

Application News

No. SCA-100-001

Food – MOSH/MOAH

Mineral Oil Residues in Food Part 1- Dry Food (Flour, Noodles and Rice)

■ 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 oil contain aromatic hydrocarbons in a concentration range from 15-35%. Food grade mineral oils (white oils) can have lower MOAH concentrations. Rice and pasta and other grain based food products including flour from different grain varieties are consumed in high amounts all over the world. The annual rice consumption per capita is over 54 kg per capita [2]. The annual pasta consumption in Italy is 26 kg and approximately 8 kg in Germany [3].

At the moment there are no legal limits in Europe for MOSH/MOAH, but MOSH concentrations up to 2 mg/kg and MOAH levels below 0,5 mg/kg are considered to be acceptable.

■ System Setup

For the determination of the two mineral oil fractions an online LC-GC –FID system was

used. The 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 1 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 Parameters:

Shimadzu LC-20AD solvent delivery pump
Column: Allure Silica 5 µm (250 × 2.1 mm)
Gradient: Start with 100 % n-Hexane (flow 0.3 ml/min), CH₂Cl₂ raised to 35 % within 2 min (hold for 4.20 min), column was flushed at 6.30 min with 100 % CH₂Cl₂ (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: D₂-lamp; 230 nm, 40 °C cell temperature

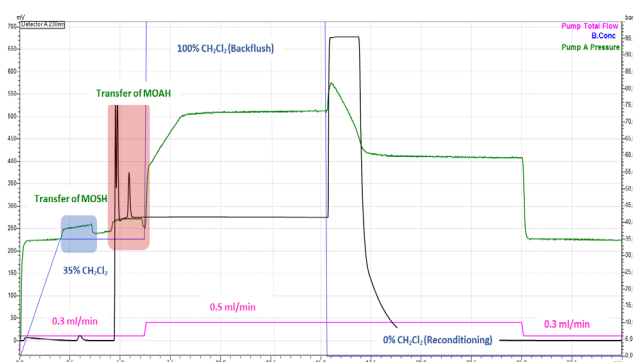


Fig. 1: LC-Chromatogram

GC Parameters:

Shimadzu GC-2010 Plus dual FID

Guard Columns: Restek MXT Siltek (10 m × 0.53 mm id)

Columns: Restek MTX ®-1 (15 m × 0.25 mm id × 0.1 µm df)

Carrier gas: Hydrogen (150 kPa analysis pressure; evaporation pressure: 87 kPa)

MOSH, 85 kPa MOAH

Temperature program:

60 °C (6 min) @ 20 °C/min to 100 °C (0 min) and followed by 35 °C/min to 370 °C (9.29 min)



Fig. 2: LC-GC-FID System

■ Experimental Work

Sample preparation

Depending on the expected mineral oil concentration 1-10 g of homogenized and finely ground samples were used. The sample was extracted in Hexane/Ethanol 1/1 after the addition of an internal standard mixture (Restek MOSH/MOAH standard Cat.#: 31070 containing 9 internal standards) at room temperature for 2 hours under

occasionally shaking the flask. After the extraction water was added and centrifuged. The washing step was repeated a second time. Afterwards the organic layer was dried over anhydrous sodium sulphate and the volume was adjusted to 1 mL in an automated solvent concentrator. The extract was transferred into a 2 mL autosampler vial and put in the autosampler rack of the LC-GC system. Aliquots of 50 µl were injected into the LC. Here the separation into the MOSH- and MOAH fraction is performed. Each fraction has a size of 450 µL and is transferred to the respective channel of the GC for parallel MOSH/MOAH determination.

Quantification

For the quantification of the MOSH fraction the internal standard Bicyclohexyl (CyCy) is used. For the MOAH fraction 2-Methylnaphtahlene (2-MN) is used. All other internal standards are used to ensure no losses of analytes and a good separation between the MOSH and MOAH fraction.

