Development of an analytical method for the determination of mineral oil aromatic hydrocarbons (MOAH) from printing inks in food packaging

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A B S T R A C T

An analysis method was developed to detect chemical markers of mineral oil aromatic hydrocarbons (MOAH) from offset printing inks in food packaging materials. 16 aromatic hydrocarbons were used as target analytes and different solid phase extraction procedures (SPE) and gas chromatography coupled to mass spectrometry (GC–MS) were tested. The concentration range studied was 0.1–7.5 μg g^{-1} with R2 higher than 0.9963, intraday RSD values below 5 %, RSD values between days lower than 12 %, recoveries higher than 80 %, LOD and LOQ lower than 0.09 μg g^{-1}. Ten of the target analytes were identified in offset printing inks at concentrations between 2.28 and 8.59 μg g^{-1}. Nine of them were also identified in the food packages examined in concentrations ranging from 0.10 to 0.33 μg g^{-1}. These compounds were: methylnaphthalene, 2-methylnaphthalene, biphenyl, 2,6-dimethylnaphthalene, acenaphthene, 3,3′,5,5′-tetramethylbiphenyl, 4,6-dimethyldibenzothiophene, 1-methylpyrene, benzo(b)naphtha(1,2-d)thiophene and 9,9′-dimethylfluorene. Mineral oil in food packaging was previously analysed by GC with flame ionization detection (FID).

1. Introduction

Recently, contamination by mineral oil aromatic hydrocarbons (MOAH) has become a public interest due to their harmful effects on human health. Scientific studies have shown that MOAH act as endocrine disruptors (Tarnow et al., 2016) and that MOAH with three or more rings can be mutagenic and/or genotoxic carcinogens (Efsa, 2012). Given this situation, the European Food Safety Authority (EFSA) suggested that food must be free of MOAH (Efsa, 2012), and the European Commission published a recommendation (EU) 2017/84, indicating the necessity to monitor the presence of mineral oil hydrocarbons (MOH), in general, in food and food contact materials (Commission, 2017).

Food contamination from MOH, that includes MOAH, might come from different sources, such as lubricating oil used in agricultural and industrial food processing machinery, food additives, processing aids, and direct or indirect contact from food packaging (Efsa, 2012). Among food packaging, paper and board tend to be the most contaminated by MOH, probably because they are often covered both with a layer of printing ink for decorative purposes or made from recycled fibres (Biedermann, Uematsu, & Grob, 2011; Pack et al., 2020; Vollmer et al., 2011).

From the chemical point of view, MOAH are formed by compounds that contain aromatic rings, mostly branched. They are usually present together with saturated hydrocarbons (MOSH). Therefore, MOAH needs to be separated from MOSH to be analysed (Efsa, 2012).

A commonly used method for analysing MOH in food matrices or food contact materials is on-line liquid chromatography with gas chromatography coupled to a flame ionisation detector (LC-GC-FID) (AENOR, 2018; Bratinova & Hoekstra, 2019; Weber, Schrag, Mildau, Kuballa, Walch, & Lachenmeier, 2018). However, the off-line separation by solid-phase extraction (SPE) of MOSH and MOAH before the chromatographic injection is an alternative option for laboratories that do not have the hyphenated instrumentation (Fiselier et al., 2013; Liu, Huang, Wu, Li, & Ouyang, 2017; Moret, Barp, Grob, & Conte, 2011).

Despite the many achievements made in the study of mineral oils, their chemical analysis continues to be a huge analytical challenge. The chemical complexity of these substances has made the chromatographic separation of their individual components very difficult. Besides, both MOSH and MOAH fractions show humps composed of a broad series of unresolved peaks (Biedermann & Grob, 2015; Gharbi et al., 2017) that could mask compounds of different nature, which could lead to false quantitative analysis, since it is impossible to distinguish between

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analytes (Buijtenhuijs & Van De Ven, 2019; Weber et al., 2018). In a recent study, Koster et al. indicated that when MOH contamination is low, current methodologies cannot reliably conclude whether or not the sample is contaminated (Koster et al., 2020). Therefore, it is necessary to apply additional confirmatory techniques, such as two-dimensional gas chromatography (GCxGC), mass spectrometry, and the identification of chemical markers (Jaén, Domeño, Alfaro, & Nerín, 2021; Moret et al., 2013; Spack et al., 2017).

Chemical markers have been proposed as indicators of MOH contamination. The use of diisopropylnaphthalene as an indicator of contamination from recycled paper or cardboard is common (Biedermann & Grob, 2015; Moret et al., 2013). Bratinova et al. recommend the use of mass spectrometry to characterise MOH when interference is suspected, as well as the use of indicative MOSH/MOAH compounds (Bratinova & Hoekstra, 2019). Spack et al. concluded that identifying a more significant number of marker substances would improve the capacity of mass spectrometry as a confirmatory technique (Spack et al., 2017). Recently, Jaén et al. found 27 substances in different mineral oil samples that could be used as markers of MOAH contamination (Jaén et al., 2021).

Therefore, the objective of this study is to develop a rapid SPE-GC–MS method to determine aromatic hydrocarbons that could be used as MOAH markers in printing inks. For this purpose, the following steps were carried out: i) To optimize and validate different SPE procedures in combination with GC–MS to analyse the compounds proposed as chemical markers of MOAH; ii) To identify and quantify these markers in mineral oil-based offset printing inks. iii) To apply the method to food packaging samples contaminated with MOAH.

