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Application News

No. SCA_100_002

Food – MOSH/MOAH

Mineral Oil Residues in Food
Part 2 - Automated Removal of Natural Interferences by Online Epoxidation

▪ Introduction

Mineral oil (MO) residues in food raised public concern due to some elevated concentrations up to several thousand milligrams per kilogram food [1]. Due to the chemical structures two groups of MOs can be differentiated. Mineral oil saturated hydrocarbons (MOSH) consist of linear and branched alkanes, and alkyl-substituted cyclo-alkanes, whilst mineral oil aromatic hydrocarbons (MOAH) include mainly alkyl-substituted polyaromatic hydrocarbons. Technical grades of mineral oils contain aromatic hydrocarbons in a concentration range from 15-35%. The determination of MOSH and MOAH in food can be done by an automated LC-GC-FID system for routine analysis. Unfortunately some food material contain natural occurring olefins (eg. Squalene, sterenes, carotenoids...), which can interfere with the analysis of the aromatic fraction. These interferences can be removed by epoxidation with 3-chloroperbenzoic acid (mCPBA)

▪ Epoxidation with 3-Chloroperbenzoic acid [2]

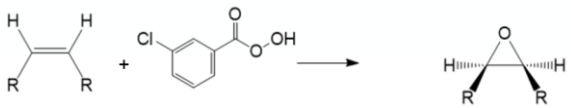

Fig. 1: Reaction scheme of epoxidation

By the epoxidation process of double bonds the polarity is changed in a way that the epoxidized substances show a different retention behavior in a way that they are removed from the MOAH transfer window [3].

▪ System Setup

For the epoxidation and determination of the two mineral oil fractions an online LC-GC –FID system equipped with Agitator and Centrifuge was used. LC is directly connected to two high temperature GC columns with retention gaps which are installed in one GC oven. MOSH and MOAH fractions are separated on a silica gel column using a n-hexane /dichloromethane gradient. The interface between LC and GC is controlled by Axel Semrau LC-GC Chronect interface. After transferring the MOSH fraction on column 1 and MOAH on column 2 the temperature programme is started and both fractions are separated simultaneously and detected by FID. Figure 2 shows a typical LC-Chromatogram with UV-signal in black, pump pressure in green, CH2Cl2 concentration in blue and total flow in purple. Figure 2 shows the LC-GC-FID system.

LC Parameter:

- Shimadzu LC-20AD
- Column: Allure Silica 5 µm (250 × 2.1 mm)
- Gradient: Start with 100% n-Hexane (flow 0.3 ml/min), raised to 35% CH_2Cl_2 within 2 min (hold for 4.20 min), column was backflushed at 6.30 min with 100% CH_2Cl_2 (flow 0.5 ml/min; hold for 9 min) and reconditioned to 100% n-Hexane (flow 0.5 ml/min; hold for 10 min). Flow was decreased afterwards to 0.3 ml/min until next injection.
- UV-Detector: D_2-lamp; 230 nm, 40 ºC cell temperature

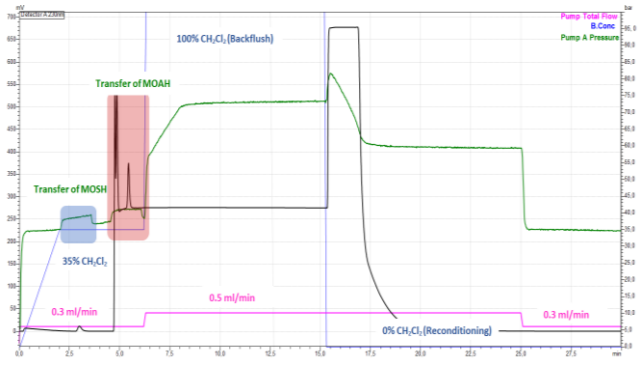

Fig. 2: LC-Chromatogram of Shimadzu LabSolutions

GC Parameter:

- Shimadzu GC-2010 dual FID
- Guard Columns: Restek MXT Siltek (10 m × 0.53 mm id)
- Columns: Restek MXT ®-1 (15 m × 0.25 mm id × 0.1 µm df)
- Carrier gas: Hydrogen (150 kPa analysis pressure; evaporation pressure: 75 kPA MOSH, 80 kPA MOAH
- Temperature program:
- 60 ºC (6 min) @20 ºC/min to 120 ºC (0 min) and followed by 40 ºC/min to 400 ºC (9 min)