According to a proposed method published by the German Bundesinstitut für Risikobewertung (BfR) quantification is done by integration of the hump for different molecular weight regions. They propose for food contact materials three ranges for the MOSH fraction (C₁₀-C₁₆, C₁₆-C₂₅ and C₂₅-C₃₅) and two ranges for the MOAH fraction (C₁₀-C₂₅, C₂₅-C₃₅). For dry food only the ranges up to C₂₅ are used [4]. Figure 3 shows the MOSH (black trace) and MOAH (purple trace) of a spaghetti sample with a MOSH concentration of 12.7 mg/kg from C₁₆-C₃₅ and the marked regions of C₁₆-C₂₅ (blue) and C₂₅-C₃₅ (red). The internal standards are marked with symbols (black squares internal standards MOSH: C₁₁, Bicyclohexyl, C₁₃ and Cholestane eluting with the MOSH hump, purple stars internal standards MOAH: Pentylbenzene, 1 & 2-Methylnaphthalene, Tri-tert-butylbenzene and Perylene). The rice sample (Figure 3) shows also additional peak in the rear part of the chromatogram. These are naturally occurring odd-numbered alkanes with a chain length of C₂₁ to C₃₅.

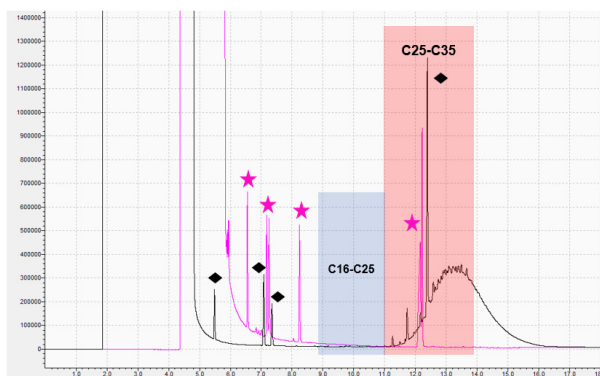


Fig. 3: Spaghetti sample

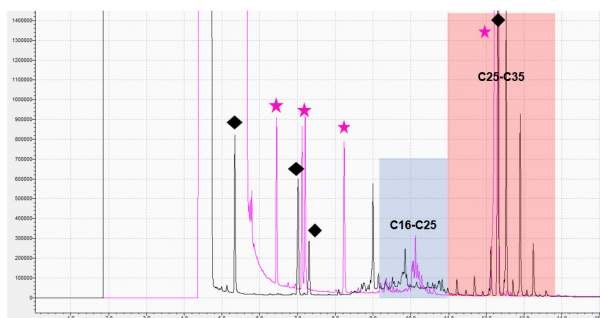


Fig. 4: Rice sample

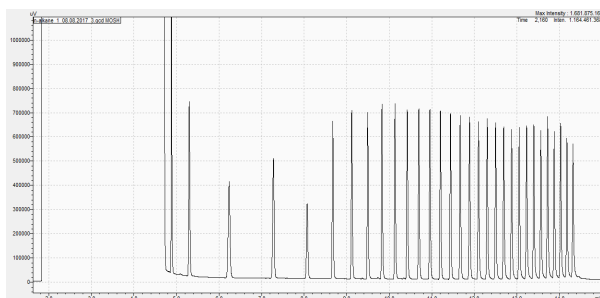


Fig. 5: n-Alkane standard C10-C40 as retention time marker

■ Conclusion

The new LC-GC-FID provides a tool for fast and reliable routine analysis of MOSH and MOAH in dry non fatty food. After the extraction step the samples are analysed fully automated with a high sample through put.

■ 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) Link:
<https://www.statista.com/statistics/256002/global-per-capita-rice-use-since-2000/>
- 3) Link:
<http://www.internationalpasta.org/index.aspx?idsub=118>
- 4) Link:
<http://www.bfr.bund.de/cm/343/bestimmung-von-kohlenwasserstoffen-aus-mineraloel-oder-kunststoffen.pdf>

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]

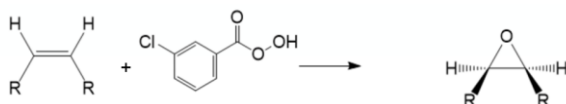


Fig. 1: Reaction scheme of epoxidation

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% CH₂Cl₂ within 2 min (hold for 4.20 min), column was backflushed at 6.30 min with 100% CH₂Cl₂ (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: D₂-lamp; 230 nm, 40 °C cell temperature

