

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

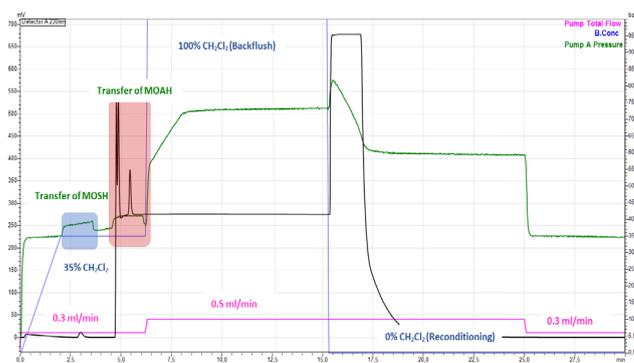


Figure 2: LC- Chromatogram

■ LC Parameters

Instrument: Shimadzu LC 20AD
Column: Allure Silica 5 μm (250 \times 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: 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 $\text{\textcircled{R}}$ -1 (15 m \times 0.25 mm id \times 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)

■ 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 μm -200 μm 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 μL 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 μl were injected into the LC and 450 μL 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 C_{25} - C_{35} 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.

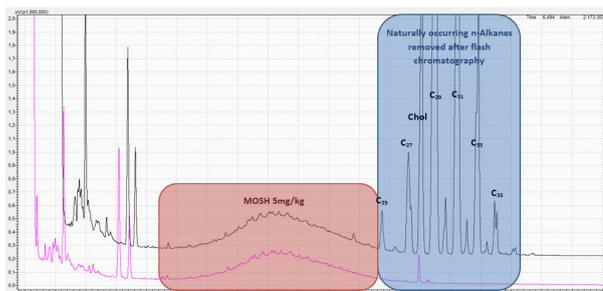


Figure 3: Chromatograms of Rice sample before and after flash chromatography with Alox.

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

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