Migration of mineral oil aromatic hydrocarbon (MOAH) from hot melt adhesives used in food packaging materials

Janira Jaén, Celia Domeño, Paula Vera, Cristina Nerín[*]

Analytical Chemistry Department, GUIA Group, I3A, EINA, University of Zaragoza, Mª de Luna 3, 50018 Zaragoza, Spain

A B S T R A C T

This article studies the migration of MOAH from hot melt adhesives used in multilayer laminates into food simulants. First, the initial concentration of a group of compounds selected as MOAH markers in several adhesives was determined by headspace solid phase microextraction coupled to gas chromatography mass spectrometry (HS-SPME-GC-MS), using the previously optimised method. Then, the migration of the MOAH fraction and MOAH markers from the laminates was studied. The MOAH fraction was analysed by gas chromatography with flame ionisation detection (GC-FID), and the MOAH markers were analysed by HS-SPME-GC-MS. Twelve MOAH markers were detected, and their initial concentrations were between 0.46 and 33.8 µg g^{-1}. Only eight were identified after migration, ranging between 0.62 and 21.33 µg dm^{-2}, with a migration percentage of 12–75%. The fraction of MOAH that migrated eluted mainly in the C16-C25 range and reached concentrations of 19.65 µg dm^{-2} from the laminate.

1. Introduction

Hot melt adhesives are widely used to assemble materials in multi-layer laminates commonly found in food packaging. Adhesives are formulated using a combination of chemicals with specific functions. The main component is the base polymer, which gives its name to the adhesive type. Ethylene/vinyl acetate copolymer (EVA), is the most common hotmelt adhesive, but block copolymers of styrene, such as styrene-butadiene-styrene (SBS) and styrene- or amorphous polyolefins (APAO) are also used. Besides, additives as paraffin wax or tacky resins are added to reduce the viscosity and improve the wetting. Also, pigments, fillers and other additives are used to optimise the physical, chemical or mechanical properties of the adhesive (Mildenberg, Zander, & Collin, 1997; Petrie, 2000).

They are originally solid polymers (granules, pellets, powers…) at temperature below 80ºC but when they are heated at 150 ºC − 200 ºC they soften and melt. Once the adhesive is melted, it is applied over the substrates to be joined and the adhesive hardens by cooling. The substrates more commonly used with hotmelt adhesives for food contact materials are paper, cardboard, selected plastic films, metals and glasses.

Even though in most cases, the adhesives are not in direct contact with food, scientific studies have shown that many compounds migrate from the adhesives to the food; both additives used in its manufacture and NIAS (non-intentionally added substances) coming from the degradation of components or impurities (Aznar et al., 2011; Nerín et al., 2012; Vera, Canellas, & Nerín, 2014; Yan, Hu, Tong, Lei, & Lin, 2020). However, there is no specific regulation about the use of adhesives as food contact materials and their possible migrations. Only a general Framework Regulation about objects and materials intended to come into contact with food (European Commission, 2004); and the guidelines of the Commission Regulation (EC) 2023/2006 to regulate manufacturing practices (European Commission, 2006) that adhesives producers have to be followed.

Mineral oil hydrocarbons (MOH) are complex mixture of substances that come from the distillation of crude oil and contain mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH), which can be separated by liquid chromatography. The presence of MOAH in food is of concern due to the genotoxic carcinogenic potential of MOAH with three or more aromatic rings. MOAH can reach food from recycled paper and cardboard, and printing inks applied to paper and cardboard, as MOH are used in the manufacture of plastic materials, waxes, lubricants and adhesives, among others (EFSA, 2012).

To the best of our knowledge, there are a few publications that relate the presence of this type of compounds in the adhesives. Biedermann et al. showed typical MOSH and MOAH chromatograms for hot melt

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adhesives (Biedermann & Grob, 2012). Barp et al. investigated the migration of MOHs from cardboard to food and found that a significant percentage of total MOHs contamination came from adhesives (Barp, Suman, Lambertini, & Moret, 2015a, 2015b). Lommatzsch et al. separately analysed the main components of hot melt adhesives and concluded that the primary source of hydrocarbons in adhesives with the ability to migrate to dry foods is tacky resin C5 (piperylenes, cyclopentene, cyclopentadiene (CPD), C4–6 species), C9 (styrene, α-methylstyrene, vinyltoluene, dicyclopentadiene, indene, methylindenes, non-reactive aromatics) and modified aromatic resins (DCPD) (Lommatzsch, Biedermann, Grob, & Simat, 2016).

