Research Article

Quantification of Mineral Oil Aromatic Hydrocarbons (MOAH) in Anhydrous Cosmetics Using 1H NMR

Sandra Weber,[1] Tamina Schmidt,[1] Paul Schumacher,[2] Thomas Kuballa [iD],[1] Gerd Mildau,[1] Stephan G. Walch,[1] Andrea Hartwig,[2] and Dirk W. Lachenmeier [iD] [1]

[1]*Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Strasse 3, Karlsruhe 76187, Germany*
[2]*Karlsruher Institut für Technologie (KIT), Institut für Angewandte Biowissenschaften, Lebensmittelchemie und Toxikologie, Karlsruhe 76131, Germany*

Correspondence should be addressed to Dirk W. Lachenmeier; lachenmeier@web.de

In cosmetic products, hydrocarbons from mineral oil origin are used as ingredients in a wide variety of consistency, from liquid oil to solid wax. Refined mineral oil hydrocarbons consist of MOSH (mineral oil saturated hydrocarbons) and a low proportion of MOAH (mineral oil aromatic hydrocarbons). MOSH and MOAH comprise a variety of chemically similar single substances with straight or branched chains. In the context of precautionary consumer protection, it is crucial to determine hydrocarbons from mineral oil origin of inferior quality quickly and efficiently. This publication presents a rapid method for quantifying MOAH by proton nuclear magnetic resonance spectroscopy (1H qNMR) in anhydrous cosmetics such as lipstick, lip gloss, and lip balm. A sample clean-up using solid-phase extraction (SPE) was developed for the complete removal of interfering aromatic substances to improve the robustness of the method for analysing compounded cosmetics. In preliminary trials using silica gel thin-layer chromatography, the retention behaviour of 21 common aromatic compounds was tested in eluents with different solvent strength including EtOAc, MeOH, cyclohexane, and dichloromethane. Based on these results, the SPE sample cleanup with silica gel and cyclohexane as an eluent was suggested as best suitable for the purpose. The SPE cleanup was successfully achieved for all tested potentially interfering aromatic cosmetic ingredients except for butylated hydroxytoluene. The recovery for lipophilic cosmetics is more than 80% based on naphthalene as calculation equivalent. Furthermore, a specific sample preparation for the examination of lipsticks was implemented. The SPE cleanup was validated, and the robustness of the method was tested on 57 samples from the retail trade. The 1H qNMR method is a good complement to the LC-GC-FID method, which is predominantly used for the determination of MOSH and MOAH. Chromatographic problems such as migration of MOSH into the MOAH fraction during LC-GC-FID can be avoided.

1. Introduction

The term "mineral oil hydrocarbons" summarizes a complex combination of numerous saturated and aromatic chemically similar hydrocarbons. Mineral oil raw materials are used in cosmetics in different consistencies, for example, as liquid oil or solid wax. They are classified according to their consistency into the groups listed in Table 1.

Hydrocarbons from mineral oil origin are often used in cosmetics due to their different positive properties, such as good skin compatibility, good cleaning performance, and high stability. These substances are subject to mandatory declaration requirements and are indicated in the ingredients list as mineral oil, paraffin, paraffinum liquidum, petrolatum, cera microcristallina (microcrystalline wax), ceresin, or ozokerite (Table 1). In addition, hydrocarbons from mineral oil origin are inexpensive and can be produced in consistent quality. These properties make them interesting for a wide range of cosmetic products such as skin care products, face and body cleanser, sun screens, and hair and lip care products [1–5].

Analytically, hydrocarbons are usually divided into two groups: MOSH (mineral oil saturated hydrocarbons) and MOAH (mineral oil aromatic hydrocarbons). MOSH consists of saturated aliphatic and cyclic hydrocarbons and MOAH of aromatic partially hydrogenated and highly alkylated compounds. In general, MOSH and MOAH are

TABLE 1: Classification of mineral oil raw materials according to their consistency.

Common INCI name of mineral oil raw materials	Description (considering EC CosIng database)
Mineral oil	Typically used as a general term for derived products from raw oil refinement (often petrolatum).
Paraffinum liquidum	"White mineral oil (petroleum), a highly refined petroleum mineral oil consisting of a complex combination of hydrocarbons." It mainly consists of saturated hydrocarbons (having carbon numbers in the range of C15–C50).
Hydrogenated mineral oil/ distilled petrolatum	The end product of the controlled hydrogenation of hydrocarbons from mineral oil origin.
Paraffin	"A solid mixture of hydrocarbons obtained from petroleum characterized by relatively large crystals."
Cera microcristallina/ microcrystalline wax	Hydrocarbon waxes and paraffin waxes of long, branched chain hydrocarbons. Predominantly saturated straight and branched chain hydrocarbons > C35.
Petrolatum	Complex combination of hydrocarbons. It mainly consists of saturated crystalline and liquid hydrocarbons (carbon number predominantly > C25).
Ozokerite	A complex combination of hydrocarbons. It mainly consists of saturated straight chain hydrocarbons (carbon numbers predominantly in the range of C20–C50).
Ceresin	"A complex combination of hydrocarbons produced by the purification of ozokerite."

determined as sum parameters using convention methods (e.g., by LC-GC-FID). Toxic polycyclic aromatic compounds may not be contained, specifically potentially carcinogenic 3–7 ring polycyclic aromatic hydrocarbons (PAH). They need to be removed by a comprehensive refining process to fulfil the prerequisites of the European cosmetics regulation EC/1223/09. Mineral oils used in cosmetics often also meet the purity requirements for medicinal products, i.e., they are in compliance with Pharmacopeia standards [6].

