

Technical Report

Determination of MOSH Contamination in Baby Foods by Using LC–GC and LC–GC×GC–MS

Advances in food contaminant analysis

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Abstract:

The present research is based on an investigation directed towards the use of a rapid heart-cutting multidimensional LC–GC–FID method for the analysis of mineral oil saturated hydrocarbons (MOSH), contained in different types of homogenized solid baby food. Sixteen commercial baby food samples were subjected to analysis. The results were confirmed, in qualitative terms, by collecting the LC fractions, relative to some of the food samples, and subjecting them to comprehensive two-dimensional GC–quadrupole mass spectrometry.

Keywords: multidimensional liquid-gas chromatography, comprehensive 2D gas chromatography, baby food

1. Introduction

Mineral oil is derived from crude oil and consists, mainly, of saturated and aromatic hydrocarbons. The former class of compounds, generically defined mineral oil saturated hydrocarbons (MOSH), is formed of straight and branched alkanes, as well as cyclic constituents (naphthenes). The contamination of foods with MOSH is a common occurrence, and many works have been published. For example, Grob *et al.* in 1991 found a series of contamination sources, namely lubricating oils and release agents exploited in the food industry, or mineral batching oil used for the production of jute^[1, 2]. In a later study, it was found that mineral oil can be transferred to dry baby food products *via* ink printed on cardboard containers^[3].

At present, there are no legal limits related to the amounts of MOSH contained in foods; a single exception (50 mg/kg) is that related to Ukrainian sunflower oil, following a severe case of contamination^[4]. A limit of 0.6 mg/kg for MOSH (from C₁₀ to C₂₅) contamination in foods, as a consequence of migration from cardboard packaging, has been recently proposed by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

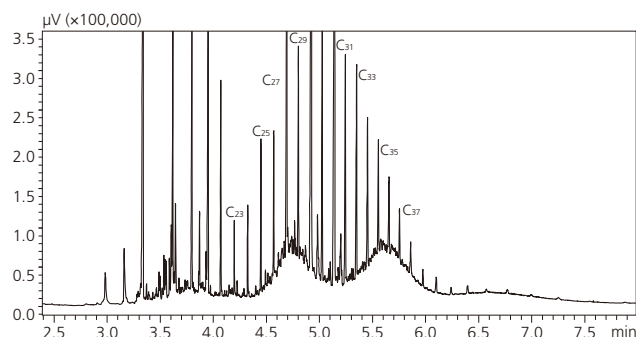


Fig. 1 LC–GC–FID chromatogram for the salmon (I) baby food sample.

Such a limit was derived from a calculation made considering an adult of 60 kg, consuming 1 kg of contaminated food per day.

Heart-cutting LC–GC, with a flame ionization detector (FID), is a prime choice for the quantification of MOSH. The first LC dimension is always exploited to separate the hydrocarbons from the lipid matrix.

The present investigation is based on the development and validation of an LC–GC–FID method for the analysis of mineral oil saturated hydrocarbons in baby food. The main novelties of the research, are related to the high analysis speed and to the use of a novel comprehensive LC–GC (LC×GC) interface, capable of working in both heart-cutting or “comprehensive” (LC×GC) conditions. In the latter configuration, continuous and sequential LC fractions are transferred to the GC column.

The MOSH fraction is transferred and analyzed in the GC dimension, where it appears typically as a “hump”. Moreover, the use of LC–GC×GC–qMS appears to be an interesting option to “see and identify” what is beneath anonymous LC–GC–FID hydrocarbon humps.

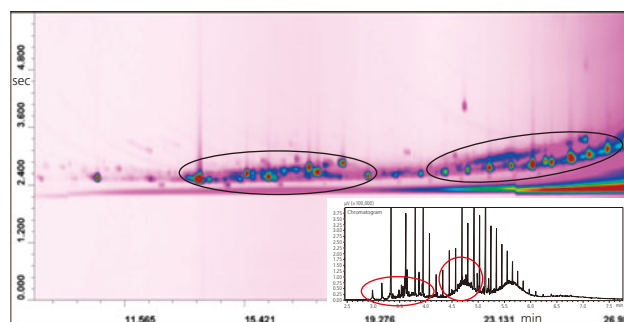


Fig. 2 TIC GC×GC–MS chromatogram relative to the MOSH fraction derived from the salmon (I) baby food.

2. Experimental

2-1. Sample Preparation

All the samples (500 mg) were extracted for three times with hexane (1 mL each, for 15 min), and then each time filtered. After, the filtrates were added together and dried under a gentle nitrogen stream. The extract was weighed (to derive the % of extract) and then diluted to a final concentration of 25% v/v in hexane. Quantification was achieved through a calibration curve, constructed through external standardization. The MOSH hump was integrated through a manual software function, performing the subtraction of the linear alkanes.