2. Materials and methods

2.1. Reagents, materials and working solutions

Paraffin oil., Ph. Eur., BP, viscous liquid, silica gel high-purity grade (pore size 60 Å, 70–230 mesh) for chromatographic columns, silver nitrate on silica gel (~10 wt% loading, 230 mesh), Supelclean EZ-POP NP SPE cartridges, 1-methylnaphthalene (1-MN), 2-methylnaphthalene (2-MN), biphenyl (BP), 2,6-dimethylnaphthalene (2,6-DMN), acenaphthene (ACE), 2,6-diisopropylnaphthalene (2,6-DIPN), 3,3′,5,5′-tetramethylbiphenyl (3,3′,5,5′-TMBP), 4-methyldibenzothiophene (4-MDBT), 4,6-dimethyldibenzothiophene (4,6-DMDBT), 1-methylpyrene (1-MPYR), benzo(b)naphtha(1,2-d)thiophene (BNT), chrysene (CHRY), benzo(b)fluoranthene (BbF), perylene (PER), undecane (n-C11), *n*-tridecane (n-C13), bicyclohexyl (Cycy), 5α-cholestane (Cho), pentylbenzene (5B), 1,3,5-tri-*tert*-butylbenzene (TBB) were purchased from Sigma-Aldrich (Madrid, Spain). While, 3,6-dimethylphenanthrene (3,6-DMP) was from Dr. Ehrenstorfer (Augsburg, Germany) and 9,9′-dimethylfluorene (9,9′-DMF) was from Tokyo Chemical Industry CO., ltd.

Silanized glass wool was acquired from Supelco (Bellefonte, USA). Anhydrous sodium sulphate extra pure Ph. Eur. and solvents of HPLC grade such as toluene, *n*-hexane, methanol, ethanol absolute, isopropanol, methylene chloride (DCM), acetone and acetonitrile (ACN) were from Scharlab SL (Barcelona, Spain).

MOSH/MOAH kit provided by European Union Reference Laboratory for Food Contact Materials (EURL-FCM) was used to help in the development and validation of the analytical methods for the determination of MOH in food and FCM (Food contact materials). The test items used were: a C10-C50 mixture of *n*-alkanes in *n*-hexane (QC05), Engine oil + Gravex in *n*-hexane (QC06) prepared at JRC and recycled cardboard (QC08). Each test item was accompanied by MOSH/MOAH chromatograms and their respective indicative values.

The vacuum manifold system was purchased from Waters Corporation (Massachusetts, USA). The "Vibromatic" mechanical laboratory shaker was obtained from J.P Selecta (Spain), and the Ultramatic GR system for the purification of ultrapure water type I (reactive grade) was

obtained from Wasserlab (Spain).

Individual standard solutions of all compounds were prepared at 6000 µg g⁻¹ in toluene, except PER and 2,6-DIPN, which were prepared at 1200 µg g⁻¹ in toluene, and in *n*-hexane respectively. Two mixed stock solutions were prepared through appropriate dilutions of the individual standards. Solution A was prepared by dilution in *n*-hexane of the following aromatic hydrocarbons: 2-MN, 1-MN, BP, 2,6-DMN, 2,6-DIPN, ACE, 3.3′,5.5′-TMBP, 4-MDBT, 4,6-DMDBT, 1-MPYR, 3,6-DMP, 9,9-DMF, BNT, CHRY, BbF and PER, up to 100 µg g⁻¹. The second standard mixture (solution B) contained aromatic and saturated compounds with the following concentrations: n-C13, Cycy, Cho, 5B, TBB, 1-MN, 2-MN at 350 µg g⁻¹, n-C11 at 175 µg g⁻¹ and PER at 650 µg g⁻¹; this solution was prepared by dilution in toluene and used to verify the separation of MOSH and MOAH. All solutions were stored at −4° C, and working solutions were prepared daily.

2.2. Selection of MOAH markers

The substances used as target analytes in this study were proposed in a previous study as MOAH markers (Jaén et al., 2021). Furthermore, these substances were identified by different authors in mineral and crude oil samples. A total of sixteen aromatic hydrocarbons were selected: three heterocyclic aromatic sulphur compounds (4-MDBT, 4, 6-DMBT, and BNT), commonly used to track crude oil contamination (Fang et al., 2017, 2016; Yang et al., 2016) and which were also detected in mineral oils by two-dimensional gas chromatography GCxGC-MS (Biedermann & Grob, 2009, 2015); diisopropylnaphthalene (2,6-DIPN) to confirm contamination from recycled cardboard (Biedermann & Grob, 2015; Zhang, Noonan, & Begley, 2008) biphenyl (BP) and alkylbiphenyl (3.3′,5.5′-TMBP) identified in petroleum products (Bundt, Herbel, Steinhart, Franke, & Francke, 1991; Paschke, Herbel, Steinhart, Franke, & Francke, 1992) and used as crude oil indicators (Ogbesejana, Oluwasesan, & Ali, 2017; Trolio, Grice, Fisher, Alexander, & Kagi, 1999); four unbranched PAHs (ACE, CHRY, BbF and PER) and six branched PAHs (1-MN, 2-MN, 2,6-DMN, 3,6-DMP, 1-MPYR and 9,9′-DMF), belonging to the homologous series of naphthalene, phenanthrene, anthracene, fluorene, chrysene and pyrene which were previously identified in mineral oils (Bartsch, Hutzler, Vieth, & Luch, 2017; Biedermann & Grob, 2015; Gmbh & Kg, 2009; Koch, Becker, Päch, Kühn, & Kirchhoff, 2020). Another important criterion in the selection of these compounds was the commercial availability of certified reference standards.

2.3. Samples

This study used two different types of mineral oil-based offset printing inks and nineteen virgin cardboard food packaging. The evaluated cardboard containers were: three decorated cardboard plates, two white cardboard plates, four decorated cardboard cups, three boxes for pasta, two boxes for raisins, two paper packaging for wheat flour, and three boxes of tea. All samples were purchased from the local market and analysed in triplicate.