The recommended technique for MOAH analysis is online liquid chromatography with gas chromatography combined with flame ionisation detection (HPLC-GC-FID) (AENOR, 2018; Bratinova & Hoekstra, 2019). The chromatographic analysis of MOAH is characterised by the formation of broad chromatographic humps (Biedermann & Grob, 2012), which could encompass other substances (Koster et al., 2020). Some researchers argue that GC-FID should be supplemented with confirmatory techniques such as mass spectrometry, two-dimensional gas chromatography (GCXGC) or nuclear magnetic resonance (NMR) spectroscopy (Lachenmeier et al., 2017; Spack et al., 2017; Weber et al., 2018).

Given the complexity of mineral oils and the expensive instrumentation required for their analysis, the use of chemical markers is an alternative to certainty identify the presence of MOAH and its reliability of the source. It is also recommended to identify chemical markers to determine the origin of contamination (Spack et al., 2017). Recently, Jaén et al. identified 16 compounds that can be used as MOAH markers (Jaén, Domeño, Alfaro, & Nerín, 2021).

The main objectives of this study were: (i) to develop and optimise a novel solid phase microextraction method coupled to gas chromatography (HS-SPME-GC-MS) to analyse MOAH markers (ii) to identify and quantify the initial concentration of MOAH chemical markers in commercial samples of hot melt adhesives that had MOH among their components (iii) to study the migration of MOAH markers and MOAH fraction from some laminates manufactured with these adhesives to modified polyphenylene oxide (Tenax®) as food simulant.

2. Materials and methods

2.1. Reagents

Acetone, n-hexane, toluene, dichloromethane and ethanol absolute, all HPLC grade were from Panreac (Barcelona, Spain). 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); and standard mixture of saturated alkanes (C7-C40) of 1000 μg mL^{-1} were purchased from Sigma-Aldrich (Madrid, Spain). 3,6-dimethylphenanthrene (3,6-DMP) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) and 9,9'-dimethylfluorene (9,9'-DMF) was supplied by Tokyo Chemical Industry CO., LTD.

SPME fibers, Tenax TA 60/100 mesh and salinised glass wool were supplied by Supelco (Bellefonte, USA). Anhydrous sodium sulphate extra pure Ph. Eur was from Scharlab SL (Barcelona, Spain). The "Vibromatic" mechanical laboratory shaker was obtained from J. P. Selecta (Spain). Silica gel high-purity grade (pore size 60 Å, 70–230 mesh), silver nitrate on silica gel (~ 10 wt% loading, 230 mesh) were purchased from Sigma-Aldrich (Madrid, Spain).

Two standard solutions were prepared. Solution A contained 16 standards (1-MN, 2 -MN, BP, 2,6-DMN, ACE, 2,6-DIPN, 3,3',5,5'-TMBP,

4-MDBT, 4,6-DMDBT, 1-MPYR, BNT, CHRY, BbF, PER, 3,6-DMP and 9,9'-DMF) at 100 μg g^{-1} in hexane. Solution B contains the standards used to verify the separation of MOSH and MOAH in the following concentrations: n-C13 (175 μg g^{-1}), n-C11 (350 μg g^{-1}), Cycy (366 μg g^{-1}), Cho (350 μg g^{-1}), 5B (355 μg g^{-1}), 1-MN (342 μg g^{-1}), 2-MN (355 μg g^{-1}), TBB (357 μg g^{-1}), and PER (699 μg g^{-1}) in toluene. All solutions were stored at − 4 °C.

2.2. MOAH markers

The compounds used as chemical markers in this study were identified and selected in a previous investigation, as MOAH markers for food packaging (Jaén et al., 2021). In addition, these compounds include branched and unbranched polycyclic aromatic hydrocarbons (PAHs) and benzothiophenes, which other authors have identified in mineral oils (Biedermann & Grob, 2009, 2015; Li et al., 2012; Yang et al., 2016).

The compounds evaluated as MOAH markers in hot melt adhesives were: 1-MN, 2-MN, BP, 2,6-DMN, ACE, 9,9'-DMF, 2,6-DIPN, 3,3',5,5'-TMBP, 4-MDBT, 4,6-DMDBT, 3,6-DMP, 1-MPYR, BNT, CHRY, BbF and PER. The molecular structure of these compounds and their molecular masses are shown in Table S1.

2.3. Samples

2.3.1. Hot melt adhesives

Eight adhesives supplied by different adhesive companies were studied. They were commercially used as food contact materials to join cardboard forming the geometric shape of the packages.

Six of them were hotmelt adhesives based on EVA and two were a special kind of hotmelt adhesives called pressure sensitive adhesives (PSA) based on SBS.

All of them contained the parent polymer or copolymer, paraffins or waxes, resins, fillers or pigments, but their formulation was unknown due to confidential reason.

The EVA base adhesives were AD1, AD2, AD3, AD5, AD6 and AD8, and the PSA were AD4 and AD7. Besides, a mineral oil free base hot melt adhesive also supplied by one adhesive company was used for the construction of the calibration curve.

