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Determination of Mineral Paraffins in Feeds and Foodstuffs by Bromination and Preseparation on Aluminium Oxide: Method and Results of a Ring Test

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Introduction

The contamination of animal feed with PCBs and dioxins in Belgium (early 1999) brought to our attention the danger of poisoning animals and human foodstuffs through waste disposal into fats used for the production of mixed feeds. One of the roots of the danger is that many oils and fats for feed production are considered as wastes, and since traditionally various kinds of wastes are fed to animals, not everybody is sufficiently aware of the limits of such practices.

Oils and fats as well as mixed feeds were analyzed for non-edible wastes using mineral paraffins as markers according to the assumption that most of the wastes contain mineral oil products (1). Indeed, in Switzerland, oils and fats from public waste collection sites were contaminated at concentrations of up to many percents. In the meantime, their use for feed production was stopped. More surprisingly, many other oils used for feeding animals also contained mineral hydrocarbons. Particularly high concentrations were found in the by-products from edible oil refining, principally consisting of free fatty acids and the condensate from deodorization. The sources of such contamination still await being identified.

The analysis of mineral oil material, first of all isoalkanes with 12 to 40 carbon atoms, is demanding. In fact, several laboratories produced poor results, with deviations reaching a factor of 100, and thus caused widespread confusion and annoyance, particularly among those who wanted to do their best to solve the problems. Deviations resulted from different understanding of what is meant by "mineral paraffins", but also from inadequate analysis. Since there is no official method, various in-house methods were adapted to the task, apparently with insufficient valida-

tion. In the interest of avoiding further problems, a method was developed and tested by eight laboratories. It does not involve on-line LC-LC-GC as used in (1) and should be amenable for any laboratory disposing of a gas chromatograph equipped with a flame ionization detector and an on-column injector.

For the numerous samples analyzed by both methods, the quantitative results corresponded well. The detection limit of on-line LC-GC is slightly better (an aliquot corresponding to 20 mg fat or oil is injected, rather than 10 mg); the removal of the interfering olefins (without bromination) is similar provided two well retaining HPLC columns are used. The main advantage of fully automated on-line LC-GC is relevant when large numbers of analyses are to be performed: some 25 samples can be analyzed per day, including blanks and check samples, without fully occupying a person.

The method is also useful for the control of foodstuffs. In fact, many foods are contaminated by mineral oil components (2). Sources are lubricating oils (3), release agents (4), dust binding agents, printing inks (5), and packaging materials (6–10), but also admixture of diesel oil to palm oil or the contamination in containers or ships previously used for technical products.

Fats for feed production

Premixed feeds for animals, e.g. layer hens, chicken, beef, milk cows, or pigs, usually contain 3–5 % fat. The characteristics of the fats and oils, such as the degree of unsaturation, are adjusted to the application. In practice, the following oils and fats seem to be important:

- used frying fats from restaurants, fast food chains, and industrial food production (e.g. chips);
- used edible oils from public collection sites (some countries only);
- free fatty acids and condensates from edible oil refining;
- raw plant oils;
- animal body fats from slaughter houses, bone fat, sometimes hydrogenated (recently forbidden in many countries);
- hydrogenated fish oil.

Paraffins, mineral oil fractions

Saturated hydrocarbons are the main components of mineral oil and of most of its products. Since the complete chemical analysis of the materials contaminating feeds and foods, including the aromatics, additives and their degradation products, heavy metals etc. is difficult, the components accompanying the mineral paraffins must be deduced through the identification of the source.

The composition of the hydrocarbons may provide some information about the source. The following mineral oil fractions may be encountered:

1. Diesel and heating oil (hardly distinguishable): high proportion of n-alkanes, typically reaching from C8 to C25.

2. Kerosene: similar to diesel oil, but only reaching to about C18.
3. Gas oils: high vacuum distillates with a Gaussian type volatility distribution and consisting of a range of hydrocarbons comprising 10–15 carbon atoms. In GC, white oils, also called paraffin oils, are commonly centered at the retention time of the C21–24 n-alkanes, base oils for lubricants (e.g. motor oils) at C28–32, and hydraulic oils at C23–30. Usually the n-alkanes and the aromatics are removed from the gas oils, such that in the gas chromatograms the remaining branched and cyclic hydrocarbons form a fairly symmetric hill of Gaussian shape with hardly any peak on it (see (1)).
4. Vaseline: volatility range of gas oils; aromatics are removed, but not the n-alkanes.
5. Waxes: n-alkanes from deparaffination of gas oils, used for certain food packaging materials.
6. Poly-alpha-olefines (PAOs): condensates of unsaturated mineral oil cracking products or oligomers of polyethylene; usually hydrogenated to eliminate residual double bonds. Condensed cracking products result in mixtures with a broad range of volatility, in GC forming a hump of unresolved iso-alkanes reaching from C25 to beyond C45. They serve, e.g., as additive to gasoline used for two-stroke engines. The PAOs from polyethylene oligomers have a more defined composition, in GC forming 2–3 rather narrow humps, typically around the C28 and C34 n-alkanes. They are used for synthetic motor oils, but also for food-compatible lubricating oils.

The main types of mineral oil products can be distinguished by their GC finger prints. Particularly for the gas oils, however, it is hardly possible to conclude on the application: the same fraction may serve as base oil for different products, merely distinguished by the additives. Feeds and foodstuffs primarily contained hydrocarbons with a composition corresponding to that of gas oils (1), in GC often forming several overlapping humps.

Being restricted to the saturated hydrocarbons, the method described here reveals concentrations which are lower than the real contents of contaminants, and the toxicologically most relevant components are likely to be overlooked.

