



Comparison of carrier gases for the separation and quantification of mineral oil hydrocarbon (MOH) fractions using online coupled high performance liquid chromatography-gas chromatography-flame ionisation detection

Matthias Groschke^{a,b,*}, Roland Becker^b

^a Axel Semrau GmbH, Stefansbecke 42, 45549 Sprockhövel, Germany

^b Bundesanstalt für Materialforschung und -prüfung (BAM), Unter den Eichen 87, 12205 Berlin, Germany

ARTICLE INFO

Keywords:

Mineral oil hydrocarbons
Nitrogen carrier gas
Food
Vegetable oil
Infant formula

ABSTRACT

On-line coupled high performance liquid chromatography-gas chromatography-flame ionisation detection (HPLC-GC-FID) was used to compare the effect of hydrogen, helium and nitrogen as carrier gases on the chromatographic characteristics for the quantification of mineral oil hydrocarbon (MOH) traces in food related matrices. After optimisation of chromatographic parameters nitrogen carrier gas exhibited characteristics equivalent to hydrogen and helium regarding requirements set by current guidelines and standardisation such as linear range, quantification limit and carry over. Though nitrogen expectedly led to greater peak widths, all required separations of standard compounds were sufficient and humps of saturated mineral oil hydrocarbons (MOSH) and aromatic mineral oil hydrocarbons (MOAH) were appropriate to enable quantitation similar to situations where hydrogen or helium had been used. Slightly increased peak widths of individual hump components did not affect shapes and widths of the MOSH and MOAH humps were not significantly affected by the use of nitrogen as carrier gas. Notably, nitrogen carrier gas led to less solvent peak tailing and smaller baseline offset. Overall, nitrogen may be regarded as viable alternative to hydrogen or helium and may even extend the range of quantifiable compounds to highly volatile hydrocarbon eluting directly after the solvent peak.

1. Introduction

The occurrence of mineral oil hydrocarbons (MOH) traces in food and especially in edible oils and fats has raised public concern [1]. Hydrocarbons of petrogenic origin are composed of varying ratios of saturated mineral oil hydrocarbons (MOSH) and aromatic mineral oil hydrocarbons (MOAH). The MOSH group consists of numerous mostly branched but also unbranched open-chain hydrocarbons, also named paraffins, and of cycloalkanes with varying alkylation patterns, the so-called naphthenes [2,3]. MOAH comprise aromatic hydrocarbons with one or more aromatic ring systems which are nearly exclusively alkylated [2,4]. MOH sources of relevance include depending on the food matrix printing inks released from recycled packaging, as well as lubricating oils and greases [5–9] either used as processing aids or present as general background contamination in the environment [10]. In cases, the determination of the respective contamination origin may pose a challenge [10]. MOH tend to accumulate in the human body [5–7] and the MOAH fraction is suspected to display toxicity [11].

Currently, there are no legal limits for MOH in foodstuff but recommendations have been issued by the European Food Safety Agency and the German Federal Institute for risk assessment (BfR) to minimise mineral oil residues in foods [6,8] and the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) of the European Commission [12]. The SCoPAFF recommendations are expected to be adopted as official regulation. MOH quantification is meanwhile done routinely using automated liquid chromatography-gas chromatography-flame ionisation detection (LC-GC-FID) procedures including integrated sample preparation for the epoxidation of interfering natural olefins and aluminium oxide treatment for the removal of biogenic n-alkanes, enabling high sample-throughput at reduced contamination risk [13]. Standardisation and improvement of sample preparation are currently ongoing [14–17].

MOSH and MOAH are typically separated by LC on a normal phase and then either transferred consecutively onto the same short (10–15 m) GC column with a thin film (0.1–0.25 μm) via on-column-type interfacing [2,10,18,19,20] or separately onto two similar column

* Corresponding author.

E-mail address: groschke@axelsemrau.de (M. Groschke).

<https://doi.org/10.1016/j.chroma.2024.464946>

Received 16 February 2024; Received in revised form 22 April 2024; Accepted 24 April 2024

Available online 28 April 2024

0021-9673/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

configurations. Accordingly, either one or two flame ionisation detectors are employed. Though a number of alternative LC-GC interfacing concepts involving programmed temperature vaporising (PTV) have been described [21], commercial laboratories apply exclusively the standardised procedure according to EN 16995 [14] and ISO/DIS 20122 [15]. While some workers in the field use hydrogen as carrier gas [13, 17] as suggested by EN 16995 [14] and that is considered superior due to the higher gas velocity in the precolumn [13], others, including some routine laboratories use helium [10,16,22], especially if FID is used in parallel to MS detection [23]. Instead, nitrogen is generally considered as a poor carrier gas. According to the van Deemter equation nitrogen has a low optimum of linear velocity which tends to increase the run time for sufficient separations and apparently has never been considered as carrier gas for LC-GC-FID analysis of MOSH/MOAH traces in the literature. Cost benefit considerations triggered the quest for a investigation of nitrogen as alternative to the usual carrier gases for the LC-GC-FID quantification of MOSH and MOAH with sufficient accuracy and sensitivity. For this purpose, neat mineral oils, (highly refined mineral oil, gear oil, engine oil with significant amounts of MOAH, two coconut oils and two infant formula samples each displaying MOSH/MOAH trace concentrations of practical relevance were employed. GC parameters were optimised for each carrier gas and the separation and quantification characteristics were compared and are discussed in regard to practical implications.

