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#### **RESEARCH ARTICLE**

### Separation of the mineral oil aromatic hydrocarbons of three and more aromatic rings from those of one or two aromatic rings

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An analytical method was developed for the quantitation of the mineral oil aromatic hydrocarbons in cosmetic raw materials separating those of one or two aromatic rings from those of three and more aromatic rings. Normal phase high performance liquid chromatography was used with donor-acceptor complex chromatography. The composition of both fractions and the quantities of respective compounds were determined by comprehensive two dimensional gas chromatography with time of flight mass spectrometry and by liquid chromatography coupled to gas chromatography with flame ionization detection.

#### **KEYWORDS**

donor-acceptor complex chromatography, mineral oil aromatic hydrocarbons, polyaromatic hydrocarbons, triaromatic hydrocarbons, two dimensional gas chromatography

#### **1 | INTRODUCTION**

Mineral oil products are complex mixtures of thousands of compounds, produced from crude oil by various physical and chemical processes. The mineral oil hydrocarbons (MOH) can be subdivided into mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). Unlike the commonly known polycyclic aromatic hydrocarbons (PAH), the majority of MOAH is highly alkylated and depending on the degree of refinement partially hydrogenated MOAH are expected [1,2].

Human exposure to MOSH and MOAH may be by the oral route, either via foods or cosmetic and pharmaceutical products, such as lip care products and laxatives, which can almost entirely consist of MOH [3].

Required by EU-Regulations [4-6], manufacturers of MOH based lubricants, oils in food contact, cosmetic and

pharmaceutical raw materials reduce the amount of MOAH in their products mainly in order to remove genotoxic constituents. The resulting raw materials should comply with the European Pharmacopoeia requirements or pass the IP346 test method [7]. The criterion of the IP346 test is that the gravimetrically determined total DMSO extractables are below 3 wt% of the product, which is a threshold for emerging carcinogenic effects in the mouse skin painting assay.

After fractionation by distillation, the raw materials usually undergo hydrogenation for further removal of MOAH. However, the hydrogenation of polycyclic MOAH is usually incomplete, leaving constituents containing isolated aromatic rings. For their complete hydrogenation, more severe conditions are needed. Hence, MOAH may still be present in these products [8].

According to the 2012 opinion of the European Food Safety Authority, "MOAH with three or more, non- or simplealkylated, aromatic rings may be mutagenic and carcinogenic, and therefore of potential concern" [9].

Therefore, and supported by others [8,10], there is a need to differentiate between the mono-/diaromatic fraction (MDAF) and the tri-/polyaromatic fraction (TPAF).

Presently, normal phase HPLC coupled on-line to GC with flame ionization detection (FID) is the most commonly used method for determining MOAH in mineral oil products

Article Related Abbreviations: DACC, donor acceptor complex chromatography; FID, flame ionization detection; GCxGC, comprehensive two dimensional gas chromatography; HOMO, highest occupied molecular orbital; MDAF, mono- and diaromatic fraction; MOAH, mineral oil aromatic hydrocarbons; MOH, mineral oil hydrocarbons; MOSH, mineral oil saturated hydrocarbons; PAH, polycyclic aromatic hydrocarbons; TPAF, tri- and polyaromatic fraction.

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or products containing MOAH [11]. However, this method does not enable to separate between the MDAF and the TPAF. Other modifications of this method, for example proposed by Zoccali et al. [12] are also lacking separation power for determination of TPAF. More detailed separations are achieved by comprehensive two-dimensional gas chromatography (GCxGC) [1]. In combination with time of flight (TOF)-mass spectrometry (MS), it provides the most efficient way of compound identification. The interpretation of GCxGC plots of MOAH is mostly qualitative [13], but there are different approaches for quantitative determinations of compound groups [1,14,15]. Yet, GCxGC separations alone are mostly still insufficient, since neither MOSH and MOAH nor MDAF and TPAF are completely separated [14]. It should also be taken into account that the concentration of the TPAF within the MOAH fraction is in cosmetic raw materials usually far below (at least by the factor of 100) that of the MDAF, which presupposes an effective MDAF/TPAF separation and often causes problems of detectability of the TPAF when analyzed together with MDAF.