Distinction from feed and food components

Certain hydrocarbons are also natural components of feeds and foods, and the mineral paraffins must be distinguished from them. Most of the natural hydrocarbons are olefines, primarily in the ranges of C16–20 and C25–30. They can be eliminated by suitable preseparation if the analysis is restricted to the saturated hydrocarbons. Natural paraffins, primarily n-alkanes with prevailing odd numbers of carbon atoms (predominating n-C27, n-C29, and n-C31) and some defined branched alkanes forming clear signals, cannot be removed in this way and must be distinguished from mineral hydrocarbons in GC.

Principals of the method

Mineral paraffins consist of an enormous number of components and individual calibration of detector responses is impossible. Flame ionization detection (FID) provides approximately constant response per unit of weight for all of them, which is one of the reasons to use GC as analytical method.

GC neither separates mineral oil paraffins into individual components, nor does it combine them to sharp, well defined signals. Thus, the broad humps of unresolved material do not enable identification by classical means, such as retention times – they could in fact represent any type of highly isomerized material. Also mass spectrometry provides little information about material identity. The specificity of the method must, therefore, be based on a preseparation which is sufficiently selective that practically only paraffins reach GC.

In the method described below, mixed feeds or foods are extracted with an apolar solvent, possibly after drying with the help of sodium sulfate. As certain natural olefines are difficult to separate from the paraffins, their polarity is increased by bromination. The brominated extract or fat is passed through a column packed with active aluminium oxide. The fraction containing saturated hydrocarbons is reconcentrated to about 500 µl and analyzed by GC-FID injecting a 50 µl volume by the on-column/retention gap technique (11).

Large volume injection is a prerequisite for reaching the required detection limit, because the ratio of signal to background noise of a broad hump is poor and the sensitivity of FID is modest anyway. As the Swiss limit for mineral paraffins in fats and oils for animal feed is 30 mg/kg (12), the method must enable the determination of a concentration of 10 mg/kg. This is a sensitivity also appropriate for food analysis. The alternatives to large volume injection are inelegant. Reconcentration to ca. 10 µl is tedious and results in severe loss of the more volatile components. To avoid the handling of such small volumes, the amount of sample material had to be increased by a factor of about 10, which would proportionally increase the bromine consumption and the required size of the aluminium oxide column.

Three internal standards are added to the sample. Usually the n-alkane C14 (100 mg/kg sample) is used for calculation of the mineral oil paraffins. When this large peak exceeds the capacity of the integration system, n-C15 is used, of which only 10 mg/kg is added. 1-Hexadecene (1-C16:1, 100 mg/kg) is brominated and should not be visible in the chromatogram. It serves as a control of the completeness of bromination and the retention power of the aluminium oxide column.

A short GC column (e.g. 10 m × 0.25 mm i.d.) is used in order to minimize the baseline drift: a straight baseline up to a high oven temperature is a prerequisite for the sensitive determination of a hump of unresolved material. The smaller the amount of stationary phase in the column, the less column bleed is obtained. Short columns provide reduced separation efficiency, i.e. may be less deep separation between the unresolved peaks above the hump. However, the concentrations of mineral oil paraffins determined with a 7 m and a 30 m column were identical, i.e.

separation reached the same contour line of the hump. This experiment was performed on fatty acids from edible oil refining, hence a sample containing an extremely high load of interfering natural components.

Reagents and materials

Aluminium oxide 60 or 90 basic, activity stage I, packed in a glass bottle, e.g. Alumina Woelm B Super 1; internal standards, n-tetradecane (n-C14), n-pentadecane (n-C15), 1-hexadecene (1-C16:1), all from Fluka (Buchs, Switzerland); pentane as extraction solvent, hexane as solvent and eluent, heptane to prepare the standard solution, chloroform, bromine, purity to be checked by blanks; paraffin oil or standard motor oil (assuming an 85 % paraffin content) as external standard and to check the method; edible oil free from mineral oil, e.g. refined rape seed or sunflower oil (check by analysis).

Internal standard solution (IS solution, 1 mg/ml each of n-C14 and 1-C16:1, 0.1 mg/ml of n-C15): weigh 100 mg each of n-C14 and 1-C16:1 into a 10 ml measuring flask, and 10 mg n-C15 into another 10 ml measuring flask; fill them up with heptane. Combine 2 ml of each of these two solutions into a 20 ml measuring flask and fill up with heptane. Transfer IS-solution into autosampler vials and store these in the refrigerator (no limit for storage, control through the check sample).

Check sample (30 or 100 mg/kg paraffin oil in edible oil free from mineral oil): weigh 9 mg paraffin oil and add 300 g edible oil (30 mg/kg) or weigh 20 mg paraffin oil and add 200 g edible oil (100 mg/kg); homogenize with a magnetic stirrer during at least 2 h; no limit for storage.

Bromine solution: 5 (±1) % bromine in chloroform. Storable for about 2 weeks (limited by the formation of disturbing peaks in the early part of the chromatogram).

Tube for aluminium oxide column: ca. 75 × 12 mm i.d., made of glass (plastic releases interfering material), preferably with frit, e.g. Bakerbond SPE tubes with PTFE-frit.

Gas chromatograph with on-column injector and FID, autosampler being optional; ca. 10 m × 0.25 mm i.d. separation column coated with a methyl silicone of ca. 0.3 µm film thickness; 8 m × 0.32 mm i.d. uncoated precolumn, may consist of raw fused silica, attached to the separation column by, e.g., a press-fit connector. 50 or 100 µl on-column syringe.

Procedure

Sample preparation

From foods or feeds, the fat is extracted since the hydrocarbons are mainly dissolved in this phase. To wet samples, such as fish, meat, or egg yolk, sodium sulfate is admixed until lumps disappear (2–3 times the sample weight). The granulate is covered with pentane (or hexane) and allowed to stand overnight (with some occa-

sional shaking). The extract is decanted, centrifuged if necessary, the solvent evaporated by a rotary evaporator, and the fat weighed in for analysis. Results are calculated related to the fat. Fat extraction may be incomplete, but the yield is assumed to be the same for fat and the hydrocarbons.