The glassware was sequentially rinsed with methanol, acetone, and *n*-hexane, then dried in an oven at 100 °C and covered with aluminium foil to avoid contamination of the samples. The cardboard samples were also protected with aluminium foil, and blank samples were analysed throughout the process.

2.4. Instrumental analysis

2.4.1. GC–MS analysis

An Agilent 6890 N gas chromatograph equipped with a Combi PAL automatic sampler (CTC Analytics, Zwingen, Switzerland), coupled to a quadrupole mass spectrometry detector (5975, Agilent) was used for the analysis. The temperature of the injector was 250 °C. The injection volume was 1 µL, and the splitless mode was used. The separation of the

analytes was performed with a DB-5 column (30 m × 0.25 mm ID, 0.25 μm internal thickness) supplied by Agilent Technologies. The oven temperature was initially set at 50 °C for 1 min, then raised at the rate of 10 °C min^{-1} to 300 °C, and maintained at this temperature for 10 min. The total analysis time was 36 min. The solvent delay was 8 min. The carrier gas was helium (99.999 %) at a constant flow rate of 1 mL min^{-1}. Ionisation was performed by electronic impact (EI) at 70 eV. The transfer line and ion source temperatures were 280 °C and 250 °C, respectively.

The acquisition was performed in full scan mode (*m/z* range 45–400) and the selective ion monitoring (SIM) mode for quantitative analysis. SCAN mode was used to select the monitored ions and confirm the retention times of the standards. The monitored ions (Table S1 in supplementary material) were selected based on their relative abundance in the SCAN mass spectrum. The data were acquired and processed using the MSD ChemStation data analysis software (version F.01.00.1903, Agilent Technologies).

2.4.2. GC-FID analysis

GC-FID analysis was performed in a Trace GC Ultra chromatograph (Thermo Electron Corporation, Milan, Italy) with an HP-5 analytical column (60 m × 0.25 mm ID, 0.25 μm film thickness) from Agilent Technologies and a flame ionisation detector (FID). A volume of 5 μL of the sample was injected in splitless mode using an AS 300 autosampler, and the injector temperature was 250 °C. To improve the LOD limits, the sample was concentrated before injection and further injections of 5 μL were performed. This injection volume was possible using a 105 mm liner with a 5 mm diameter, injection in splitless mode, injector temperature of 250 °C, with a carrier gas inlet pressure of 10.1 psi and hexane as solvent. It should be noted that prior to sample analysis the maximum injection volume under these conditions was calculated using an online solvent expansion calculator to avoid liner overfilling. The oven temperature was programmed as follows: start at 50 °C for 2 min, then increased at 30 °C min^{-1} to 310 °C and held for 15 min. The total run was 26 min. The flow rate of the carrier gas (helium, 99.999 %) was 2 mL min^{-1}. The FID detector temperature was 350 °C. The Chrom-Card GC (Thermo Electron) software was used to analyse the data.

2.5. Sample preparation

The extraction of mineral oil and target analytes from offset printing inks was carried out with *n*-hexane. In a glass vial, 0.050 g of offset ink were weighed and 5 mL of *n*-hexane was added; then, the sample was placed in an ultrasonic bath with constant stirring for 1 h. The supernatant was then decanted into a clean vial and concentrated under a gentle stream of nitrogen gas to 1 mL.

In the case of cardboard containers intended for food contact, mineral oil extraction was carried out following the conventional methodology reported in the literature by several authors (Barp, Purcaro, Moret, & Conte, 2013; BfR & KLZH, 2012; Dima, Verzera, & Grob, 2011; Lorenzini et al., 2010; Vollmer et al., 2011) with slight changes. The cardboard was cut into small pieces, approximately 0.5 cm on each side, excluding the parts containing adhesives. Next, 2 g of the sample were placed in a 20 mL glass vial, and 15 mL of an *n*-hexane/ethanol (1:1) mixture were added. The vial was shaken for two hours by sonication at room temperature. Subsequently, the solvent mixture was decanted, and 5 mL of water were added. The proportion of solvents and water used was experimentally tested in the laboratory. It should be noted that although 15 mL of the hexane:ethanol mixture (1:1) is added, after extraction only 12.5 mL can be decanted. To determine the amount of water needed to separate the hexane, we tested different amounts of water (4, 5, 6, and 7 mL of water) and each time around 6 mL of hexane were collected. The supernatant layer of *n*-hexane was separated and transferred to another vial where it was concentrated to 1 mL under a gentle stream of nitrogen at 40 °C. It is important to note that 10 μL of solution B were added to the cardboard replicas intended for MOAH

analysis by GC-FID.

2.6. MOAH fraction analysis

Manual separation of MOSH and MOAH was performed using a mixture of silver nitrate-coated silica gel, conventionally used to separate aromatic and aliphatic hydrocarbons from crude oils and derivatives (Bennett & Larter, 2000; BfR & KLZH, 2012; Moret et al., 2011). This mixture was prepared by mixing 33 g of high purity silica gel (previously activated in a muffle furnace at 400 °C for 24 h) with 1 g of silver nitrate mixed in silica gel (~10 % by weight), and it was homogenised for 12 h on a mechanical laboratory shaker. 3 g of this mixture were placed in a glass column (160 mm × 0.8 mm ID) which contained a piece of glass wool at the bottom and then 1 g of sulphate of anhydrous sodium on top of the silica layer. The column was covered with aluminium foil to prevent oxidation of the stationary phase.

The MOSH and MOAH fractions were separated following the workflow recommended by (BfR & KLZH, 2012). After separation, both fractions were concentrated to 0.4 mL under a gentle stream of nitrogen at 40 °C. All extracts were gravimetrically controlled. The analysis was carried out by GC-FID.

