

Determination of hydrocarbons from mineral oil (MOSH & MOAH) or plastics (POSH & PAO) in packaging materials and dry foodstuffs by solid phase extraction and GC-FID

Remark:

This analytical method was exclusively developed and established to determine mineral oil components in the field of food contact materials. The data listed in this document were jointly prepared by the Kantonales Labor Zurich (KLZH; Official Food Control Authority of the Canton of Zurich) and the National Reference Laboratory for Food Contact Materials at the Federal Institute for Risk Assessment (BfR). The integration cuts of the chromatograms up to n- C25 outlined in this document does not prejudge planned legal regulations on the migration of mineral oil from recycled paper and cardboard to food and has no influence on method development.

1. Definitions

The following hydrocarbons can be determined analytically:

- **Mineral oil saturated hydrocarbons (MOSH):** paraffins (open chain hydrocarbons) and naphthenes (cyclic hydrocarbons). The latter are mostly highly alkylated and originate either directly from mineral oil or are formed during refining by hydrogenation of aromatic rings or other conversion processes.
- **Polyolefin oligomeric saturated hydrocarbons (POSH):** oligomers consisting of saturated hydrocarbons from polyolefins (e.g. polyethylene, polypropylene) and related products, which are able to migrate into foodstuffs.
- **Poly alpha Olefine (PAO):** Isoparaffins with short main chains and long side chains. Precursor substances are either short-chain polyethylenes (e.g. hexene or octene), or olefinic fractions from stream-cracking which were separated into narrow ranges of volatility by distillation. For instance, low molecular weight PAOs are the main constituents of synthetic motor lubricant oil or lubricants in the food sector. Higher molecular PAOs (resins) are used in glues (e.g. hotmelts).

REMARK: With the analytical method described in this test procedure, no reliable chromatographic separation of MOSH, POSH and PAO is possible.

- **Mineral oil aromatic hydrocarbons (MOAH):** highly alkylated mono- and/or poly-aromatic hydrocarbons from mineral oil. In partially hydrogenated mineral oils both, saturated and aromatic rings can be found. Hydrocarbons having at least one aromatic ring are considered as MOAH, even if they predominantly consist of saturated carbons.

2. Principle

The method describes the extraction, pre-separation and quantitative determination of hydrocarbons from mineral oils (MOSH and MOAH) or plastics (POSH and PAO) in packaging materials and dry foodstuffs. Extraction of the hydrocarbons is followed by separation of the MOSH-, POSH- and PAO-containing fraction from the MOAH-containing fraction by a silver nitrate/silica gel solid phase extraction cartridge. The final quantitative determination of both fractions is carried out by GC-FID with large-volume on-column injection.

3. Reagents und Instruments

3.1 Analytical standards

Substance	CAS No.	Abbreviation	Fraction
n-Undecane	1120-21-4	n-C 11	MOSH, POSH, POA
n-Tridecane	629-50-5	n-C 13	
Bicyclohexyl	92-51-3	Cycy	
5 α -Cholestane	481-21-0	Cho	
1-Methylnaphthalene	90-12-0	1 MN	MOAH
2-Methylnaphthalene	91-57-6	2 MN	
1,3,5-Tri-tert-butylbenzene	1460-02-2	TBB	
Perylene	198-55-0	PER	
Pentylbenzene	538-68-1	5 B	

3.2 Chemicals

- Acetone
- Deionized water
- Dichloromethane
- Hexane
- Silica gel 60 (0.063 - 0.200 mm), approx. 300 g in 20 cm crystallizing dish for 24 h at 400 °C in a muffle furnace
- Silver nitrate (pro analysi)
- Toluene

3.3 Instruments and support materials

- 500 mL glas-bottle
- 60 - 70 mL glass test tubes with screw top (PTFE – septum)
- Aluminum foil
- Brown glass GC-vials with glass inserts and cap with PTFE-septum
- Centrifuge (5000 rpm)
- Drying cabinet (maximum temperature 400 °C)
- Gas chromatograph with on-column injector or PTV injector with on-column insert and FID
- Glass cartridges for solid phase extraction (e.g. from 15 mL glass syringes)
- Glass fiber frits
- Glass Pasteur pipettes
- Glas-syringe: 10 μ L und 0.5 mL,
- Graduated pipettes; glass; various capacities
- Mill (with metal container and -lid)
- Pear-shaped flasks 10 mL; 25 mL