All of them were originally solid polymers (granules or pellets) at room temperature.

To be cured, 1 g of each adhesive was placed in a sealed glass vial; the vial was then heated for approximately 10 min at 160–180 °C (upon recommendation from the manufactures). Three replicates of each one were prepared. Although PSA adhesives only needed a slight pressure instead of heating to be cured, they were also heated under the same conditions as the other adhesives in order to identify the presence of MOAH in these adhesives and to know the initial concentrations of the compounds selected as MOAH markers.

2.3.2. Laminates

Before manufacturing the laminates for the migration test, a previous assay was developed to check the lack of MOAH or marker compounds in the cardboard used to make these laminates. For this purpose, the cardboard was subjected to the mineral oil extraction process described by Vollmer et al. (2011) with slight modifications which are summarized below: in a 20 mL glass vial, 2.0 g of the cardboard cut into pieces of 0.5 cm on each side were placed, then 15 mL of hexane: ethanol (1:1) mixture was added, and it was left in the ultrasound bath for two hours, Next, the extract was decanted into another vial, and 5 mL of water were added. The supernatant hexane layer was separated and carefully concentrated under nitrogen gas to 1 mL, and later, it was analysed by HS-SPME-GC-MS.

Laminates with a sandwich structure (substrate-adhesive-substrate) were prepared for migration tests. The cardboard used as substrate had a grammage of 412 g m^{-1} and was obtained from the local trade.

The first step in the laminate manufacturing was to cut the cardboard

into rectangular sheets with 4 × 2 cm. The adhesive was then melted, and a homogeneous film of the melted adhesive was placed on the cardboard sheet. Finally, the structure was completed, placing a second sheet of cardboard over the melted adhesive.

The amount of adhesive used was calculated as the difference between the laminate mass and the cardboards used as substrates. The average mass of the adhesives in the laminate was 0.10 ± 0.01 g in 8 cm².

2.4. Instrumental

2.4.1. GC-MS
GC-MS analysis was performed on an HP 6890 chromatograph coupled to an HP 5975 mass selective detector. It was equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). The column was a DB-5 (30 m x 0.25 mm × 0.25 mm) from Agilent Technologies (Madrid, Spain). The temperature program applied to the oven was: initially 50 ºC, then raised to 310 ºC at 10 ºC min^{-1} and was kept at 310 ºC for 5 min, the splitless mode was selected, and the acquisition was carried out in SIM mode. Helium was used as carrier gas at a flow rate 1 mL min^{-1} and the injection temperature was 250 °C. The quantifier ions and their corresponding retention times are shown in Table S1. Data were processed using MSD ChemStation software (version F.01.00.1903, Agilent Technologies).

2.4.2. GC-FID
GC-FID analysis was performed on a Trace GC Ultra chromatograph equipped with a flame ionisation detector (FID) and an AS 300 autosampler (Thermo Electron Corporation, Milan, Italy). The temperature of the FID was 350 °C. The analytical column was an HP-5 (60 m x 0.25 mm ID, 0.25 μm film thickness) from Agilent Technologies. The oven temperature program was as follows: at 50 °C for 2 min, then increased to 30 °C min^{-1} up to 310 °C and held for 15 min. The total run was 26 min. The carrier gas flow rate (helium, 99.999%) was 2 mL min^{-1}, and the inlet pressure was 70 kPa. The injector temperature was 250ºC, and 5 μL of the MOAH fraction was injected in splitless mode. The data were acquired and processed using the Chrom-Card GC software (Thermo Electron).

Repeated solvent injections determined the position of the baseline. The area of the MOAH c-fractions was defined by the retention times of n-alkanes injected under the same conditions as the samples. The sharp peaks at the top of the MOAH hump were subtracted from the area of the corresponding sub-fraction, and the quantification was carried out through the internal standard method (Bratinova & Hoekstra, 2019).

2.5. Optimisation of the HS-SPME method

Optimisation tests were performed by directly injecting 10 μL of a standard solution containing all analytes (10 μg g^{-1}) in hexane. The optimised parameters were: type of fiber, extraction temperature (30–80 ºC) and extraction time (5–30 min).

The first step was the selection of the best fiber, that is, the one providing the highest response of the different analytes. For this purpose, the fibers tested were: polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR, 50/30 μm film thickness), Carboxen/polydimethylsiloxane (CAR/PDMS, 85 μm film thickness) and Polydimethylsiloxane (PDMS, 100 μm film thickness). And the extraction conditions were 15 min at 50ºC and a desorption temperature of 250ºC during 2 minThe second step was the performance of Face Centred Central Composite Design to select the optimum extraction temperature and time. The statistical design was carried out with the Modde 6.0 software from Umetrics (Umea, Sweden). For this statistical analysis, the total chromatographic areas obtained from the random variation of the temperature and the extraction time were used. The experiments to optimise these conditions were carried out with the selected fiber.