2. Experimental

2.1. Chemicals

The reference standard (Lot#AO165807) used for MOSH/MOAH quantification (internal standard, IS) containing nine compounds in toluene was purchased from Restek (Germany): n-undecane (C11), 300 µg/mL, n-tridecane (C13), 150 µg/mL, bicyclohexyl (Cycy), 300 µg/mL, cholestane (Cho), 600 µg/mL, 1-methylnaphthalene (1-MN), 300 µg/mL, 2-methylnaphthalene (2-MN), 300 µg/mL, n-pentylbenzene (5B), 300 µg/mL, perylene (PER), 600 µg/mL, 1,3,5-tri-tert-butylbenzene (TBB), 300 µg/mL. The MOSH/MOAH retention time standard (Lot #A0166787) containing ten compounds in cyclohexane (100 µg/mL) was purchased from Restek (Germany): n-decane (C10), n-undecane (C11), n-tridecane (C13), n-hexadecane (C16), n-eicosane (C20), n-tetracosane (C24), n-pentacosane (C25), n-pentatriacontane (C35), n-Tetracontane (C40), n-pentacontane (C50). n-Hexane and dichloromethane (SupraSolv®) were from Merck, Darmstadt (Germany). Ethanol (>99 %) was from VWR Germany. KOH (p.a.) was from Merck, anhydrous Na₂SO₄ (>99 %), anhydrous Na₂S₂O₃ (p.a., >98 %), NaHCO₃ >99.7 % and 3-chloroperbenzoic acid (mCPBA, > 77 %) were from Sigma-Aldrich, Germany.

Table 1

Optimised chromatographic conditions, resulting baseline drifts and separation characteristics of critical pairs in the internal standard.

| Gas ^a | Oven program | Baseline solvent current offset ^b [pA] | | | Resolution of critical pairs ^c | | | |
|------------------|---|---|---|----------------------|---|----------------------------|-------------------------------|--------------------|
| | | <i>t_{x-1 min}</i> ^d | <i>t_{y+1 min}</i> ^d | offset | 2-MN & 1-MN | | CyCy & C13 | |
| | | <i>R_s</i> | <i>W</i> _{0.5} | <i>R_s</i> | <i>W</i> _{0.5} | <i>R_s</i> [C13] | <i>W</i> _{0.5} [C13] | |
| H ₂ | 60 (6 min) $\xrightarrow{10\text{ K/min}}$ 110 °C $\xrightarrow{30\text{ K/min}}$ 380 °C (10 min) | 1.60 | 24.90 | 23.30 | 3.29 | 0.033 | 4.94 | 0.036 |
| He | 60 (6 min) $\xrightarrow{10\text{ K/min}}$ 110 °C $\xrightarrow{30\text{ K/min}}$ 380 °C (10 min) | 2.80 | 15.77 | 12.97 | 3.04 | 0.037 | 3.97 | 0.041 |
| N ₂ | 60 (6 min) $\xrightarrow{15\text{ K/min}}$ 130 °C $\xrightarrow{30\text{ K/min}}$ 380 °C (11 min) | 2.89 | 3.10 | 0.21 | 1.84 <i>1.85</i> | 0.045 <i>0.044</i> | 1.94 <i>1.80</i> | 0.041 <i>0.047</i> |

^a Carrier gas flow: 5 mL/min (He, H₂), 4 mL/min (N₂).

^b Offset between baseline levels one minute before and one minute after the respective solvent peak (see Fig. 2).

^c Resolutions (*R_s*) and half width (*w*_{0.5}) were determined for all gases using Shell SN 500 (2 µg) containing absolute masses of 30 ng (1-MN and 2-MN, Cycy) and 15 ng (C13).

The *R_s* and *w*_{0.5} given in italics were obtained with the internal quantification standard using absolute injected masses of 100 ng (1-MN and 2-MN, Cycy) and 50 ng (C13).

^d *t_x*, *t_y*: Retention times at a current of 500 pA (solvent peak, see Fig. 2).

2.2. Test samples

The infant formula samples were shared by a commercial laboratory in Germany, the coconut samples were obtained in food retail in Germany and the mineral oils SN 500, Ondina X430, Omala 68 and 220 from Shell are available in the common market as well.

2.3. Sample preparation

2.3.1. Mineral oils

50 mg of a mineral oil sample was dissolved in 1 mL of n-hexane. 4 µL of this solution were diluted with 10 µL of the internal standard and 986 µL of n-hexane. The mineral oil samples were analysed according to the procedure of the German Federal Institute for Risk Assessment and Kantonales Labor Zürich, Switzerland [24].

2.3.2. Infant formula

Following the standard operating procedure developed by the EU Joint Research Centre [25] 2 g of infant formula were spiked with 50 µL of a 1:10 dilution of the internal standard, then 2 mL of water were added and the sample was vortexed for 2 min, 2 mL of ethanol and 4 mL of KOH solution (50 %) were added and vortexed again for 2 min, further treated for 30 min at 60 °C and 750 rpm to saponify the sample. To extract the mineral oil hydrocarbons 1 mL of ethanol and 5 mL of n-hexane were added and the organic phase was collected. A similar second extraction was done, and the organic phases were combined, washed with 5 mL of water/ethanol (1:1) and concentrated to 2 mL by a nitrogen flow. Afterwards, the epoxidation was started by adding 1 mL mCPBA solution (0.2 mg/mL in ethanol) at 40 °C for 15 min while shaken thoroughly at 750 rpm. The epoxidation was stopped by adding 2 mL Na₂S₂O₃/NaHCO₃ (1:1, 10 % in water). Then 2 mL of ethanol were added to support the phase separation and the sample was centrifuged for 3 min at 3000 rpm. The organic phase was dried with Na₂SO₄. 50 µL were injected into the LC-GC-FID system. A schematic summary can be found in supplementary Fig. S1.

2.3.3. Coconut oils

1 g of the coconut oil was diluted in 2 mL of n-hexane and spiked by 30 µL of internal standard for quantification. The required epoxidation was done similar to the infant formula treatment. A schematic summary can be found in supplementary Fig. S2.