In terms of precautionary consumer health protection and in the light of a large variety of mineral oil containing cosmetics, there is a need for efficient methods for characterizing the profile of mineral oil in cosmetic products. The aim is to distinguish high-quality mineral oil hydrocarbons (pharmaceutical, cosmetic, or food-grade mineral oil) from less refined hydrocarbons (technical-grade mineral oil material) and to check the compliance with the European regulations. According to the current state of the art, different analytical methods are used, for example, online coupled high-performance liquid chromatography-gas chromatography-flame ionization detection (LC-GC-FID), comprehensive two-dimensional gas chromatography-mass spectroscopy (GCxGC-MS), and nuclear magnetic resonance spectroscopy (NMR). A recently published review

provides an overview of the current state of literature about the methods for detecting MOSH and MOAH in food, food contact materials, tissues, and cosmetics [1].

From the available methods, LC-GC-FID is currently the most widely used procedure for the analysis of mineral oil hydrocarbons. This method uses the excellent separation performance of liquid chromatography (HPLC) to separate the hydrocarbons into saturated and aromatic hydrocarbons, predominantly MOSH and MOAH. Each single fraction is then separated by online coupled gas chromatography and detected by flame ionization detection (GC-FID) according to the volatility and carbon amount of the substances. As a result of the complex composition of mineral oils, no separated peaks but so-called humps are obtained, which still provide a relatively characteristic profile for the different mineral oil compositions [2, 7]. For individual samples, GCxGC-MS after offline HPLC preseparation may be used to further characterize MOAH by ring number and degree of alkylation [3, 8].

The chromatographic methods described above separate mineral oil hydrocarbons by the different elution force of the compounds. The proton NMR spectroscopy separates a sample into different signal areas based on the individual atom's electronic environment. For the MOAH fraction, the spectral aromatic region between δ 9.2 and 6.5 ppm can be integrated (excluding the solvent signals) and for non-mineral oil compounds the spectral region between δ 6.5 and $-$ 3.0 ppm. A detailed description of the high-resolution ^{1}H quantitative NMR method (^{1}H qNMR) to evaluate MOSH and MOAH in pure mineral hydrocarbon-based cosmetics and cosmetic raw materials is given in Reference [3]. Furthermore, low-field NMR may offer a more economic approach for MOSH/MOAH screening [1].

In our experience, the quantitative ^{1}H NMR spectroscopy is a good complement to the LC-GC-FID method. It is useful as a simple screening tool to get information about the MOSH/MOAH distribution. By this evaluation, the MOSH/MOAH ratio can be estimated and migration of MOSH into the MOAH fraction as evident in the LC-GC method can be circumvented. The LC-GC-FID method elutes firstly MOSH and then MOAH. The typically large excess of MOSH in cosmetic products (>99.5% [3]) can lead to carryover of the MOSH fraction into the MOAH fraction. By prior estimation of the MOSH/MOAH ratio using NMR, the sample weight for LC-GC-FID can be adjusted accordingly or a depletion of MOSH can be carried out as sample preparation [1]. Additionally, the determination of mineral oil via NMR spectroscopy provides a retention behaviour-independent validation of the LC-GC-FID results and additional information on further minor components that are visible in the ^{1}H NMR chemical shift range (δ 6.5–3.0 ppm) is possible.

Nevertheless, the previously described NMR procedure [1, 3] was only suitable for pure mineral oil-based cosmetic products. However, compounded cosmetic products containing hydrocarbons from mineral oil origin are often composed of many ingredients including aromatic substances, e.g., preservatives such as parabens, UV-filters, perfumes, antioxidants such as BHT, or active compounds such as tocopherol. These aromatics must be

removed completely by a reliable cleanup to avoid false-positive MOAH results by ^{1}H NMR spectroscopy because all aromatic compounds are detected in the spectral region between δ 9.3 and 6.5 ppm. This publication is a follow-up of the publication by Lachenmeier et al. [3] which focused on the determination of MOSH/MOAH in mineral oil raw materials and mineral oil-based cosmetic products without other aromatic ingredients. Using the sample preparation presented in this article, the method can be expanded to anhydrous cosmetic products (e.g., lip products) containing other aromatic ingredients. Furthermore, a specific sample preparation for the examination of lipsticks was implemented. A homogeneity experiment of 12 lipsticks and 12 lip care sticks showed that choosing a specific sample preparation for solid lip products is imperative. The procedure was validated for lip cosmetics (liquid, creamy, and solid), and the robustness of the method was tested on 57 samples from the retail trade. The entire process from sample preparation to the evaluation of the NMR data was validated.

2. Materials and Methods

2.1. Chemicals and Materials. Cyclohexane SupraSolv, TLC silica gel 60 F254 plates, and silica gel (40, 0.063–0.200 mm) were purchased from Merck (Darmstadt, Germany). Acetone-d$_6$ from Eurisotop D009H (Gif sur Yvette Cedex, France). Syringe filters with PET membranes (Chromafil Xtra PET-20/25 0.2 μm) and syringe filters with GF/PET membrane (Chromafil Xtra GF/PET-20/25 0.2 μm) were obtained from Macherey Nagel (Düren, Germany).

2.2. Thin-Layer Chromatography (TLC). TLC was used to determine the appropriate eluent for the solid-phase extraction (SPE) cleanup on the silica 60 stationary phase. As a test system, 21 common aromatic compounds were used (Table 2), and their elution behaviour compared to naphthalene (as calculation equivalent for MOAH in NMR) was investigated in eluents with different solvent strength. As a matrix, a retail-available petroleum jelly was used to simulate a real product cleanup. The TLC plates were analysed under an UV lamp (Camag, Muttenz, Switzerland) at 254 nm.

2.3. NMR Method. All ^{1}H NMR measurements were performed using a Bruker Ascend 400 spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm SEI probe PA BBI 400S1 with Z-gradient coils and a Bruker automatic sample changer (Sample Xpress, Bruker Biospin). All spectra were acquired at 300.0 K. The spectra (for samples dissolved in 120 μL acetone-d$_6$/TMS + 480 μL cyclohexane sample solution) were acquired using the Bruker noesygppr1d pulse program with 32 scans, 2 prior dummy scans (DS), and receiver gain of 45 (RG). This pulse program is used to suppress the H$_2$O signal and has been further optimized for the cyclohexane parameters. The ^{1}H NMR method largely corresponded to the one described in Reference [3]. For investigations in cyclohexane, cyclohexane suppression was applied. ^{1}H quantitative NMR in this

TABLE 2: Test compounds for the TLC investigation.