Here, a first liquid chromatographic step is proposed for pre-separation and enrichment of the TPAF. The most common type of stationary phase, when dealing with ring specific separation of aromatic hydrocarbons, is silica-based amino phase. This type of phase is utilized, for example, in the IP 391 HPLC-method for the determination of aromatic hydrocarbon types in middle distillates [16]. Other methods in the past used this stationary phase as well [17]. However, as a silica-based amino phase column did not provide sufficient separation for aromatics with a high degree of alkylation, donor-acceptor complex chromatography (DACC) [18] was applied. Isolated MOAH, for example, from cosmetic raw materials, underwent DACC separation and the MDAF and TPAF were quantified by on-line HPLC-GC-FID as described in [19]. GCxGC-TOF-MS was used to check the MDAF/TPAF separation and to characterize the composition of the fractions.

#### **2 | MATERIALS AND METHODS**

#### 2.1 | Samples, standards, and chemicals

Two samples of petrolatum (vaseline) and two samples of paraffinum liquidum (paraffin oil) were provided from a cosmetic manufacturer.

For the control of the separation conditions and the quality of the MDAF/TPAF separation, a mixture of standard substances was used. It contained 33 aromatic compounds in *n*-hexane with: a custom-synthesized mixture of octylphenan-threnes (four isomers) obtained through Friedel Crafts acylation of phenanthrene with octanoyl chloride and subsequent Wolf-Kishner reduction; 2-methylnaphthalene (2MN; Sigma-Aldrich, Steinheim, Germany); PAH 20 Mix, containing

16 EPA PAH plus dibenzo[a,e]-, dibenzo[a,h]-, dibenzo[a,i]and dibenzo[a,l]pyrene (Neochema, Bodenheim, Germany); a mixture of 11 alkylated and hydrogenated PAHs: benzo[a]fluorene, benzo[b]fluorene, 9,10-dihydroanthracene, 9,10-dimethylanthracene, 3,6-dimethylphenanthrene, 2-ethylanthracene, 1-methylanthracene, 2-methylfluoranthene, 1-methylfluorene, 1-methylphenanthrene, and 1-methylpyrene purchased as solutions of single compound (Neochema, Bodenheim, Germany).

LC–GC analysis involved the following MOSH/MOAH standards [1]: *n*-undecane (C11), *n*-tridecane (C13), bicyclohexyl (CyCy),  $5-\alpha$ -cholestane (Cho), *n*-tetracontane (C40), 1,3,5-tri-*tert*-butyl benzene (TBB), pentylbenzene (5B), 1-methylnaphthalene (1-MN), 2-methylnaphthalene (2-MN), perylene (Per), and a mixture of C12-C60 *n*-alkanes (Sigma-Aldrich, Steinheim, Germany).

For the control of the GCxGC separation, a mixture of mineral oil products, 16 EPA PAHs plus carbazole as well as the above-mentioned MOSH/MOAH standards plus octadecylbenzene and 1,4-bis(2-ethylhexyl)benzene was kindly provided by Official Food Control Authority of the Canton of Zurich (Zurich, Switzerland).

Silica gel 60 (0.06–0.2 mm) was purchased from Macherey-Nagel (Düren, Germany), *n*-hexane for residue analysis from LGC Standards (Wesel, Germany), dichloromethane for pesticide residue analysis from Honeywell (Th. Geyer, Renningen, Germany) and silver nitrate for loading of the silica gel from Sigma-Aldrich (Steinheim, Germany).

#### 2.2 | Experimental overview

The cosmetics raw materials petrolatum (vaseline) and paraffinum liquidum (paraffin oil) were used for the experiments on determining the TPAF. The samples underwent the preparation and measurements summarized in Chart 1. First, the MOAH were separated from the MOSH through a preparative column packed with silver-ion-loaded (0.3%) silica gel. Using a donor-acceptor-chromatography HPLC column, the isolated MOAH fraction was separated into MDAF and TPAF.



CHART 1. Outline of the separation process

To avoid overloading of the DACC column and to enrich the TPAF, fractions of five separations were pooled. The aromatic fractions were characterized using GCxGC-TOF-MS; concentrations were determined by LC-GC-FID.