2-2. LC–GC–FID Analyses

All sixteen samples were analyzed by using an LC×GC system (Shimadzu) consisting of:

(1) A Shimadzu Prominence LC-20A system, equipped with a CBM-20A communication bus module, two LC-20AD dual-plunger parallel-flow pumps, a DGU-20A degassing unit, an SPD-M20A photodiode array detector, a CTO-20A column oven, and an SIL-20AC autosampler. Data were acquired by the LCsolution software (Shimadzu). LC conditions: a 100 mm L. × 3 mm I.D. × 5 μm *d_p* silica column (SUPELCOSIL LC-Si, Supelco, Milan, Italy) was operated under isocratic conditions, using hexane as mobile phase (0.35 mL/min). Injection volume: 20 μL. At the end of the heart cut, the column was backflushed using CH₂Cl₂.

(2) A Shimadzu AOC-5000 autoinjector equipped with a dedicated dual side-port syringe, employed as a transfer device. Chromatography band transfer is achieved, in the stop-flow mode, through a modified 25-μL syringe. The lower part of the syringe is connected, *via* two transfer lines, to the LC detector exit and to waste. A Teflon plug is located at the end of the syringe plunger; the latter is characterized by a lower OD with respect to the barrel ID, thus enabling mobile phase flow inside the syringe. In the waste mode, the syringe plug is located below both lines and the effluent is directed to waste. In the cut position, the syringe plug is located between the upper and lower line and the effluent flows to the GC. From 0 to 1.5 min the LC effluent was directed to waste, while from 1.5 to 2.0 min the LC effluent was directed to the GC.

(3) A Shimadzu GC-2010 Plus, equipped with an Optic-3 PTV injector (GL Sciences B.V., Eindhoven, The Netherlands). The Optic-3 injector was temperature-programmed as follows: from 75 °C (1 min) to 360 °C at 250 °C/min. Injection mode: split, at a ratio of 200:1 for 1 min during sample introduction and solvent vent, then splitless for 1 min during sample desorption and transfer, then 50:1 for the remaining analysis time.

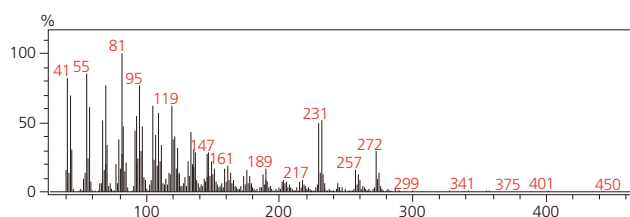


Fig. 3 Mass spectrum extracted from the first “cycloalkane zone”.

Data were acquired by the GCsolution software. GC conditions: an SLB-5 ms [silphenylene polymer, virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)] 15 m L. × 0.10 mm I.D. × 0.10 μm *d_f* column (Supelco) was heated from 50 °C (1 min) to 360 °C (4 min) at 70 °C/min. Carrier gas, hydrogen, was supplied at an initial pressure of 529 kPa (constant linear velocity: 100 cm/s). FID (360 °C) sampling frequency was 50 Hz.

A dedicated LC×GC software enabled the control of each instrument through the respective native software. The LC×GC software controlled the transfer process. All parameters, including the LC and GC methods, can be saved with the LC×GC method.

2-3. GC×GC–MS Analyses

All GC×GC applications were carried out on a Shimadzu GC×GC–MS system, consisting of a GC-2010 gas chromatograph, and a GCMS-QP2010 Plus quadrupole mass spectrometer. The primary column, an SLB-5 ms 30 m L. × 0.25 mm I.D. × 0.25 μm *d_f* column, was connected to an uncoated capillary segment (1.0 m L. × 0.25 mm I.D., used to create a double-loop), by using an SGE SilTite mini-union (SGE, Ringwood, Victoria, Australia). The uncoated capillary was then connected to a segment of BPX50 (50% phenyl polysilphenylene–siloxane) 1.5 m L. × 0.10 mm I.D. × 0.10 μm *d_f* column (SGE), by using another union. Modulation was carried out every 6 s, by using a loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (350 °C) was 375 ms.

Table 1 Samples analyzed, total levels of contamination, and contamination considering an upper boundary defined by C₂₅.