2.4. Online coupled high performance liquid chromatography-gas chromatography-flame ionisation detection (LC-GC-FID)

LC-GC-FID experiments were performed on a CHRONECT workstation MOSH/MOAH from Axel Semrau (Sprockhövel, Germany) composed of a 1100 HPLC system (binary pump and variable

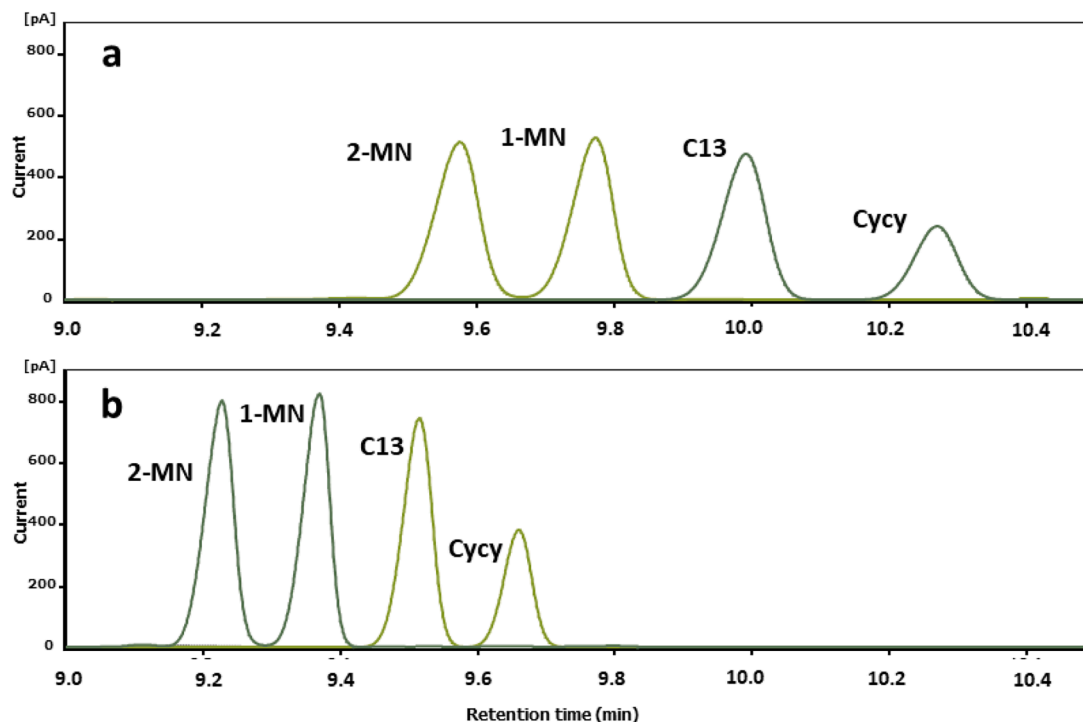


Fig. 1. Resolution of critical pairs 1- and 2-methylnaphthalene (1-MN, 2-MN) and n-tridecane and bicyclohexyl (C13, Cicy) using nitrogen as carrier gas (see Table 1).

a: Chromatographic starting conditions as used for H₂ and He applied to N₂.
 b: Optimised chromatographic conditions for N₂.

wavelength detector from Agilent Technologies, Waldbronn, Germany), an Agilent 7890A gas chromatograph (GC) with a flame ionisation detector (FID) and a robotics platform based on a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Three rotatory switching valves (VICI AG International, Schenkon, Switzerland) were used to guide the eluent from the HPLC into the GC. The GC was equipped with one on-column interface and one solvent vapor exit. A range of 5 μ L to 100 μ L of the prepared samples were injected onto an Allure Silica HPLC column (250 mm x 2.1 mm, 5 μ m, 60 Å , Restek, Bellefonte, PA, USA) without additional column temperature control. The mobile phase consisted of n-hexane and dichloromethane. Starting at 100 % n-hexane with 300 μ L/min, the mobile phase was changed to 65 % n-hexane 1.0 min after injection and held for additional 5.0 min. MOSH was eluted between 2.0 – 3.5 min (450 μ L). After elution of the MOAH fraction (4.3 – 5.8 min, 450 μ L), the silica gel HPLC column was backflushed with dichloromethane at 500 μ L/min for 9 min. Afterwards, the column was reconditioned with n-hexane at 500 μ L/min for 15 min. The MOAH elution window was verified by UV detection at 230 nm. 1,3,5-tri-tert-butylbenzene (TBB) and perylene (Per) marked the starting- and endpoint of this window. LC-GC transfer was done by the retention gap technique and partially concurrent solvent evaporation [18]. An uncoated precolumn (deactivated stainless steel, 10 m x 0.53 mm, Restek, Germany) was followed by a steel T-piece union (modified butt-to-butt connector, Trajan Scientific and Medical, Ringwood, Australia) connecting to the solvent vapor exit and a separation column coated with a 100 % dimethyl polysiloxane film (MXT-1, Siltek-treated stainless steel, 15 m x 0.25 mm x 0.25 μ m, Restek, Bellefonte, PA, USA). From HPLC, the MOSH and MOAH fractions were transferred to GC at an oven temperature of 60 $^{\circ}$ C. The solvent vapor exit was opened 0.5 min prior to elution of each fraction and was closed 0.3 min after the fraction was transferred. The oven temperature program and gas flow were adjusted for the respective carrier gas as reported in the discussion below. The FID base temperature was set to 380 $^{\circ}$ C. The carrier gas flows were subject to optimisation as given in Table 1. The FID gas flows for air,

hydrogen, and nitrogen were set to 300, 30, and 30 mL/min, respectively. Data processing was performed with Clarity 8.5 (DataApex, Prague, Czech Republic). Quantitation by use of internal standards was based on bicyclohexyl (cyclohexyl cyclohexane, Cicy) for MOSH and 2-methyl naphthalene (2-MN) for MOAH, and TBB for MOAH in case of epoxidated samples.

