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Development of a manual method for the determination of mineral oil in foods and paperboard

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ABSTRACT

So far the majority of the measurements of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) were obtained from on-line high performance liquid chromatography–gas chromatography–flame ionization detection (on-line HPLC–GC–FID). Since this technique is not available in many laboratories, an alternative method with more easily available tools has been developed. Preseparation on a small conventional liquid chromatographic column was optimized to achieve robust separation between the MOSH and the MOAH, but also to keep out the wax esters from the MOAH fraction. This was achieved by mixing a small portion of silica gel with silver nitrate into highly activated silica gel and by adding toluene into the eluent for the MOAH. Toluene was also added to the MOSH fraction to facilitate reconcentration and to serve as a keeper preventing loss of volatiles during solvent evaporation. A 50 μl volume was injected on-column into GC–FID to achieve a detection limit for MOSH and MOAH below 1 mg/kg in most foods.

1. Introduction

Mineral oil hydrocarbons (MOH) might be the quantitatively most important food contaminant. Their presence is almost ubiquitous and concentrations may be high. Recently EFSA published their scientific opinion on mineral oil hydrocarbons and identified numerous sources [1]. Mineral oil products consist of complex mixtures. Here two fractions are analyzed: the mineral oil saturated hydrocarbons (MOSH), comprising paraffins (open chain alkanes) and naphthenes (hydrocarbons with at least one saturated ring), and the mineral oil aromatic hydrocarbons (MOAH). The MOAH include polyaromatic compounds, but in contrast to the polyaromatic hydrocarbons (PAH) widely analyzed, they almost exclusively exist as alkylated species and, therefore, exist in enormous numbers of isomers.

Most data on mineral oil concentrations in food was generated by on-line coupled high performance liquid chromatography–gas chromatography–flame ionization detection (HPLC–GC–FID), recently reviewed in [2,3]. An analogous method with another HPLC–GC interface was described in [4]. On-line HPLC–GC for the determination of mineral oil is from the early 1990s [5] and was extended to the MOAH in 2009 [6]. Since it requires special equipment and expertise there has been the request to develop a technically less demanding alternative.

The standard method applied in the far past, primarily in environmental analysis, determined the sum of the mineral oil hydrocarbons by infrared (IR) spectroscopy. Extracts in carbon tetrachloride were purified by retention of polar constituents on Florisil or silica gel and analyzed by quantitative IR in the C—H stretching region. Detection limits were reported as 1 mg/kg for feeds and 10 mg/kg for tissue [7]. However, this method does not distinguish the mineral hydrocarbons from hydrocarbons endogenous in foods.

Present methods are based on FID because of calibration problems encountered with other detection methods, particularly mass spectrometry (MS) [2]. FID is the only method available for a quantitative determination of mixtures of hydrocarbons which are not available as standards. As it provides virtually the same response per mass of hydrocarbons, any standard can be used for determining any mineral oil. However, FID is of modest sensitivity, which is a particularly severe drawback for MOSH and MOAH analysis as they form broad humps. In fact, 50–100 ng MOSH or MOAH is required to be measurable [2].

GC is the separation technique of choice because it enables the distinction of the mineral oil hydrocarbons from hydrocarbons naturally occurring in food. It also enables the characterization of the

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mineral oil hydrocarbons by the molecular mass distribution and the pattern of peaks on top of the hump of unresolved components. However, MOSH and MOAH cannot be separated by GC, i.e. this must be achieved by preseparation.

In mineral oil analysis by GC–FID, the challenges are (i) the preseparation of the MOSH and MOAH, (ii) the removal of interfering material, in particular the wax esters, olefins and lipids, (iii) the reconcentration to achieve a limit of quantitation in the range between 0.1 and 1 mg/kg food as required from present toxicological evaluation [8] and (iv) elevated fat contents in the samples.

Almost all previously described sample preparation methods were limited to the analysis of the MOSH. Early methods started with the saponification of lipids and were derived from those developed for minor components in edible fats and oils, e.g. [9,10]. This step can be avoided when the liquid chromatographic column has sufficient capacity to retain the lipids, first of all the triglycerides.

A method designed for isolating the MOSH from up to 100 mg lipids involved a small liquid chromatography column containing 3.5 g activated aluminum oxide [11]. Of the reconcentrated eluate, 50 μl was injected into GC-FID by the on-column/retention gap technique to obtain a detection limit of around 1 mg/kg fat. If olefins from the edible oil, such as squalene isomerization products and sterenes, interfered, the edible oil was brominated prior to preseparation to increase the retention of the olefins. Later it was found that activated silica gel not only retains olefins beyond the MOSH, rendering bromination unnecessary, but also has increased capacity to retain lipids. 250 mg edible oil or fat could be loaded onto 2 g silica gel activated at 400 °C overnight [12]. Fiorini et al. [13] proposed using non-activated silica gel to obtain a narrower fraction and to inject in standard splitless mode.

After the finding of high mineral oil concentrations in Ukrainian sunflower oils early in 2008 [14], many control laboratories started analyzing MOSH, though at a rather high detection limit, as the European Commission had set a 50 mg/kg legal limit [15]. To support control, the EU Joint Research Centre (JRC)/Institute for Reference Materials and Measurements (IRMM) organized a proficiency test. Totally 55 laboratories provided results [16]. Of those specifying the method, the majority worked with columns with 10–30 g packing and a correspondingly high solvent consumption.