Baby food	MOSH (mg/kg)	≤ C ₂₅ alkanes (mg/kg)
1 - Salmon I	13.8	2.0
2 - Plaice	3.5	0.9
3 - Chicken	3.0	1.4
4 - Beef I	1.9	0.8
5 - Beef II	5.6	2.2
6 - Beef-Ham	4.9	2.0
7 - Turkey	3.2	1.1
8 - Sea bass	1.4	0.9
9 - Calf	2.5	0.7
10 - Rabbit	3.5	1.0
11 - Ostrich	1.6	—
12 - Salmon II	1.1	—
13 - Prune I	0.6	—
14 - Pear	3.6	0.6
15 - Fruit mix	0.3	—
16 - Prune II	10.5	1.8

MS parameters: the samples were analyzed in the full scan mode, using a mass range of 40–460 m/z ; spectra generation frequency: 25 Hz; interface and ion source temperatures were 300 °C and 200 °C, respectively. MS ionization mode: electron ionization. GC oven temperature program: 80–360 °C at 10 °C/min. Carrier gas, helium, was supplied at an initial pressure of 250 kPa (constant linear velocity). Injection temperature: 350 °C. Injection mode and volume: split (1:10), 4 μL . Data were collected by the GCMSsolution software; bi-dimensional visualization was carried out by using the Chrom-Square v. 2.0 software.

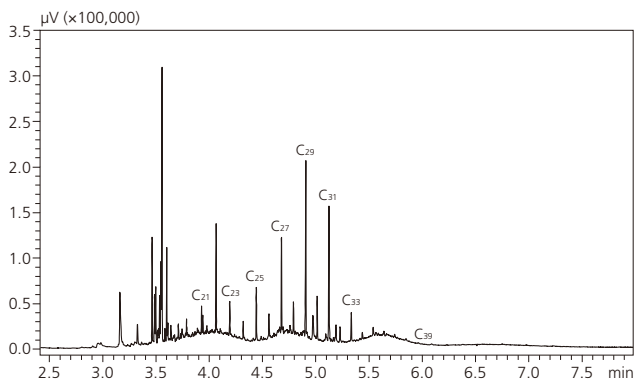


Fig. 4 LC-GC-FID chromatogram for the beef (II) baby food sample.

3. Method Validation

A seven-point calibration curve was constructed using solutions of mineral oil saturated hydrocarbons in hexane at the 1, 5, 10, 20, 50, 100, and 200 ppm levels ($n = 3$). The area of each MOSH hump was integrated by using the GC-FID “manual integration” software function; the n -alkane areas on top of the hump were subtracted from the final result. The derived calibration curve was linear in the range of concentrations considered and was characterized by a regression coefficient (R^2) of 0.9994. Intra-day precision was calculated by analyzing one sample, six times consecutively. The resulting standard deviation equaled ± 1.5 mg/kg (average value: 35.2 mg/kg), while the coefficient of variation was 4.1%. Inter-day precision was calculated by analyzing sample no. 9 three times, on five consecutive days. The resulting standard deviation equaled ± 0.34 mg/kg (average value: 14.5 mg/kg), while the coefficient of variation was 2.4%. Limits of detection and quantification were calculated by multiplying the standard deviation of the area of the “blank” olive oil sample ($n = 3$) three and ten times, respectively, and then dividing the result by the slope of the calibration curve. The values extrapolated, namely 0.15 and 0.5 mg/kg, gave final LOD and LOQ values of 0.6 and 2 ppm, respectively. Such LOD and LOQ values can be considered as acceptable.

4. Results and Discussion

Initially, the scope of the present research was to apply an LC-GC method to baby foods, prepared using vegetable oil. Twelve homogenized solid baby foods, containing either meat or fish (and vegetable oil), were subjected to analysis (Table 1). Three products were fish-based (2 salmon, sea bass, plaice), while all the others contained meat as the main ingredient (chicken, 2 beef, calf, beef-ham, turkey, rabbit, ostrich). Hexane baby food extraction was performed three times; the fourth extraction gave a negligible analytical response. The vegetable oil used as an ingredient was, in all twelve cases, sunflower oil and on the basis of its position in the ingredient list it was certainly present in low amounts (most probably less than 5%). A hexane blank was analyzed periodically throughout the period of research, by drying a 3 mL volume of hexane (the volume used for sample extraction), by adding a 50 μL volume of hexane to the residue, and then subjecting a 20 μL quantity to LC-GC analysis. The resulting chromatograms were always characterized by a negligible response. As can be observed in Table 1, the meat and fish baby foods were all contaminated (the results are the average of two applications), to various degrees. Also reported in the table are (approximate) MOSH concentrations considering the upper alkane boundary on the basis of the BMELV proposal (MOSH up to C_{25}). The highest MOSH level, namely 13.8 mg/kg, was found in the salmon I sample.

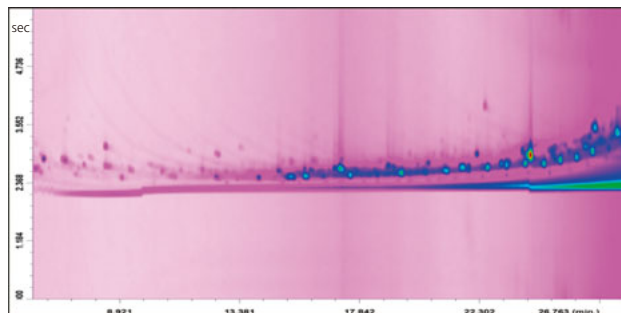


Fig. 5 TIC GCxGC-MS chromatogram relative to the MOSH fraction derived from the beef (II) baby food sample.