#	Substance	Function
1	BHT	Antioxidant
2	α-Tocopherol	Skin care agent
3	Retinol	Skin care agent
4	Coenzyme Q$_{10}$	Skin care agent
5	Dibutyl phthalate	Banned plasticizer
6	Benzophenone-3	UV filter
7	3-(4-methylbenzylidene)camphor	UV filter
8	Ethylhexyldimethyl PABA	UV filter
9	Amylcinnamal	Perfuming
10	Benzyl alcohol	Perfuming, preservative
11	Methyl eugenol	Perfuming
12	Coumarin	Perfuming
13	Sorbic acid	Preservative
14	Phenyl salicylate	Perfuming
15	Benzophenone	UV filter
16	Methylparaben	Preservative
17	Butylparaben	Preservative
18	CI 11710	Colour
19	CI 26100	Colour
20	CI 47000	Colour
21	CI 61565	Colour

publication has been established using the ERETIC methodology (electronic reference to access in vivo concentrations) based on the PULCON principle (pulse length-based concentration determination) [9]. The ERETIC factor was established using a quantification reference (quantref) sample containing ethyl benzene (10.24 mg mL^{-1} in cyclohexane/acetone-d$_6$) and diethyl phthalate (8.86 mg mL^{-1} in cyclohexane/acetone-d$_6$). The quantref sample was prepared by dissolving 54 mg diethyl phthalate in 5 mL cyclohexane/acetone-d$_6$. The recovery had to be $100 \pm 5\%$.

2.4. Samples and Sample Preparation. For the robustness of the ^{1}H qNMR method, it is crucial that all aromatic compounds except mineral oil aromatic hydrocarbons are removed during sample preparation. The sample cleanup depends on the matrix, as shown in Table 3.

Sample preparation for pure hydrocarbons from mineral origin and hydrocarbon-containing cosmetics without further aromatic ingredients can be conducted as previously published [3]. In short, 50 mg of cosmetic product was dissolved in 1.5 mL CDCl$_3$ in an ultrasonic bath. To enhance the solubility of the sample, the ultrasonic bath was heated up to an elevated temperature (Table 3). When turbidity occurred, syringe filters with PET membrane or GF/PET membrane were used for membrane filtration depending on the degree of it. A given amount of sample solution (Table 3) was then taken for the NMR measurement.

Most cosmetic products contain, in addition to mineral oil aromatic hydrocarbons, other aromatic compounds, such as BHT, which would interfere with the ^{1}H qNMR measurement (Table 2). A matrix-dependent cleanup was developed to remove a large number of these components. The cleanup can be universally applied to anhydrous cosmetics (e.g., lip care products and lipsticks). For this, 50 mg of cosmetic product was dissolved in 1.5 mL cyclohexane in an

TABLE 3: qNMR-analysis of pure mineral oil cosmetics and lipophilic cosmetics with further aromatic ingredients.

	Pure mineral oil cosmetics [3]	Lipophilic cosmetics with further aromatic ingredients
Cleanup step (SPE)	No	Yes. For lipstick/solid lip products, an additional preparation step to ensure homogeneity has to be carried out in advance.
Solvent	$CDCl_3$/TMS[a]	Cyclohexane
Temperature	55°C (bp $CDCl_3$: 61°C)	75°C (bp cyclohexane: 81°C)
Sample solution for NMR	600 μL	480 μL diluted with 120 μL acetone-d_6/TMS[a]
Analytes	MOSH and MOAH	MOAH[b]

[a]0.1% tetramethylsilane. [b]MOSH signals are interfered by cyclohexane suppression.

ultrasonic bath. Subsequently, an SPE cleanup step was carried out. An SPE-glass column (3 mL capacity) was provided with a PTFE frit, 750 mg ± 10 mg silica gel-40 was added, and the SPE column was sealed with a second frit. The SPE column was conditioned with 1 mL of cyclohexane. Afterwards, 1.5 mL of the sample solution was applied to the SPE column and eluted with cyclohexane into a 5 mL glass volumetric flask. For the NMR measurement, 480 μL of sample solution was taken from the volumetric flask and diluted with 120 μL of acetone-d_6/TMS (Table 3).

As additional preparation step for lipstick, the following two steps have to be carried out to ensure homogeneity of the sample. A cross section of the lipstick is cut from the core of the product (2-3 mm from the edge), and a peanut-sized potion is scraped out (Figure 1(a). The obtained mass was filled with a spatula to homogeneity on a watch glass (Figure 1(b)).

2.5. Validation Concept. For the validation of lip cosmetics, 3 lip cosmetics with different consistency were chosen: an intensely colored lipstick (solid product), a lip balm (creamy product), and a colored lip gloss with glitter particles (liquid product). First, all cosmetic agents were analysed for their MOAH content by ^{1}H qNMR spectroscopy. For all 3 products, this was below the detection limit of 0.03 g MOAH/100 g sample. Therefore, all 3 products were suitable for spiking experiments according to the preparation specified in Table 4. The INCI list for each matrix is listed in Table 5.

A vaseline of known MOAH content was added to each matrix (additive weighing). The vaseline was stirred into the cosmetic product by melting the matrix (Table 4). Vaseline was added in nonequivalent proportions (9 points in the range of 0.00–1.19 g MOAH/100 g sample) [10] (the results are summarized in Tables 6–8). The validation series was prepared as described in the sample preparation (Section 2.4).

