SHIMADZU
Excellence in Science

Technical Report

Determination of Mineral Oil Saturated and Aromatic Hydrocarbons in Edible Oil by Liquid-liquid-gas Chromatography with Dual Detection

Mariosimone Zoccali[1], Giorgia Purcaro[1], Luigi Mondello[1, 2]

Abstract:

Nowadays, food contamination by mineral oil (MO) is a very important issue and its presence in edible oils is widely documented. Mineral oils are hydrocarbons mixture of petrochemical origin containing mainly two families of compounds, namely mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). The most used approach for mineral oil analysis is liquid chromatography (LC) coupled to gas chromatography (GC) with flame ionization detection. Using a silica column, triglycerides can be retained and the separation of MOSH and MOAH can be achieved. However, in the case of vegetable oils, the presence of olefins (mainly squalene and isomers) does not allow the correct quantification of the MOAH fraction. For such a reason additional tools are needed. In the present research, a silica column was coupled to a silver-ion column to retain olefins. The proposed method was applied for the analysis of different edible oils, obtaining satisfactory performance characteristics.

Keywords: liquid-gas chromatography, mass spectrometry, MOSH/MOAH, food safety, edible oils

1. Introduction

Mineral oil hydrocarbons (MOH) are complex mixtures deriving from crude petroleum, which is composed by two major classes of compounds, namely mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). Food are contaminated with mineral oil through different sources: environmental contamination, food grade mineral oils (which is used during food processing for different purposes), and food contact materials. In the specific case of edible oils, fraudulent admixture with technical grade products cannot be completely ruled out. Moreover, the analysis can be complicated by the presence of high amount of olefin, deriving from squalene (SQ) isomerization, co-eluting in the MOAH fraction.

Due to the complexity of the mixture and the high number of isomers the MOH contamination appears as a hump in the chromatographic trace. Furthermore, the lack of standards makes the calibration of the detector response impossible, thus mass spectrometry (MS) detection is not suitable for quantitative determination, while the use of flame ionization detector, which provides virtually the same response for all hydrocarbons is the most suitable choice.

The aims of the research were to improve purity of the MOAH fraction (removing olefin) by using an additional silver-ion column and to confirm the petrogenic origin of the MOH contamination evaluating the presence of hopanes as markers. Three LC columns in series, two silica ones (to separate MOSH from MOAH and both from the bulk of triglycerides) and a silver-ion one, on-line coupled to a GC equipped with a double detection [FID and triple quadrupole (QqQ) MS] were employed.

2. Experimental

2-1. Sample and sample preparation

Eleven samples were analyzed [6 extra virgin olive oils (EVOO), 2 olive oils (OO), 2 pomace olive oils, and the Ukrainian sunflower oil (provided by a local control laboratory)]. The samples of vegetable oils were diluted 1:5 (*w/v*) in *n*-hexane prior to injection.

2-2. LC-GC-QqQ MS/FID analysis

All applications were carried out using Shimadzu's Online HPLC Cpmprehensive Two Dimensional Gas Chromatograph Triple Quadrupole Mass Spectrometer "5D Ultra-e". In this study, the GC separatoin was performed in one dimensional analytical approach.

LC configuration	: 150 × 3 mm ID, 5 µm d_p + 250 × 2.1 mm ID, 5 µm d_p silica columns (SUPELCOSIL LC-Si, Sigma-Aldrich/Supelco) connected through a six-port valve to a 150 × 1 mm ID, 5 µm d_p Nucleosil SA, 100 Å (Sigma-Aldrich/Supelco) lab-silvered.
LC conditions	: MOSH (from 4.50 min to 6.15 min) and MOAH (from 7.0 to 20.85 min) fractions were eluted with a gradient starting with 100% *n*-hex (5.00 min) and reaching 50% of CH_2Cl_2 after 7 min, the initial flow rate of 300 µL min^{-1} was reduced to 150 µL min^{-1} after 5.59 min, at the same time (5.60 min) the six-port valve was switched (Fig. 1), diverting the flow to the silver-ion column.
Inj vol	: 50 µL.

After the transfer of the fractions of interest the columns were washed with CH_2Cl_2 and then reconditioned with *n*-hex.