Preseparation

100 mg fat (homogenized by fusion at 50–60° C in an oven), oil, or extract are weighed into a 3 ml screw cap vial. If fat tends to solidify (e.g. animal body fat), the vial is placed on a warm plate. Bromine solution is admixed in portions under repeated shaking until the solution remains slightly colored; up to 600 µl bromine solution may be required, mainly depending on the unsaturation of the fatty acids. 10 µl of IS solution is added by dipping the needle tip of a 25 µl syringe into the solution. After 15 min, the excess of bromine and the chloroform are removed in a stream of nitrogen.

3.5 (± 0.2) g dry aluminium oxide are packed into the tube, removing cavities by tapping the column. The sample is picked up in 0.3 ml hexane and transferred onto the dry column, rinsing the vial with another 0.2 ml hexane. The paraffins are eluted with hexane collecting the first 2.0 ml of eluate. The eluate is reconcentrated to about 0.5 ml in a stream of nitrogen, dipping the vial into a bath of warm water. Complete evaporation must be avoided because there is no longer any oil or fat preventing loss of volatile components.

GC-analysis

Carrier gas inlet pressure: 60 kPa for hydrogen or 1.2 kPa for helium. Injection of a 50 µl volume at 5 µl/s (autosampler) or 1–5 µl/s (manual injection) and a column temperature of 60° C (4 min, i.e. sufficient to complete solvent evaporation). Temperature is programmed at 20°/min to 320° C (3 min).

An analytical sequence comprises the following steps:

1. GC-blank: 50 µl injection of hexane; a freshly installed, deactivated uncoated precolumn usually releases some "ghost peaks", possibly polymerized silylation material.
2. Check sample adjusted to the task of the analysis: analysis of a fat containing 30 or 100 mg/kg mineral paraffins (see above). Verify whether the concentration of the paraffins calculated from the internal standard corresponds to that added.
3. Total analysis blank: the whole procedure is performed with an edible oil free of mineral oil paraffins (e.g. that used for preparing the check samples). If the analysis involves an extraction step, it should also be included.
4. Samples.
5. GC-blank after a strongly contaminated sample, including an estimation of the memory effect.

Quantitative determination

The baseline is transferred from a blank and the gas chromatogram inspected for the presence of mineral paraffins, looking out for unresolved humps and n-alkanes with carbon numbers balanced between even and odd. Well shaped peaks are considered only if they belong to a homologous series of mineral n-alkanes or to isoalkanes between dominating mineral n-alkanes. The regions of the n-alkanes C16–C19 and C26–C30 often contain interfering natural components (the largest peaks frequently being n-C29 and n-C31).

Integration

Quantitative determinations can be performed by integration of the internal standard n-C14 (100 mg/kg) and the mineral oil material without applying a response factor. If the peak of n-C14 is too high to be fully integrated (attenuate to check the complete peak), reduce the signal size delivered to the integrator or use n-C15 (10 mg/kg).

Integration of a hump of unresolved paraffins, as shown below for some examples, occurs in two steps. First the total area is determined from the beginning to the end of the hump including the peaks on top. The baseline is set manually or transferred from a blank chromatogram. Then the peaks on top of the hump are integrated, either selecting the parameters such that the new baseline follows the top of the hump, or the contour line is manually approximated in a few sections as straight lines. The summed peak areas are subtracted from the total area of the hump.

Estimation

The content of mineral oil paraffins can also be estimated by comparison of areas in terms of mm^2 in the printed chromatograms: the area of a hump observed for a sample is compared with that of an integrated chromatogram or a check sample containing a known concentration of mineral paraffins. This procedure is usually faster and adequate if a maximum accuracy is not required or anyway impossible. It also serves for the control of results obtained by integration, but is not suitable if large peaks of n-alkanes belonging to mineral oil material must be included since it is difficult to estimate the area of such signals.

First the baseline and the contour line towards the peaks on top of the hump are drawn into the chromatogram, surrounding the area to be assessed. Then the area of the hump is approximated by triangles or rectangles, including as much extra area as is excluded, and the area calculated in units of mm^2. Results are corrected for differences in the concentrations of the sample injected through the integrated internal standards. It must be kept in mind that the area in terms of mm^2 depends on the attenuation of the chromatogram and the chart speed (range shown in the printed chromatogram).

Verification of the results

The principal verification tool for the method is the check sample consisting of edible oil with a known amount of mineral paraffin (see above). For the verification of the results the following potential problems must be kept under control:

1. Contamination of the sample by the packaging material used to bring it to the laboratory (e.g. a fat sample poured into a plastic beaker while hot). In case of doubt, an extract from the packing material is analyzed and the composition compared with the sample. Polyethylene provides predominating peaks for n-alkanes or 1-alkenes with an even number of carbon atoms, but usually also contains some mineral oil material.
2. Inhomogeneous sample: fats and crystallizing samples (e.g. free fatty acids) must be liquefied.
3. Contamination during sample preparation, e.g. by grease from glass ware or plastic stoppers, paraffins from the aluminium oxide container, aged bromine solution, aluminium oxide column packed with a plug of wool, hand cream, gloves. Check by total analysis blank.
4. Contamination of the sample solution by the cap of the autosampler vial. Often it occurs inconsistently and typically forms a hump centered at C30–C35 topped by large peaks of n-alkanes. Check by repeating the analysis with a new solution. Only a single injection should be performed from a vial since penetration of the septum pierces the PTFE membrane protecting the septum against the sample liquid. It may help to cover the vial by aluminium foil before closure with the cap.
5. The 1-C16:1 standard checks the completeness of the bromination and should not show up in the chromatogram. 1-C16:1 may, however, contain some n-C16, which is eluted at almost the same retention time.
6. Insufficient activity of the aluminium oxide results in breakthrough of the brominated olefines through the column (see also comment below). Results tend to be too high since the olefins are included in the analysis as their bromo derivatives. Breakthrough is recognized by the dibromo 1-C16:1 forming a large peak in the region of the n-C22 alkane.
7. Deformation of the GC baseline or broad signals in the region of the mineral oil paraffins to be analyzed. The problem may result from incomplete elution of the previous sample or from material released by the on-column injector. Check with a GC blank. In case of severe problems, rinse the GC column with dichloromethane.
8. Inaccurate addition of the standards or reconcentrated IS solution. Control through the analysis of the check sample.
9. Too small peaks for the internal standards, typically with losses being higher for n-C14 than for n-C15: evaporation during reconcentration of the fraction from the aluminium oxide column (do not evaporate to dryness!).