#### **2.3** | Sample preparation

Of cosmetic raw materials, 1 g was weighed into a 40 mL EPA screw neck glass vial. After addition of 20 mL n-hexane and 50 µL MOSH/MOAH internal standard solution in n-hexane  $(50 \,\mu\text{g/mL})$ , the vial was closed with a screw cap, shaken and placed in a bead bath at 60°C for 5 min. The clear solution was cooled for 20 min in a refrigerator at 4°C to precipitate high molecular weight compounds. The influence of the precipitation step upon the compounds in the boiling range below C50 was investigated by experiments with a mixture of C12-C60 n-alkanes. The comparison of the peak area ratios of n-alkanes C50/C20 after LC-GC-FID measurements of precipitated and not precipitated samples showed no significant losses of C50 (recovery: 97%) after precipitation step. After a centrifugation at 2000 rpm, 1 mL of the clear solution was subjected to preparative separation of the MOSH from the MOAH, using a glass SPE column with a glass fiber frit filled with 6 g silica gel, loaded with 0.3% silver nitrate according to [20]. The MOSH were eluted with 18 mL n-hexane, after that the column was conditioned with 5 mL mixture of 30% dichloromethane in n-hexane. The elution of MOAH was performed with 25 mL dichloromethane.

#### 2.4 | Instrumental analysis

#### 2.4.1 | Normal phase HPLC

The MOAH fraction was separated into MDAF and TPAF using a Nucleosil Chiral-2 5 µm, 250 mm x 4 mm column (Macherey Nagel, Düren) mounted in an 1100 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a fluorescence detector. This column utilizes a Pirkle-Phase [21] consisting of an N-(3,5-dinitrobenzoyl)-D-phenylglycine moiety bonded through a spacer to the silica-surface. The gradient of *n*-hexane/dichloromethane at a flow rate of 1 mL/min started with 100% n-hexane up to 8.7 min, decreased to 30% n-hexane up to 9 min, maintained it until 15 min, returned to 100% n-hexane at 15.2 min and reconditioned the column with 100% *n*-hexane up to 25 min. MOAH-fractions obtained through the separation described in 2.3 were concentrated (maximum 4.6 mg/mL) by the means of vacuum evaporation at 300 mbar and 40°C, 100 µL were injected for each separation run.

#### 2.4.2 | GCxGC-TOF-MS

For GCxGC-TOF-MS, a Leco Pegasus 4D (Leco Instrumente, Mönchengladbach, Germany) was used, controlled by Leco ChromaTOF acquisition software. The instrument EPARATION SCIENCE

consisted of a 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless injector, a PAL combi XT autosampler (CTC Analytics AG, Zwingen, Switzerland), a secondary internal oven, a cryogenic consumable-free (CF) nitrogen-cooled (FC100 chiller from SP Scientific-FTS Systems, Warminster, PA, USA) jet modulator and a TOF mass spectrometer. The column configuration was of the reversed polarity type, with a 30 m  $\times$  0.25 mm i.d.  $\times$  0.15  $\mu$ m DB-17HT (Agilent Technologies, Waldbronn, Germany) first dimension column connected via the ultimate union connection system (Agilent Technologies, Waldbronn, Germany) to a 1.5 m  $\times$  0.25 mm i.d.  $\times$  0.1  $\mu$ m DB-5HT (Agilent Technologies, Waldbronn, Germany) second dimension column. These columns were temperatureprogrammed from 60 to 370°C at 3°C/min without secondary oven offset. The modulator offset was 20°C. Helium was used as a carrier gas in constant flow mode (1 mL/min). Modulation was in staged mode, from 9 to 14 s at the end of chromatographic separation in order to avoid the wrap-around of high boiling compounds. Spectra were collected in the m/z range from 35 to 650, with a scan rate of 50 spectra/s. The ion source was at 250°C, the transfer-line at 340°C; a detector voltage of 1600 V was applied after the solvent delay of 450 s. To lower the LOD, pooled TPA fractions (2.2) were evaporated (at 300 mbar and 40°C) to 40 µL. Injection volumes were between  $1-3 \mu L$  in pulsed splitless mode.