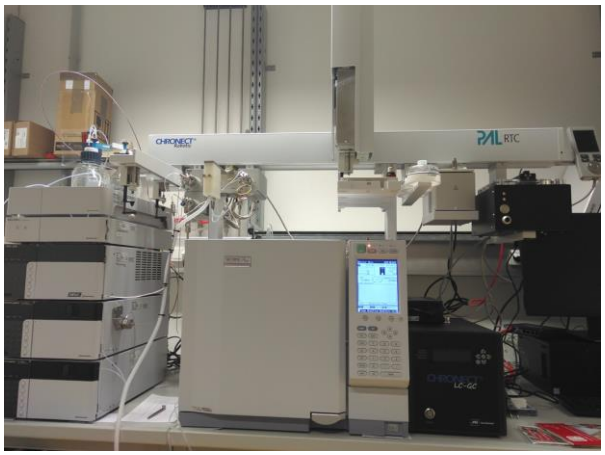

Fig. 3: LC-GC-FID System with automated epoxidation unit

- **Experimental Work**

Sample preparation

300 mg of oil were weight into a 10 ml glass vial. 600 µL of n-Hexane and an internal standard mixture (Restek MOSH/MOAH standard Cat.#: 31070 containing 9 internal standards) were added, the vial closed and shaken. The vial was placed in the PAL RTC autosampler rack of the LC-GC system and a 2 mL autosampler vial with Na_2SO_4 prepared. Epoxidation is done completely automatic: The autosampler PAL RTC transfers the 10 ml vial into the agitator, which is heated to 40 ºC and adds 0.5 ml of 200 mg/L meta-Chloroperbenzoic acid solution in Ethanol. The reaction takes place at 40 ºC for 15 min in the agitator. Afterwards 2 ml of a 100 g/L Na_2SO_3 solution in water and 1 ml of Ethanol are added and the vial shaked at 750 rpm for 1 min. The vial is transported into the centrifuge and centrifugated for 1 min at 2000 rpm. The autosampler PAL RTC transfers the vial back to the tray and 0.5 ml of the upper hexane phase into the prepared 2 ml autosampler vial with Na_2SO_4. After 5 min of drying time an aliquot of 50 µL of this phase are injected into the LC and 450 µL were transferred directly on the pre-columns for the MOSH and MOAH fraction respectively.

Effect of Epoxidation

Figure 4 shows the chromatogram of the MOAH fraction of an extra virgin olive oil sample. The marked area indicates the retention range of the internal standards. Due to the high concentration of squalene the chromatography is severely disturbed in a way that the internal standards cannot be used for quantification. Figure 5 shows a chromatogram of the same sample after successful epoxidation. After the removal of over 90% of squalene the internal standards show perfect peak symmetry.

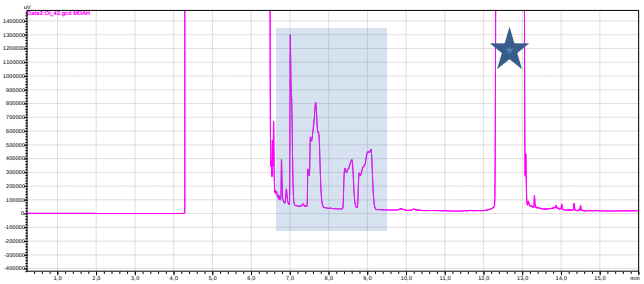

Fig. 4: MOAH Fraction of extra virgin olive oil sample BEFORE epoxidation (squalene is marked)

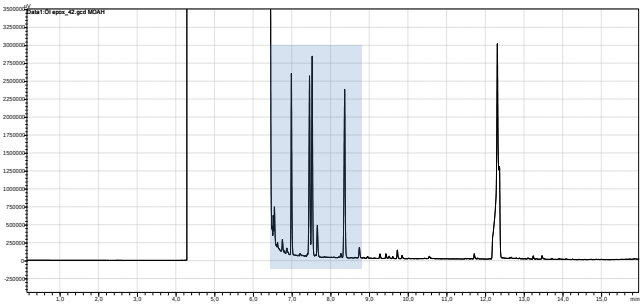

Fig. 4: MOAH Fraction of extra virgin olive oil sample AFTER epoxidation

- **Conclusion**

The new LC-GC-FID system with automated epoxidation enables the removal of natural interferences for routine analysis with high sample throughput.

- **References**

1) EFSA Panel on Contaminants in the Food Chain (CONTAM) Scientific Opinion on Mineral Oil Hydrocarbons in Food DOI: 10.2903/j.efsa.2012.2704

2) M. Biedermann et al., Aromatic Hydrocarbons of Mineral Oil Origin in Foods: Method for Determining the Total Concentration and First Results J. Agric. Food Chem. 2009, 57, 8711–8721

3) M. Nestola et al., Determination of mineral oil aromatic hydrocarbons in edible oils and fats by online liquid chromatography–gas chromatography–flame ionization detection – Evaluation of automated removal strategies for biogenic olefins, Journal of Chromatography A, 1505 (2017) 69–76

Shimadzu Europa GmbH

www.shimadzu.eu