10. Peak areas of n-C14 and n-C15 are not in a ratio of 10:1. Either the area of n-C14 is too small since the (high!) signal was cut by the integration system (strongly attenuate to see the top of the peak), or the area of n-C15 is excessive because of overlapping with material from the sample (prepare sample without addition of the standards).
11. Errors during integration, such as by subtraction of peaks outside the window of the integrated mineral oil material or neglect of peaks within the window, e.g. because of a height exceeding the maximum of the integration system. Check results by comparison with other chromatograms or check samples.

Confirmation of mineral oil

The conclusion that the material detected in a sample represents mineral paraffins can be supported by the following arguments:

1. Bromination and preseparation is selective for paraffins.
2. The hump of components without significant separation and with hardly any peaks on top represents a mixture of such an enormous number of components as it is encountered nowhere in living nature.
3. A hump of Gaussian shape, i.e. a composition with a regular, symmetric distribution and a width corresponding to about 15 carbon atoms is indicative of a distillation (GC depicts volatility).
4. The same matrices, such as oils or other foods, also exist without mineral paraffins, and the humps vary from one sample of given type to another.

Mass spectrometry is of modest usefulness. The total ion chromatogram is not specific for hydrocarbons; the ions m/z 57 or 71 used in single ion monitoring are observed in the spectra of many organic compounds of elevated molecular mass. Quantitative data obtained with the ion m/z 57 corresponded to that obtained by FID provided the response was calibrated with a gas oil.

Validation

The method was designed for the control of a 30 mg/kg limit. The lower detection limit is 3–20 mg/kg, depending on the distribution of the mineral paraffins and the interferences from the sample. A mixture of several mineral oil products with a broad distribution in volatility results in a broad hump which is relatively difficult to quantitate (see examples discussed below). There is no upper detection limit; if GC or the integration system is overloaded, the sample solution must be diluted.

Quantitative data

The predominating uncertainty in quantitative determinations originates from the interpretation of the chromatograms (integration of a broad hump). It depends on the sample and must be estimated from the chromatograms. The definition of the baseline and the contour line towards the peaks on top of the hump must be varied

between reasonable extremes in order to determine the maximum and minimum possible area.

Another problem may occur for samples containing large amounts of components overlapping with the internal standard, first of all samples containing mineral n-alkanes of the type encountered in diesel or heating oil. The maximum contribution to the peak area of the internal standard can be estimated from the n-alkanes surrounding the internal standards. Overlapping with the standards causes the results to be too low.

For "easy" samples containing around 30 mg/kg of mineral paraffins, a realistic assumption of uncertainty is 20 %, for extremely "difficult" samples it is a factor of 2. Unless uncertainty is defined differently, the limit of 30 mg/kg should be considered exceeded with certainty only when the analytical result exceeds 50 mg/kg. On the other hand, the content of mineral oil paraffins is certainly below the limit only when the analytical result does not exceed 20 mg/kg.

Results

The following examples and chromatograms illustrate the results obtained. They are from the late phase of method development when it was still experimented with various internal standards.

Preseparation

The results in figure 1 are from a sample of fatty acids and condensate from edible oil refining. The gray surface in the top chromatogram was interpreted as mineral paraffins. The components producing the peaks on top of the hump are typical for vegetable oils. The n-alkanes are alternating, those with an odd number of carbon atoms being predominant (main peak, n-C29). The shape of the hump suggests a mixture of deparaffinated gas oils. The material is centered at n-C23, i.e. at lower molecular weight than typical for lubricating oil. The n-alkanes C13–C15 were added as internal standards, but the concentration chosen, 10 mg/kg, was too low (interferences). The shaded area corresponded to a concentration of 1010 mg/kg. The estimated uncertainty is around 20 % and primarily originates from the separation between the hump and the peaks on top.

The chromatograms in the center and at the bottom (F2 and F3) are from the subsequent 1.5 ml fractions of the aluminium oxide column and show isolated peaks of somewhat more polar compounds, but no hump which could be misinterpreted as mineral paraffins. It is concluded that the cut (volume) of the paraffin fraction is rather uncritical. With the first 1.5 ml of hexane, over 98 % of the material of interest is included.