- Pipettors with filter
- Precision balance, analytical balance
- Test tubes with ground glass joint
- Thermometer
- Vacuum rotary evaporator
- Various spatulas and funnels
- Volumetric flasks 10 mL; 20 mL
- Volumetric pipettes, glass; various capacities

3.4 Minimization and control of hydrocarbon blank values

The entire workflow has to be controlled for possible contamination. Blanks have to be carried along in the daily routine in order to prevent contamination during sample processing. In hydrocarbon analysis, the use of hand lotion (by lab staff), grease from ground glass joints, rotary evaporators and syringes have been identified as most important sources. Various laboratory glassware (e.g. GC glass vials or Pasteur pipettes) are commonly packed in cardboard packages or polyolefin foils. Thus, laboratory glassware packed in these materials may have to be baked out prior use.

Testing for contamination of glass ware may use the same solvent to rinse a series of pieces (e.g. vials or pipettes), which enhances the sensitivity and the chance of detecting incidentally contaminated pieces.

In order to prevent subsequent hydrocarbon contaminations, it has to be assured that previously baked out laboratory glassware is kept protected (e.g. packed in aluminium foil) until use.

Duplicate measurements should not be processed simultaneously, but rather in two consecutive series to prevent identical contaminations.

The following cleaning procedure provides an alternative way to obtain non-contaminated laboratory glassware:

1st rinse: acetone/ water (9/1; v/v)

2nd rinse: acetone

3rd rinse: hexane

Subsequently, the treated laboratory glassware is kept in beakers covered with baked out aluminium foil.

3.5 Standard Solutions

3.5.1 Preparation of stock solutions

3.5.1.1 Stock solution A

Exactly 120 mg n-undecane and 60 mg n-tridecane are weighed in a 20 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.1.2 Stock solution B

Exactly 120 mg 5 α -cholestane is weighed in a 10 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.1.3 Stock solution C

Exactly 120 mg bicyclohexyl is weighed in a 20 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.1.4 Stock solution D

Exactly 120 mg of each, 1-methylnaphthalene, 2-methylnaphthalene and pentylbenzene are weighed in a 20 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.1.5 Stock solution E

Exactly 120 mg TBB is weighed in a 20 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.1.6 Stock solution F

Exactly 24 mg perylene is weighed in a 20 mL volumetric flask, dissolved in toluene and filled to the marking.

3.5.2 Preparation of the internal standard – mix

Initially, exactly 5 mL of stock solution F (item 3.5.1.6) are transferred in a 10 mL volumetric flask. Subsequently, exactly 0.5 mL of each of the stock solutions A – E (items 3.5.1.1 - 3.5.1.5) are pipetted into the same volumetric flask and filled with toluene to the marking (between pipetting the respective stock solutions, the glass syringe has to be flushed 5 times using toluene!).

Remark 1: With dry storage in darkness and at cool temperature, the solution can be stored for about 6 months.

3.6 Solvents for sample extraction

3.6.1 Extraction solvent for paper and cardboard samples

A solution of ethanol and hexane with a mixing ratio of 1:1 (v/v) is prepared.

3.6.2 Extraction solvent for foodstuff samples

For foodstuff samples, the extraction is carried out with hexane.

3.7 Eluents for sample prepreparation

3.7.1 Eluent for solid phase extraction (Eluent A)

Hexane is used as eluent A. It serves for the conditioning of the solid phase cartridge and for the elution of the MOSH fraction.

3.7.2 Eluent for solid phase extraction (Eluent B)

Using a volumetric pipette, 20 mL dichloromethane and 5 mL toluene are transferred into a 100 mL graduated cylinder and filled up with hexane. This solution has to be prepared fresh on a daily base. Eluent B is used for the elution of the MOAH fraction.

3.7.3 Preparation of a 0.3 % silver nitrate/silica gel mixture

For a column packed with 0.3 % silver nitrate/silica gel, a blend of highly activated silica gel with silver-nitrate-coated silica gel is needed. Hence, in a 500 mL glass bottle (wrapped with aluminum foil) a mixture of 66 g silica gel (previously baked out at 400 °C), and 33 g of a 1% silver nitrate/silica gel mixture is homogenized using a head-over-heads shaker for 12 hours.

3.7.4 Coating of silica gel with 1% silver nitrate

In 50 mL deionized water, 0.5 g silver nitrate is dissolved and the solution transferred to a round bottom flask (due to the light sensitivity of silver nitrate wrapped with aluminium foil or made out of brown glass) containing 49.5 g silica gel. Subsequently, the water is evaporated initially for 1 hour at 70 °C, followed by 1 hour at 80 °C and 12 hours at 90 °C in a drying cabinet (after this process the silica gel has to be completely dry).