2.5. Quality control of the sample preparation and the instrumental analysis

The comparison of carrier gases was based on the usual quality control measures done in MOSH/MOAH analysis. This included in particular the correct consecutive transfer of the two LC fractions to the gas chromatograph, eluate restriction avoiding the loss of the more volatile components, sufficient separation between n-hexane and C11, a suitable peak resolution and shape over the whole boiling range and completion of the GC run within 30 min regardless of the carrier gas. Correct separation of relevant time markers and relevant compounds were achieved using the internal standards for quantification and the retention time standard. The JRC Guidance [2] was adopted to set a target recovery between 80 and 120 % of the C10/C20 and C50/C20 ratios. In addition, the baseline offsets, linear ranges, and carrier over were investigated as relevant characteristics. Chemicals, glassware, septa, and seals were checked for sufficiently low sample blank values. The maximum sample blank value to be tolerated, including all further processing, was one-third of the targeted determination limit of 1 mg/kg. The integration of MOSH and MOAH humps were done as prescribed by the standard procedure [13,26].

3. Results and discussion

3.1. Carrier gas related optimization of GC condition

For comparing carrier gases regarding appropriate separation of

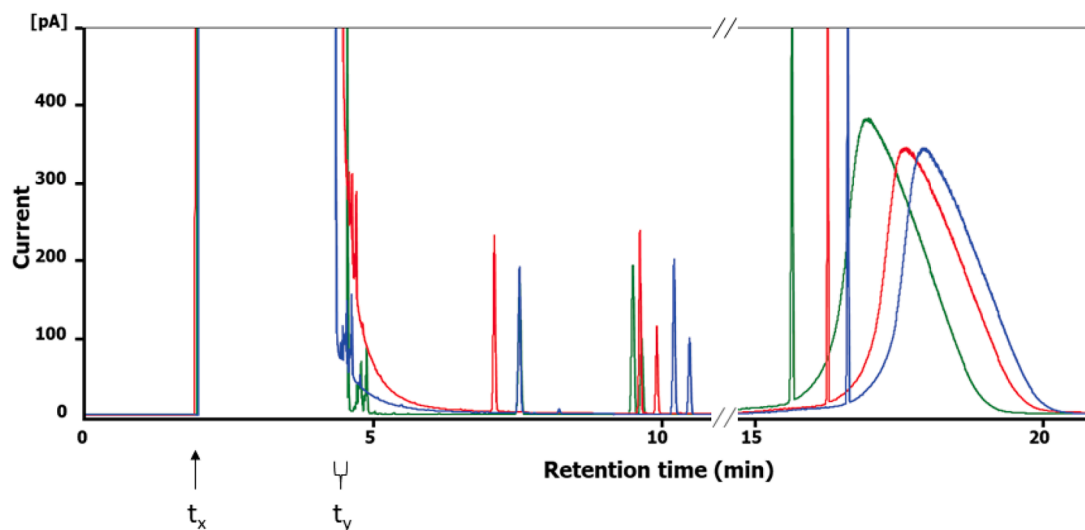


Fig. 2. Overlaid excerpts of chromatograms obtained after injection of 2 μ g of medical white oil Ondina X430 with the three carrier gases. Green = N₂, Blue = He, Red = H₂.

t_x : Retention time at a current of 500 pA (start of solvent peak).

t_y : Retention time at a current of 500 pA (end of solvent peak).

MOSH and MOAH fractions and subsequent accurate quantification of the fractions, a one-channel system was used that requires consecutive separation of MOSH and MOAH on the same GC column.

The configuration of the system complies with EN 16995 and ISO/DIS is depicted in supplementary Fig. S3. The chromatographic conditions suggested for hydrogen as carrier gas [14] were used as starting point. Then, the carrier gas flow and the oven program were optimised such that regardless of the carrier gas the GC run was confined to 30 min and minimum separations requirement were met. Table 1 shows the GC conditions employed for the respective carrier gases. The conditions adopted for hydrogen were appropriate for helium as well while nitrogen required the given adjustments of ramp and heating rate. As described by the van Deemter equation a lower gas flow should favour peak separation in case of nitrogen. In order to retain the overall run time limit the heating rate had to be increased. Supplementary Table S1 comprises the gas flow/oven program combinations tested for nitrogen and supplementary Table S2 shows the gas flows at the FID with the three carrier gases.

Table 1 reveals that the helium and hydrogen display similar separation characteristics of the critical pairs 2-MN/1-MN and C₁₃/C₁₀ of the internal standard which are used to evaluate the GC column performance. While nitrogen differs significantly in this regard, the optimised conditions allow for sufficient resolution of the pairs both from an IS solution and a real-world oil spiked with the IS. The resulting gas flows at the flame ionisation detector are given in supplementary table S2. b: Optimised chromatographic conditions for N₂

Fig. 1 reveals that the rather broad peaks could be narrowed significantly using the optimised conditions. It should be noted that the baseline drift offsets for the three carrier gases are quite different (Table 1). Fig. 2 shows overlaid excerpts of the chromatograms obtained with the three carrier gases using a solution containing 2 μ g of Ondina X430 and the IS for quantification. For highly volatile contaminations, e. g. contaminations based on ink colours, a straight baseline in the volatile range is required to ensure accurate quantifications. Therefore, the effect of the three carrier gases on the behaviour of the solvent band was compared. To define the tailing of the solvent band the baseline current was determined one minute before the solvent peak start (t_x) as well as 1 min after the significantly dropping of the respective solvent peak (t_y). The offset between both values is defined as the baseline solvent drift and enables an objective comparison of the performance influence of the carrier gases. Nitrogen exhibits the lowest offset and therefore

Table 2

Retention times (RT) in min and peak areas for the *n*-alkanes in the retention time standard.