3. Results and Discussion

3.1. Initial TLC Experiments for Solvent Selection. The retention behaviour of 21 frequently used aromatic ingredients (Table 2) was tested by a TLC experiment. As a TLC system, silica gel and eluents with different solvent strength (ethyl acetate, methanol, tert-butyl methyl ether, cyclohexane, dichloromethane, water/methanol (25/75), tert-butyl ethyl ether/cyclohexane (20/80), and tetrahydrofurane/cyclohexane (20/80)) were investigated experimentally. All 24 compounds
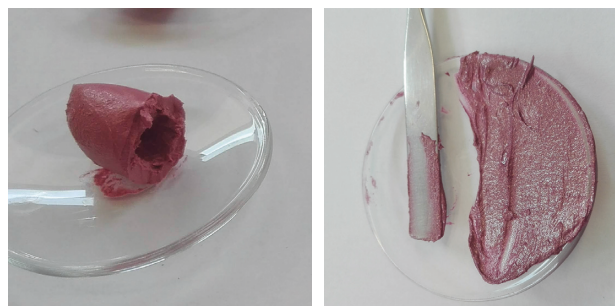

FIGURE 1: Additional preparation step for lipstick. (a) Removal from the lipstick; (b) filled with a spatula to homogeneity.

TABLE 4: Creation of the validation series samples.

Matrix	Spiking procedure
Lip balm	Adding and melting in a water bath at 60°C.
Lip gloss	Adding and melting in a water bath at 60°C.
Intensive colored lipstick	1. Weigh lipstick, melt, and cool 2. Weigh vaseline and adding to the lipstick 3. Melting together in a water bath at 70°C.

TABLE 5: INCI for the matrices used for validation.

Matrix	INCI
Lip balm	*Butyrospermum parkii* butter, paraffinum liquidum, lanolin, *Ricinus communis* seed oil, caprylic/capric triglyceride, *Simmondsia chinensis* seed oil, cera alba, *Persea gratissima* oil, octocrylene, cetyl alcohol, ozokerite, zinc stearate, tocopheryl acetate, tocopherol, *Helianthus annuus* seed oil, BHT
Lip gloss	Polyisobutene, hydrogenated polyisobutene, paraffin, cera alba, cera microcristallina, tocopheryl acetate, parfum, limonene, linalool, [±]mica, silica, tin oxide, calcium aluminium borosilicate, aluminium hydroxide, CI 15850, CI 75470, CI 77491, CI 77492, CI 77499, CI 77891
Intensive colored lipstick	Pentaerythrityl tetraisostearate, polybutene, octyldodecanol, bis-diglyceryl polyacyladipate-2, tridecyl trimellitate, cera microcristallina, polyethylene, trimethylsiloxyphenyl dimethicone, mica, *Argania spinosa* kernel oil, ethyl vanillin, sodium saccharin, pentaerythrityl tetra-di-t-butyl hydroxyhydrocinnamate, benzyl alcohol, aroma, CI 42090, CI 45410, CI 77492, CI 77891

retard slightly with polar eluents. There is no sufficient elution difference between polar compounds and MOAH. A desired low retention for MOAH and sufficiently good retention for

TABLE 6: Validation lip balm.

Validation points	Target value (MOAH) (g/100 g)	Weighted sample (mg)	Actual value (MOAH) (g/100 g)	Recovery (%)
Measurement series: 1; technician 1; NMR spectrometer 2				
P1	0.00	50.90	0.00	100.00
P2	0.08	49.70	0.07	88.71
P3	0.14	49.80	0.13	88.83
P4	0.22	50.60	0.20	89.64
P5	0.37	51.90	0.35	95.41
P6	0.51	49.40	0.49	94.57
P7	0.71	51.60	0.70	98.75
P8	0.90	51.00	0.86	95.90
P9	1.19	51.30	1.15	96.38
Measurement series: 2; technician 2; NMR spectrometer 2				
P1	0.00	50.90	0.00	100.00
P2	0.08	51.10	0.07	88.58
P3	0.14	50.00	0.12	86.34
P4	0.22	50.50	0.20	89.02
P5	0.37	49.90	0.33	89.47
P6	0.51	50.90	0.47	92.13
P7	0.71	51.10	0.67	94.48
P8	0.90	50.80	0.79	87.47
P9	1.19	51.40	1.15	96.51
Measurement series: 3; technician 1; NMR spectrometer 1				
P1	0.00	51.20	0.00	100.00
P2	0.08	50.90	0.04	50.31
P3	0.14	50.10	0.10	65.86
P4	0.22	52.30	0.18	79.93
P5	0.37	51.60	0.32	86.83
P6	0.51	51.00	0.48	93.54
P7	0.71	50.50	0.68	95.97
P8	0.90	51.90	0.91	100.62
P9	1.19	51.30	1.14	95.72

TABLE 7: Validation lipstick.

Validation points	Target value (MOAH) (g/100 g)	Weighted sample (mg)	Actual value (MOAH) (g/100 g)	Recovery (%)
Measurement series: 1; technician 1; NMR spectrometer 2				
P1	0.01	48.70	0.01	96.59
P2	0.05	50.50	0.05	102.21
P3	0.14	51.70	0.15	106.20
P4	0.20	50.90	0.18	94.97
P5	0.34	49.60	0.34	100.11
P6	0.51	50.00	0.48	95.22
P7	0.70	52.30	0.66	95.63
P8	0.90	50.40	0.84	93.38
P9	1.19	51.50	1.08	91.09
Measurement series: 2; technician 2; NMR spectrometer 1				
P1	0.01	51.20	0.01	72.89
P2	0.05	50.30	0.02	42.31
P3	0.14	51.00	0.07	53.03
P4	0.20	50.50	0.14	69.88
P5	0.34	52.50	0.30	86.73
P6	0.51	50.20	0.43	84.59
P7	0.70	51.10	0.59	84.70
P8	0.90	51.20	0.72	80.21
P9	1.19	50.80	0.95	80.16
Measurement series: 3; technician 1; NMR spectrometer 1				
P1	0.01	50.20	0.01	126.14
P2	0.05	50.20	0.04	90.86
P3	0.14	51.70	0.11	75.07
P4	0.20	51.30	0.14	74.08
P5	0.34	48.60	0.29	87.17
P6	0.51	50.70	0.48	95.50
P7	0.70	51.10	0.68	97.58
P8	0.90	52.30	0.88	97.78
P9	1.19	53.20	1.07	90.17

the remaining compounds is achieved for the eluents cyclohexane, dichloromethane, and n-hexane/cyclohexane. The result reflects the elution behaviour corresponding to the eluotropic series of silica gel. For the SPE cleanup, cyclohexane was selected as the preferred eluent, because it is suitable for ^{1}H qNMR spectroscopy. In addition to the correct elution behaviour, the signal ranges of the eluent in the ^{1}H NMR spectroscopy were a decisive decision criterion, with cyclohexane (1.44 ppm), dichloromethane (5.30 ppm), and n-hexane/cyclohexane (1.28 and 0.88 ppm).