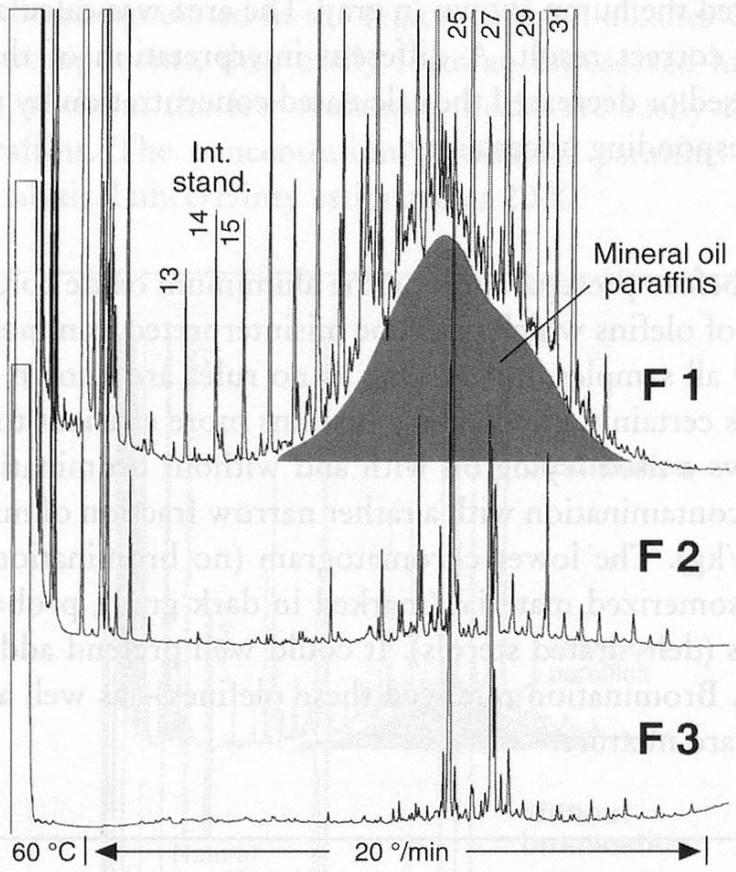

Figure 1 **Fatty acids from edible oil refining with about 1000 mg/kg mineral paraffins. Fractions 1 to 3 from the aluminium oxide column**

Detection limit

The chromatogram in figure 2 is from a used frying oil which was considered rather difficult to analyze. It contained less than 5 mg/kg mineral paraffins, but was

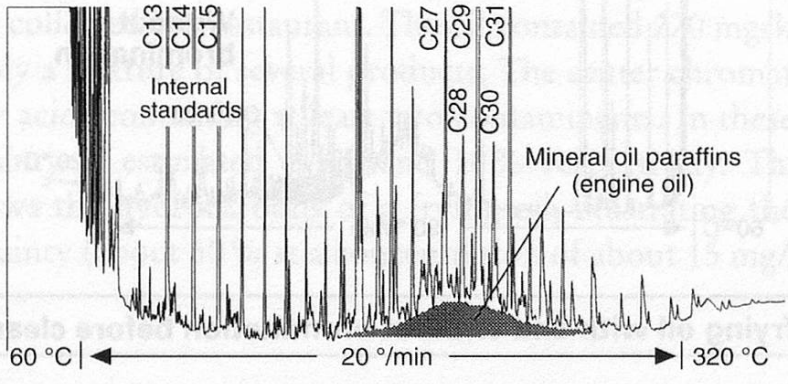

Figure 2 **Used frying oil to which 15 mg/kg of an engine oil was added**

spiked with 15 mg/kg of an engine oil (of which about 85 % are paraffins). This engine oil produced the hump shown in gray. The area was calculated as 13 mg/kg, i.e. provided the correct result. A different interpretation of the chromatogram could have increased or decreased the calculated concentration by up to some 30 %, suggesting a corresponding uncertainty.

Bromination

Bromination before preseparation on the aluminium oxide column should facilitate the removal of olefins which could be misinterpreted as mineral paraffins. It is not necessary for all samples, but as long as no rules are known to determine for which sample it is certainly unnecessary it seems more efficient to always apply it.

Figure 3 shows a used frying oil with and without bromination. In the upper chromatogram a contamination with a rather narrow fraction of mineral paraffins is observed (25 mg/kg). The lower chromatogram (no bromination) contains additional, strongly isomerized material (marked in dark gray), probably largely consisting of sterenes (dehydrated sterols). It could well pretend additional 15 mg/kg mineral paraffins. Bromination removed these olefines – as well as the 1-C14:1 of the internal standard mixture.

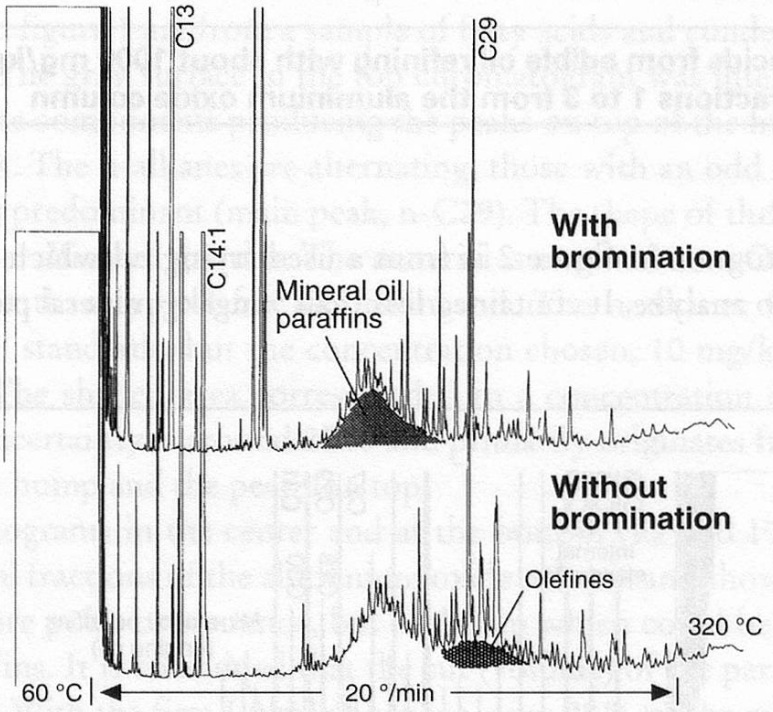

Figure 3 **Used frying oil with and without bromination before clean up**

Figure 4 shows a sample of fatty acids from an edible oil refinery with disturbing natural hydrocarbons eluted in the region of the n-alkanes C16–C19. As they comprise many components, they easily form an unresolved hump. Bromination eliminates most of them; the few remaining peaks are easily distinguished from the mineral paraffins. The concentration of mineral paraffins was calculated as 32 mg/kg, the analytical uncertainty estimated as 20 %.