| Carrier gas Alkane | Retention times ^a | | | Peak area ^b | | |
|-----------------------|------------------------------|-----------------|------------------|------------------------|----------------|----------------|
| | H ₂ | He | N ₂ | H ₂ | He | N ₂ |
| C10 | 5.82 | 6.00 (0.18) | 6.22 (0.40) | 2179 (0.94) | 2361 (0.94) | 1994 (0.93) |
| C11 | 7.19 | 7.58 (0.39) | 7.54 (0.35) | 2220 | 2368 | 2045 |
| C13 | 10.01 | 10.52 (0.51) | 9.66 (-0.35) | 2317 | 2464 | 2119 |
| C16 | 12.63 | 12.91 (0.28) | 11.87 (-0.76) | 2342 | 2499 | 2133 |
| C20 | 14.13 | 14.40 (0.27) | 13.42 (-0.71) | 2311 | 2517 | 2146 |
| C24 | 15.27 | 15.55 (0.28) | 14.57 (-0.70) | 2328 | 2475 | 2143 |
| C25 | 15.53 | 15.81 (0.28) | 14.83 (-0.70) | 2238 | 2388 | 2097 |
| C35 | 17.65 | 17.95 (0.30) | 16.96 (-0.69) | 2178 | 2357 | 2054 |
| C45 | 18.49 | 18.80 (0.31) | 17.80 (-0.69) | 2182 | 2406 | 2052 |
| C50 | 19.91 | 20.24 (0.33) | 19.23 (-0.68) | 1993 (0.86) | 2225 (0.88) | 1920 (0.89) |
| RSD ^c [%] | - | - | - | 4.5 | 3.4 | 3.3 |

^a in brackets: difference of respective RT to the RT with hydrogen as carrier gas in min.

^b in brackets: C10/C20 and C50/C20 ratios.

^c Relative standard deviations of the area of the alkanes.

potentially the best performance for early eluting compounds. It is assumed that nitrogen as carrier gas leads to less solvent soaking of the dimethyl siloxane phase of MXT-1 column film.

Table 2 comprises the retention times and peak areas obtained for the *n*-alkanes in the retention time standard. It is seen that as a result of the initial heating rate the retention times for the *n*-alkanes tend to be shorter in case of nitrogen compared to the other two carrier gases. Nevertheless, the individual compounds are sufficiently separated (see supplementary Fig. S4). As demanded in [2] the response ratio C50/C20 is greater than 80 % and the respective standard deviations of all alkanes responses (C10 – C50) are below 5 % in all cases which is regarded as practically appropriate for a performant system while [2] demands even

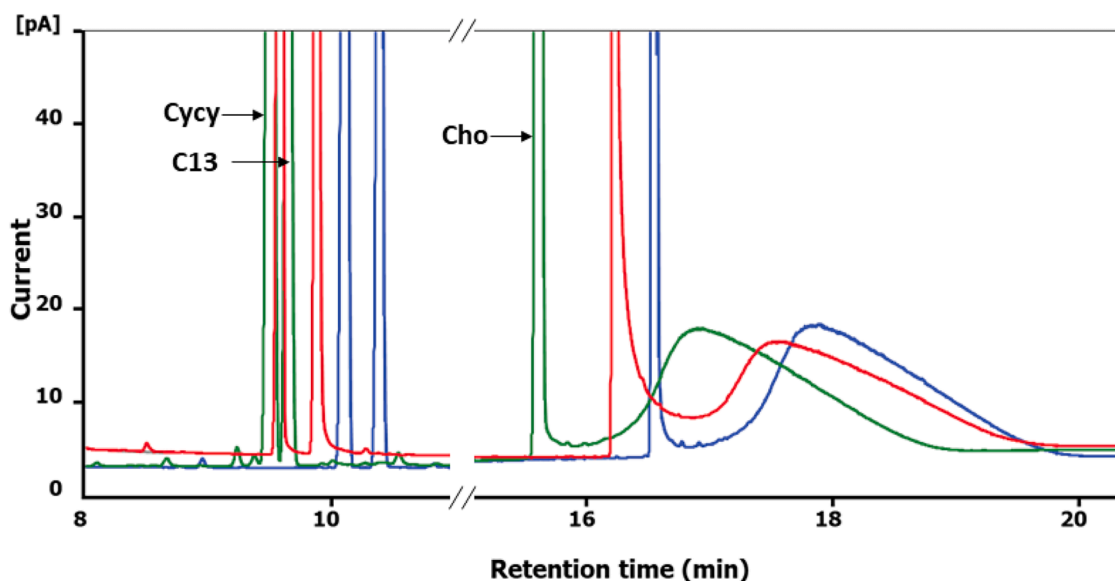


Fig. 3. Injection of 50 ng medical white oil (=Shell Odina X430, pure MOSH), overlay of chromatograms obtained with the three carrier gases. Green = N₂, Blue = He, Red = H₂.

Table 3

Recovery of MOSH and MOAH fractions from the neat mineral oils and associated variability ($n = 6$).

| Oil | Carrier gas | Recovery ^a MOSH (%) | Variability ^b MOSH (%) | Recovery ^a MOAH (%) | Variability ^b MOAH (%) |
|--------------------------|----------------|-----------------------------------|--------------------------------------|-----------------------------------|--------------------------------------|
| SN 500 | H ₂ | 55.49 | 0.15 | 33.38 | 0.23 |
| | He | 56.65 | 0.09 | 33.20 | 0.56 |
| | N ₂ | 57.17 | 0.31 | 34.14 | 0.84 |
| Omala 68 | H ₂ | 72.65 | 0.19 | 17.06 | 0.40 |
| | He | 75.04 | 0.17 | 16.68 | 0.99 |
| | N ₂ | 74.49 | 0.32 | 17.54 | 0.40 |
| Addinol M50 ^c | H ₂ | 58.81 | 0.91 | 33.61 | 1.40 |
| | He | 60.54 | 0.38 | 32.01 | 1.72 |
| | N ₂ | 61.00 | 0.58 | 33.16 | 2.14 |
| Omala 220 ^c | H ₂ | 58.22 | 0.15 | 25.07 | 1.07 |
| | He | 59.53 | 0.32 | 19.32 | 10.93 |
| | N ₂ | 60.07 | 0.15 | 25.08 | 1.34 |
| Ondina X430 ^c | H ₂ | 94.53 | 0.19 | – | – |
| | He | 97.25 | 0.07 | – | – |
| | N ₂ | 97.16 | 0.17 | – | – |

^a Portion of injected sample (2 μ g) quantified as MOSH or MOAH, respectively.