1 Chromaleont s.r.l., c/o University of Messina, - Polo Annunziata - viale Annunziata, 98168 – Messina, Italy
2 "Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali", University of Messina, - Polo Annunziata - viale Annunziata, 98168 – Messina, Italy

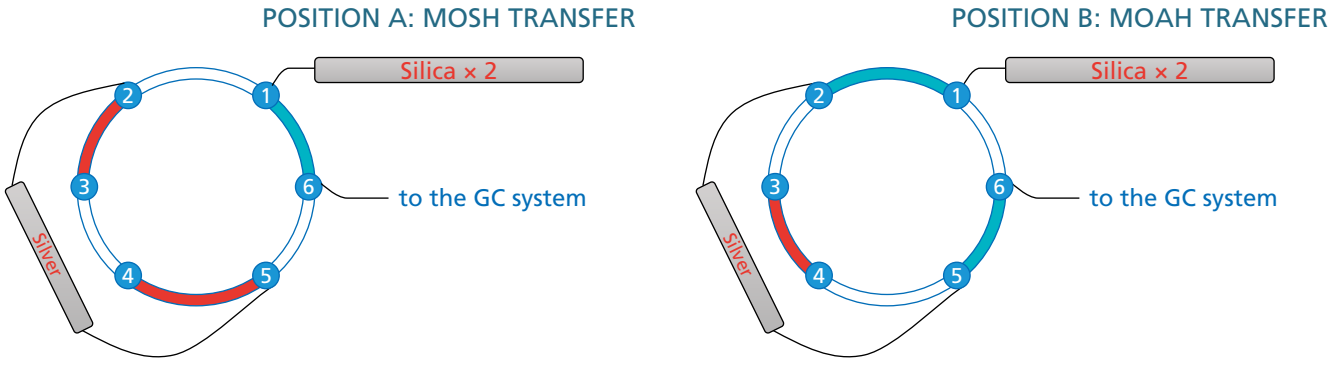

Fig. 1 Six-port valve configuration.

PTV conditions	: Temperature started from 35°C (held for all the transfer period plus 10 s) to 360°C at 20°C/s. Split Program started by a ratio of 240:1 and 180:1 during the LC transfer period plus 6 s respectively for MOSH and MOAH fraction, then changed to a split ratio of 0 for 2.4 min, followed by a split ratio of 200:1 for the remaining analysis time.
GC conditions	: column SLB-5ms 30 m × 0.25 mm ID × 0.25 µm *df* (Sigma Aldrich/Supelco). The outlet of the column was connected by using a "Y"-Union to a 0.5 m × 0.10 mm ID (for FID analysis) and to a 1.3 m × 0.10 mm ID uncoated column (for MS analysis).
	First and second oven temperature started at 35°C (3.0 min) reaching 350°C at 40°C min^{-1} (5 min). Carrier gas He, initial pressure of 255 kPa.
FID parameters	: Temperature was 360°C and sampling frequency was 250 Hz
MS parameters	: Ionization mode: electron ionization (70 eV). Interface and ion source temperatures: 300°C and 280°C. Ar was used as collision gas at 200 kPa.
	Full Scan Acquisition mode: mass range; *m/z* 45–360; spectral production frequency; 20 Hz
	MRM mode: Hopanes transitions were (*m/z*) 370>191 for C_{27} (Ts= 18α(H)-22,29,30-trisnorhopane and Tm= 17α(H)-22,29,30-trisnorhopane); 384>191 for C_{28} (28,30-BNH = 28,30-bisnorhopane); 398>191 for C_{29} (C29$\alpha\beta$= 17α(H), 21β(H)-30-norhopane); 412>191 for C_{30} (C30$\alpha\beta$= 17α(H), 21β(H)-hopane); 426>191 for C_{31} (31$\alpha\beta$S= 17α(H), 21β(H), 22S-homohopane and 31$\alpha\beta$R= 17α(H), 21β(H), 22R-homohopane); 440>191 for C_{32} (32$\alpha\beta$S= 17α(H), 21β(H), 22S-bishomohopane and 32$\alpha\beta$R= 17α(H), 21β(H), 22R-bishomohopane); 454>191 for C_{33} (33$\alpha\beta$S= 17α (H), 21β(H), 22S-trishomohopane and 33$\alpha\beta$R= 17α(H), 21β(H), 22R-trishomohopane); 468>191 for C_{34} (34$\alpha\beta$S= 17α(H), 21β(H), 22S-tetrak-ishomohopane and 34$\alpha\beta$R= 17α(H),21β(H), 22R-tetrak-ishomohopane); 482>191 for C_{35} (35$\alpha\beta$S= 17α(H), 21β(H), 22S-pentakishomohopane and 35$\alpha\beta$R= 17α(H), 21β(H), 22R-pentakishomohopane); collision energy (CE) 20 eV.

