EFFICIENT REMOVAL OF OLEFIN INTERFERENCES BY ON-LINE LIQUID-LIQUID CHROMATOGRAPHY PRIOR TO THE GAS CHROMATOGRAPHY DETERMINATION OF MINERAL OIL CONTAMINATION IN VEGETABLE OILS

Mariosimone Zoccali[1], Giorgia Purcaro[1], Laura Barp[2], Marco Beccaria[1], Danilo Sciarrone[2], Luigi Mondello[1,2]

[1] *Chromaleont S.r.l., Viale Boccetta 70, 98122 Messina, Italy*

[2] *Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali - University of Messina - Polo Annunziata, Viale Annunziata, 98168 Messina, Italy*

mariosimone.zoccali@chromaleont.it

INTRODUCTION

Mineral oils are a complex mixture of compounds, primarily manufactured from crude petroleum through distillation processes and various refining steps, mainly consisting of saturated (MOSH) and aromatic hydrocarbons (MOAH). MOAH can reach 10-30% of the MOSH content and are of potential concern for human health due to their carcinogenic effect. Vegetable oils can be contaminated with mineral oils from different sources. Liquid chromatography coupled to gas chromatography (LC-GC) with flame ionization detection (FID), represents the method of choice for the analysis of these two families. However, it is important to highlight that, the lack of mass spectrometry (MS) detection, due to the frequent controversies about the effective nature of the chromatographic hump, makes these determinations an hard task. Moreover, a correct quantification of the MOAH fraction can be affected by the presence of olefins, particularly squalene and its isomers, which can reach 5000 mg/kg in olive oils. In the present work, a novel on-line LC-GC method with a double detection [FID and triple quadrupole (QqQ) MS] were employed for the determination of hydrocarbon contamination in edible oils. Two different LC columns were coupled in series: a silica column to retain the bulk of the matrix (triglycerides) and to separate MOSH and MOAH, followed by a silver-ion one, which retains olefins allowed to obtain a MOAH hump free of interfering peaks. The QqQ MS system was used to evaluate the presence of hopanes as markers of petrogenic origin of the MOH contamination.

EXPERIMENTAL

Chemicals

A C_7-C_{40} alkanes standard mixture was used to investigate the elution order in different LC columns. A working standard solution, obtained by mixing 5-α-cholestane (Cho) and perylene (Per) at 0.6 mg/mL, undecane (n-C_{11}), cyclohexyl cyclohexane (CyCy), squalene (SQ), 1,3,5-tri-tert-butylbenzene (TBB), pentyl benzene (5B), 1-methyl naphthalene (1MN), 2-methyl naphthalene (2MN) and anthracene (A) at 0.30 mg/mL and n-C_{13} at 0.15 mg/mL in toluene, was used to optimized the method and added to the samples as internal standard.

LC-GC-MS/FID system

All applications were carried out on an 5D Ultra-e (Shimadzu) system (Figure 1).

- LC columns: 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) + 150 × 1 mm ID × 5 µm d_p silver-ion column (lab-made). 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-hexane (0.3 min) and reaching 50% of dichloromethane after 7 min, at 300 and 150 µL/min, respectively. Injection volume: 40 µL. After the transfer of the fractions of interest the columns were washed with dichloromethane and then reconditioned with n-hexane.
- Transfer device: AOC-5000 auto injector with a dedicated dual side-port syringe.
- GC column: the analytical column was an SLB-5ms (30 m × 0.25 mm ID × 0.25 µm *df*). The outlet of the column was connected to a MXT "Y"-Union (Restek); the latter was then linked 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). Carrier gas: helium, initial pressure of 255 kPa (constant linear velocity). The second column was connected to the FID (360° C) and to the MS through two uncoated capillaries. MS conditions: full scan mode, 45-360 m/z mass range; electron ionization (EI) mode; frequency 10 Hz; interface 300° C, ion source 280° C.