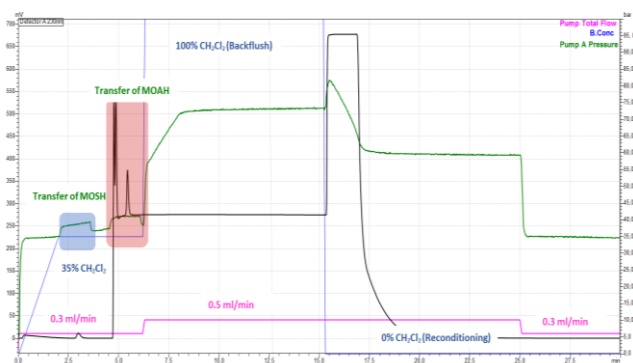


Fig. 2: LC-Chromatogram of Shimadzu LabSolutions

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 °C (6 min) @20 °C/min to 120 °C (0 min) and followed by 40 °C/min to 400 °C (9 min)

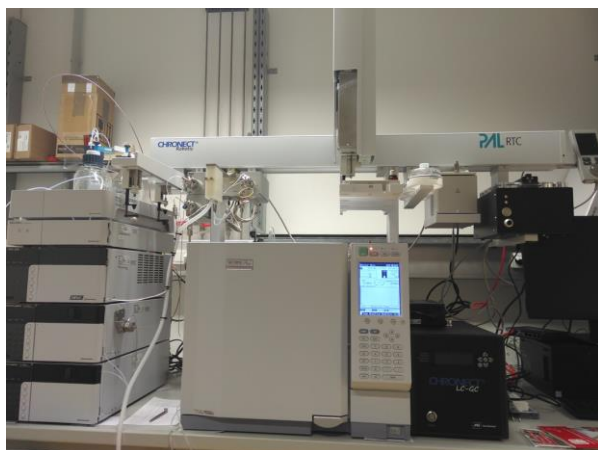


Fig. 3: LC-GC-FID System with automated epoxidation unit

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 Na₂SO₄ prepared. Epoxidation is done completely automatic: The autosampler PAL RTC transfers the 10 ml vial into the agitator, which is heated to 40 °C and adds 0.5 ml of 200 mg/L meta-Chloroperbenzoic acid solution in Ethanol. The reaction takes place at 40 °C for 15 min in the agitator. Afterwards 2 ml of a 100 g/L Na₂SO₃ solution in water and 1 ml of Ethanol are added and the vial shaken 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 Na₂SO₄. 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.

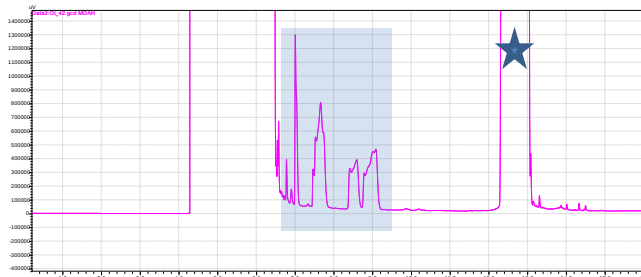


Fig. 4: MOAH Fraction of extra virgin olive oil sample
BEFORE epoxidation (squalene is marked)

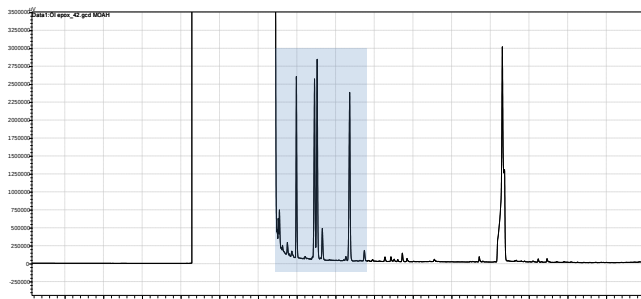


Fig. 4: MOAH Fraction of extra virgin olive oil sample
AFTER epoxidation

■ 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

Application News

No. SCA_100_003

Food – MOSH/MOAH

Mineral Oil Residues in Food Sample Preparation Part 3 Fract and Collect

■ Introduction

Mineral oil residues in food gain a lot of public concern in the recent years. No matter if talking about rice, noodles, olive oil or chocolate, there are numerous examples of mineral oil contaminations in food. At the moment the challenges we face



Figure 1: LC-GC-FID System with automated epoxidation unit

are the ubiquitous presence of mineral oils from raw material to the finished product and the big lack of information concerning composition, health effects, analysis and regulatory limits. Mineral oil hydrocarbons (MOH) are divided into the two groups mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH).

