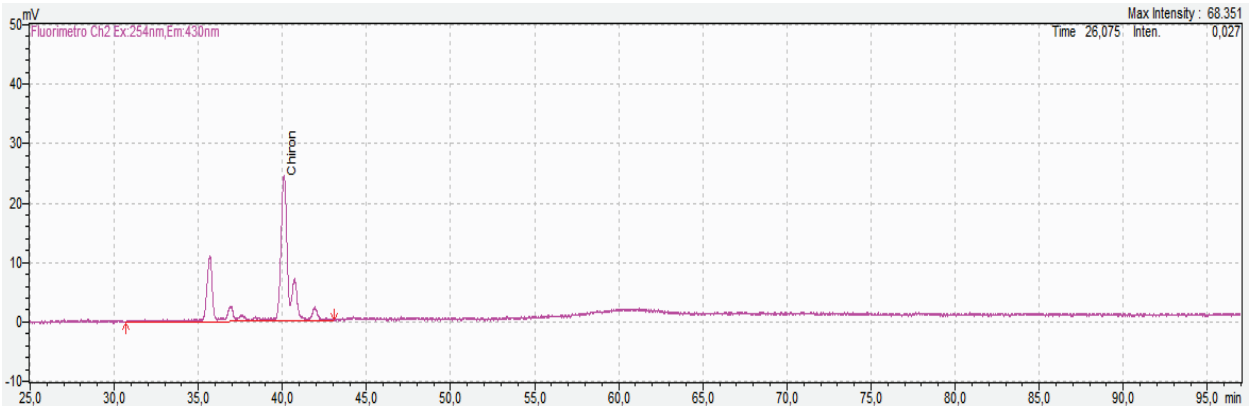
	Range	LOQ max (mg/kg)	LOQ-t (mg/kg)	Recovery (%)	Intermediate precision (%)	BIAS (%)
HPLC-FLUO	C10-C50	0,5	0,1	83-106*	3**	8-19
JRC 2019	C10-C50	2	0,5	70-120	20	-
JRC 2023	C10-C50	2	-	80-110	20	-

**Referred to a spiked range of 0,5-20 mg/kg MOAH*
*** referred to a PT sample with an assigned value of 4 mg/kg MOAH*

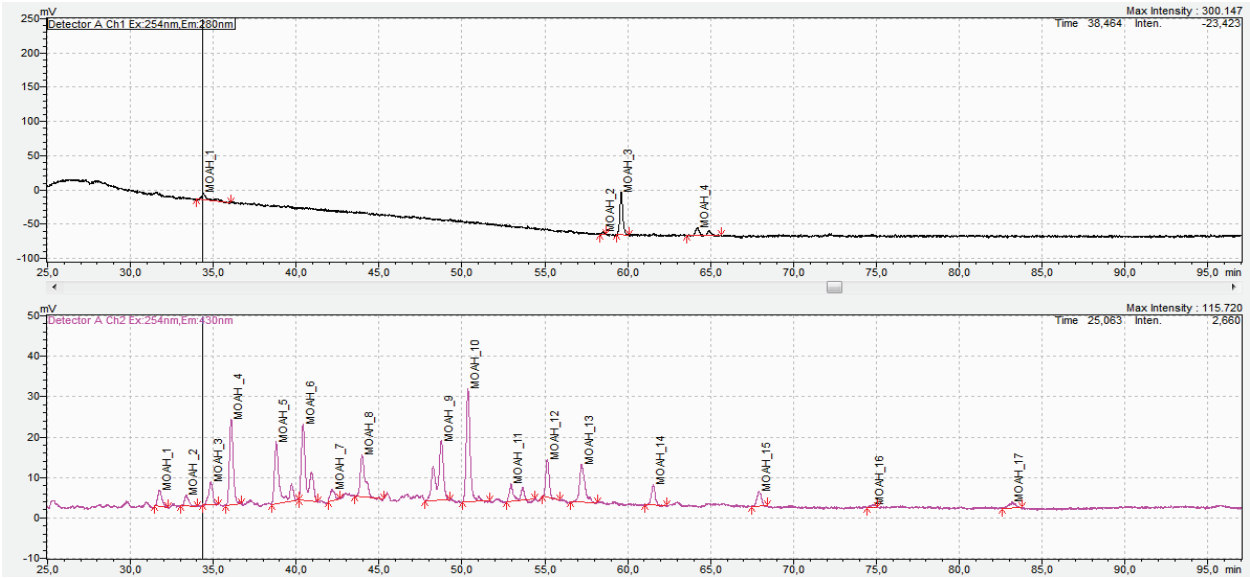
Chromatogram traces obtained by HPLC- FLUO method



Chromatogram 4-Ethyl-Toluene Standard Channel 1



Chromatogram Chiron AS Standard Channel 2



Chromatogram Sample (grapeseed oil) content of 12 mg/kg
MOAH acquired on Channel 1 and 2

and precision values in accordance with the JRC prescription, reaching the LOQ of 0,5 mg/kg. The accuracy was evaluated obtaining good Z scores with international and national proficiency test participation. The validated method was applied to a total of 109 samples, detecting MOAH in most of them.
The higher MOAH content was found in refined grapeseed, palm, and sunflower oil. Moreover, a comparison between samples analysed with on-line LC-GC-FID and HPLC-FLUO was performed showing that the method is in agreement with the official one. The method is simple and easily applicable to carry out a pre-assessment in the routine control of the MOAH content in vegetable oil and fat samples using instrumentation commonly present in analytical laboratories (HPLC-FLUO) and a simple sample preparation.

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