Moret et al. [17] optimized the approach for MOSH analysis toward small column size (1 g silica gel for 250 mg oil) with a low solvent consumption. For the complete removal of the olefins without derivatization, the silica gel contained 10% silver nitrate. Of the 1.5 ml fraction of hexane containing the MOSH, 40 μl was injected by the on-column/retention gap technique, resulting in a limit of quantitation in vegetable oil of 15 mg/kg.

Moret et al. [18] extended this method to MOAH analysis: the MOAH were eluted from same column packed with silica gel/silver nitrate using hexane/dichloromethane 1:1. Fast programming of the oven temperature (50°/min) accelerated the elution and resulted in a gain in sensitivity.

The method described in this paper aimed at separate MOSH and MOAH analysis with a performance and sensitivity comparable to on-line HPLC–GC, but only requiring conventional GC equipment. The target was a limit of quantitation suitable for the control of a 0.6 mg/kg limit for MOSH in the majority of the foods. Since the aliquot of sample loaded onto the column is limited by the fat content, the same compromise was chosen as for on-line HPLC–GC: instead of using a large packed bed adjusted to the samples with highest fat content, the method was configured for samples containing up to 20% fat, applying separate enrichment for those of a higher fat content [2,3].

2. Experimental

2.1. Materials

Hexane from Brenntag (Schweizerhall AG, Basel, Switzerland) was redistilled. Alternatively "Baker Ultra Resi-Analyzed" hexane 9262 (J.T. Baker, Deventer, The Netherlands) or hexane Chromasolv (Sigma–Aldrich, Buchs, Switzerland) was directly used. Toluene Pestanal 34494 from Fluka (Buchs, Switzerland) was purified by passing 10 ml through a column packed as for the samples. Dichloromethane was either Ultra Resi-Analyzed 9264 from Baker or Chromasolv from Sigma–Aldrich. Ethanol 8462 from Baker or Lichrosolve from Merck was used. 1,1,2-Trichloroethane was from Fluka, silver nitrate and silica gel 60, 0.063–0.200 mm, from Merck, Darmstadt, Germany. The silica gel/10% silver nitrate from Sigma–Aldrich/Fluka contained hydrocarbons (presumably olefins from the closure). It was purified by packing into a column and rinsing with toluene, then dichloromethane and hexane, followed by drying in a rotary evaporator at 70 °C.

Standards for MOSH analysis were *n*-undecane (*n*-C_{11}), *n*-tridecane (*n*-C_{13}), cyclohexyl cyclohexane (Cycy) and 5-alpha-cholestane (Cho); those for MOAH analysis were pentyl-benzene (5B), 1- and 2-methyl-naphthalene (MN), 1,3,5-tri-tert-butyl-benzene (TBB) and perylene (Per; all from Fluka/Sigma–Aldrich). Verification standards used in the context of method development were hexaethyl-benzene, 1,4-bis(2-ethylhexyl)-benzene, 1,4-bis(3,7-dimethyloctyl)-benzene, 1-phenyloctadecane and 1,2,3,5-tetracyclohexyl-benzene (all from Fluka/Sigma–Aldrich). The wax ester stearyl heptadecanoate (WE35) was a gift from Carlo Mariani (Milan). Gravex 913, a hydrotreated naphthenic mineral oil product used in printing inks from Shell was a gift from Shell Hamburg (Germany).

Stock solutions of 100 mg C_{11}, C_{13}, Cycy, 5B, 1MN, 2MN and TBB were prepared in 10 ml toluene or 1,1,1-trichloroethane. For standard solution 1, 12 mg each of Per and Cho was weighed into a 20 ml measuring flask to which 600 μl of each stock solution (only 300 μl of C_{13}) was added. The flask was filled up with toluene or 1,1,1-trichloroethane, resulting at 0.3 mg/ml except for C_{13} (0.15 mg/ml) as well as Cho and Per (0.6 mg/ml). For standard solution 2, solution 1 was diluted to 10 μg/ml (1:30).

2.2. Extraction of food and paperboard samples

Extraction of the various types of samples was described in detail in [2]. Dry food samples were extracted by immersion in hexane, mostly overnight at room temperature. Homogenized paperboard samples were extracted by immersion in ethanol/hexane (1:1) at room temperature for 2 h. Completeness of the extraction was checked by re-extraction at accentuated conditions, as extractability of solid samples may strongly vary. Wet samples were first blended into ethanol and allowed to stand for 1 h to largely exchange the water in the pores against ethanol. Then the ethanol was decanted and the residue extracted by immersion in hexane overnight. The ethanol and the hexane were combined and the hydrocarbons collected in the hexane phase by addition of water.

2.3. Preseparation by column liquid chromatography

For the preseparation, 30 cm × 11 mm i.d. glass columns with a frit (Fisher Scientific, article B53275, Wohlen, Switzerland) were packed with silica gel containing 0.3% silver nitrate. Thirty-three grams of silica gel coated with 1% silver nitrate were mixed with 67 g activated silica gel. Silica gel was coated with 1% silver nitrate by dissolving 0.5 g silver nitrate in 50 ml water and addition to 49.5 g silica gel (round flask protected with aluminum foil against