#### 2.4.3 | Online HPLC–GC–FID

MOAH, MDAF, and TPAF were measured by an on-line HPLC-GC-FID system (Axel Semrau, Sprockhövel, Germany), using a PAL CTC sampler (CTC Analytics, Zwingen, Switzerland) on a 1260 Infinity HPLC instrument (Agilent Technologies, Waldbronn, Germany). A silica gel column (Restek Allure Silica 5  $\mu$ m, 250 mm  $\times$  2.1 mm) was connected via an Y-interface [22] to a DANI Master GC (DANI Instruments, Cologno Monzese, Italy) equipped with an uncoated precolumn (Restek MXT 10 m × 0.53 i.d.) followed by a steel T-piece union connecting it to SVE (solvent vapor exit) and a nonpolar separation column (Restek MXT-1,  $15 \text{ m} \times 0.25 \text{ mm}$  i.d.  $\times 0.25 \text{ \mu m}$ ). A gradient of n-hexane with dichloromethane was used with backflush after the elution of the MOAH, started at 0.3 mL/min with 100% n-hexane, reaching 35% dichloromethane after 1.5 min, backflush initiated after 6.2 min with 100% dichloromethane at 0.5 mL/min for 9 min, followed by a recondition with 100% *n*-hexane for 10 min at a flow rate of 0.5 mL/min and 5 min at 0.3 mL/min. The injection volume was 90 µL for the TPAF (40 µL were dissolved to 100 µL after the GCxGC-TOF-MS injection), and 10-50 µL for MOAH and MDA fractions. Hydrogen was used as a carrier gas with 90 kPa applied during the fraction transfer from LC to GC through the Y-interface and 150 kPa after the partially concurrent solvent evaporation [23] and



FIGURE 1 HPLC-FLD (Ex = 280, Em = 380) chromatograms of (A) n-hexane isocratic elution of a mixture of phenanthrene (1), 1-methylphenanthrene (2), 3,6-dimethylphenanthrene (3) and isomers of octylphenanthrene (4) with retention time to cut MDAF/TPAF; (B) gradient elution (2.4.1) of the aromatic compound mixture (2.1) including isomers of octylphenanthrene (4) with marked retention time windows for MDAF and TPAF

closure of the SVE valve. GC started at 58°C (11 min), followed by a temperature program of 5°C/min to 80°C, then at 15°C/min to 110°C and at 25°C/min to 370°C (7 min), resulting in a total run time of 34 min.

#### **2.5** | Verification through internal standards

After the preparative MOSH/MOAH separation, the MOAH fraction contained following internal standards 5B, TBB, 1MN, 2MN, and perylene. Since only perylene contains more than two aromatic rings out of the mentioned compounds, the MDAF/TPAF separation resulted in the presence only of perylene in the tri- and polyaromatic fraction. For the quantification purpose 2-methylnaphthalene was added to the TPAF prior to the evaporation step.

#### **3 | RESULTS AND DISCUSSION**

#### 3.1 | Determination of the separation cut between mono and diaromatic and tri- and polyaromatic fractions

DACC separates aromatic hydrocarbons according to their ring number [24]. Therefore, any simple triaromatic compound, e.g. phenanthrene would theoretically mark the beginning of the TPA fraction. However, a high degree of alkylation of MOAH compounds must be taken into account. For that reason mix of custom-synthesized octylphenanthrenes (2.1) was used to investigate chromatographic behavior of higher alkylated triaromatics on the used column.

The experiments showed, that *n*-hexane, as a non-polar solvent allowing intense donor acceptor interactions, eluted methyl- and dimethyl-substituted phenanthrenes after the parent compound, as reported for another DACC-phase [24], but higher alkylated before (Figure 1A). The earlier elution of octylphenanthrene is presumably due to weaker donoracceptor complex formation caused by the increased steric hindrance of the aromatic core through the alkyl moiety.

Hence, the octylphenanthrene was used as a marker to establish the cut: the start of the TPAF was positioned at the onset of the peak of the first eluted isomer of octylphenanthrene (Figure 1A). The gradient elution was designed to let dichloromethane reach the detector shortly after the elution of octylphenanthrene on the cut (as seen by the raise of the signal through dichloromethane-forced elution of polyaromatics on Figure 1B). This allowed satisfying separation of diaromatics from triaromatics by n-hexane elution before the cut and accelerated the elution of triaromatics after the cut through dichloromethane to lower the runtime.

A mixture of aromatic compounds including octylphenanthrene (2.1) was used to determine the selectivity of the MDAF/TPAF separation on the DACC column (Figure 1B). Eluate was collected before and after the cut retention time, concentrated by evaporation and analyzed by GCxGC-TOF-MS for further investigation of the selectivity at the cut (Figure 2).