The MOSH consist of branched and unbranched open chain hydrocarbons called paraffines and cyclic hydrocarbons with at least one saturated ring called naphthenes. They can accumulate in the human body and cause granulomas. The MOAH consist of aromatic substance classes, that can be highly alkylated and have 1-4 rings. They

make up 15-30% of the whole mineral oil fraction and contain potential mutagenic and carcinogenic substances. [1]

The determination of MOs in food can be done by an automated LC-GC-FID system for routine analysis which is shown in Figure 1. However, using this method with flame ionisation detection gives only information about the total concentration of MOH, without any further information about the constituents.

The Fract & Collect-tool gives the opportunity to collect MOSH and MOAH fractions after the HPLC fractionation and to use them for further analysis on GC-MS or 2D-Comprehensive GCxGC-MS.

■ System Setup

For determination of whole mineral oil concentration 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.

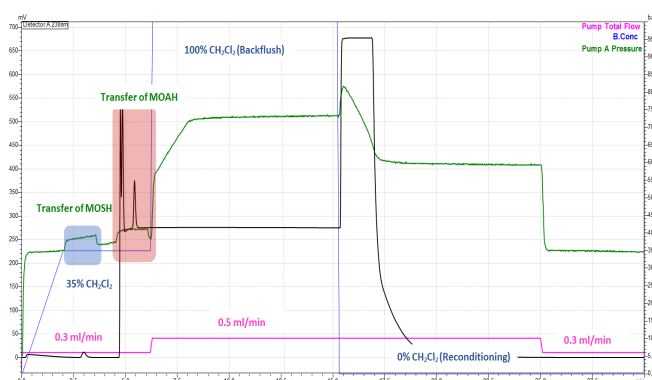


Figure 2: LC Chromatogram

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.

■ LC Parameters

Instrument: 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% CH₂Cl₂ within 2 min (hold for 4.20 min), column was backflushed at 6.30 min with 100% CH₂Cl₂ (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: D₂-lamp; 230 nm, 40 °C cell temperature

■ GC Parameters

Instrument: Shimadzu GC 2010 dual FID
 Columns: Restek MXT ®-1 (15m × 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 °C (6 min) @20 °C/min to 120 °C (0 min) and followed by 40 °C/min to 400 °C (9 min)

■ GCMS Parameters

Instrument: Shimadzu GCMS-TQ8050, Q3 scan, 50-400 amu, 5 scans/s
 Column: 30 m Restek RXi5MS 0.25*0.25
 60 °C (1 min) @5 °C/min to 340 °C (5 min)

■ Fract and Collect

For Fract & Collect only the LC is used. The samples are injected as usual (50 µL on LC) and the two fractions separated on the normal phase column. A switching valve is responsible for the separated transfer of MOSH and MOAH. It can either transfer the two fractions on the GC-Columns or redirect it to the Fract & Collect Tool. The tool, which consists of a transfer line and a needle, collects the fractions in prepared autosampler vials in the rack.

■ Experimental Work

The following chromatogram shows the MOAH fraction of a recycled paper sample. Five fractions were collected as described above, combined and brought to a final volume of 500 µL in an automated solvent concentrator. Aliquots of 1 µL were injected in the GCMS- TQ8050 system.

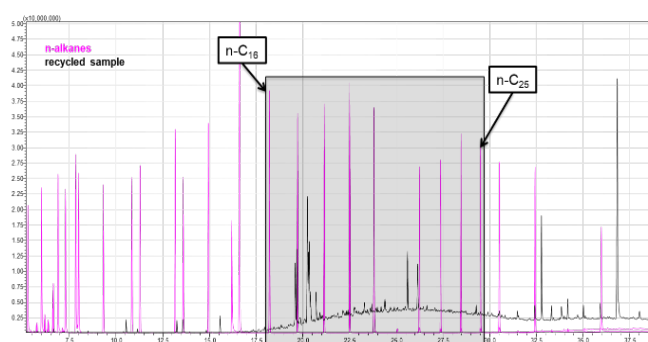


Figure 3: TIC of MOAH extract (black) and standards (purple)

Using extracted ions a more detailed information of the composition of the fractions can be generated.