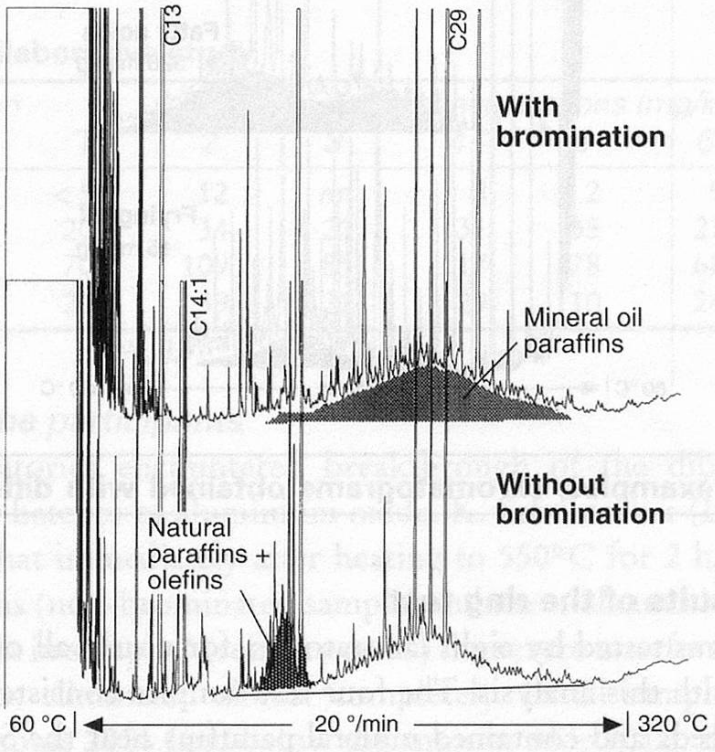

Figure 4 **Fatty acids from edible oil refining, containing about 30 mg/kg mineral paraffins as well as natural hydrocarbons which may be misinterpreted as mineral components if co-eluted. Bromination removes most of these**

Some further examples are shown in figure 5. The top chromatogram is from a used frying oil collected in a restaurant. The oil contained 270 mg/kg mineral paraffins, presumably a mixture of several products. The center chromatogram is from a sample of fatty acids containing at least two contaminants. In these two cases, analytical uncertainty is estimated as 20 and 10 % respectively. The bottom chromatogram shows the hydrocarbons of a frying oil, illustrating the detection limit and the uncertainty (about 50 % at a concentration of about 15 mg/kg).

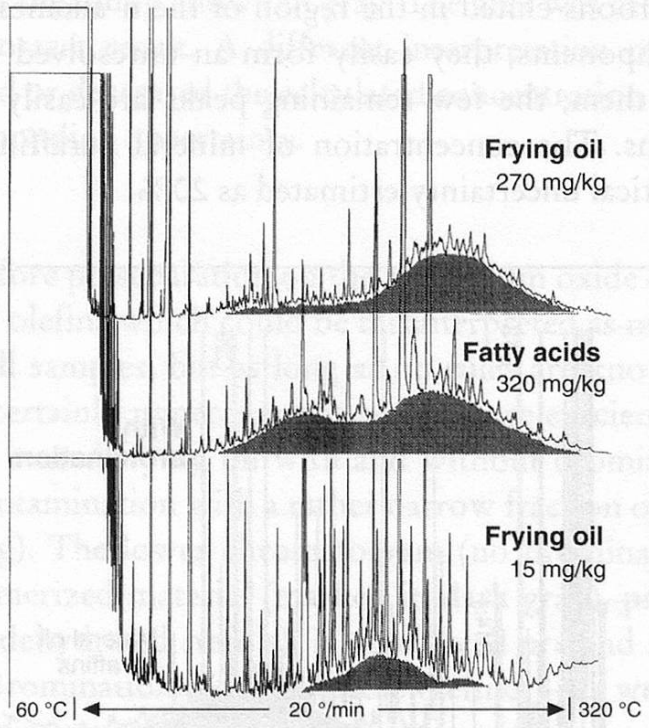

Figure 5 **Further examples; chromatograms obtained with differing attenuation**

Appendix 1: Results of the ring test

The method was tested by eight laboratories, for nearly all of which it was the first experience with this analysis. The four test samples consisted of fats typically used for animal feeds and contained mineral paraffins near the Swiss limit for fats for feed production, i.e. 30 mg/kg.

1. Mixture of used frying oils ("Fritmix") with less than 3 mg/kg of mineral paraffins according to measurements by this method as well as by on-line LC-GC.
2. Same matrix to which two gas oils were added at a total concentration of 25 mg/mg ("Fritmix +"), namely a paraffin oil used as release agent by a candy producer with the hydrocarbons centered at C22 and a base oil for motor oils centered at C29. This mixture resulted in a broad distribution of the hydrocarbons and an irregular contour line, which rendered its analysis relatively difficult.
3. Fatty acids/refinery condensate with a low content of mineral paraffins but a high content of natural hydrocarbons, resulting in strong interferences.
4. Lard with less than 2 mg/kg of contaminants to which 20 mg/kg of a base oil for lubricants was added. Being almost free of interferences by natural components, it should have been the easiest sample.