The MDAF GCxGC plot (Figure 2B) reveals a weak signal for phenanthrene/anthracene, but as it was also observed in the blank, it must have been due to background contamination. The TPAF (Figure 2C) includes a signal for acenaphthylene that was not found in blanks. As it is a diaromatic compound with an additional double bond, it does not fit into the TPAF. Its proportion in the TPAF fraction varied between 4 and 25% and had to be accepted as an insufficient selectivity.

The recovery rates of the correctly separated standards in the TPAF compared to the same substances prior to the separation varied between 93 and 118%, with a mean recovery of 106%, indicating essentially loss-free separation procedure.

The signals of the octylphenanthrene isomers (peak group 4, Figure 1A) indicate that the retention time of alkylated aromatic compounds depended not only on the chain length of the alkyl moiety, but also on their position on the aromatic ring system. Hence, compounds with a given number of carbon atoms in the alkyl groups may be eluted in fairly broad retention windows, possibly overlapping with others. Therefore, triaromatic species containing more than eight carbon atoms in the alkyl chain may be eluted before as well as after the cut, depending on position of the alkyl groups.

The mixture of mineral oil products plus PAH standards (2.1) was separated using the cut established above (Figure 3).



FIGURE 2 Sections of GCxGC-TOF-MS contour-plots of the test-mixture, XIC (extracted-ion chromatogram) of the molecular ions m/z 128, 142, 152, 154, 166, 178, 180, 192, 202, 206, 216, 228, 252, 276, 278, 302. (A) prior to separation; (B) MDAF; (C) TPAF. 1 naphthalene, 2
2-methylnaphthalene, 3 acenaphthylene, 4 acenaphthene, 5 fluorene, 6 9,10-dihydroanthracene, 7 1-methylfluorene, 8 phenanthrene, 9 anthracene, 10 1-methylanthracene, 11 1-methylphenanthrene, 12 3,6-dimethylphenanthrene, 13 2-ethylanthracene, 14 9,10-dimethylanthracene, 15 fluoranthene, 16 pyrene, 17 2-methylfluoranthene, 18 benzo[a]fluorene, 19 benzo[b]fluorene, 20 1-methylpyrene, 21 benzo[a]anthracene, 22 chrysene, 23 benzo[b]fluoranthene, 24 benzo[k]fluoranthene, 25 benzo[a]pyrene, 26 indeno[1,2,3-cd]pyrene, 27 dibenzo[a,h]anthracene, 28 benzo(g,h,i)perylene, 29 dibenzo(a,e)pyren, 30 dibenzo(a,i)pyren, 31 dibenzo(a,h)pyren, 32 dibenzo(a,l)pyren, 33 octylphenanthrenes



**FIGURE 3** Sections of GCxGC-TOF-MS contour-plots of the mixture of mineral oil products (2.1) in TIC (total ion current). (A) prior to separation; (B) MDAF; (C) TPAF. 1 acenaphthylene, 2 acenaphthene 3 fluorene, 4 dibenzothiophene, 5 phenanthrene / anthracene, 6 carbazole, 7 fluoranthene, 8 pyrene, 9 benzo[a]anthracene, 10 chrysene, 11 benzo[b]fluoranthene / benzo[k]fluoranthene, 12 benzo[a]pyrene, 13 perylene, 14 indeno[1,2,3-cd]pyrene / dibenzo[a,h]anthracene, 15 benzo(g,h,i)perylene

Mass filtering (for description see 3.3) showed the presence of higher than C8-alkylated phenanthrenes/anthracenes in both MDAF and TPAF, confirming the above interpretation.

## **3.2** | Quantification of aromatic fractions isolated from cosmetic raw materials

The preparative MOSH/MOAH separation (2.3) allowed isolation of larger amounts of MOAH and avoided tailing of MOSH into the MOAH that known to occure on a conventional NP-HPLC column with comparable sample loads.

LC-GC-FID analysis of the pre-separated MOAH fractions showed that MOAH in the petrolatum used for cosmetics was of a higher molecular mass than those in paraffin oils, with maxima approximately at the retention times of n-alkanes  $C_{35}$  for petrolatum 1,  $C_{40}$  for petrolatum 2 (Figure 4 A and B) and  $C_{20}$  for paraffin oil 1,  $C_{25}$  for paraffin oil 2 (C and D).