Figure 4 shows the Total Ion Chromatogram (TIC) and five different ions (m/z 218, 232, 246, 260, 274 & 288). These ions are the molecular peaks of alkylated benzenes with a chain length from C₁₀-C₁₅.

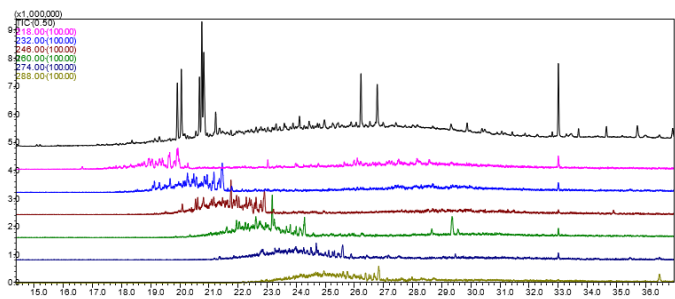


Figure 4: TIC of MOAH and extracted ions for alkylated benzenes

Rice	1.8-160 mg/kg
Fish (related to fat content)	10-1200 mg/kg
Cocoa and chocolate	5-1300 mg/kg
Baby food (packed in paperboard)	up to 33 mg/kg
Products stored in jute bag (rice, coffee, cocoa)	up to 500 mg/kg
Bakery products	up to 2800 mg/kg
Edible oil	up to 6000 mg/kg

Table 1: Found levels of MOHs in food [2]

▪ Conclusion

The new LC-GC-FID with automated MOH analysis and the possibility of collecting pre-separated fractions gives the opportunity for additional analysis of complex sample mixtures using GC-MS or GCxGC-MS.

▪ References

[1] Bundesinstitut für Risikobewertung (BfR), Stellungnahme Nr. 008/2010, 09.12.2010.

[2] EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal, 2012 10(6), 2704-2889.



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Mineral Oil Residues in Food Part 4 Removing Natural Occurring Alkanes

▪ 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 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 like rice or chocolate contain natural occurring (odd numbered) alkanes in the range of C_{23} - C_{33} which can interfere and heavily disturb the analysis of the MOSH fraction. These interferences can be removed by flash chromatography on aluminium oxide columns.

• Removing natural occurring Alkanes with aluminium oxide (Alox)

It has been shown that activated aluminium oxide is feasible to retain long-chain Alkanes with a chain length of $>C_{20}$, while iso-alkanes can pass nearly undisturbed. The retention was the biggest when using n-Hexane as solvent and was destroyed under the presence of polar substances like water. Therefore polar interferences have to be removed e.g. by covering the alox with silica gel (which is not retaining Alkanes).

The retention mechanism is relatively unknown and retention power of the alkanes on the alox is low: only about 1 mg of alkanes is retained by 30 g

of Alox. Furthermore, only the MOSH fraction elutes from the column, the MOAH is retained. Nevertheless it can be a useful tool for decreasing of overloaded samples and to lower detection limits [3,4].

▪ System Setup

The 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, CH_2Cl_2 concentration in blue and total flow in purple. Figure 2 shows the LC-GC-FID system [5,6].



Figure 1: LC-GC online system

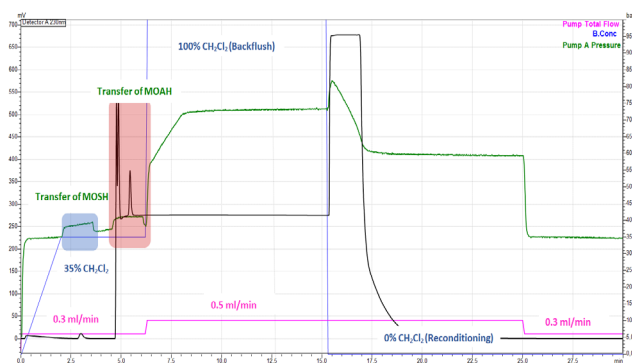


Figure 2: LC- Chromatogram

LC Parameters

Instrument: Shimadzu LC 20AD
Column: Allure Silica 5 μ m (250 $\times$ 2.1 mm)
Gradient: Start with 100% n-Hexane (flow 0,3 ml/min), raised to 35% CH₂Cl₂ within 2 min (hold for 4.20 min), column was backflushed at 6.30 min with 100% CH₂Cl₂ (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: D₂-lamp; 230 nm, 40 °C cell temperature