Quantitative analysis was performed using the internal standard method. Cycy was used to quantify MOSH fraction, and TBB was used to quantify the MOAH fraction. The area of each fraction was determined by integrating the entire hump and subtracting the sharp peaks found in the hump MOAH as described in the Joint Research Center (JRC) publication [7] and repeated injections of solvent blanks obtained the baseline. The limit of quantification of the method was calculated using the signal-to-noise ratio (S/N) generated by direct injection of a sample of pure mineral oil at low concentrations and taking into account the amount of sample analysed and the enrichment steps during sample treatment. The limit was lower than 2.5 mg kg^{-1}.

The efficiency of the analytical methodology for analysing the MOSH and MOAH fraction was verified with the MOSH/MOAH kit provided by FCM-EURL. The test items of the kit were analysed and compared with the indicative values of the kit.

2.7. Analysis of MOAH markers

The SPE cartridges used were double-layered, containing one layer of florisil (top) and another layer that was a mixture of Z-sep/C18 (bottom). The method described by the manufacturer was optimized to work with samples of mineral oil. The composition and volume of the eluent were the parameters evaluated to optimize the method. The solvent combinations tested were: C1) 15 mL of ACN alone; *C*2) 14 mL of ACN followed by 1 mL of DCM; C3) 14 mL of ACN followed by 1 mL of isopropanol; C4) 13 mL of ACN and 2 mL of DCM; C5) 13 mL of ACN and 2 mL of isopropanol. Actual samples of mineral oils spiked with a standard 5 μg g^{-1} solution of the target analytes were used in all optimization experiments.

The final protocol consisted of conditioning the cartridge with 10 mL of acetone, then vacuum-dried at −15 mmHg for 10 min at room temperature. Subsequently, 0.4 mL of sample was added to the cartridge, and penetration through the solid phase was allowed to complete. The target compounds were eluted with 13 mL of ACN, followed by 2 mL of isopropanol. The eluate was collected and concentrated under a gentle stream of nitrogen at 40 °C until 0.5 mL was obtained. These concentrated extracts were directly analysed by GC–MS. The extraction was carried out in a vacuum collecting system from Waters Corporation (Massachusetts, United States).

Once the above factors were optimized, the behaviour of the aromatic compounds proposed in this research as MOAH markers was studied. For this purpose, a mineral oil sample free of MOAH (paraffin oil) was spiked with 50 μg g^{-1} of solution A and loaded into the cartridge. The eluate was sequentially collected in 1 mL fractions and analysed by GC–MS.

The possibility of evaporation losses of the target analytes during the concentration step of the samples under nitrogen current was also studied, analysing standard solutions of 5 µg g^{-1} of the aromatic compound mixture before and after concentration.

2.7.1. Analytical parameters

The validation of the analytical method for qualitative and quantitative analysis by GC–MS of the target analytes was performed by determining the linearity, linear range, recovery, precision, robustness, detection limits (LOD), and quantification limits (LOQ). Linearity and linear range were determined through external standard calibration using seven solutions prepared in a concentration range from 0.1 to 7.5 µg g^{-1}. Recovery experiments were carried out by fortifying blank samples (paraffin oil) with two different concentrations (1 and 5 µg g^{-1})
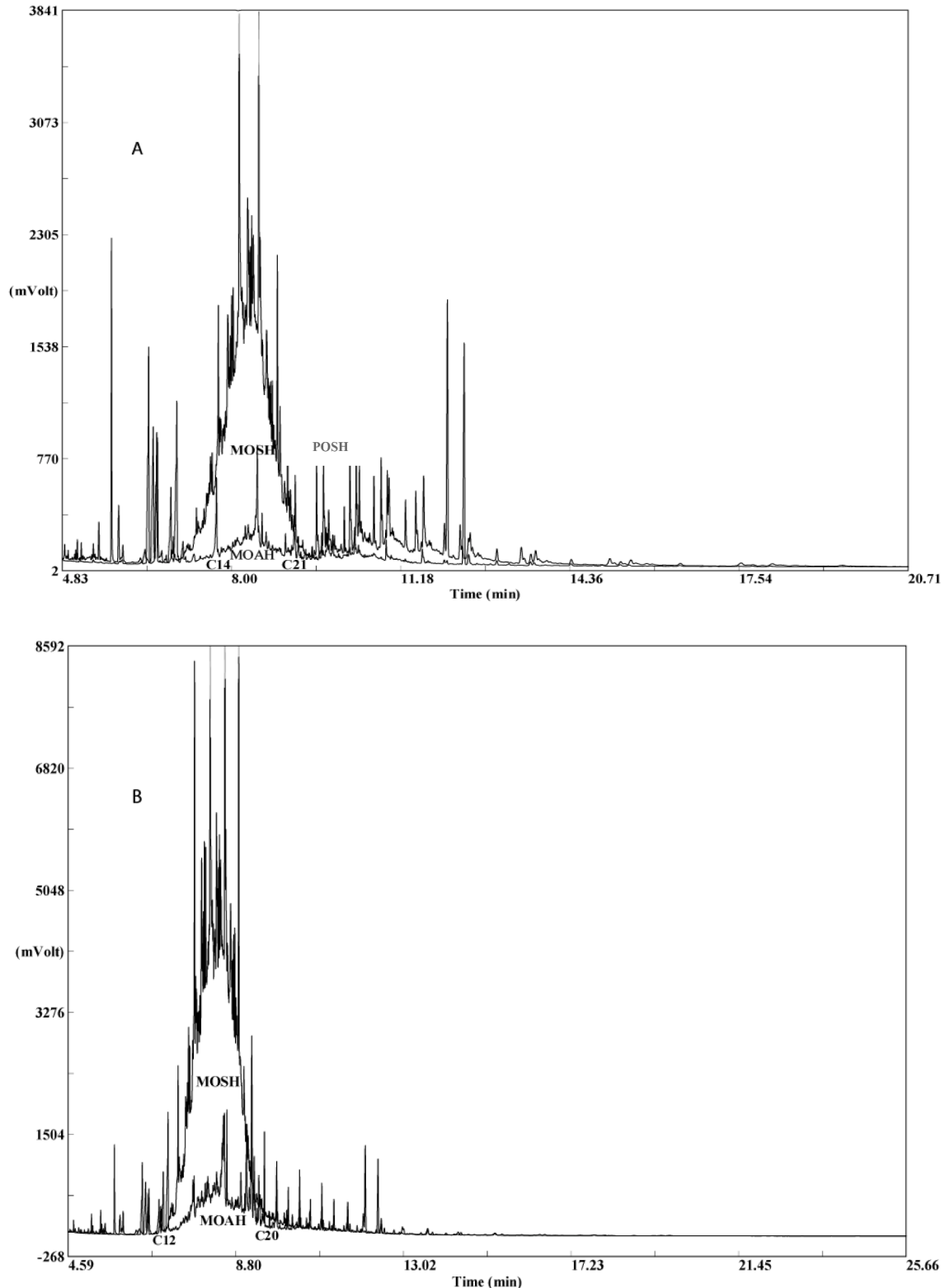