Remark 1: With dry storage in darkness and at ambient temperature, the 1 % silver nitrate/silica gel can be stored for about 1 year. The 0.3% silver nitrate/silica gel mixture is only stable for approximately 14 days because of the limited stability of the activated silica gel.

4. Procedure

4.1 Sample preparation

4.1.1 Paper and board

A representative sample of the paper or board is cut to pieces of no more than 2 cm edge length, followed by homogenisation in an adequate inert glass or metal vessel. If cardboard packaging (e.g. boxes) is investigated, the representative sample should not include glued parts (e.g. hotmelts), as these often consist of hydrocarbons of high molecular mass disturbing GC analysis.

2 g (\pm 0.1 g) of the homogenised paper or board sample is weighted into a 70 mL screw cap glass test tube (with PTFE septum). 20 μ L internal standard mix (item 3.5.2) and 10 mL ethanol/hexane (1:1; v/v) (item 3.6.1) are added. The tube is vigorously shaken, then allowed to stand at room temperature for 2 hours. Prior to sampling the extract, the tube is agitated over again. In order to remove the ethanol, approximately 4 mL of the extract are taken and shaken with 10 mL water. Finally, an aliquot of the supernatant hexane phase is taken for separation on a solid phase extraction cartridge.

4.1.2 Dry foodstuffs

The entire foodstuff sample is homogenised in an inert glass or metal vessel. If required by the composition of the foodstuffs sample (e.g. pasta, pastry, cereals, chocolate), an aliquot of the sample may be ground initially. 10 g (\pm 0.1 g) of the homogenised sample is weighed into a centrifuge tube with PTFE-lined screw cap. After spiking with 10 μ L internal standard mix (item 3.5.2), 10 mL hexane is added.

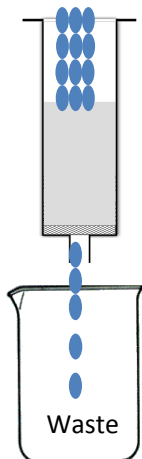
The sample is vigorously shaken and allowed to stand for 12 hours. Prior to withdrawal of the extract, the mixture is shaken once more. In order to separate the extract, the sample is allowed to settle or centrifuged. Finally, an aliquot of the supernatant hexane phase is taken for separation on the solid phase extraction cartridge.

4.2 Solid phase extraction procedure

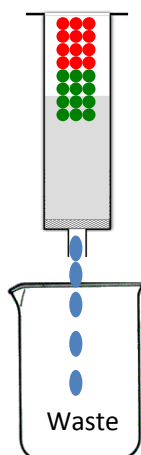
After insertion of a fitting glass fibre frit (alternatively baked out glass wool), the glass cartridge (chromatography column) is baked out in a drying cabinet at 400°C for 24 hours. In order to fill the glass cartridge, it is placed in a high-walled beaker lined with aluminium foil. Exactly 3 g of the 0.3 % silver nitrate/silica gel mixture (Item 3.7.3) is weighted into the glass cartridge and slightly compacted by tapping the cartridge.

The subsequent work flow for separation of the MOSH from the MOAH fraction is illustrated in the following figures.

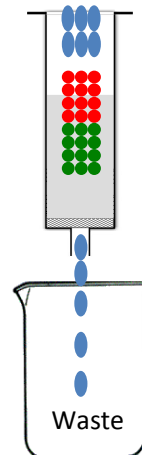
Step 1
Conditioning
10 mL eluent A
(If necessary 2 x 5 mL)



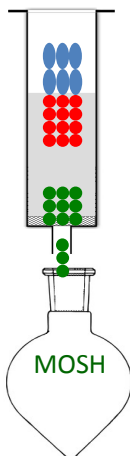
Step 2
Loading the sample
1 mL for food extracts
0.2 mL for paper/board
extracts



Step 3
Elution of dead volume
2 mL eluent A

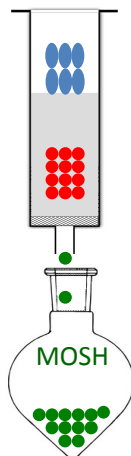


Step 4
Elution of MOSH fraction
4 mL eluent A



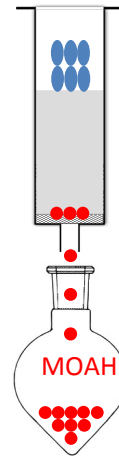
10 mL pear-shaped flask

Step 5
Elution of MOSH fraction
2 mL eluent B



10 mL pear-shaped flask

Step 6
Elution of MOAH fraction
12 mL eluent B
(If necessary 2 x 6 mL)



25 mL pear-shaped flask

4.3 Enrichment of the respective MOSH- and MOAH-fraction using a vacuum rotary evaporator

To the MOSH fraction, 270 µL toluene is added that has previously been purified on a 0.3 % silver nitrate/silica gel column. Then the MOSH and the MOAH fractions are concentrated to a volume of 250-300 µL using a rotary evaporator.