2.6. Initial concentration of MOAH markers in adhesives

The initial concentration of MOAH markers in the adhesives were determined using 1 g of different cured adhesives. To quantify their concentrations, 1 g of mineral oil free cured hot melt adhesive was placed in a 20 mL vial and 10 μL of different concentrations of solution A were added. The concentration range studied was 0.5–32 μg g^{-1} and the linearity of the calibration curve for each compound is shown in Table 1. Three replicates of each sample were prepared and analysed by HS-SPME-GC-MS method that was previously optimised.

2.7. Migration tests

To evaluate the migration potential of MOAH markers and confirm the presence of MOAH with the ability to migrate to food under the conditions studied, migration tests were performed using Tenax (60/100 mesh) as food simulant.In accordance with the provisions of the UNE-EN 14338 standard, Tenax was previously purified with acetone in a Soxhlet for 6 h and dried in an oven at 160ºC (AENOR, 2004).

2.7.1. Optimisation of extraction of MOAHs from Tenax
Previous to migration tests, the methodology for the extraction of these markers from Tenax was optimised. For this, 100 μL of solution A were added to three samples containing 0.32 g of Tenax. The concentration of the analytes was 23 μg of compound per g Tenax. The analytes were extracted four consecutive times with hexane. Each extraction was carried out with 4 mL of hexane under constant stirring by ultrasound for 1 h at room temperature. The extracts were concentrated in a nitrogen gas stream to 0.4 mL at 40 °C. After that, 10 μL of each concentrated extract was analysed by HS-SPME-GC-MS. After establishing the optimal number of extractions, recovery experiments were performed in triplicate to evaluate the extraction efficiency.

2.7.2. Migration test analyses and quantification
As shown in Fig. S1, for the migration tests, one of the sides of the laminates was covered with 0.32 g of Tenax according to the 4 g dm^{-2} ratio established by the UNE-EN-14338 standard. Next, the assembly (laminate-Tenax) was carefully wrapped in aluminium foil and placed inside a glass Petri dish with a diameter of 6 cm. The whole set was kept

Table 1
Analytical parameters of the HS-SPME-GC-MS method.

Compounds	Linearity (μg g^{-1})	R^2	Slope	LOD (μg g^{-1})	LOQ (μg g^{-1})	RSD (%)
2-MN	0.524–14.7	0.995	9.40E+02	0.113	0.377	10.1
1-MN	0.572–16.0	0.994	1.48E+03	0.103	0.345	5.2
BP	0.551–31.6	0.994	1.69E+03	0.050	0.167	3.4
2,6-DMN	0.526–25.1	0.995	1.68E+03	0.073	0.244	3.5
ACE	0.534–30.6	0.994	1.99E+03	0.014	0.048	3.0
9,9'-DMF	0.550–21.9	0.988	4.31E+03	0.030	0.099	2.2
2,6-DIPN	0.542–25.8	0.992	4.61E+03	0.012	0.040	5.6
3,3',5,5'-TMBP	0.54–31.0	0.997	3.25E+03	0.013	0.042	6.3
4-MDBT	0.538–25.5	0.991	1.95E+03	0.040	0.134	3.6
4,6-DMDBT	0.5321–14.9	0.995	1.99E+03	0.024	0.078	1.7
3,6-DMP	0.526–14.8	0.991	1.71E+03	0.038	0.125	8.9
1-MPYR	0.534–15.0	0.995	2.56E+03	0.022	0.074	3.4

in an oven for 10 days at 40 °C. The migration conditions were selected following Regulation EU 10/2011(European Commission, 2011). Three replicates and a blank were prepared for each assay.

After migration, the analytes were extracted from Tenax with hexane and concentrated following the optimised protocol described in Section 2.7.1. For the study of MOAH markers, 10 µL of the concentrated extract were taken and analysed directly by HS-SPME-GC-MS. For their quantification, 10 µL of increasing concentration of solution A in the range of 0.5–30 µg g^{-1} were analysed using the same methodology.

To study the migration of the MOAH fraction, 20 µL of solution B were added to Tenax before MOH extraction. Next, the MOSH and MOAH fractions were separated following the protocol developed by BfR & KLZH which consisted of passing 0.4 mL of the concentrated migration extraction through a glass column (160 mm × 8 mm internal diameter) filled with 3.0 g of activated silica gel coated with 0.3 % silver nitrate. The column was conditioned with 10 mL of n-hexane, then the 0.4 mL of sample was loaded and 2 mL of n-hexane were added and discarded as dead volume. The MOSH fraction was eluted with 4 mL of hexane and 2 mL of a second eluent prepared by adding 5 mL of toluene, 20 mL of dichloromethane, and 75 mL of hexane in a 100 mL volumetric flask, were added. MOAHs were eluted with 12 mL of the second eluent (BfR & KLZH, 2012). Subsequently, the MOAH fraction was concentrated under a gentle stream of nitrogen gas to 0.4 mL and analysed by GC-FID. MOAH was quantified using the internal standard method described by Bratinova & Hoekstra (Bratinova & Hoekstra, 2019).