^b Relative standard deviation of the recovery.

^c $n = 3$.

a limit of just 10 %. The slightly lower area counts in case of nitrogen is believed to be due to the dependence of ion yield in the FID on the respective gas composition.

3.2. Comparison of carrier gases using neat mineral oils

The selected mineral oil samples were chosen to cover different boiling ranges, hump shapes and MOSH to MOAH ratios. Shell SN 500 is a high refined mineral oil and used as a quality control sample by the JRC [2]. Shell Omala 68 and Omala 220 are gear oils with wide boiling range of mineral oil hydrocarbons and representatively for common contaminations in tropical oils. Shell Ondina X430 as a medical white oil is free of MOAH and useful for LoQ determinations.

Fig. 3 shows the chromatograms obtained with the three carrier gases after injection of 100 ng medical white oil Shell Odina that consists of 100 % MOSH. It is again seen that nitrogen is most favourable in regard for tailing and that the detector response is largely unaffected by the carrier gas. Thus, the requirement to reach a LoQ of 1 mg/kg – the

Table 4

Linearity and carry over.

| Oil | Carrier gas | R ² ^a MOSH | R ² ^a MOAH | Response variability ^b MOSH [%] | Response variability ^b MOAH [%] | Carry over ^c [%] |
|----------|----------------|-------------------------------------|-------------------------------------|---|---|--------------------------------|
| SN 500 | H ₂ | 0.9998 | 0.9998 | 4.21 | 3.18 | 0.15 |
| | He | 0.9997 | 0.9997 | 3.04 | 4.85 | 0.26 |
| | N ₂ | 0.9998 | 0.9996 | 2.92 | 3.49 | 0.11 |
| Omala 68 | H ₂ | 0.9996 | 0.9996 | 3.45 | 8.47 | 0.28 |
| | He | 0.9997 | 0.9996 | 2.70 | 9.61 | 0.39 |
| | N ₂ | 0.9998 | 0.9998 | 2.49 | 3.08 | 0.16 |

^a Coefficient of determination of the linear regression obtained after injection of dilution series (0.20 μ g, 0.50 μ g, 1 μ g, 2 μ g, 5 μ g, 10 μ g).

^b Relative standard deviation of the ratio of detector response to injected sample mass of the dilution series.

^c Carry over of MOSH after injection of 20 μ g of the respective oil.

detectability of 50 – 100 ng MOSH - [3,26] is therefore easily met with all carrier gases. The signal to noise ratio is sufficient to allow quantifying even lower amounts. It should also be noted that nitrogen does not display a significant hump widening compared to the other two carrier gases. In fact, the humps are not different among carrier gases.

Table 3 shows that the recovery of MOH fractions from the investigated mineral oils. After separation by LC the MOSH and MOAH fractions were quantified using the described procedure and the respective internal standards. The differences between fraction recoveries obtained with the carrier gases are well below 10 % which may be considered as practical within-laboratory variability.

Table 4 comprises the response variabilities using the three carrier gases and two mineral oils. The coefficients of variation show that the observed data are satisfactorily explained by the assumption of linearity in the investigated range. The carry-over was determined by comparing the MOSH humps observed after injection of 20 μ g of the mineral oil with the MOSH hump observed after the following injection of pure solvent. For this purpose, only the respective MOSH fractions were considered ~~only~~ because the very low amounts of MOAH were hardly detectable.