FIGURE 4 LC-GC-FID chromatograms of the MOAH fractions. (A) petrolatum 1; (B) petrolatum 2; (C) paraffin oil 1; (D) paraffin oil 2. Internal standards: pentylbenzene (5B), 1- and 2- methylnaphthalene (1MN, 2MN), 1,3,5-tri-tert-butylbenzene (TBB) and perylene. n-alkanes C16, C20, C35, C50 used as retention time markers



FIGURE 5 LC-GC-FID chromatograms of the TPAF fractions. (A) petrolatum 1 (B) petrolatum 2. For abbreviations see Figure 4

The many peaks on top of the MOAH hump of petrolatum 1 indicate low isomerization of certain types of MOAH. A similar pattern is usually observed in the aromatic fraction of waxes and originates from alkylated benzenes. In fact, the same pattern was observed in the MDAF.

Figure 5 shows the LC-GC-FID chromatograms of the TPAF of the two petrolatum samples. The retention time of the humps is similar to the entire MOAH-fractions, that is, also similar to the MDAF. The paraffin oils did not contain a detectable TPAF.

The concentrations of the MOAH, MDA, and TPA of the petrolatum and paraffin oil samples, determined by LC-GC-FID, are listed in Table 1. The mono and diaromatic compounds were the main part of the MOAH. The measurement uncertainty was in the range of 20%.

evaporation steps after MOSH/MOAH-The and MDAF/TPAF-separation resulted in partial loss of 2methylnaphthalene (60% recovery), which led to overquantification of MDAF. The measurements of MDAF with 2MN added only after the separation and directly before the injection to LC-GC-FID showed no evaporation losses, since the determined concentrations were as expected comparable to MOAH amounts.

MDAF amounts determined indicate no large losses of analytes during the separation procedures on the one hand and support the accuracy of the small amounts of the TPAF determined on the other hand, since they were in the range below 1% of the MOAH fraction.

The limit of quantification for TPAF was 10 mg/kg based on the measurements of blank samples, as they delivered values in the range of 3 mg/kg.

#### 3.3 | Characterization with GCxGC-TOF-MS

The characterization of the MOAH by GCxGC-TOF-MS used the mass-filtering approach proposed by Biedermann et al. [1]. In accordance with this method, the substance classes were identified by their retention behavior and corresponding mass spectra.

#### **TABLE 1** Summary of quantification results

Sample	MOAH-amount [%]	MDAF-amount [%]	TPAF-amount [mg/kg]
Petrolatum 1	2.0	1.7	110
Petrolatum 2	2.3	2.3	50
Paraffin oil 1	0.014	0.013	<10
Paraffin oil 2	0.071	0.057	<10



FIGURE 6 A section of GCxGC-TOF-MS (TIC) contour plot of a standard mixture with marked groups of compounds

Figure 6 gives a notion about the arrangement of different compound groups within the used so called reversed phase GCxGC chromatographic setup with medium polar column in the first and non-polar column in the second dimension. The aromatics are found in the lower part of the contour plot, their retention time in the first dimension grows with their increasing ring number. The alkylation gives a shift towards later elution in both dimensions, so that alkylated congeners are situated to upper right of the parent compound forming slanted bands of isomeric compounds with the same degree of alkylation as shown in Figures 6 and 10.

The normal phase GCxGC chromatographic setup with a non-polar column in the first and medium polar column in the second dimension enables a better separation of the aromatic hydrocarbons than the above mentioned reverse phase column arrangement. However, Jennerwein et al. [25] found the reverse phase approach to have sufficient resolving power for the aromatics, to deliver more precise quantification results and improved sensitivity due to smaller peak width of aromatic hydrocarbons compared to normal phase column combination.

The GCxGC-TOF-MS MOAH measurements contourplots of the samples are shown in Figure 7, the MDAF results were very similar and are therefore not shown.

Figure 7 underlines the observation done on the corresponding LC-GC-FID chromatograms (Figure 4) of the petrolatum samples. Petrolatum 1 has a compact MOAHregion with the most intense signals situated in the area of alkylbenzenes with some prominent single peaks. Whereas petrolatum 2 sample shows a distinct MOAH region having a spread of continuous signals into the lower part of the plot indicating high degree of isomerisation of present compounds.