GC Parameters

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

Experimental Work

Sample preparation

Depending on the expected mineral oil concentration 1-10 g of homogenized and finely ground samples were used. The samples were extracted in Hexane, after the addition of an internal standard mixture (Restek MOSH/MOAH standard Cat.#:31070 containing 9 internal standards) at room temperature for 2 hours or overnight under occasionally shaking the flask. The glass columns with inserted filters were filled with 10 g of aluminium oxide (90, basic, 0.063 mm - 0.2 mm, activated for 16h at 500 °C) and 3 g of silica gel (60, 60 μ m-200 μ m or 70mesh-230mesh respectively, activated for 16h at 40 °C). The columns were washed with 20 ml of n-Hexane prior to loading the sample. The extracts were centrifuged, if necessary, concentrated to a volume of about 1 ml under a stream of nitrogen. About 100 μ L were taken and filled into an autosampler vial with micro insert for analysis of MOAH, the remaining samples was loaded onto the column. The MOSH fraction was eluted with 20 ml of n-Hexane, concentrated in an automated solvent concentrator and transferred into a 2 mL autosampler vial. This was placed in the autosampler rack of the LC-GC system. Aliquots of 50 μ L were injected into the LC and 450 μ L were transferred directly on the pre-columns for the MOSH fraction only.

Example

Figure 3 shows two chromatograms of a MOSH fraction of a rice sample spiked with 5 mg/kg of a mineral oil. Before flash chromatography with Alox n-Alkanes in the range of C₂₅-C₃₅ are present. "False positive" integration would give a MOSH value of 10.8 mg/kg. After flash chromatography the n-alkanes were completely removed and data evaluation is possible without any interferences. The result for the MOSH value is 4.31 mg/kg this time. The MOAH value that was measured prior to flash chromatography gives a concentration of 0.64 mg/kg and results in a total MOH recovery of 4.95 mg/kg of the spiked 5 mg/kg.

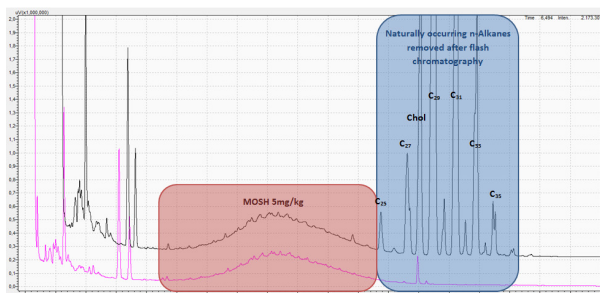


Figure 3: Chromatograms of Rice sample before and after flash chromatography with Alox.

Conclusion

Flash chromatography with aluminium oxide and silica gel gives an important tool for the removal of naturally occurring odd-numbered n-Alkanes.

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] K. Fiselier, D. Fiorini, K. Grob, Activated aluminum oxide selectively retaining long chain n-alkanes. Part I, description of the retention properties, *Analytica Chimica Acta*. 634 (2008) 96-101. doi:10.1016/j.aca.2008.12.007.

[3] K. Fiselier, D. Fiorini, K. Grob, Activated aluminum oxide selectively retaining long chain n-alkanes: Part II. Integration into an on-line high performance liquid chromatography–liquid chromatography–gas chromatography–flame ionization detection method to remove plant paraffins for the determination of mineral paraffins in foods and environmental samples, *Analytica Chimica Acta*. 634 (2008) 102-109. doi:10.1016/j.aca.2008.12.011.

[4] Biedermann, M.; Grob, K. On-line coupled high performance liquid chromatography–gas chromatography for the analysis of contamination by mineral oil. Part 2: Migration from paperboard into dry foods: Interpretation of chromatograms. *Journal of Chromatography A* 2011, 1255, 76-99, doi:10.1016/j.chroma.2012.05.096.

[5] Biedermann, M.; Grob, K. On-line coupled high performance liquid chromatography–gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis. *Journal of Chromatography A* 2011, 1255, 56-75, doi:10.1016/j.chroma.2012.05.095.

Mineral Oil Residues in Food: Part 5 - How to choose the right sample preparation

■ 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 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, sample preparation of the “food-matrix” is complex and difficult.