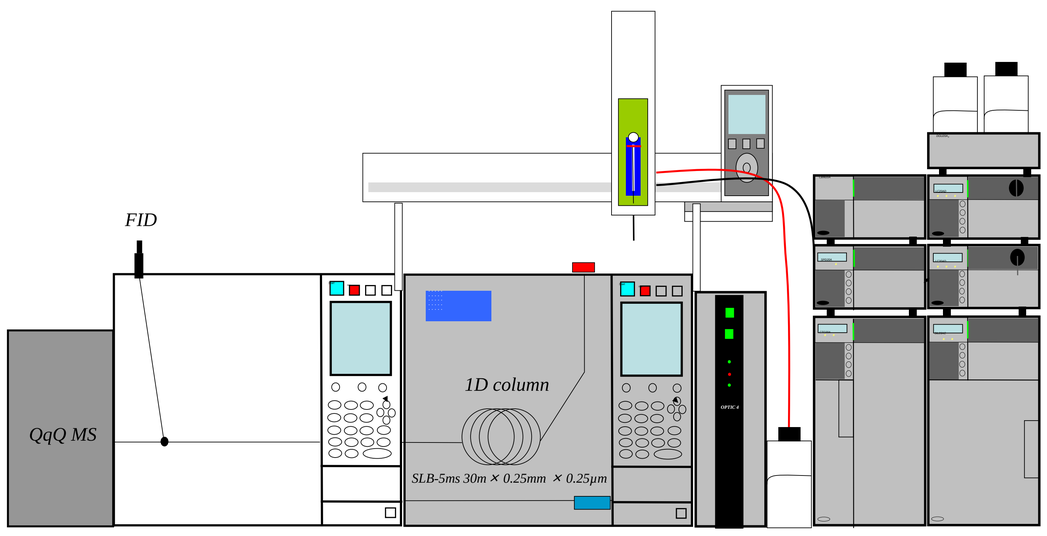


Figure 1. LC-GC-MS/FID system.

METHOD OPTIMIZATION

MOSH MOAH separation

Initially, a silica column coupled in series with a silver ion column were used to obtain the separation of the MOSH and MOAH fractions and the retention of the olefins, but the inverted elution order on the silver column caused a partially co-elution of the MOSH and MOAH fraction previously separated on the silica column. As known the longer n-alkanes eluted before the shorter one in the silica column, while, it was noticed, that the order was reversed in the silica-ion column. Figure 2 shows three consecutive 20 seconds-fractions (Fr) transferred to the GC system: hydrocarbons from about C_{37} to C_{40} are more present in the third fraction (Fr3) indicating that they finished to elute later than hydrocarbons with a lower molecular mass.

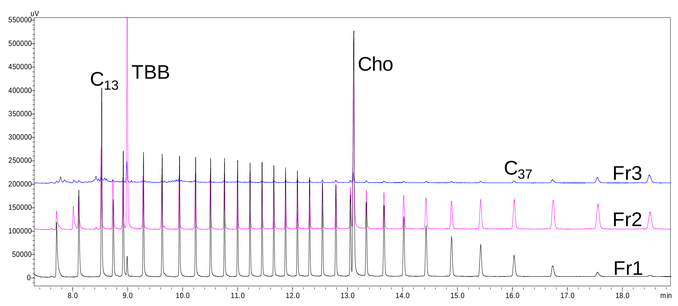


Figure 2. Alkanes elution order on a silver-based LC column.

Thus, to avoid any possible co-elution, a six port valve installed in the LC system was used to isolate the silver ion column (Figure 3). Furthermore, to improve the chromatographic separation between MOSH and MOAH, two silica columns were coupled in series. The valve configuration is as follows: in position 1, the MOSH fraction separated on the silica columns was directly transferred to the GC system, and immediately after, the valve was switched in position 2 transferring the MOAH fraction (including SQ) into the silver-ion column, where the SQ is retained while the aromatic hydrocarbons are eluted and transferred to the GC.

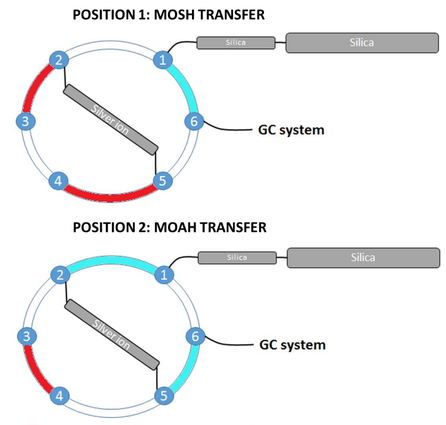


Figure 3. Six port valve configuration.

An extra virgin olive oil (EVOO) spiked with 100 mg/kg (8% MOAH) of vacuum pump oil was analyzed by using just the LC silica column and applying the final configuration (silica + silver-ion columns) above described. In **Figure 4** the MOSH and MOAH chromatograms that point out the efficiency of the silver ion column to retain SQ and its isomers are reported.