3.2. SPE Cleanup. Based on the results of thin-layer chromatography, the SPE cleanup for silica gel/cyclohexane was developed. To check the robustness of the SPE cleanup, the breakthrough rate was screened for some frequently used cosmetic ingredients (Table 9).

The breakthrough rates of these critical ingredients provide information about the robustness of the implemented SPE cleanup step. As shown in Table 9, most of the investigated polar aromatic compounds are well retained due to their interactions with the hydrophilic groups of the deployed silica gel and showed no interfering signals in the ^{1}H NMR spectra between δ 9.3–6.5 ppm after SPE cleanup. Only a few substances, such as benzyl benzoate and

tocopherol, elute partially with breakthrough rates of less than 1%. Furthermore, some perfuming agents such as eugenol, coumarin, benzyl alcohol, and several cinnamic acid derivates were tested as mixtures in different commercially available perfume oils. The breakthrough rates determined were generally below 8%. Usually, preservatives, antioxidants, and perfume oils are used in percentages below 2% in cosmetics while these experiments were performed with weights similar to cosmetics sample preparation (50 mg ± 5 mg) to achieve significant results. The actual signals in real cosmetic products would therefore be most probably below the detection limit for NMR measurements. Figures 2 and 3 show two application examples of a mineral oil containing an UV filter and a lip gloss sample.

This situation is different with butylated hydroxytoluene (BHT), a frequently used antioxidant with additional masking functions. It can reduce oxidant reactions and the intrinsic smell and taste (lipstick) of a product to improve its storage life and scent. Owing to the two tert-butyl groups strongly shielding the hydroxyl group, BHT elutes almost entirely resulting in more than 90% breaking through. The steric hindrance leads to visible signals for BHT even at the commonly low concentrations in cosmetic products. A comparison to the structural and functional similar butylated

TABLE 8: Validation lip gloss.

Validation points	Target value (MOAH) (g/100 g)	Weighted sample (mg)	Actual value (MOAH) (g/100 g)	Recovery (%)
Measurement series: 1; technician 1; NMR spectrometer 2				
P1	0.00	51.70	0.00	185.71
P2	0.04	53.70	0.02	50.60
P3	0.11	49.60	0.06	52.03
P4	0.19	50.30	0.14	74.14
P5	0.34	49.80	0.30	89.09
P6	0.49	51.30	0.41	84.12
P7	0.69	52.60	0.60	87.44
P8	0.88	50.90	0.79	89.25
P9	1.19	53.70	1.03	86.91
Measurement series: 2; technician 1; NMR spectrometer 1				
P1	0.00	49.40	0.00	64.29
P2	0.04	52.20	0.02	44.80
P3	0.11	51.20	0.09	81.92
P4	0.19	50.00	0.15	79.02
P5	0.34	51.70	0.29	86.12
P6	0.49	49.60	0.47	96.10
P7	0.69	49.80	0.63	91.07
P8	0.88	51.20	0.78	88.33
P9	1.19	50.30	1.07	90.29
Measurement series: 3; technician 2; NMR spectrometer 2				
P1	0.00	51.40	0.00	314.29
P2	0.04	50.90	0.02	39.68
P3	0.11	51.20	0.07	64.49
P4	0.19	51.90	0.15	74.91
P5	0.34	51.20	0.26	77.18
P6	0.49	50.30	0.40	81.00
P7	0.69	50.50	0.55	79.79
P8	0.88	51.00	0.68	77.24
P9	1.19	51.60	1.00	83.97

TABLE 9: Breakthrough rates of aromatic ingredients frequently used in cosmetics with signals in the chemical shift region integrated for MOAH determination (δ 9.3–6.5 ppm) in ^{1}H NMR spectra.

INCI	Function in cosmetics	Breakthrough rate (%)[a]
Benzyl benzoate	Antimicrobial, perfuming, solvent	0.2
BHA	Antioxidant, masking	0.0
BHT	Antioxidant, masking	90
Tocopherol	Antioxidant, masking, skin conditioning	0.5[b]
Phenoxyethanol	Preservative	0.0
Methylparaben	Preservative	—[c]
Ethylparaben	Preservative	—[c]
Propylparaben	Preservative	—[c]
Benzophenone-3	UV filter/absorber	0.0
Octocrylene	UV filter/absorber	0.0
Ethylhexyl methoxycinnamate	UV filter/absorber	0.0

[a]Determined by comparison of ^{1}H NMR results with and without SPE cleanup. [b]Determined in a mixture of 70% tocopherol and 30% *Helianthus annuus* (sunflower) seed oil. [c]Mainly insoluble in cyclohexane.
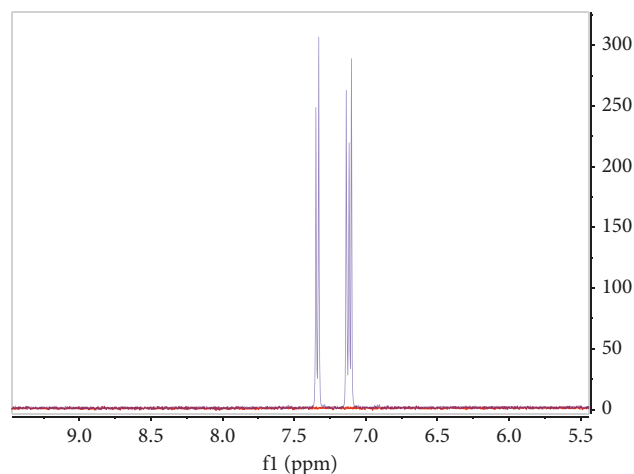