■ Challenge “Food”- Matrix

The analysis of mineral oil hydrocarbons in food needs for every type of food an adequate sample preparation. The analysis starts with the question, if contaminations only occur on the surface (via migration), or if MOH is also incorporated. Therefore, quantitative extraction of the mineral oil hydrocarbons from the matrix needs varying conditions and strategies. Depending on the matrix, long extraction times are used and extraction efficiency needs to be controlled for every matrix. However, also when extraction is finished, there are several problems. Many food types include a large amount of fat, that needs to be removed prior analysis. Other food types, contain naturally occurring olefins (eg. Squalene, sterenes, carotenoids..), which can interfere with the analysis of the aromatic fraction and need

epoxidation or naturally occurring n-Alkanes that interfere with the MOSH and need to be removed by column chromatography with aluminium oxide.

The following schemes show a summary of possible extraction ways and extract clean up and enrichment for several food types.

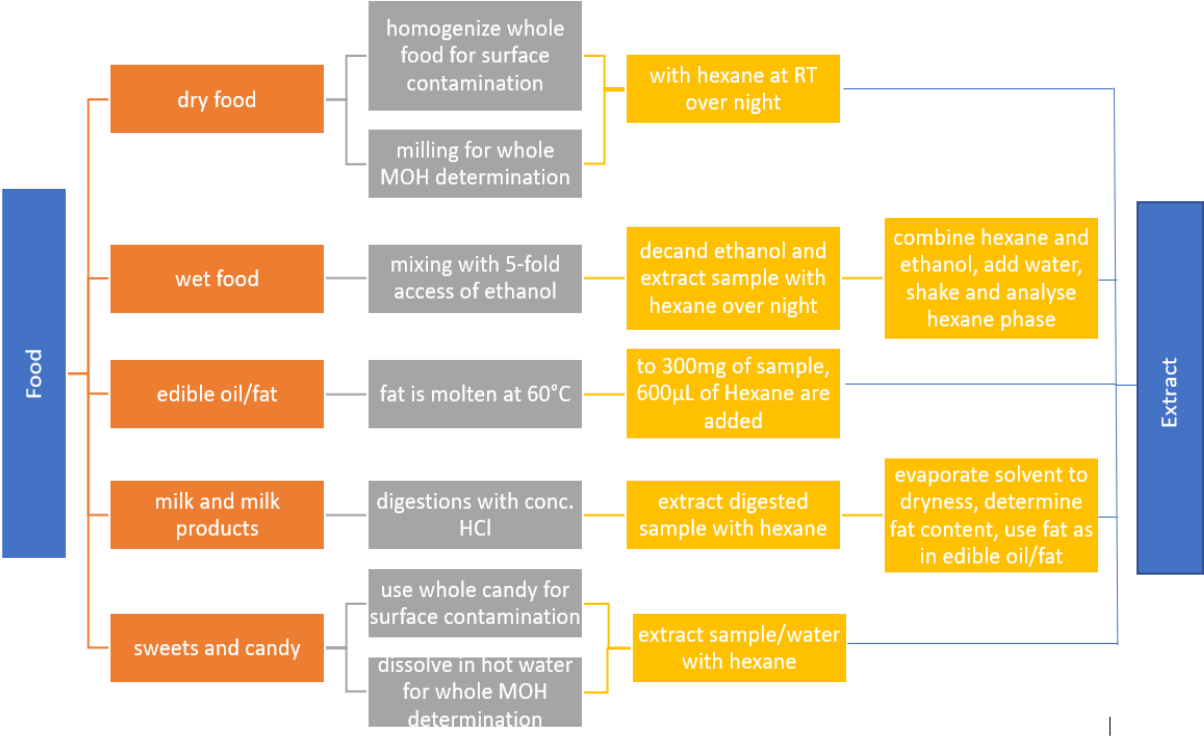
A detailed summary of extraction conditions for different food types can be found in [1,2]. Clean up and enrichment strategies can be found in the Shimadzu application notes #2 and #3 and in [3,4,5].