For a water bath set to 55 °C the following conditions were found suitable:

	Time [minutes]	Vacuum [mbar]
Step 1	3	520 – 530
Step 2	3 – 4	350 – 360

These settings have to be verified for each vacuum rotary evaporator and adjusted if necessary

To prevent backflow of condensate at the end of the evaporation process, it is recommended to install a PTFE or glass tube from the entrance of the air ventilation to slightly above the pear-shaped flask.

Cleaning of the rotary evaporator is performed by evaporating hexane on a daily base. Then the blank is tested by the evaporation of a mixture of 6 mL hexane and 270 µL toluene.

The MOSH and MOAH fractions, concentrated to a final volume of 250-300 µL, are transferred to GC autosampler vials using baked out glass Pasteur pipettes. If necessary, these samples can be temporarily stored in a freezer.

4.4 GC conditions

4.4.1 Chromatographic conditions

Initial temperature: 75 °C

Isothermal time: 9.0 minutes

Temperature program:

Rate	Temperature	Isothermal time
22 °C/min	240 °C	0.0 min
30 °C/min	380 °C	12.0 min

Injector: Cool on-column

Injection volume: 40 µL

Injection velocity: slow

Inlet pressure: 60 kPa for 4 minutes followed by 35 kPa constant

Carrier gas: Hydrogen

Mode: Ramp pressure

4.4.2 Separation system

Uncoated pre-column

Raw or deactivated fused silica
Length: 7 m
Inner diameter: 0.53 mm

Separation column

Stationary phase: 100% dimethyl polysiloxane
Length: 10-15 m
Inner diameter: 320 µm
Film thickness 0.1 µm
Initial flow: 2.8 mL/min
Average velocity: 66 cm/sec

Detector settings – FID:

Temperature: 365 °C
Hydrogen: 40 mL/min
Air flow rate: 450 mL/min
Flow mode: Constant makeup
Makeup flow rate: 45 mL/min
Makeup gas: Nitrogen

Remark 1: The uncoated pre-column must be of low retention power, as can be verified by the absence of peak broadening at elevated oven temperatures, e.g. for a series of n-alkanes.

Remark 2: High temperature separation columns with low bleed are preferred.

4.4.3 Data system

Desirable features for the software

- Integration of broad humps of unresolved components with vertical cuts
- Subtraction of peaks to be defined by the operator
- Compensation for baseline drifts
- Free establishment of a baseline

4.4.4 Measurement procedure

Gas chromatographic conditions have to be identical when measuring blanks, sample extracts, hydrocarbon standard solutions and controls.

A solvent mixture of equal composition to the samples (hexane/toluene) has to be included in each sample schedule. The resultant chromatogram is used to correct the blank, sample extracts, hydrocarbon standard solutions and control chromatograms with regards to column bleed prior to integration.

5. Quality control

5.1.1 Internal standards

The internal standards have two functions: measurement of the target material and verification of method performance.

5.1.1.1 MOSH and POSH fraction

The following standards are relevant for the MOSH and POSH analysis:

- Bicyclohexyl (Cycy) is the main internal standard. It does not occur in relevant amounts in mineral oils and polyolefins.
- n-Tridecane (n-C13) ensures the separation from Cycy: coeluted n-C13 from the sample would increase the peak area for Cycy and, hence, result in too low concentrations calculated for the target material. This separation tends to be incomplete for polysiloxanes containing 5 % phenyl as stationary phase. Further, n-C13 helps the safe recognition of Cycy, as n-C13 and Cycy form a characteristic pair. n-C13 is added at half the concentration to rule out that the wrong peak is taken for Cycy.
- The even more volatile n-undecane (n-C11) serves as a guard: if volatiles were lost during sample evaporation or injection, the losses would be higher for n-C11 than for Cycy. Hence, the peak area of n-C11 should not be significantly reduced compared to Cycy. However, the area of Cycy may also be increased due to co-elution with a sample component.
- For samples without an overcrowded region around n-C28, also cholestane (Cho) can be used for verification of the Cycy area, but Cho primarily serves the verification of the separation between the MOSH and MOAH.