A dedicated "5D Solution" software (Shimadzu) enabled the control of each instrument through each native software.

2-3. Results and discussion

MOH contamination in vegetable oils is often deriving by an environmental source, thus it is usually centered around the *n*-alkane C_{30} (typical of an environmental contamination source). SQ and its isomers, eluted from the LC within the MOAH fraction, elute at similar retention time in the GC run, thus causing a rather high uncertainty in the quantification procedure, which is carried out by subtracting the areas of the peak sitting on the top of the hump.

The strategy applied in the present research was to improve the LC separation to obtain a more reliable quantification, in particular of the MOAH fraction and to confirm the petrogenic origin by detecting the possible presence of hopanes by QqQ MS. An additional silica and a silver-ion columns were added before the on-line transfer into a GC system, which was equipped with a dual detection (FID/QqQ MS). About 67% and 33% of the effluent reached the FID and MS at 35°C, respectively. Quantification was carried out using the information obtained from the FID detection, while the QqQ MS data (set in the simultaneous full scan/MRM mode) was used to confirm the chemical class of the hump and possible interferences, and to selectively detect the presence of hopanes hindered in the MOSH fraction.

2-4. LC separation optimization

As already proved in previous research, the use of silica LC columns is the best choice to efficiently retain triacylglycerols (TAGs), and to separate MOSH and MOAH, but olefins were not retained enough to be separated from the MOAH fraction. Therefore, the separation occurring in silver ion column was explored. In fact, the separation occurring in this type of column is based on the formation of weak complex between silver ion and the π-bonds in unsaturated compounds. Such a separation mechanism can be exploited to delay olefins elution beyond that of the MOAH.

However, the combination of the these two columns (silica and silver-ion) needed to solve some technical issues, since a lower flow rate was required for the silver-ion column (150 µL min^{-1}) compared to the flow applied using only the silica columns (300 µL min^{-1}), to avoid a too extensive leaching of the silver-ion column; furthermore an inversion of the elution order in the silver-ion column, which caused a partial coelution of the MOSH and MOAH fraction previously separated, required the addition of a six-port valve to isolate the columns (Fig. 1).

The result obtained using the optimized final configuration was compared with the conventional system, equipped with only one silica column. The systems were tested using an EVOO sample spiked with 100 mg kg^{-1} of vacuum pump oil. The FID chromatograms of both MOSH and MOAH fractions obtained using the two different configurations are reported Fig. 2. Silver-ion column proved to be very effective in removing SQ, allowing a more precise quantification of the MOAH fraction. Comparing values of the hump area obtained by integration performed subtracting the olefin peaks (Fig. 2-1) and the area of the hump obtained physically removing the olefins (Fig. 2-2).