FIGURE 2: NMR spectra of a mineral oil sample containing 5% of 3-(4-methylbenzylidene)camphor. The blue ^{1}H NMR spectra show the signals of a direct measurement in cyclohexane/acetone-d$_6$, while the red spectra represent the result of the same sample following SPE cleanup. The aromatic light filter is not detectable following SPE.
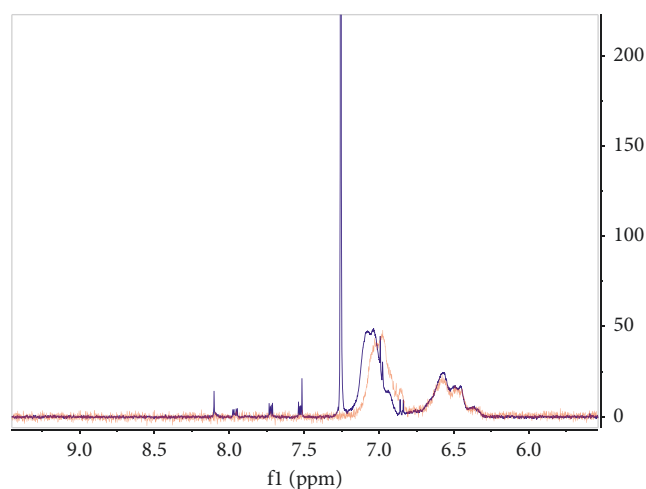

FIGURE 3: NRM spectra of a lip gloss sample. The blue ^{1}H NMR spectra show the signals of a direct measurement in cyclohexane/acetone-d$_6$, while the red spectra represent the result of the same sample following SPE cleanup. The interfering signals in the range 8.2–7.2 ppm are completely removed.

hydroxyanisole (BHA) shows that the steric hindrance is obviously only given with more than one tert-butyl group as the aromatic BHA signals were missing after the cleanup (Figure 4). BHT can be detected by the characteristic singlets at δ 6.88 ppm and δ 5.32–5.29 ppm (shifting signal) and therefore needs to be taken into account for MOAH detections in cyclohexane/acetone-d$_6$ to avoid false positive results.

3.3. Special Preparation Step for Lipstick and Solid Lip Products.

Lipsticks were found to be challenging to analyse due to homogeneity issues. These may arise during the production process of lipsticks. The shape of lipsticks is
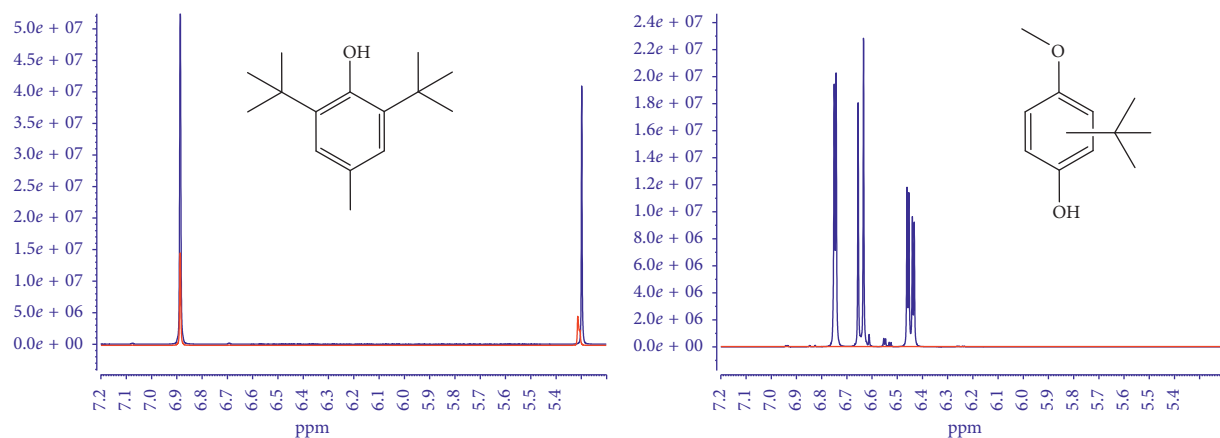
FIGURE 4: Comparison of BHT (a) and BHA (b). The blue ^{1}H NMR spectra show the signals of a direct measurement in cyclohexane/acetone-d_6, while the red spectra represent the result of the SPE cleanup. While BHA is completely removed by SPE, a considerable amount of BHT remains.

achieved by pouring the hot mass into special silicone or metal casting molds. When the lipstick cools down in the casting mold, different temperature zones are created, which can lead to inhomogeneity with respect to the composition of the pencil as a result of the flow behaviour. The process of shaping itself can also contribute to the inhomogeneity of the surface. The potential inhomogeneity within a lipstick is irrelevant to the user of the product, but may be relevant to the comparability of analytical results. In an experiment, 12 lipsticks and 12 lip care sticks were examined with regard to the homogeneity of MOSH and MOAH. For this purpose, for each lip product, at 3 different points of the lipstick, a sample was taken (from the surface of the lipstick to the core). Figure 5 shows the results of the 36 samples from 12 lipsticks. It becomes apparent that the results regarding MOSH led to a highly significant inhomogeneity of the results, whereas the MOAH values did not lead to inhomogeneity. In the process of making a lipstick, the ingredients are melted and poured into metal or silicone molds. The sticks are then cooled to freezing. The solidification occurring during this process does not lead to a perfect homogeneous distribution of oils and waxes within the lipstick. During the cooling process, there is a temperature gradient from the core of the lipstick to the edge. The different temperature zones lead to surface phenomena at the edge zone of the lipstick. A uniform distribution of oils and waxes in the pencil is not to be expected. The MOSH value recorded by ^{1}H NMR spectroscopy encompasses all saturated hydrocarbon building blocks, including side chains. An inhomogeneity of the stick is therefore mainly visible in the MOSH value. The homogeneity experiment was repeated using the additional sample preparation step for lipstick (Section 2.4).