Fig. 1. MOSH/MOAH chromatograms of cardboard food packaging samples: of CFPS04 (A) and CFPS02 (B).

of solution A. The spiked samples were analysed following the procedures described in section 2.7. Intra-day precision was evaluated by analysing spiked samples during the same day and under the same working conditions. Inter-day precision was done by analysing fortified samples on three different days. Precision was expressed as a relative standard deviation (RSD). The S/N (signal to noise) was used to determine LOD and LOQ. The S/N value was calculated at very low and known concentrations of the analytes, close to the noise. LOD was established as the minimum analyte concentration detected at three times S/N and LOQ at ten times S/N. Also, the method quantification limit was estimated considering the amount of sample and the pre-concentration and dilution factors applied in each case.

All samples were done in triplicate. The results are expressed as the mean $\pm$ standard deviation of three independent measurements (n = 3) for all experiments.

3. Results and discussion

3.1. MOAH fraction analysis

The efficiency of the methodology used for the analysis of MOAH was verified by the analysis of two test elements of the MOSH/MOAH kit. Fig. S1 shows the MOSH and MOAH chromatograms of test item QC06, confirming the presence of internal standards in the appropriate MOHs fraction. Table S2 shows the MOSH/MOAH values obtained after analysing the QC06 and QC08 test elements, the indicative value of the kit, and the error percentage. The relative error was below 10 % in all cases.

The GC-FID analysis of the cardboard revealed mineral oils in nine samples, but only in five of them MOAH was detected. The samples contaminated with MOAH were: two decorated cardboard plates (CFPS01 and CFPS02), two decorated cardboard cups (CFPS03 and CFPS04) and a tea box (CFPS05) with coloured prints on the outside.

Fig. 1 shows the MOSH/MOAH chromatograms of samples CFPS02 and CFPS04. The MOSH chromatograms were characterised by a large narrow hump of unresolved peaks corresponding to a complex mixture of hydrocarbons; that probably in this case, in addition to MOSH, contained polyolefinic oligomeric saturated hydrocarbons (POSH) derived from the polyolefin film that covered the decorated surface of the contaminated samples (Dima et al., 2011; Pack et al., 2020), and that elute together with MOSH, forming humps in the same chromatographic region (Biedermann-Brem, Kasprick, Simat, & Grob, 2012; Pack et al., 2020). In comparison, the MOAH fraction generated a smaller hump.

The MOAH content found in food cardboard packaging ranged from 8.4 to 32.9 mg kg^{-1} (see Table 1). The decorated cardboard cup (CFPS03) had the highest concentration, and the lowest concentration was found in the tea box (CFPS05). MOAH concentrations represent between 5 and 9 % of the total mineral oil concentration in the samples, while the MOSH content was higher than 90 % in all samples with concentrations between 85.9 and 332.6 mg kg^{-1}.

The hydrocarbon distribution in the samples was in the typical range of mineral oils for printing inks (Dima et al., 2011; Lorenzini et al., 2010; Pack et al., 2020). MOAH was detected in the decorated plates from n-C12 to n-C20 centred on n-C17, in the case of decorated cups between n-C11 to n-C20 centred on n-C18 and for the tea box from n-C14 at n-C21 also centred on n-C18. As expected, the protrusions detected were found

in the range of printing inks; and typical recycled cardboard chromatograms, with extensive humps, were not observed as a result of contamination of the cardboard by various types of mineral oils (Dima et al., 2011; Lorenzini et al., 2010). It confirms the fact that mineral oil comes from the printing inks used to decorate cardboard food packaging.

3.2. Analysis of MOAH markers

3.2.1. Optimization of the extraction method

Fig. 2 shows the recovery results of the target analytes related to the volume and eluent composition. The first option evaluated was the use of ACN as the only eluent because ACN is a solvent of intermediate polarity with little affinity for the oily matrix, which allows the elution of aromatic compounds without dragging the interferences coming from the matrix. However, as shown in Fig. 2, ACN alone (C1) obtained low analytes recoveries, while a moderate decrease in eluent polarity significantly improved the recovery percentage (C2–C5 combinations). The affinity of aromatic compounds for less polar solvents such as DCM and isopropanol facilitated their extraction; nevertheless, an excess of DCM (combination C4) caused the extraction of the matrix from the sample, with the formation of tiny drops of oil that prevented obtaining a clean enough extract to be directly analysed by gas chromatography. For this reason, a small aliquot of the supernatant liquid was taken for chromatographic analysis, thus reducing the percentage of recovery of the analytes. Instead, the C5 combination, which consisted of adding 13 mL of ACN followed by 2 mL of isopropanol, showed the best results, with recoveries greater than 85 %, and was considered the optimal option in this study.