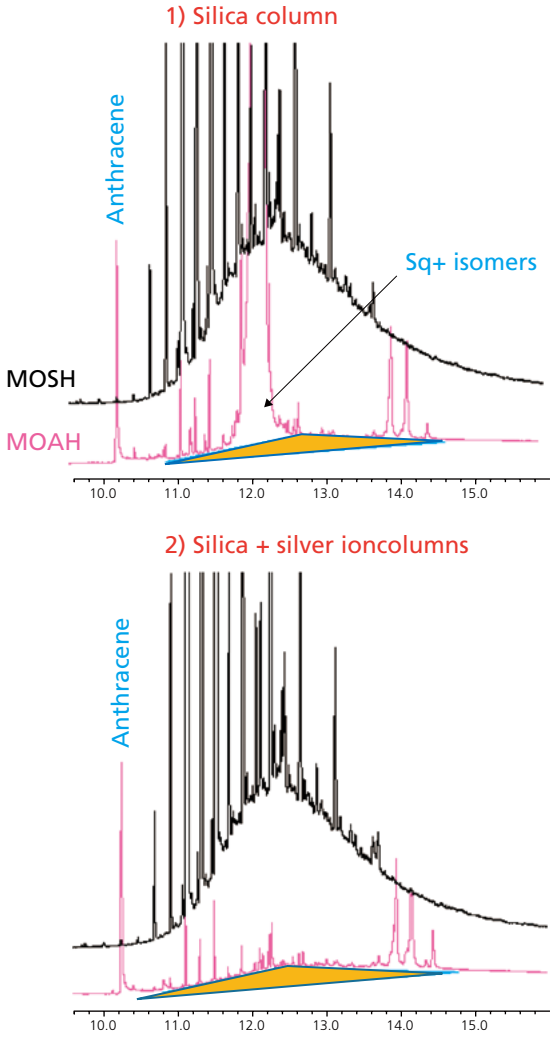

Fig. 2 LC-GC-FID chromatograms of spiked EVOO using 1) the silica column alone, and 2) the silica+silver-ion columns.

2-5. Analysis of commercial products

The amount of MOSH and MOAH found in all 11 samples are reported in Table 1. The sunflower oil was the most contaminated sample, follow by olive pomace oils, presenting MOSH values above 200 mg kg^{-1} and MOAH above 30 mg kg^{-1}. The OO samples had different contamination levels, corresponding to 205.6 (OO-1) and 11.5 mg kg^{-1} (OO-2) of MOSH, respectively; while MOAH was found only in OO-1 (8.0 mg kg^{-1}). EVOO samples were the least contaminated, with a MOSH level in the 4–20 mg kg^{-1} range, except for EVOO-1, which was slightly above (21.8 mg kg^{-1}), and a MOAH amount always below LOQ.

Table 1 Amount of MOSH and MOAH in the samples analyzed

Samples	MOSH (mg kg^{-1})	MOAH (mg kg^{-1})
Sunflower oil	2540	355
Pomace oil-1	445	66
Pomace oil-2	230	32
olive oil-1	206	<LOQ
olive oil-2	12	8
Extra virgin olive oil-1	22	<LOQ
Extra virgin olive oil-2	20	<LOQ
Extra virgin olive oil-3	6	<LOQ
Extra virgin olive oil-4	16	<LOQ
Extra virgin olive oil-5	10	<LOQ
Extra virgin olive oil-6	4	<LOQ

The 30% of the effluent flow diverted to the QqQ MS was used to possibly detect the presence of hopanes (petrogenic markers) hidden beneath the MOSH hump, during the same analysis performed for quantification purposes. An absolute quantification of the hopanes was not possible, due to a lack of standards; therefore a "qualitative" sensitivity, based on the MOSH content, was roughly evaluated by injecting consecutive dilution of the motor oil, vaseline and vacuum pump oil.

A "limit of confirmation" (S/N=3 for the highest hopane) of the origin of the mineral oil hump, related to the content of MOSH, was obtained for the petrochemical source evaluated, corresponding to 6, 22, and 30 mg kg^{-1} of MOSH for motor oil, vaseline and vacuum pump oil, respectively.

Hopanes were found in sample OO-1, the olive pomace oils and the sunflower oil, while traces of hopanes were found in EVOO 1, 2 and 4, which presented MOSH contamination at the "limit of confirmation" range (Table 1). In Fig. 3 is reported the QqQ MS chromatogram of OO-1 sample and an expansion of the hopane region. Although not statistically significant, the source of contamination of the real-world samples can be hypothesized comparing the relative hopane content values.