The results are shown for 3 lipsticks that in the first experiment exhibited results strongly deviating from each other. It turns out that the same lipstick leads to homogeneous results with suitable sample preparation (Figure 6). To achieve comparable results, the sample preparation of lipstick or solid lip care products therefore requires the additional step as described to ensure comparable results.

3.4. Validation Results and Applicability in Routine Analysis.

Three experimental runs were carried out, each at nine concentration levels with varying different parameters (first, matrix; second, NMR spectrometer; and third, sample preparation). For each particular run, an individual blank matrix was used for spiking: a liquid product (lip gloss), a creamy lip balm, and a solid product (lipstick). The validation series were measured on two different NMR spectrometers (400 MHz) to include the variation of the devices during validation (second robustness criteria). The 3rd robustness criterion takes into account the sample cleanup (as described in Section 2.4) by two different technicians. In order to calculate the performance characteristics (recovery, 0.95 prediction band, and critical limits), all obtained results were regressed on the spiked amounts by weighted least squares regression [10] (Figures 7–9).

A 95% prediction band of 0.02 to 0.05 g MOAH/100 g for a spiked MOAH content of 0.05 g/100 g and a 95% prediction band of 0.91 to 1.27 g MOAH/100 g for a spiked MOAH content of 1.19 g/100 g are shown in Figure 7. Recovery was sufficient above 0.2 g MOAH/100 g (Figure 8). The measurement uncertainty is sufficient below 20% also at concentration above 0.2 g MOAH/100 g (Figure 9).

3.5. Comparison with Reference UV/VIS Methods.

In order to determine the purity and quality of raw materials from mineral oil origin, photometric methods are still often applied, e.g., Ph. Eur. [6]. These methods are used for refined products to ensure a minimized content of polyaromatic compounds. In the present work, the Ph. Eur. method [6] was used as a comparison method. The method is based on UV/Vis measurements of DMSO extracts that need to fulfil certain requirements depending on the nature of a mineral oil raw material. Thus, e.g., vaseline can be checked for conformity to the established standards. The DMSO extracts are being compared to naphthalene solutions. In the case of white and yellow vaseline, the extracts' absorbance needs to be below a certain threshold predetermined by the absorbance maximum of different naphthalene concentrations in DMSO. Naphthalene therefore has also been chosen as calculation
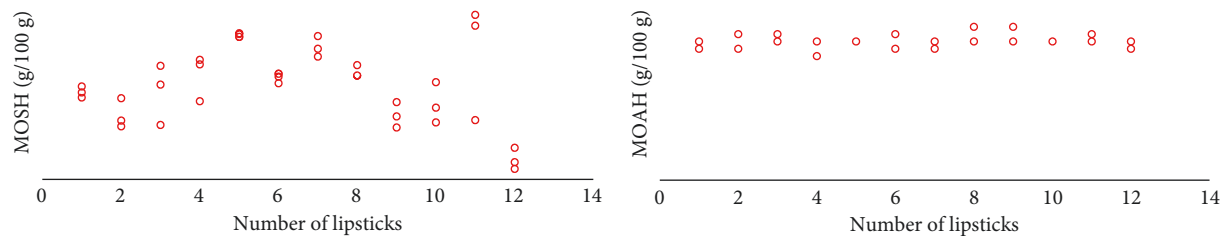
FIGURE 5: Homogeneity experiment of 36 samples from 12 lipsticks. MOSH (a) test statistic = 7.27, f(0.95) = 2.22, and the result is highly significant inhomogeneous; MOAH (b) test statistic = 1.09, f(0.95) = 2.22, and the result is homogenous.
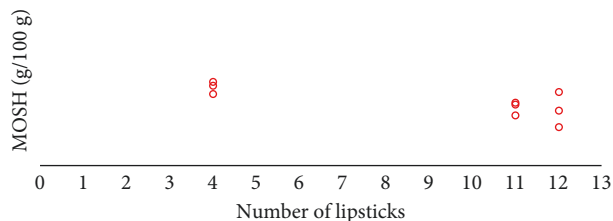

FIGURE 6: Homogeneity experiment of 3 strongly scattering lipsticks from the previous experiment (Figure 5) for MOSH. Test statistic = 3.55, f(0.95) = 5.14, result is homogenous.
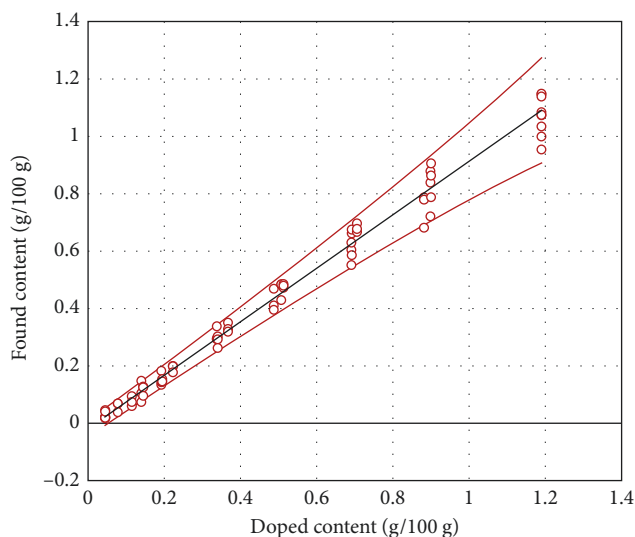