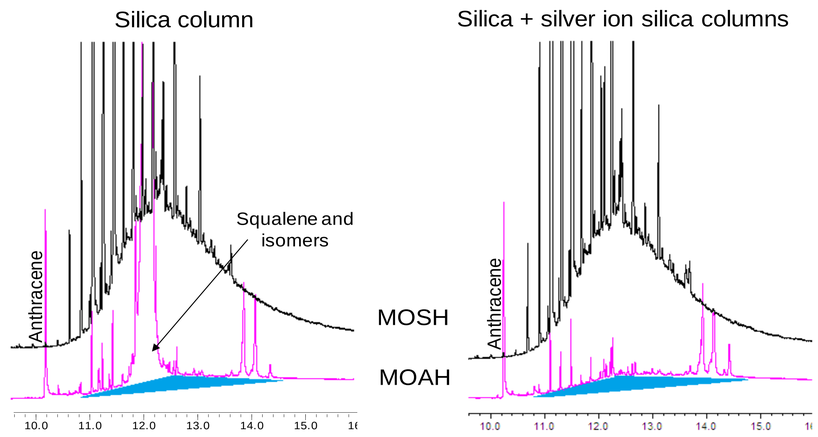
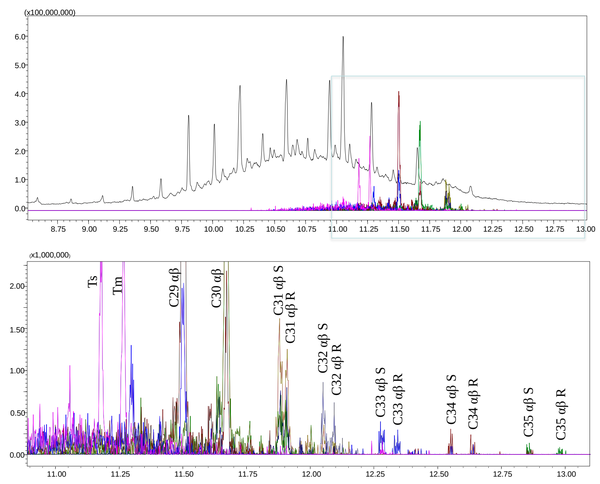


Figure 4. LC-GC-FID chromatograms of spiked EVOO.

Hopanes detection

The use of a split between the FID and the QqQ-MS, diverting about 30% of the effluent to the QqQ MS allowed to confirm, during the same analysis, the petrochemical origin of the contamination [1]. Totally 14 hopanes were evaluated to confirm the petrochemical origin. In Figure 5 is reported the QqQ-MS chromatogram of OO sample and an expansion of the hopane region. Hopanes identification was based on previously published profiles [2, 3].



Compounds	Abbreviations	MRM Transitions
18(H)-22,29,30-trisnorhopane	Ts	370>191
17(H)-22,29,30-trisnorhopane	Tm	370>191
17(H), 21(H)-30-norhopane	C29 αβ	398>191
17(H), 21(H)-hopane	C30 αβ	412>191
17(H), 21(H), 22S-homohopane	C31 αβ S	426>191
17(H), 21(H), 22R-homohopane	C31 αβ R	426>191
17(H), 21(H), 22S-bishomohopane	C32 αβ S	440>191
17(H), 21(H), 22R-bishomohopane	C32 αβ R	440>191
17 (H), 21(H), 22S-trishomohopane	C33 αβ S	454>191
17(H), 21(H), 22R-trishomohopane	C33 αβ R	454>191
17(H), 21 (H), 22S-tetrakishomohopane	C34 αβ S	468>191
17(H),21(H), 22R-tetrakishomohopane	C34 αβ R	468>191
17(H), 21(H), 22S-pentakishomohopane	C35 αβ S	482>191
17(H), 21(H),22R-pentakishomohopane	C35 αβ R	482>191

Figure 5. LC–GC–QqQ-MS trace of an OO. 1) Full scan and MRM; 2) expansion of the hopanes zone with identification and MRM transitions.

CONCLUSION

The use of the silver ion column enabled the retention of olefins (SQ and its isomers) allowing a more precise and reliable integration and quantification of the MOAH fraction. In fact, the presence of the olefin peaks on the top of the MOAH hump leads to a quantification error of about 10%. Furthermore, the use of a dual detection, and in particular of 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

References:

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