Participants were Labor Haase-Aschoff, Bad Kreuznach; Staatliches Medizinal-, Lebensmittel- und Veterinäruntersuchungsamt Südhessen, Wiesbaden, both Germany. Interlabor, Belp; Labor Veritas, Zurich; Swiss Quality Testing Services

(SQTS), Dietikon; Coop Qualitätscenter, Pratteln; Official Food Control Authorities of St. Gallen and Zurich, all Switzerland.

The results are shown in table 1. As concluded from a meeting discussing the results, the concentrations found in the frying oil without addition of mineral paraffins primarily reflected uncertainty from an unstable baseline. For sample 3 (refinery acids), the contour line separating the mineral paraffins from the natural compounds was the key problem: high values were obtained when this line was set high.

Table 1
Results of the collaborative study

Sample	*Addition*	*Laboratory, concentrations (mg/kg)*							
	mg/kg	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Fritmix	none	< 5	12	nn	8	2	9	15	nn
Fritmix +	25	20	34	20	31	35	21	37	24
Acids	none	70	109	85	117	78	68	88	60
Lard	20	20	39	21	29	10	24	19	17

Comments by the participants

Several laboratories encountered breakthrough of the dibromo hexadecane when using older batches of aluminium oxide. K. Schumacher (Laboratory Haase-Aschoff) found that immediately after heating to 550° C for 2 h, the packing even retained the olefins (non-brominated sample) and the n-alkanes beyond about C24, with the effect that in sample 3 (refinery acids) the severe interference by the natural hydrocarbons was eliminated almost completely. In all samples, concentrations were too low by some 20 % (and below the amount added), i.e. also some branched hydrocarbons were lost. After the adsorbent was allowed to stand for two days, the natural hydrocarbons were back in the chromatogram and the quantitative results were excellent.

R. Kunz (Coop) obtained peaks with a shoulder at their onset, indicative of some sample liquid flooding into the separation column (precolumn of 7 m only). The problem was solved by reducing the injection volume to 40 µl. This suggests that the proposed 8 m uncoated precolumn is used close to the capacity limit.

P. Egli (Veritas) tested the use of an ion trap mass spectrometer as an alternative detector, summing up the ions from 50 to 100 amu. For a paraffin oil, a response factor related to the internal standard of 1.09 was determined.

D. Herren (Interlabor) suggested to add the internal standard before bromination in order to prevent the tip of the syringe needle from being attacked by the bromine. As small amounts of liquid can only be added by introducing the needle tip into the liquid (accurate transfer), about 100 µl of a more diluted solution should be added.

Several participants experienced problems regarding the baseline in the range of the C16-C25 hydrocarbons as well as in the region of the column bleeding. The for-

mer disturbance may result from a contaminated on-column injector and can be eliminated by backflushing the latter with carrier gas. The baseline also tends to improve during use of the instrument and column.

General evaluation was positive. The procedure was considered simple, of adequate sensitivity, and substantially shorter than alternative methods.

Appendix 2: Remarks on the performance of aluminium oxide

The selectivity of aluminium oxide was tested using mixtures containing n-alkanes C16 to C40, a motor oil with isoalkanes centered at C29, 1-C16:1, and 1,2-dibromo hexadecane. The following results were obtained:

1. When the aluminium oxide was taken from a freshly opened bottle, the regular 2 ml fraction merely contained the n-alkanes up to C22 (the latter at about half of the correct size, n-C24 at about 10 %). The subsequent 2 ml fraction contained part of the n-C22-C26 material (60 % of n-C24), whereas n-C28 was not even eluted in the third fraction. The paraffin oil (of larger molecular weight) was eluted to more than 90 % in the first fraction, 1-C16:1 to 100 %, the 1,2-dibromo C16 not at all.
2. Losses of the longer chain n-alkanes did not significantly depend on the amount (concentration) brought onto the column, i.e. there was no sign of an adsorption effect or of limiting solubility.
3. After heating to 530° C for 2 h, 1-C16:1 was fully retained, while the performance regarding the saturated alkanes did not change significantly: n-alkanes above C22 remained on the column, whereas at least 90 % of the isoalkanes were eluted.
4. After storage of the heated aluminium oxide during a night in an open beaker, all test components were eluted, i.e. a sufficient amount of water had been absorbed for the retention power to collapse.
5. Storage of the heated aluminium oxide in a tight flask over four days reduced activity to the extent that 20 % of 1-C16:1 was eluted.
6. Addition of 0.2 % water to the freshly heated aluminium oxide caused some 40 % of 1-C16:1 to be eluted. With 1 % water, the whole 1-C16:1 was eluted, but no n-alkane beyond C24. With 5 % water, all components broke through the column.
7. Slurry packing of the column (3.5 g aluminium oxide) with 20 ml hexane/ethanol showed that with 1 % ethanol all 1-C16:1 passed the column, but no n-C26, whereas with 1.25 % ethanol all components passed. With 1.1 % ethanol, about 50 % n-C40 and about 30 % of the dibromo-C16 were recovered.
8. Neutral and acidic aluminium oxide showed the same performance.
9. Silica gel released all n-alkanes while retaining the 1-C16:1 and the dibromo-C16.

Since long-chain n-alkanes are more strongly retained on active aluminium oxide than shorter chain alkenes and retention of n-C40 is similar to that of dibromo-C16, it seems impossible to adjust retention power such that the long-chain n-alkanes are eluted while the brominated olefines are retained. For the isoalkanes, however, no serious losses were observed even when freshly heated aluminium oxide was used.