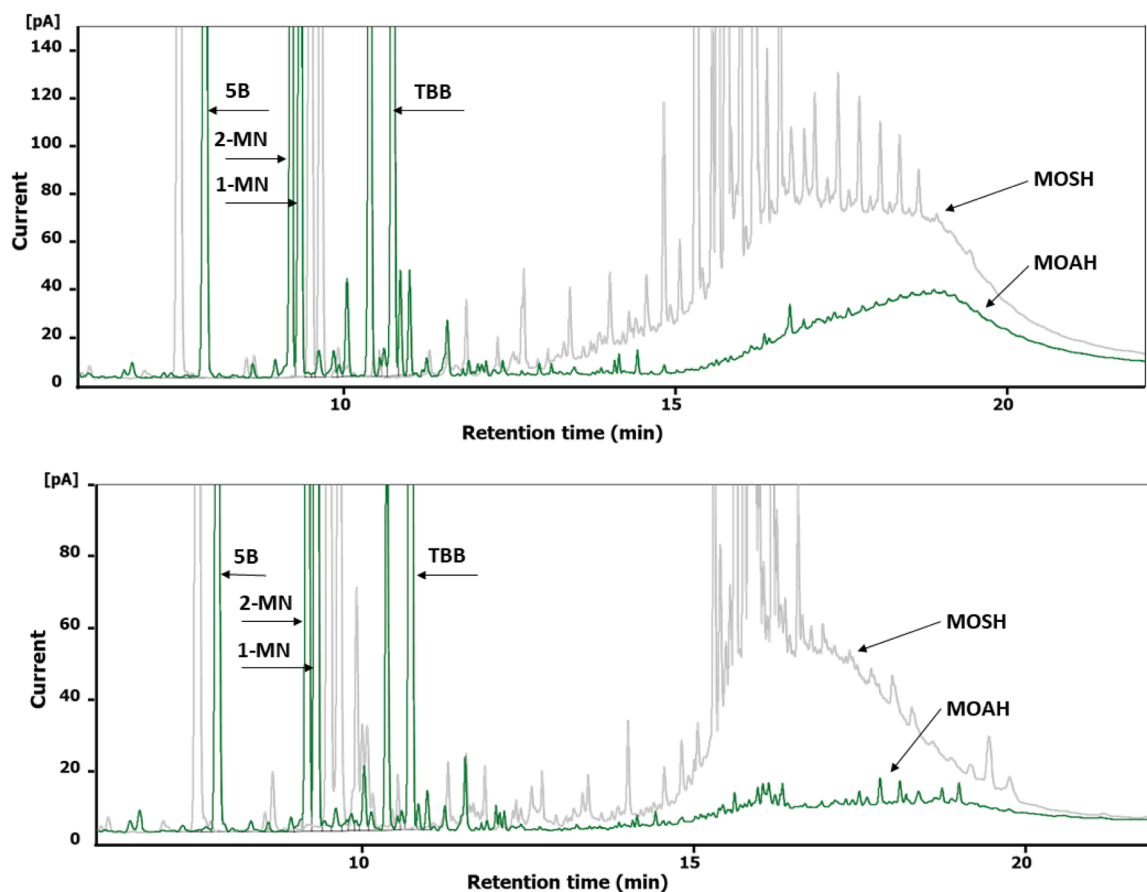


Fig. 4. MOH fractions of infant food formula 1 (a) and coconut oil 2 (b). Overlay of MOSH and MOAH fractions, carrier gas: nitrogen.

Table 5

MOSH and MOAH mass fractions in mg/kg of the coconut oils and infant formula determined with the three carrier gases.

| Sample | Carrier gas MOH fraction | N ₂ | He | H ₂ | RSD ^a [%] |
|------------------|--------------------------|----------------|-------|----------------|----------------------|
| Coconut oil 1 | MOSH | 2.56* | 2.41* | 2.89* | 7.58 |
| | MOAH | 0.15* | 0.12* | 0.76* | 86.5 |
| Coconut oil 2 | MOSH | 16.98 | 15.86 | 17.67 | 4.42 |
| | MOAH | 3.48* | 3.14* | 3.81* | 7.92 |
| Infant formula 1 | MOSH | 43.62 | 37.22 | 41.29 | 6.49 |
| | MOAH | 15.47 | 15.31 | 15.96 | 1.79 |
| Infant formula 2 | MOSH | 39.89 | 37.30 | 38.50 | 2.74 |
| | MOAH | 15.63 | 15.30 | 15.23 | 1.13 |

^a Relative standard deviation among carrier gas quantification results.

* Below the LoQ of 10 mg/kg according to EN 16995:2017.

3.3. Comparison of carrier gases for vegetable oils and infant formula analyses

Fig. 4 depicts by example the overlaid MOSH and MOAH chromatograms obtained from a coconut oil and an infant formula using nitrogen as carrier gas. The standard compounds and the MOSH and MOAH humps are all well separated and elute within the required time frame of 30 min. Supplementary Fig. S5 shows for comparison the overlaid MOSH and MOAH chromatograms obtained from coconut oil 2 with the three carrier gases. Again, the chromatograms are nearly indistinguishable, especially regarding the standard compounds and the MOSH and MOAH fraction humps. Conspicuously, nitrogen leads to the least solvent tailing and allows for detection of highly volatile compounds which extend the information on MOH contamination obtainable by this analytical approach. Table 5 comprises all MOSH and

MOAH quantification data obtained. Obviously, the results obtained with the different carrier gases are not distinguishable against an assumed uncertainty range of $\pm 10\%$ (see discussion of Table 3).

4. Conclusion

After optimisation of chromatographic settings nitrogen can be considered as suitable carrier for the determination of mineral oil contaminants in food related matrices by LC-GC hyphenation. Changing to nitrogen allows retaining the GC runtime while still sufficient peak resolutions and appropriate peak shapes are obtained. Recoveries, repeatabilities, LoQs, linear ranges and carry over were equivalent with all carrier gases. This and the lower solvent band tailing and baseline offset might well encourage the use of nitrogen as alternative carrier gas in cases where this may economically favourable.

CRediT authorship contribution statement

Matthias Groschke: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roland Becker:** Writing – review & editing, Writing – original draft, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

This work has been partially supported by the project “20NET02 Food-MetNet”, which has received funding from the EURAMET EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.chroma.2024.464946](https://doi.org/10.1016/j.chroma.2024.464946).