The characterization of the MOAH and MDA fractions of petrolatum samples proved the presence of alkylated and partially hydrogenated aromatics with one or two aromatic rings. Compound classes detected comprised alkylbenzenes, alkylated tetralines, alkylated tetrahydro and octahydro anthracenes/phenanthrenes as well as partially hydrogenated and alkylated benzanthracenes/chrysenes containing not more than two aromatic rings. The alkyl chains counted up to 40 carbon atoms for benzenes and up to 30 carbons for other compound classes.

Moreover, petrolatum 1 sample was found to contain alkylated benzothiophenes and dibenzothiophenes (Figure 8). This fact was unexpected since sulfur-containing compounds should have been removed during the petrolatum refining procedures.

The MOAH-regions of paraffin oils (Figure 7C and D) are located in a considerably lower part of the plots, well apart from the region of alkylated monoaromatics. The characterization of the MOAH and MDA fractions revealed the lack of detectable signals for alkylated benzenes and alkylated tetrahydro naphthalenes, which is very unusual for MOAH and implies extensive hydrogenation. However, partially hydrogenated and alkylated homologs of higher

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**FIGURE 7** Sections of GCxGC-TOF-MS contour plots of the MOAH fractions, TIC. (A) petrolatum 1; (B) petrolatum 2; (C) paraffin oil 1; (D) paraffin oil 2. For abbreviations see Figure 4



**FIGURE 8** Sections of GCxGC-TOF-MS contour plot of MOAH fraction of petrolatum one sample. (A) benzothiophenes (XIC of *m*/z 162, 176, 190, 204); (B) dibenzothiophenes (XIC of *m*/z 184, 198, 212, 226). Dashed lines are marking the regions of the alkylated benzothiophenes and dibenzothiophenes with the number of carbon atoms attached



1st dimension, 110-370 °C, 3 °/min

**FIGURE 9** Sections of GCxGC-TOF-MS contour plots of the TPA fractions, XIC of *m/z* 142 (2MN), 178, 202, 228, and 252 (Per) for better depiction of the TPAF region. (A) petrolatum 1; (B) petrolatum 2

aromatic compounds like phenanthrenes and benzanthracenes were still present. This can be explained through incomplete hydrogenation, since the full hydrogenation of the PAH skeleton requires more drastic hydrogenation conditions than those for benzenes and naphthalenes. The alkylation degree of detected compounds was in the range of up to 20–25 attached carbon atoms.

The analysis shown in Figure 9 confirms the findings of the LC-GC-FID measurements (Figure 5) - the TPA fractions of the petrolatum samples show clear signals located in the lower right part of the plots with an extension into high boiling range. TPA fractions of the paraffin oils showed only a few single signals of triaromatic compounds which were also present in blank samples.

The characterization of the petrolatum TPA-fractions showed presence of high alkylated tri- and polyaromatic compounds. Petrolatum 1 contained alkylated anthracenes/phenanthrenes, fluoranthenes/pyrenes and benzanthracenes/chrysenes with up to 30 carbon atoms attached. The second petrolatum sample contained the



**FIGURE 10** A section of GCxGC-TOF-MS (XIC of *m/z* 178, 192, 206, 220, 234, 248) contour plot of TPA fraction of petrolatum 1 sample. Dashed lines are marking the regions of the alkylated phenanthrenes/anthracenes with the number of carbon atoms attached

mentioned compounds in a range of up to 20 carbon atoms. This findings correspond with the assumption under 3.1, that the higher alkylated triaromatic compounds could elute after the established TPAF cut.

Figure 10 provides an example of distribution of the alkylated congeners of anthracenes/phenanthrenes in petrolatum 1 sample showing very well the above mentioned slanted propagation of alkylated compounds from lower left to upper right region of the plot with increasing degree of alkylation.

Furthermore, the TPAF of petrolatum 1 sample revealed the presence of dibenzothiophenes with high degree of alkylation. This finding is very interesting due to the fact, that these are actually diaromatic compounds and they do not fit into the TPAF. This seems to be a limitation of the used HPLC column as it is not selective enough for separation of triaromatics from diaromatics if the latter are containing heteroatoms. This phenomenon was also observed for the tetrachlorophthalimide based DACC phase [24] and seems to be common for DACC.