The concentrations of migrating substances were calculated as absolute µg of the compound that migrated to Tenax, and these values were divided by dm^2 of laminate in contact with it.

3. Results and discussion

3.1. Optimisation of the HS-SPME method

As shown in Fig. S2, the CAR / PDMS fiber was the one that showed the worst results in the extraction of the analytes. The PDMS / DVB / CAR fiber showed good efficiency extracting the most volatile analytes, but it provided lower extraction values with the highest molecular mass compounds compared to PDMS fiber. On the other hand, the PDMS fiber showed good efficiency in extracting most of the analytes, except CHRY,

BbF and PER. An explanation of the poor "extraction efficiency" of CHRY, BbF and PER (for all types of fibres) could be the low volatility of these components at the incubation temperature of 60 °C. They possibly did not enter into the gas phase and consequently could not be transferred to the fiber. In summary, the extraction efficiency of the tested fibers decreased with increasing molecular mass, and the PDMS fiber was the one that showed the best results. It can be also emphasized that the migration of MOAHs to food takes place via vapour phase, as was demonstrated in several publications. Thus, the HS-PDMS-GC-MS analysis of MOAHs gives a better information of the real migration of MOAHs to food.

Fig. 1 shows the plot of the Face Centred Central Composite Design. The validity of the model was measured based on two parameters: the ability to interpret the responses obtained (R2) and the ability to predict responses within the range studied (Q2). Both R2 and Q2 showed values close to 1, which confirms that the response surface is adequate to predict the optimal extraction conditions using the PDMS fiber. These conditions were as follows:Extraction time 17.5 min and extraction temperature 60 °C.

3.2. Initial concentration of MOAH markers in adhesives

The first step in determining MOAH markers in the adhesives under study consisted of subjecting the cured adhesives to rapid detection by HS-SPME-GC-MS. The analysis revealed that only five out of the eight adhesives studied had an unresolved chromatographic hump (UCM), which could be MOH. The adhesives that showed this UCM were: AD1, AD5, AD6, AD7 and AD8. As a next step, MOAH markers were identified in the samples by comparing the analytes mass spectra and retention time with pure standards. Fig. 2 shows the chromatogram of the MOAH markers in the AD7 sample after the curing process.

Twelve of the proposed MOAH markers were identified in the adhesives that had a UCM. The identified compounds were: 2-MN, 1-MN, BP, 2,6-DMN, ACE, 9,9'-DMF, 2,6-DIPN, 3,3',5,5'-TMB, 4-MDBT, 4,6-DMDBT, 3,6-DMP and 1-MPYR.

For the quantitative study, only the five adhesives that presented UCM and some of the MOAH markers were considered; as these adhesives were more likely to be contaminated with mineral oils and contain MOAH. The analytical method used was HS-SPME-GC-MS, and the
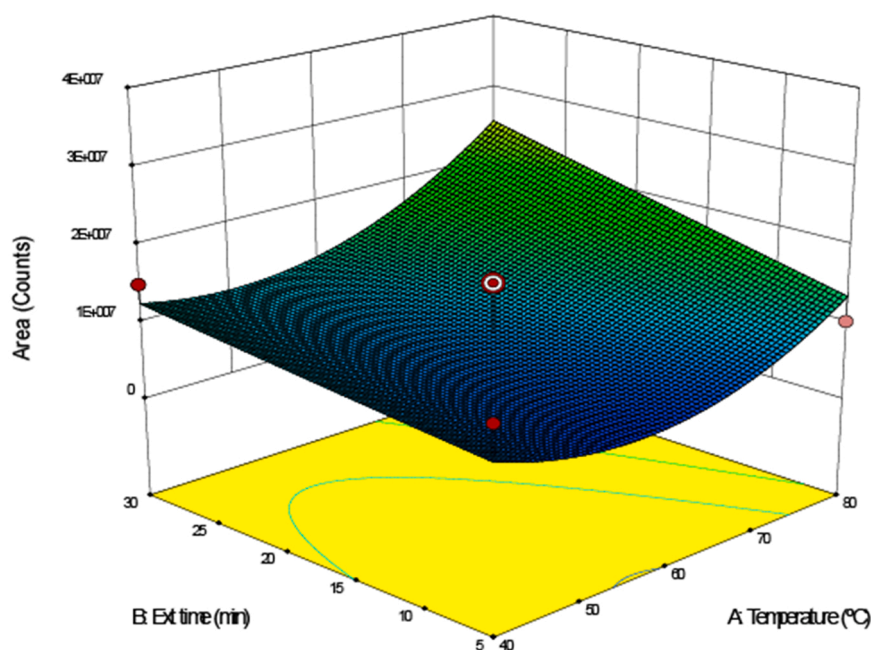