Considering contamination up to the C_{25} point, the MOSH concentration was approximately 2.0 mg/kg, well over the proposed maximum limit (0.6 mg/kg). The GC chromatogram, relative to the salmon I baby food, is reported in Fig. 1. The chromatogram is characterized by two predominant humps (and two external smaller ones) centered roughly at n -alkane C_{27} and C_{36} . After quantification, the MOSH “humps” were subjected to a qualitative investigation. A four-dimension off-line LC-GC \times GC-qMS experiment was carried out as follows: the 175 μL MOSH fraction was collected, dried, and the residue was solubilised in 10 μL of hexane. Four microliters were injected into the GC \times GC-qMS system (split ratio: 1:10), leading to an injected MOSH quantity about 25 times lower than that analyzed by using LC-GC-FID. However, the enhanced sensitivity of cryogenic modulation was something that was counted on. The LC-apolarGC \times polarGC-qMS result, which is shown in Fig. 2, was attained in a relatively short time (<30 min), but obviously could not match the speed of the LC-GC second dimension. The LC-GC-FID result, for the salmon I sample, is also included in the same figure to make a direct comparison easier.

A mass spectrum derived from the “cycloalkane zone” is shown in Fig. 3. The “cyclics” were identified on the basis of (I) bidimensional chromatogram location, and (II) information reported in the literature, related to the fragmentation of cycloalkanes^[5]: the molecular ion intensity is more abundant in cycloalkanes than in straight-chain alkanes, as is also the tendency to lose even-numbered fragment ions, producing a greater number of even-numbered mass ions. Compounds containing cyclohexyl rings tend to produce ions at m/z 83, 82 and 81 corresponding to ring fragmentation, and loss of one and two hydrogen atoms, respectively. A further example of MOSH contamination can be observed in Fig. 4 and 5, which illustrates the LC–GC–FID result for beef sample II. The MOSH levels in the beef sample were approximately one third (5.6 ppm) of that found in the salmon. However, the contamination type is different because three MOSH humps are present, with the second and third spanning the C_{25} – C_{39} range, and the first starting approximately at C_{19} and ending at C_{24} . Considering the C_{25} boundary, the MOSH concentration was 2.2 mg/kg. With regards to the other samples reported in Table 1, it can be affirmed as follows: the third most contaminated sample, namely beef-ham (4.9 ppm), generated a GC chromatogram characterized by two humps, in the C_{19} – C_{24} ($\approx 40\%$) and C_{25} – C_{42} range. Such a contamination level and type is comparable to that observed in beef sample II. Five other samples were characterized by MOSH levels around the 3 ppm mark (plaice, chicken, turkey, calf, rabbit), while the remaining four baby foods, namely beef (sample I), ostrich, sea bass, and salmon II were the less contaminated ones, with values under 2 ppm. It must be emphasized that only two baby foods, namely ostrich and salmon II, were characterized by a MOSH contamination lower than the “ C_{25} ” limit. At this point, it was decided to extend the study to fruit-based baby foods, namely products containing no vegetable oil. Four samples (2 prunes, 1 pear, 1 fruit mix) were subjected to analysis, and found to contain MOSH concentrations in the range 0.3–10.5 mg/kg (Table 1). Two baby foods, namely prune I and fruit mix, were characterized by a MOSH presence within the “ C_{25} ” limit, while the pear sample was right at the limit; the prune II sample was, on the other hand, the second most contaminated, considering all the baby foods subjected to analysis (Table 1). The results attained from the fruit products clearly indicated that the presence of MOSH was not only due to that of the vegetable oil.

5. Conclusions

All the baby foods analyzed in the present research were found to be MOSH contaminated, with considerable levels being observed (average value: 3.8 mg/kg). In fact, if one refers to the 0.6 mg/kg limit proposed by the BMELV, the contamination levels must be evaluated as high, especially considering the age and body weight of the consumers. In fact, taking into account the C_{25} boundary, the average MOSH concentration was approx. 1 mg/kg. The presence of MOSH has been found in human milk^[6], and on the basis of the present findings, there is a high probability that MOSH intake continues as soon as the infant passes on to solid food. It is clear that the origin of the MOSH contamination observed, in all samples, cannot be exactly pinpointed, though the vegetable oil probably plays a role. The hydrocarbon molecular-weight range also varied, meaning that the contamination most certainly came from different sources.

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