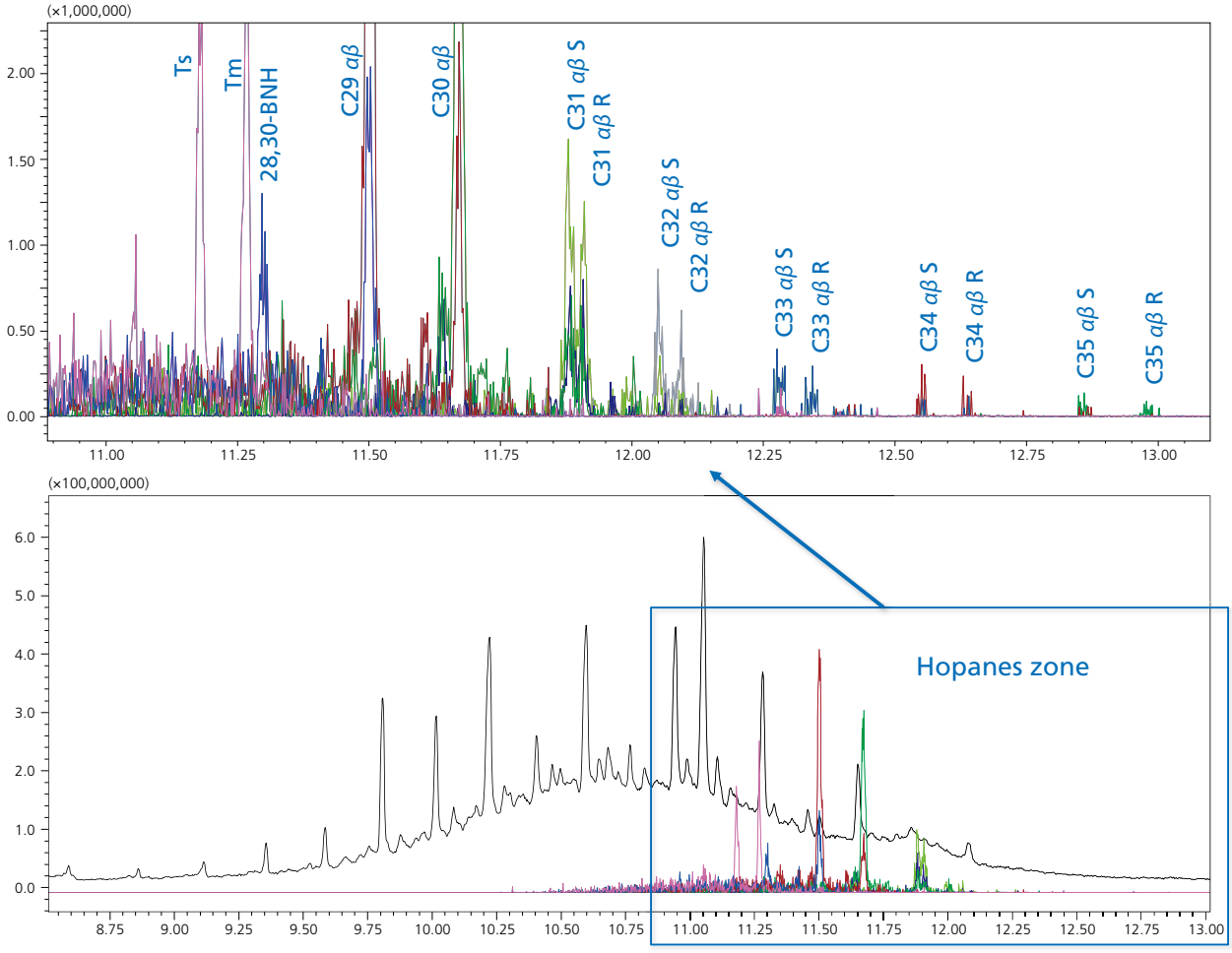

Fig. 3 LC-GC-QqQ MS trace of the OO-1 sample. Full scan and MRM (lower trace); expansion of the hopanes zone (upper trace).

3. Conclusions

The LC-GC-FID/QqQ MS method proposed can perform an accurate determination of both MOSH and MOAH fractions in edible oils. The strategy to exploit two different LC columns mechanisms was successful to retain olefins beyond Anthracene, reducing the uncertainty related to integration error in the final quantification. Moreover, diverting the eluent into two detectors, and in particular the use a powerful QqQ MS, allowed to confirm the petrogenic origin of the MOH contamination by hopanes detection, which are hindered in the MOSH fraction and present at very low concentration. Multilevel information can be obtained from a single chromatographic run, providing qualitative and quantitative information on the widespread MOH contamination.

First Edition: May, 2016

Shimadzu Corporation
www.shimadzu.com/an/

Printed in Japan 3655-05611-500NS