The elution curve shown in Fig. S2 was constructed to study the behaviour of the target analytes during the solid phase extraction. As can be seen, a high content of the analytes eluted with the first millilitres of the solvent, and most of the compounds showed a similar behaviour during solid phase extraction. However, MOAH with higher molecular weight and lower polarity eluted slower; this behaviour is likely due to their lower affinity for the eluent. Regarding the evaporation losses during the concentration step under mild nitrogen current, the results revealed no significant differences in the concentration of the target analytes in the analysed solutions; therefore, the evaporation losses are negligible in this case.

3.2.2. Analytical parameters

As shown in Table 2, method provided good precision and linearity in the range of concentrations tested (0.1–7.5 µg g^{-1}) with correlation coefficients (R^2) between 0.9964 and 0.9996 and intra-day RSD values below 6 % and inter-day RSD values less than 8 % for both spiked samples. The recovery assays of the spiked sample with 1 µg g^{-1} showed recovery values in a range from 81 to 104 %, while the sample fortified with 5 µg g^{-1} showed recovery values between 85 and 99 %. The LODs varied from 0.008 to 0.024 µg g^{-1} and the LOQs from 0.016 to 0.08 µg g^{-1}.

3.2.3. Application to real samples

The optimized extraction procedure was used to determine the target analytes in the study samples. First, the mineral oil extracted from the offset printing inks was analysed to verify the method's applicability to a natural matrix and identify the target analytes present in the mineral oils used in the oil-based offset printing inks.

The results revealed that ten of the sixteen compounds proposed as MOAH markers by Jaén et al. (Jaén et al., 2021) were found in mineral oil-based offset printing ink samples. The compounds were identified and confirmed by comparing their mass spectra and retention times with standards injected under the same conditions as the samples. The identified compounds were: 2-MN, 1-MN, BP, 2,6-DMN, 9,9′-DMF, 3,3′,5,5′-TMBP, 1-MPYR, 4,6-DMDBT, 3,6-DMP and BNT. The presence of ACE, DIPN, or 4-MBT was not detected. Neither was PAHs with more

Table 1
MOSH/MOAH concentrations (mg kg^{-1}) in cardboard food packages.

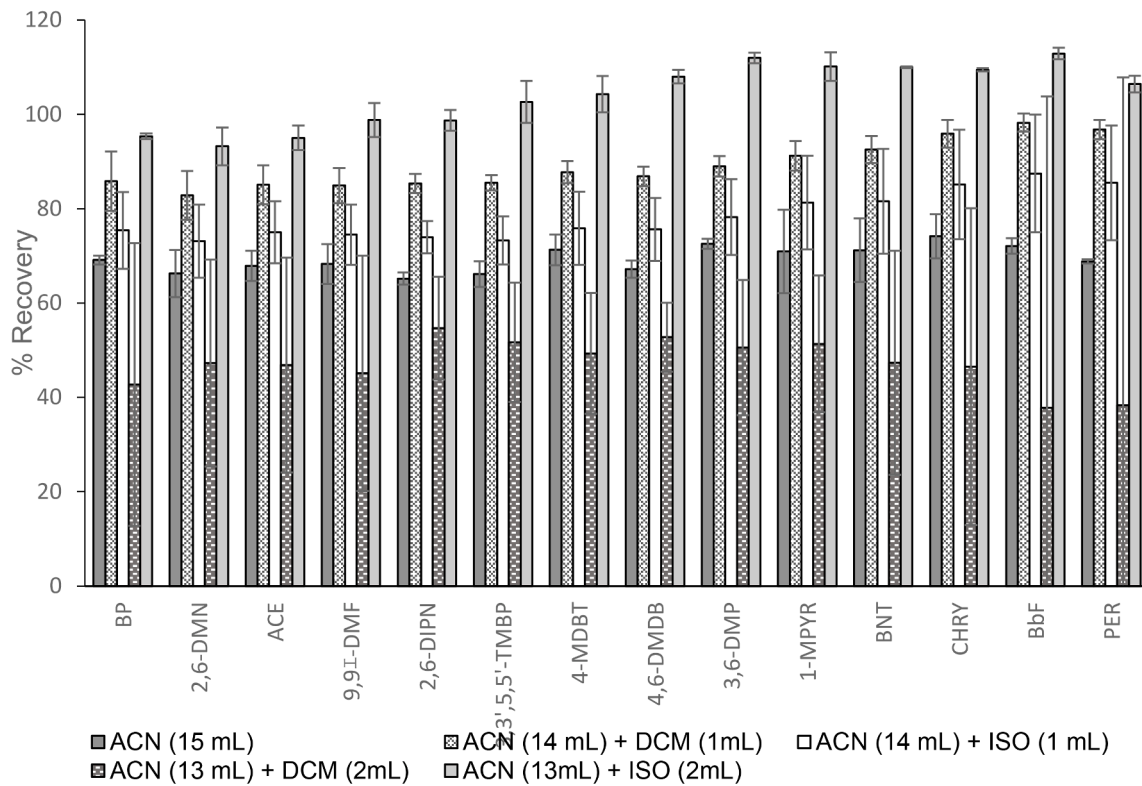| Samples	Concentration (mean $\pm$ SD)	
	MOSH (mg kg^{-1})	MOAH (mg kg^{-1})
FFC01	173.8 $\pm$ 1.3	9.7 $\pm$ 0.6
FFC02	231.0 $\pm$ 1.8	14.2 $\pm$ 1.8
FFC03	332.6 $\pm$ 1.2	32.9 $\pm$ 0.9
FFC04	301.8 $\pm$ 2.1	23.3 $\pm$ 0.63
FFC05	85.9 $\pm$ 2.0	8.4 $\pm$ 0.2

Fig. 2. Comparison of recovery of target analytes with different solvent combinations.