Fig. 1. Response surface plot for the optimal HS-SPME extraction conditions.
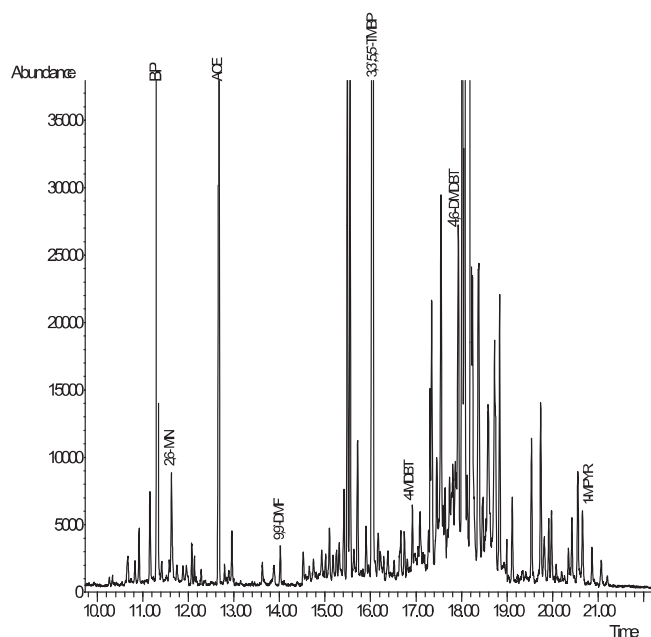
Fig. 2. Chromatogram of the MOAH markers in the AD7 sample after the curing process analysed by HS-SPME-GC-MS in SIM mode. The signal of each peak is composed of the sum of the mass fragments indicated in Table S1.

analytical characteristics of the method are shown in Table 1. The method showed good linearity, with regression coefficients between 0.988 and 0.997 for the different calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) were determined using the signal-to-noise (S/N) method. The S/N was established from the chromatograms of the analytes at low concentrations, close to noise. The limits of detection (LOD) and limit of quantification (LOQ) of the method, established as three times and 10 times S/N ratio respectively, were lower than 0.40 µg g^{-1} for all compounds and the relative standard deviation (RSD) had a value lower than 11%.

Table 2 shows the initial concentrations of MOAH markers in the five selected adhesives. The average concentration of the alkylated PAHs,

Table 2

Initial concentration of MOAH markers (mean ± SD) expressed as µg of compound per g of cured adhesive.

Compounds	MOAH markers initial concentration µg g^{-1} ± SD				
	AD1	AD5	AD6	AD7	AD8
2-MN	0.77 ± 0.01	<LOD	1.37 ± 0.08	<LOD	<LOD
1-MN	<LOQ	<LOQ	0.84 ± 0.07	<LOQ	1.03 ± 0.08
BP	7.39 ± 0.33	7.41 ± 0.38	5.21 ± 0.43	33.78 ± 0.66	3.38 ± 0.18
2,6-DMN	<LOD	<LOQ	1.05 ± 0.09	1.29 ± 0.01	<LOD
ACE	2.74 ± 0.12	2.25 ± 0.08	28.70 ± 0.79	30.23 ± 0.94	2.65 ± 0.07
9,9'-DMF	2.68 ± 0.11	<LOQ	<LOQ	0.85 ± 0.12	1.64 ± 0.03
2,6-DIPN	<LOD	<LOD	<LOQ	<LOD	<LOQ
3,3',5,5'-TMBP	2.32 ± 0.06	0.46 ± 0.02	1.23 ± 0.03	31.27 ± 2.62	<LOD
4-MDBT	4.41 ± 0.16	2.27 ± 0.04	<LOQ	2.67 ± 0.27	8.69 ± 0.72
4,6-DMDBT	2.57 ± 0.05	<LOQ	<LOQ	5.35 ± 0.44	12.58 ± 0.60
3,6-DMP	2.95 ± 0.29	2.00 ± 0.03	1.61 ± 0.15	<LOD	3.85 ± 0.05
1-MPYR	<LOD	3.77 ± 0.38	1.26 ± 0.03	2.10 ± 0.02	2.11 ± 0.12

2,6-DMN, 9,9'-DMF, 3,3',5,5'-TMB, 3,6-DMP and 1-MPYR was in the range of 0.46–31.3 µg g^{-1}; while the mean concentrations of the 2-MN and 1-MN isomers were between 0.77 and 1.37 µg g^{-1}. On the other hand, aromatic compounds with sulphur heteroatoms (4-MDBT, 4,6-DMDBT), commonly used in other studies to mark oil hydrocarbon contamination (Li et al., 2012; Yang et al., 2016), were detected in four of the samples (AD1, AD5, AD7 and AD8) at concentrations that ranged from 2.27 to 12.6 µg g^{-1}.