■ System Setup

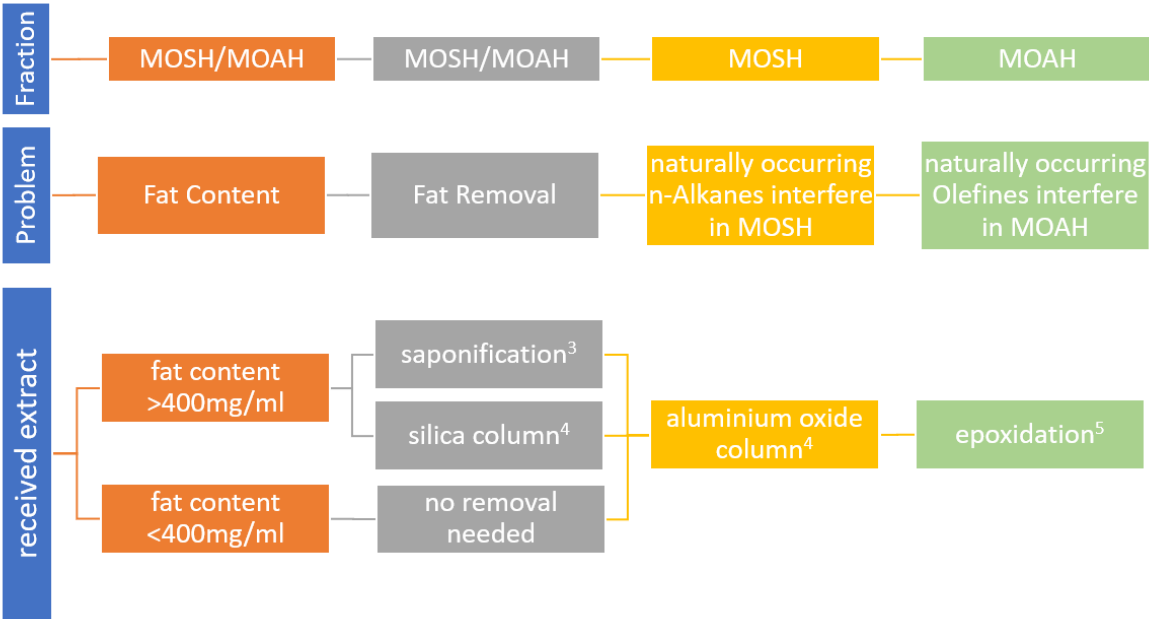
The LC is directly connected to two high temperature GC columns with retention gaps which are installed in one GC oven. MOSH and MOAH fractions are separated on a silica gel programme is started and both fractions are separated simultaneously and detected by FID. Figure 1 shows a typical LC-GC online system based on the Shimadzu LC-20AD solvent delivery pumps, the GC-2030 gas chromatograph and the LC-GC Chronect Interface by Axel Semrau.



Figure 1: LC-GC online system



Scheme 1: Extraction conditions for several food types



Scheme 2: Clean-up of received extract, depending on fraction and interferent; the limit of 400mg/ml refers to an LC column with 20mg fat limit and an injection of 50µL

▪ Conclusion

The analysis of mineral oil hydrocarbons in food is really challenging and needs a wide range of different extraction methods, clean up and enrichment steps.

▪ References

[1] Biedermann M, Grob K. 2012a. On-line coupled high performance liquid chromatography–gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis. J. Chromatogr. A. 1255:56-75.

[2] [BfR] German Federal Institute for Risk Assessment. Messung von Mineralöl – Kohlenwasserstoffen in Lebensmitteln und Verpackungsmaterialien.
<https://mobil.bfr.bund.de/cm/343/messung-von-mineraloel-kohlenwasserstoffen-in-lebensmitteln-und-verpackungsmaterialien.pdf> [14.09.2018]

[3] A. Guinda, A. Lanzón, T. Albi, 1996, 'Differences in Hydrocarbons of Virgin Olive Oils Obtained from Several Olive Varieties', Journal of Agricultural and Food Chemistry, vol. 44, no. 7, pp. 1723-1726.

[4] EN 16995:2017-06: Lebensmittel — Pflanzliche Öle und Lebensmittel auf Basis pflanzlicher Öle — Bestimmung von gesättigten Mineralöl-Kohlenwasserstoffen (MOSH) und aromatischen Mineralöl-Kohlenwasserstoffen (MOAH) mit on-line HPLC-GC-FID.

[5] Marco Nestola, Torsten C. Schmidt, 2017, '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, vol. 1505, pp. 69-76



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