Table 2
Analytical parameters of the SPE-GC–MS method applied to determine MOAH markers.

| Compound	R^2	Slope	LOD (µg g^{-1})	LOQ (µg g^{-1})	Spiked concentration (1.0 µg^{-1})			Spiked concentration(5.0 µg g^{-1})		
					Recovery (%)	RSD (%) Intraday (n = 5)	RSD (%) Interday (n = 3)	Recovery (%)	RSD (%) Intraday (n = 5)	RSD (%) Interday (n = 3)
2-MN	0.9964	1.35E + 05	0.008	0.056	82	1.4	9.4	86	1.2	7.0
1-MN	0.9989	1.26E + 05	0.016	0.056	81	1.4	5.9	85	1.6	6.1
BP	0.9980	1.73E + 05	0.016	0.064	98	2.0	7.4	99	3.0	7.0
2,6-DMN	0.9975	1.50E + 05	0.016	0.056	104	2.9	11.7	98	2.1	7.5
ACE	0.9991	1.53E + 05	0.008	0.032	94	1.8	7.9	92	1.6	6.5
9,9′-DMF	0.9996	2.38E + 05	0.016	0.040	94	3.0	9.5	93	2.8	6.4
2,6-DIPN	0.9989	1.79E + 05	0.008	0.016	95	3.2	8.3	89	1.3	4.5
3,3′,5,5′-TMBP	0.9993	2.02E + 05	0.008	0.040	91	3.2	7.5	89	2.0	5.9
4-MDBT	0.9989	1.69E + 05	0.008	0.016	94	1.0	8.7	95	2.4	6.5
4,6-DMDB	0.9995	1.76E + 05	0.008	0.024	88	2.8	6.7	92	2.0	6.2
3,6-DMP	0.9993	1.67E + 05	0.016	0.048	90	3.3	7.4	95	4.4	5.7
1-MPYR	0.9988	2.00E + 05	0.008	0.016	84	4.3	6.0	90	3.9	3.3
BNT	0.9979	2.06E + 05	0.008	0.024	90	3.8	6.7	95	3.4	7.0
CHRY	0.9974	1.87E + 05	0.024	0.072	90	2.4	7.7	97	5.2	6.6
BbF	0.9975	1.74E + 05	0.008	0.034	88	3.7	9.8	93	3.6	2.1
PER	0.9965	1.63E + 05	0.024	0.080	81	4.4	4.8	89	3.0	2.6
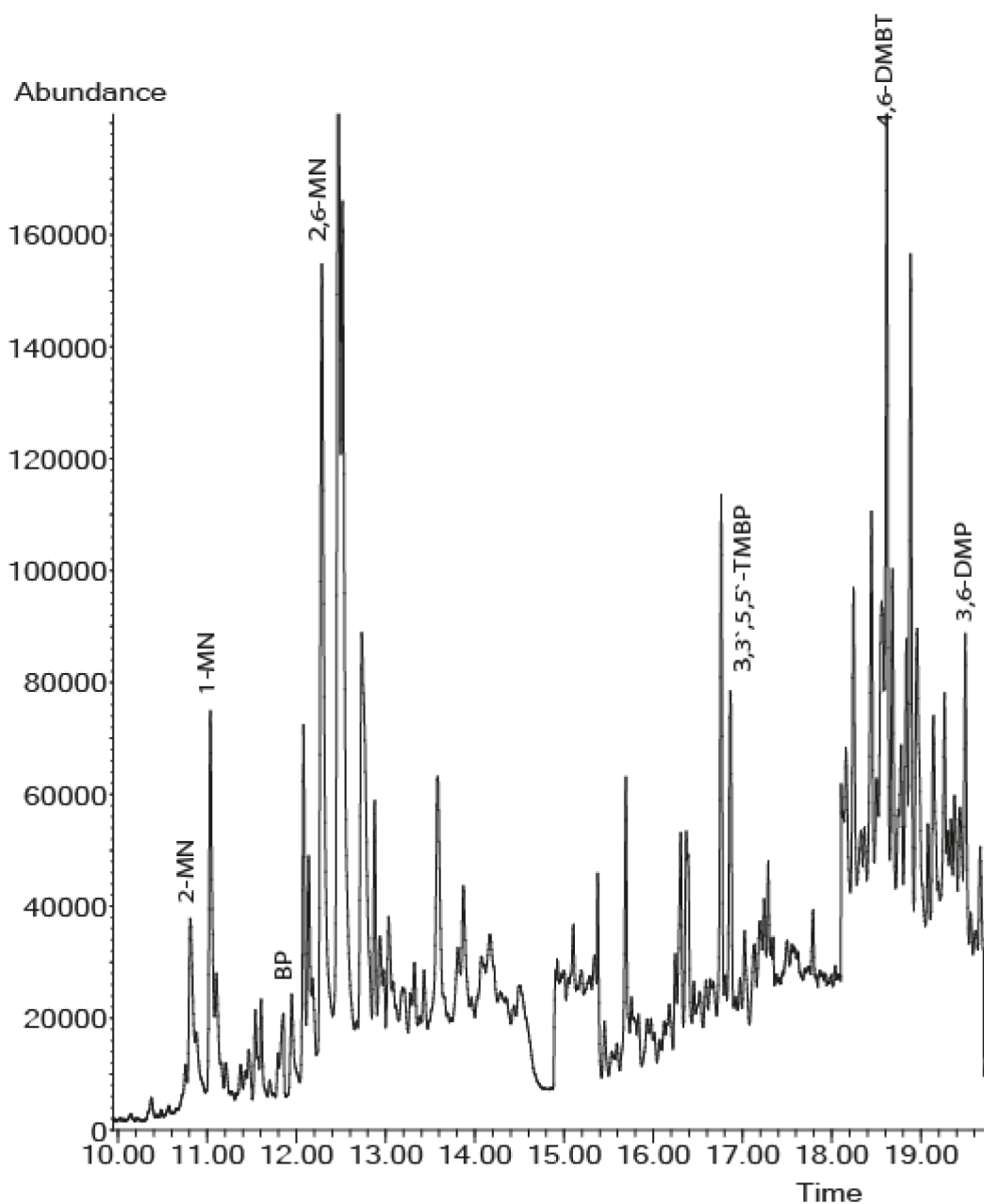
Fig. 3. Chromatogram of the target analytes identified in the OPIS01 sample of mineral oil-based offset printing inks by GC–MS.