The most abundant markers were BP and ACE. These two markers were identified in the five adhesives studied, in concentrations of 2.25–33.8 µg g^{-1}. The compound 2,6-DIPN was identified in two adhesives (AD6 and AD8); however, their concentrations were below the limit of quantification.

It is important to remember that the analysed samples belong to 5 different adhesives and therefore the markers are expected to differ from one sample to another. The presence of MOAH and MOAH markers in the adhesives may be associated with the use of modified and unmodified aromatic resins (Lommatzsch et al., 2016). Modified and unmodified aromatic resins are commonly used in some adhesives, and these resins are made from coal tar and petroleum (Mildenberg et al., 1997), being able to represent an important source of MOAH in the adhesives. Thus, for example, PSA adhesives are usually formulated with aromatic resins and plasticizers, which are compatible with SBS and improve the viscoelastic properties of the adhesive (O'Brien et al., 2007).

3.3. Migration results

Four extractions with hexane were carried out, but only the first three extracts contained significant amounts of analytes. The concentrations of the analytes in the fourth extract were below the detection limit, therefore the optimal number of extractions was set at three. The extraction efficiency was evaluated by recovery tests (see Table S2), which showed that, except for the heaviest analytes (4,6-DMDBT, 3,6-DMP and 1-MPYR), the three extractions were sufficient to achieve recoveries higher than 91%.

The migration values of MOAH markers in laminates are shown in Table 3 and are expressed as µg of compounds per dm^2 of laminate. Migration percentage was also calculated based on the initial concentration, taking into account the grammage of the adhesive in the laminate. The percentage of migration was between 12 % and 75 %.

The migration is a complex process that depends on the partition, diffusion coefficients as well as initial concentration of the migration compounds. These coefficients are strongly related with temperature of the assay as well as the characteristics of the migrating compounds such as solubility of Hildebrand, polarity, structure or molecular weight. The aromatic compounds with several aromatic rings (subject matter) are highly influenced by a lower dispersion forces that involves lower Hildebrand solubility and therefore a great tendency to remain in the adhesive (Vera, Aznar, Mercea, & Nerín, 2011). It could explain the low migration results although this would not neglect the importance of their migration studies to food simulants.

The most volatile compounds (2-MN, 1-MN and 2,6-DMN) were not detected in the migration tests as they were likely lost during curing and laminate manufacturing processes. The compounds with the highest migration values were BP and ACE. Both compounds were detected in samples AD1, AD5, AD6 and AD7. The alkylated PAHs, 9,9'-DMF, 3,3',5,5'-TMB, 3,6-DMP, 1-MPYR and the compounds 4-MDBT and 4,6-DMDBT were detected in the migration samples from AD1, AD5, AD7 and AD8.

The migration of the MOAH fraction from the laminates to Tenax was also studied. For this, MOAH was separated from MOSH and analysed by GC-FID. The results of the analysis revealed the formation of UCM in the 5 adhesives examined, thus confirming the presence of MOAH in the samples.

The migration values of the MOAH sub-fractions are shown in Table 4. These values are expressed as µg MOAH per dm^2 of the

Table 3

Migration values of MOAH markers (mean ± SD) expressed as μg of compound per dm^2 of laminate and percentage of migration calculated based on the initial concentration of the markers in the adhesive.

Compounds	MOAH marker migration μg dm^{-2} ± SD / (%)					
	LOD	Laminate AD1	Laminate AD5	Laminate AD6	Laminate AD7	Laminate AD8
2-MN	0.354	<LOD	<LOD	<LOD	<LOD	<LOD
1-MN	0.323	<LOD	<LOD	<LOD	<LOD	<LOD
BP	0.156	4.23 ± 0.23 (56)	5.64 ± 0.11 (61)	3.23 ± 0.25 (50)	21.33 ± 1.95 (42)	<LOD
2,6-DMN	0.229	<LOD	<LOD	<LOD	<LOD	<LOD
ACE	0.045	0.69 ± 0.01 (24)	1.01 ± 0.05 (36)	13.46 ± 1.48 (38)	8.52 ± 0.87 (19)	<LOD
9,9'-DMF	0.093	<LOD	<LOD	<LOD	<LOD	1.48 ± 0.19 (75)
2,6-DIPN	0.038	<LOD	<LOD	<LOD	<LOD	<LOD
3,3',5,5'-TMBP	0.040	0.62 ± 0.04 (60)	<LOD	<LOD	12.22 ± 0.48 (21)	<LOD
4-MDBT	0.126	1.54 ± 0.03 (34)	1.74 ± 0.08 (61)	<LOD	1.84 ± 0.06 (46)	1.69 ± 0.20 (16)
4,6-DMDBT	0.074	1.61 ± 0.05 (61)	<LOD	<LOD	2.54 ± 0.17 (32)	1.77 ± 0.21 (12)
3,6-DMP	0.117	2.00 ± 0.06 (65)	<LOD	<LOD	<LOD	<LOD
1-MPYR	0.069	<LOD	1.56 ± 0.11 (33)	<LOD	1.06 ± 0.07 (33)	<LOD