5.1.1.2 MOAH-fraction

The following standards are relevant for the MOAH analysis:

- 1- and 2-methylnaphthalene (1-MN and 2-MN) are the main internal standards added at the same concentration. They are easily recognized as a closely eluted pair. They should have identical areas. Otherwise, the larger peak is co-eluted with a sample component, i.e. the smaller peak should be used for calculation.
- Pentylbenzene (5B) is utilized as a guard for loss of volatile substances (analogous to n-C11).
- 1,3,5-Tri-tert-butylbenzene (TBB) assures the MOSH/MOAH separation (start of the MOAH-fraction).
- Perylene marks the ending of the MOAH fraction and assures the retention strength of the liquid chromatography.

5.1.2 Quality control sample

For analytical quality control purposes, a control solution is inserted randomly into the sequence queue and results are recorded (e.g. in a control chart). Additionally, it is

recommended to analyze appropriate certified reference substances or internal control samples in regular intervals.

6. Integration of the chromatograms

A substantial part of the integration, such as the positioning of the baseline and the integration of peaks to be subtracted, has to be carried out manually. It may be preceded by the following steps:

1. The baseline from a blank chromatogram is transferred into the chromatogram of a sample (manually or by means of suitable software). Ideally, a horizontal line can be placed to the lowest point in the chromatogram, either before or after the elution of the MOSH/MOAH fraction.
2. Defining the cuts according to molecular mass ranges:
 - a. MOSH/POSH Chromatogram
 - i. From n-C10 (start of peak) to n-C16 (end of peak)
 - ii. Packaging material: from n-C16 (end of peak) to n-C25 (end of peak);
Food: from n-C16 (end of peak) up to n-C25 (end of peak)
 - iii. Packaging material: from n-C25 to n-C35
 - b. MOAH Chromatogram
 - i. Packaging material: from n-C10 (start of peak) to n-C25 (end of peak) (retention times from MOSH/POSH chromatogram);
Food: from n-C10 up to n-C25 (end of peak)
 - ii. Packaging material: from n-C25 to n-C35
3. Integration of the total areas by mass ranges.
4. Integrate and subtract shoulder peaks from components naturally occurring in foodstuffs and components not belonging to the MOAH-fraction (e.g. diisopropylnaphthalene; DIPN) and subtract these from the total area.
5. Identify, integrate and subtract internal standards. Verify method performance (comparison of the standard areas ratio).
6. Calculate concentrations using the internal standard. As the FID response of the MOSH/POSH and of the MOAH is approximately equal and the FID response is linear (has to be verified for each GC-FID by means of calibration), no response factors are applied.

Remark 1: Chromatograms should be visually verified for adequate integration. Cuts introduced for integration should be visible.

7. Calculation und data analysis

7.1 Calculation in the paper and board

Internal standard - mix (item 3.5.2) 20.0 µl

Weighted paper sample (item 4.1.1) 2.00 g

6.00 µg Internal Standard within 2.00 g paper and board equates to 3.00 ppm

Internal Standard MOAH: e.g. 1-MN MOSH: Cycy

$$\text{Conc. Paper}_{\text{MOSH or MOAH}} (\text{ppm}) = \frac{(\text{Area}_{\text{MOSH or MOAH}} \cdot 3 \text{ ppm})}{\text{Area}_{\text{Internal-Standard}}}$$

7.2 Calculation in food

Internal standard – mix (item 3.5.2) 10.0 µl

Weighted food sample (item 4.1.2) 10.0 g

3.00 µg Internal Standard within 10.0 g food equates to 0.30 ppm

Internal Standard MOAH: e.g. 1-MN MOSH: Cycy

$$\text{Conc. Food}_{\text{MOSH or MOAH}} (\text{ppm}) = \frac{(\text{Area}_{\text{MOSH or MOAH}} \cdot 0.3 \text{ ppm})}{\text{Area}_{\text{Internal-Standard}}}$$

8. Test report

The test report has to include at least the following information:

- Reference to the extraction and clean-up procedures employed.
- Complete characterization of the sample.
- Analytical results
- Remark on the occurrence of low or high boiling substances in the chromatogram.
- All information, optional or not defined within this document, as well as all factors able to influence the results.