It was concluded that the proposed method correctly determines the isoalkanes, but the n-alkanes above C23 may largely be lost for the analysis. If these losses are a problem, silica gel is the preferable adsorbent for preseparation. On the other hand, highly active aluminium oxide which largely removes the sometimes severely interfering natural n-alkanes may even be a prerequisite for a successful analysis of mineral paraffins. The first aspect should be kept in mind when evaluating results, the second more actively be used for the analysis of difficult samples.

Summary

The method is designed for the determination of paraffins with 10 to 45 carbon atoms as typical for mineral oil products. The sample (fat or oil, food or feed extract) is brominated in order to enhance the polarity of olefins. Passage through a small column packed with active aluminium oxide only allows the paraffins to pass into the fraction analyzed by GC-FID. On-column injection of a 50 µl volume from a sample reconcentrated to 0.5 ml provides the sensitivity required for the detection of 5–20 mg/kg (sum of mineral paraffins; limit depending on the distribution of the paraffins and the interferences by paraffins of natural origin). A collaborative study with eight laboratories (7 of which inexperienced) showed that the method is suitable for the control of the Swiss limit of 30 mg/kg paraffins in fats for animal feeds.

Zusammenfassung

Die Methode ist auf die Bestimmung von Paraffinen mit 10–45 Kohlenstoffatomen ausgerichtet, wie sie typisch in Mineralölprodukten auftreten. Die Probe (Fett oder Öl, Lebensmittel- oder Futterextrakt) wird bromiert, um die Polarität der Olefine zu erhöhen. Die Vortrennung durch eine Kartusche mit aktivem Aluminiumoxid ermöglicht nur den Paraffinen, in die Fraktion zu gelangen, die anschliessend mit GC-FID analysiert wird. On-column Einspritzung eines 50 µl Volumens aus der auf 0,5 ml eingeengten Fraktion ergibt eine Empfindlichkeit, welche für eine Nachweisgrenze von 5–20 mg/kg erforderlich ist (Summe der mineralischen Paraffine; Grenze abhängig von der Verteilung der Paraffine und der Störung durch natürliche Paraffine). Ein Ringversuch mit acht Laboratorien (davon 7 ohne Erfahrung) zeigte, dass die Methode für die Kontrolle des Schweizer Höchstwerts von 30 mg/kg Paraffinen in Fetten für die Futtermittelherstellung geeignet ist.

Résumé

La méthode est indiquée pour la détermination des paraffines contenant 10–45 atomes de carbone qui sont typiques des produits à base d'huile minérale. L'échantillon (graisse ou huile, extrait d'aliment ou de nourriture pour animaux) est bromé afin d'augmenter la polarité des oléfines. La préséparation par une cartouche remplie d'oxyde d'aluminium actif permet de récupérer seulement les paraffines dans la fraction analysée par GC-FID. Le dosage «on-column» d'un volume de 50 µl d'une fraction reconcentrée à 0,5 ml est nécessaire pour atteindre une limite de détection de 5–20 mg/kg (somme des paraffines minérales; limite dépendante de la distribution des paraffines et de la perturbation par des paraffines naturelles). Une étude interlaboratoire avec huit participants (dont 7 sans expérience) démontre que la méthode convient au contrôle de la limite Suisse de 30 mg/kg de paraffines dans les graisses utilisées dans la fabrication d'aliments pour animaux.

Key words

Mineral paraffins, Fat for feed production, Contamination of edible oils, Frying oils, Aluminium oxide

References

1 *Grob, K., Vass, M., Biedermann, M.* and *Neukom, H.P.:* Contamination of animal feed and food from animal origin with mineral oil hydrocarbons. Food Addit. Contam. **18,** 1–10 (2001).

2 *Grob, K., Artho, A., Biedermann, M., Egli, J., Lanfranchi, M., Caramaschi, A., Etter, R.* und *Romann, E.:* Wieviel Mineralöl ist in den Lebensmitteln tolerierbar? Mitt. Gebiete Lebensm. Hyg. **83,** 40–54 (1992).

3 Standard DIN V 10517

4 *Grob, K., Artho, A., Biedermann, M.* and *Egli, J.:* Food contamination by hydrocarbons from lubricating oils and release agents; determination by coupled LC-GC. Food Addit. Contam. **8,** 437–446 (1991).

5 *Droz, Ch.* and *Grob K.:* Determination of food contamination by mineral oil material from printed cardboard using on-line coupled LC-GC-FID. Z. Lebensm.-Unters.-Forsch. **205,** 239–241 (1997).

6 *Grob, K., Lanfranchi, M., Egli, J.* and *Artho, A.:* Determination of food contamination by mineral oil from jute bags using coupled LC-GC. J. AOAC **74,** 506–512 (1991).

7 *Grob, K., Biedermann, M., Artho, A.* and *Egli, J.:* Food contamination by hydrocarbons from packaging materials determined by coupled LC-GC. Z. Lebensm.-Unters.-Forsch. **193,** 213–219 (1991).

8 *Castle, L., Mitchell, K.* and *Gilbert, J.:* Migration of mineral oil hydrocarbons into foods. 1. Polystyrene containers for hot and cold beverages. Food Addit. Contam. **8,** 693–700 (1991).

9 *Jickells, S.M., Nichol, J.* and *Castle, L.:* Migration of mineral oil hydrocarbons into foods. 5. Miscellaneous applications of mineral oil hydrocarbons in food contact materials. Food Addit. Contam. **11,** 333–342 (1994).

10 *Grob, K., Huber, M., Boderius, U.* and *Bronz, M.:* Mineral oil material in canned foods. Food Addit. Contam. **14,** 83–88 (1997).

11 *Grob, K., Karrer, G.* and *Riekkola, M.-L.:* On-column injection of large sample volumes using the retention gap technique in capillary GC. J. Chromatogr. **333,** 129–155 (1985).
12 Swiss Federal Research Station for Animal Production, RAP Posieux. Letter from November 26, 1999.

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