Table 4

MOAH content in adhesive samples. The values were expressed as μg of the MOAH fraction per dm^{-2} of the laminate.

	MOAH fraction migrated (μg dm^{-2} ± SD)	
Sample	C16-C25	C25-C35
AD 1	19.65 ± 1.06	5.39 ± 0.78
AD 5	9.22 ± 0.32	<LOD
AD 6	7.48 ± 0.74	<LOD
AD 7	8.19 ± 0.67	<LOD
AD 8	6.78 ± 0.28	<LOD

laminate. The limit of quantification of the method was below 2.2 μg dm^{-2}. To calculate this limit, we first estimated instrumental LOQ obtained from the S/N ratio of a mineral oil injected at low concentrations in the GC-FID; and then the amount of sample was considered, as well as the preconcentration and dilution factors applied during the preparation and treatment of the sample.

The average migration of the C16-C25 MOAH sub-fraction reached values from 6.78 to 19.65 μg dm^{-2} of laminate and the concentration of the only C25-C35 sub-fraction detected was 5.39 μg dm^{-2}.

The highest levels of MOAH migration were found in the laminates of adhesives AD1, AD5 and AD7, which were also characterised by presenting the highest number of marker compounds during migration. Fig. S3 shows the migration chromatogram of the AD5 adhesive laminate, which presents a MOAH hump in the range of the C16-C25 sub-fraction. All the laminates of the hot melt adhesives studied had a MOAH hump in this range. The AD1 adhesive, in addition to the C16-C25 hump, presented another MOAH hump in the range of the C25-C35 sub-fraction. The independent humps in this adhesive probably points out two different sources of mineral oils. In none of the laminates was MOAH found in the range C10-C16 or beyond C35.

The MOAH C16-C25 sub-fraction belongs to the volatile range of mineral oils, characterized by migrating through the gas phase at room temperature. The compounds used as chemical markers for MOAH are also within this volatility range (<C25), and their behaviour during migration is similar to the MOAH fraction.

Migration depends on several factors such as the material, temperature and nature of the migrant.(Poças, Oliveira, Pereira, Brandsch, & Hogg, 2011) The molecules <C25 have a high enough vapour pressure to migrate mainly through the gas phase, therefore their migration in the case of porous structures like paper will be faster and their concentrations in short periods of time may be favoured over heavier molecules. However, if there were heavier molecules in the adhesives (>C25) they would migrate more slowly. It is important to remember that the cardboard used to manufacture the laminate was free of MOAH. Therefore, the only source of the detected MOAH fraction and MOAH markers was the hot melt adhesive.

4. Conclusions

The HS-SPME-GC-MS method developed in this study represents a suitable alternative for the rapid, direct and reliable detection of volatile MOAH markers from solid samples, without the need for previous treatments.

Although the HS-SPME-GC-MS method showed little efficiency in the extraction of higher molecular weight analytes, under the tested conditions due to the low volatility of these compounds, the technique proved to be efficient to identify and quantify the most volatile markers, which have chemical structures and behaviour similar to MOAHs with the potential to migrate.

The identification of specific MOAH markers is important to rule out or confirm the presence of MOAH in contaminated samples. According to the results of this investigation, hot melt adhesives based on EVA and PSA adhesives represent a possible source of MOAH contamination in food; and eight of the evaluated substances (BP, ACE, 9,9'-DMF, 3,3',5,5'-TMB, 3,6-DMP, 1-MPYR, 4-MDBT and 4,6-DMDBT) are suitable for marking MOAH contamination of adhesives.

The fraction of MOAH that migrated from the hot melts was characterised as eluting mainly in the C16-C25 range, and the adhesives with highest MOAH migration also showed a higher number of markers.

GC-FID and HS-SPME-GC-MS can be considered as complementary tools to quantify and check the MOAH contamination, respectively.

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; **Paula Vera**: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – review & editing. **Cristina Nerín**: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgements

Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.fpsl.2022.100885.

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