References

- Lebensmittelverband Deutschland, e.V. Länderarbeitsgemeinschaft Verbraucherschutz, Arbeitsgruppe Lebensmittel- und Bedarfsgegenstände, Wein und Kosmetika (ALB). Aktualisierung: Orientierungswerte für Mineralölkohlenwasserstoffe (MOH) in Lebensmitteln; Lebensmittelverband Deutschland e.V., 2020. (in German)/<https://www.lebensmittelverband.de/download/orientierungswertefuer-moh-in-lebensmitteln>. (accessed Jan 14, 2022).
- S. Bratinova, E. Hoekstra (Eds.), Guidance On sampling, Analysis and Data Reporting For the Monitoring of Mineral Oil Hydrocarbons in Food and Food Contact materials, Luxembourg, Publications Office of the European Union, Luxembourg, 2019, <https://doi.org/10.2760/208879>. ISBN 978-92-76-00172-0.
- M. Biedermann, K. Grob, On-line coupled high performance liquid chromatography-gas chromatography for the analysis of contamination by mineral oil. Part I: Method analysis, *J. Chromatogr. A* 1255 (2012) 56–75, <https://doi.org/10.1016/j.chroma.2012.05.095>.
- K. Grob, M. Biedermann, A. Caramaschi, B. Pacciarelli, LCGC analysis of the aromatics in a mineral oil fraction: Batching oil for jute bags, *J. High Resolut. Chromatogr.* 14 (1991) 33–39, <https://doi.org/10.1002/JHRC.1240140109>.
- M. Nestola, T.C. Schmidt, 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, *J. Chromatogr. A* 1505 (2017) 69–76, <https://doi.org/10.1016/j.chroma.2017.05.035>.
- European Food Safety Authority. Scientific Opinion on Mineral Oil Hydrocarbons in Food; European Food Safety Authority, 2012. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2704>. (accessed June 18, 2022).
- L. Barp, C. Kornauth, T. Wuergler, M. Rudas, M. Biedermann, A. Reiner, N. Concin, K. Grob, Mineral oil in human tissues, Part I: Concentrations and molecular mass distributions, *Food Chem. Toxicol.* 72 (2014) 312–321, <https://doi.org/10.1016/j.fct.2014.04.029>, 2014.
- German Federal Institute for risk assessment (BfR) Übergänge von Mineralöl aus Verpackungsmaterialien auf Lebensmittel Stellungnahme Nr. 008/2010, 2009; BfR. <https://www.bfr.bund.de/cm/343/messung-von-mineraloel-kohlenwasserstoff-offen-in-lebensmitteln-und-verpackungsmaterialien.pdf> (accessed April 28, 2023).
- German Federation of Food Law and Food Science (BLL e.V) Toolbox for Preventing the Transfer of Undesired Mineral Oil Hydrocarbons into Food, 2017; German Federation of Food Law and Food Science (BLL e.V). <https://www.lebensmittelverband.de/download/toolbox-for-preventing-the-transfer-of-undesired-mineraloil-hydrocarbons-into-food>. (accessed Jan 14, 2022).
- M. Biedermann, K. Fiselier, K. Grob, Aromatic Hydrocarbons of Mineral Oil Origin in Foods: Method for Determining the Total Concentration and First Results, *J. Agric. Food Chem.* 57 (2009) 8711–8721, <https://doi.org/10.1021/jf901375e>.
- K. Grob, Toxicological Assessment of Mineral Hydrocarbons in Foods: State of Present Discussions, *J. Agric. Food Chem.* 66 (2018) 6968–6974, <https://doi.org/10.1021/acs.jafc.8b02225>.
- https://food.ec.europa.eu/system/files/2022-07/reg-com_toxic_20220421_sum.pdf (accessed: 19.04.2024).
- M. Biedermann, C. Munoz, K. Grob, Update of on-line coupled liquid chromatography – gas chromatography for the analysis of mineral oil hydrocarbons in foods and cosmetics, *J. Chromatogr. A* 1521 (2017) 140–149, <https://doi.org/10.1016/j.chroma.2017.09.028>.
- CEN - EN 16995:2017: Foodstuffs - vegetable oils and foodstuff on basis of vegetable oils - determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis (2017).
- ISO/DIS 20122 (under development) Vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled HPLC-GC-FID analysis. Method for low limit of quantification. ISO, Geneva, Switzerland.
- C. Albert, H.-U. Humpf, L. Brühl, Determining MOSH and MOAH with High Sensitivity in Vegetable Oil - A New, Reliable, and Comparable Approach Using Online LC–GC–FID-Evaluation of Method Precision Data, *J. Agric. Food Chem.* 70 (2022) 10337–10348, <https://doi.org/10.1021/acs.jafc.2c01189>.
- M. Nestola, Automated workflow utilizing saponification and improved epoxidation for the sensitive determination of mineral oil saturated and aromatic hydrocarbons in edible oils and fats, *J. Chromatogr. A* 1682 (2022) 463523, <https://doi.org/10.1016/j.chroma.2022.463523>.
- G. Purcaro, M. Zoccali, P.Q. Tranchida, L. Barp, S. Moret, L. Conte, P. Dugo, L. Mondello, Comparison of two different multidimensional liquid-gas chromatography interfaces for determination of mineral oil saturated hydrocarbons in foodstuffs, *Anal. Bioanal. Chem.* 405 (2013) 1077–1084, <https://doi.org/10.1007/s00216-012-6535-0>.
- G. Purcaro, S. Moret, L. Conte, Hyphenated liquid chromatography–gas chromatography technique: Recent evolution and applications, *J. Chromatogr. A* 1255 (2012) 100–111, <https://doi.org/10.1016/j.chroma.2012.02.018>.
- N. Sdrigotti, M. Collard, G. Purcaro, Evolution of hyphenated techniques for mineral oil analysis in food, *J. Sep. Sci.* 44 (2021) 464–482, <https://doi.org/10.1002/jssc.20200901>.
- P.Q. Tranchida, M. Zoccali, G. Purcaro, S. Moret, L. Conte, M. Beccaria, P. Dugo, L. Mondello, A rapid multidimensional liquid–gas chromatography method for the analysis of mineral oil saturated hydrocarbons in vegetable oils, *J. Chromatogr. A* 1218 (2011) 7476–7480, <https://doi.org/10.1016/j.chroma.2011.06.089>.
- G. Bauwens, C. Conchione, N. Sdrigotti, S. Moret, G. Purcaro, Quantification and characterization of mineral oil in fish feed by liquid chromatography-gas chromatography-flame ionization detector and liquid chromatography-comprehensive multidimensional gas chromatography-time-of-flight mass spectrometer/flame ionization detector, *J. Chromatogr. A* 1677 (2022) 463208, <https://doi.org/10.1016/j.chroma.2022.463208>.
- M. Groschke, personal perception.
- Bundesinstitut für Risikobewertung (BfR), Berlin and Kantonales Labor Zürich: Messung von Mineralöl-Kohlenwasserstoffen in Lebensmitteln und Verpackungsmaterialien (undated). <https://www.bfr.bund.de/cm/343/messung-von-mineraloel-kohlenwasserstoffen-in-lebensmitteln-und-verpackungsmaterialien.pdf> (accessed: 28.04.2023).
- S. Bratinova, G. Beldi, C. Senaldi, L. Karasek, C. Goncalves, S. Valzacchi, E. Hoekstra. Method for official control of the mineral oil aromatic hydrocarbons (MOAH) content in infant formula powder (IF), Standard Operation Procedure (Draft). European Commission, Geel, 2021.
- German Society for Fat Science (DGF), DGF Standard Method C-VI 22 (20), Mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled LC-GC-FID – Method for limit of quantitation, Supplement 26 (2) (2020).