Table 3
Concentration of individual aromatic compounds (mean ± SD) in cardboard food packaging samples contaminated with MOAH.

Compounds	Cardboard food packaging sample (µg g^{-1} ± SD)					offset printing inks sample (µg g^{-1} ± SD)	
	CFPS01	CFPS02	CFPS03	CFPS04	CFPS05	OPIS01	OPIS02
2-MN	0.17 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.16 ± 0.02	0.30 ± 0.01	7.61 ± 0.52	8.59 ± 0.60
1-MN	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.19 ± 0.01	6.59 ± 0.10	6.74 ± 0.42
BP	0.12 ± 0.02	0.13 ± 0.01	0.11 ± 0.01	< LOQ	0.22 ± 0.01	3.48 ± 0.33	4.44 ± 0.33
2,6-DMN	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	<LOQ	0.24 ± 0.01	7.37 ± 0.58	5.30 ± 0.19
9,9′-DMF	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ	<LOQ	2.49 ± 0.05
3,3′,5,5′-TMBP	0.09 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	<LOQ	0.33 ± 0.01	2.28 ± 0.16	2.77 ± 0.26
4,6-DMDBT	0.10 ± 0.02	nd	<LOQ	nd	0.17 ± 0.01	2.50 ± 0.16	2.83 ± 0.21
3,6-DMP	0.14 ± 0.03	0.12 ± 0.01	nd	nd	0.20 ± 0.01	3.85 ± 0.13	4.36 ± 0.04
1-MPYR	0.14 ± 0.02	nd	0.12 ± 0.01	nd	0.24 ± 0.01	5.37 ± 1.13	4.65 ± 0.22
BNT	0.11 ± 0.02	nd	nd	nd	nd	7.29 ± 1.83	8.09 ± 0.44

nd: not detected.

than three aromatic rings such as CHRY, BbF and PER detected. Fig. 3 shows the peaks of seven of the target analytes identified in one of the printing ink samples (OPIS01).

Except for 9,9′-DMF, all compounds identified in the printing inks were also identified and quantified in the MOAH-contaminated cardboard samples. Table 3 shows the target analytes identified in the samples and their respective concentrations. As can be seen, sample CFPS01 (decorated cardboard plate) presented the highest amount of analytes, followed by sample CFPS05 (decorated tea box); and CFPS04 (cardboard cup) was the sample with the lowest number of target analytes. The compounds most frequently found in the food packaging under study were the lowest molecular weight target analytes, such as methylated naphthalenes, detected in almost all samples. Among the aromatic hydrocarbons with three or more aromatic rings, which pose a greater risk to consumer health (Efsa, 2012), only 3,6-DMP and 1-MPYR were found.

The concentration of the analytes in the printing inks ranged between 2.28 and 8.59 µg g^{-1}. The compounds with the highest concentration were methyl naphthalenes (2-MN, 1-MN, 2,6-DMN) and BNT, followed by 1-MPYR, 3,3′,5,5′-TMBP; and the compound 4,6-DMDBT had the lowest concentrations. The concentrations of the target analytes in the cardboard food package ranged between 0.10 and 0.33 µg g^{-1}. The concentrations were evidently lower than those found in mineral oil-based offset printing inks. However, the concentrations were high enough to be detected and related to MOAH contamination.

4. Conclusions

In this study, an offline SPE-GC–MS method has been optimized to determine MOAH contamination markers in both mineral oil-based offset printing inks and cardboard food packaging samples. The results have shown that the developed method is fast, has good sensitivity, precision and linearity within the range of the studied concentrations with recovery values of the target analytes above 80 %.

Ten of the compounds proposed as MOAH markers were determined in the offset printing inks. These compounds were: 2-MN, 1-MN, BP, 2,6-DMN, 9,9′-DMF, 3,3′,5,5′-TMBP, 1-MPYR, 4,6-DMDBT, 3,6-DMP and BNT. Except for 9,9′-DMF, which was detected below the LOQ, all other compounds were also determined in MOAH contaminated cardboard food packaging samples. In our opinion, the presence of these compounds in the samples correlates with the MOAH of mineral oil-based offset printing inks.

MOAH analysis is complicated and presents a large number of analytical problems, which have not yet been resolved, such as the lack of validated samples, analysis protocols, mineral oil standards, in addition to the lack of detector selectivity and the uncertainty that produce humps. In this research, a simple, robust, easy to reproduce analytical methodology is optimized that allows the determination of compounds that could serve to mark MOAH contamination from mineral oil-based offset printing inks with the purpose of complementing the GC-FID analysis. We also consider chemical markers and mass spectrometry valuable tools, complementing the FID analysis of the MOAH fraction, especially in laboratories where other analytical hyphenated instruments such as LC-GC-MS are not available.

CRediT authorship contribution statement

Janira Jaén: Conceptualization, Methodology, Validation, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Celia Domeño:** Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – review & editing, Supervision. **Cristina Nerín:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133745.

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