According to Holstein et al. [18] the elution order of the compounds from a DACC column depends on the strength of the complex formation between acceptor on the stationary phase and the analyzed donor substance. This complex is more stable if the molecular orbital (MO)-energy difference of the lowest unoccupied molecular orbital (LUMO) of the acceptor and the highest occupied molecular orbital (HOMO) of the donor is minimal. Thus the elution order of compounds depends in our case on their HOMO energy (LUMO energy of the stationary phase remains the same). In agreement with Koopmans' theorem, the HOMO energy is equal to the ionisation potential (I<sub>P</sub>) of a molecule [26]. Therefore the ionisation potential was used as a molecular descriptor to assess the strength of donor-acceptor complex formation. Table 2 contains an overview of I<sub>p</sub> values for some aromatics with varying ring number and dibenzothiophene, based on literature (references provided). The Ip value of dibenzothiophene is situated in the range of aromatic substances containing more than three aromatic rings. This observation assumes a large affinity to DAC formation and following strong retention on the used stationary phase and explains the elution of dibenzothiophenes within the TPA fraction.

#### TABLE 2 Ionisation potential of aromatic compounds

Compound	Ionization potential, I <sub>p</sub> [eV]
Benzene	9.26
Naphthalene	8.15
Phenanthrene	8.07
Anthracene	7.42
Pyrene	7.70
Chrysene	7.82
Benzanthracene	7.52
Dibenzothiophene	7.57

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References: [27]  $I_p$  for aromatics, [28]  $I_p$  for dibenzothiophene

#### 3.4 | Discussion

The established cut retention time for the beginning of the TPAF is on the one hand situated at the limit of separation capacity and selectivity of the used column as the finding of acenaphthylene in TPAF shows. On the other hand a part of the higher than C8 alkylated triaromatic compounds elute within the MDA fraction. This limitation of the method must be taken into consideration.

The quantitative results show clearly, that the MOAH fractions of cosmetic raw materials contain mostly alkylated mono- and diaromatic compounds, which are according to toxicological evaluation of the experts [8], most likely constitute a small risk, if any.

Furthermore the determined TPAF amounts of petrolatum samples make up only about 0.6% (petrolatum 1) and 0.2% (petrolatum 2) of the MOAH fractions. Since these compounds are considered to be of potential concern, and as the adverse effect of a substance is usually a function of concentration, it would be interesting to know the amounts of each single compounds present in TPAF. This question is not that easy to answer, only some estimation could be done using GCxGC-TOF data. About 2000 peaks with different intensity were identified by the ChromaTOF software within the TPA fraction of petrolatum 1 sample. Considering this number of peaks the mean concertation for a single compound would be about 50  $\mu$ g/kg. Most probably there will be compounds which exceed or are below this value by orders of magnitudes.

Another important question is, whether all of the isolated substances are of potential concern, because the observed high degree of alkylation of tri- and polyaromatic compounds would presumably result in an alteration of their biological activity.

#### **4 | CONCLUDING REMARKS**

The present work describes an approach for separation and quantitation of MOAH in fractions according to the

#### PARATION SCIENCE

number of the aromatic rings. This technique results in toxicity-relevant analytics of the MOAH, since the only aromatic compounds containing three and more aromatic rings are believed to be potentially of toxicological concern. The need for such method arises as a consequence of the EFSA statement [9] and conclusions of the experts in the field of the MOSH/MOAH research [8].

The presented separation was performed on cosmetic raw materials by donor-acceptor complex liquid chromatography followed by quantification of corresponding fractions of MDAF as well as TPAF compounds through LC-GC-FID. The quantification of TPAF indicates that the MOAH of the analyzed samples consists to > 99% of mono- and diaromatic compounds. Respective TPAF amounts of cosmetic raw material samples were at 110 and 50 mg/kg for petrolatum samples and below the limit of quantification of 10 mg/kg for the paraffinum liquidum samples.

The separation of the tri- and polyaromatic compounds from diaromatics containing heteroatoms (e.g. dibenzothiophenes) cannot be achieved due to their similar ionization potentials and presents a selectivity limitation of the used chromatographic column. This must be taken into account for analysis of samples containing heteroaromatics.

#### 5 | OUTLOOK

The need to enrich the fraction of triaromatic compounds is making the quantification of TPAF time consuming and laborious. The automation of the separation procedures and the development of an on-line LC-LC-GC-FID separation method would improve sensitivity and reduce time for analytical procedures.

#### **CONFLICT OF INTEREST**

The authors have declared no conflict of interest.

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