FIGURE 7: Linear weighted regressions for the matrices lip gloss, lip balm, and lipstick. Plot: detected content of MOAH in g/100 g as a function of spiked content of MOAH in g/100 g, 0.95 prediction bands.
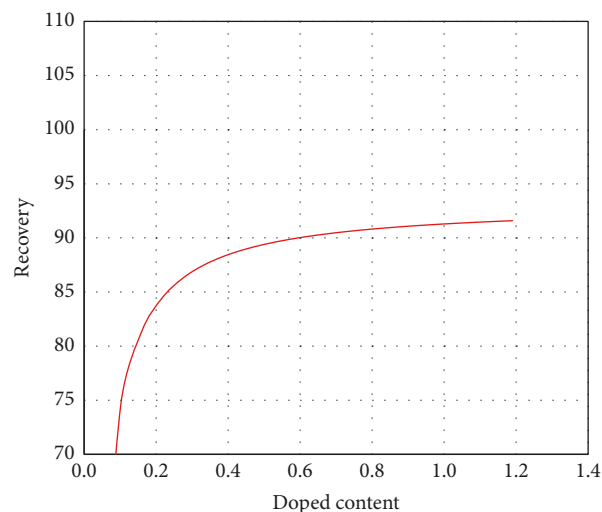

FIGURE 8: Linear weighted regressions for the matrices lip gloss, lip balm, and lipstick. Plot: MOAH recovery (%) as a function of spiked content of MOAH in g/100 g.
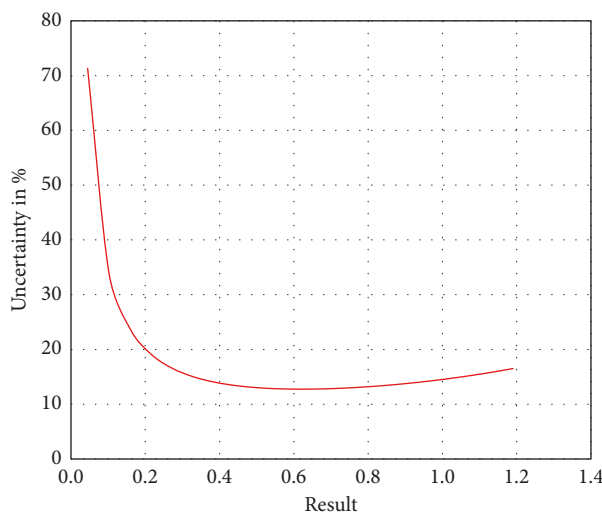

FIGURE 9: MOAH uncertainty (%) as a function of result of MOAH in lip products.

equivalent for MOAH determination of mineral oil raw materials by the previously described ^{1}H NMR method [3].

Figure 10 shows the comparison of the absorbance maxima in DMSO provided by the Ph. Eur. method and the MOAH content determined via ^{1}H qNMR in CDCl$_3$ for 13 different vaselines.

All samples appeared to be in conformity with the Ph. Eur. standard requirements although the MOAH contents varied between 0.06 and 1.10 g/100 g. This goes in line with the Ph. Eur. labelling of 9 of the examined vaseline (blue). The remaining four were of technical grade (red) usually containing more aromatic compounds than highly refined mineral oil raw materials.

There is no obvious correlation between the two differing methods as higher MOAH contents do not always correspond with high absorbance maxima and vice versa. Furthermore, technical-grade products do not stand out with higher MOAH contents as this is, e.g., also
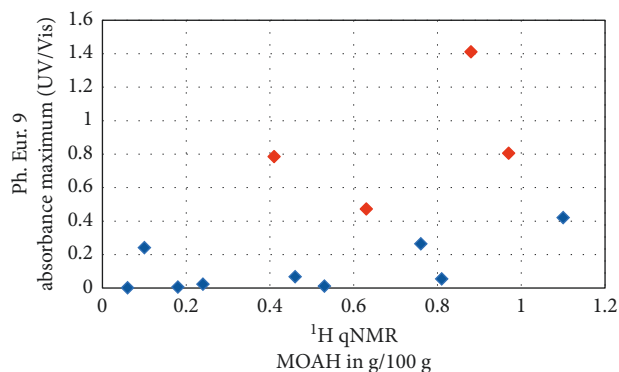
FIGURE 10: Comparison of the methods Ph. Eur. 9 and ^{1}H qNMR for 13 vaseline samples (blue: pharmaceutical quality; red: technical quality).
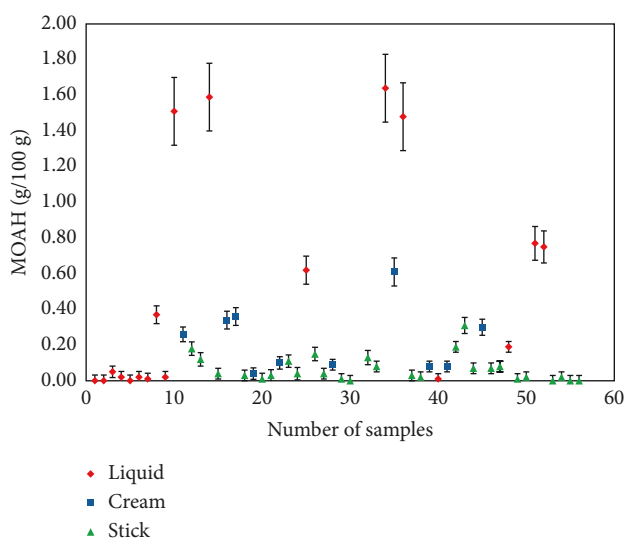

FIGURE 11: MOAH content in g/100 g sample for a selection of retail lip products. Classification according to the consistency (red–liquid; blue–creamy; green–solid).

influenced by the degree of alkylation of aromatic rings in NMR measurements.

3.6. Results of Lip Cosmetics. Different lip products from the retail trade were purchased as test products, and their MOAH content was analysed. As already presented in the validation concept (Section 2.4), the products were classified according to their consistency into liquid, creamy, and solid lip products. The results are shown in Figure 11: 19 liquid products, e.g., lip gloss (red), 10 products of creamy consistence, e.g., lip balm products (blue), and 27 solid products, e.g., lipstick or lip care stick (green).

Figure 11 also illustrates the wide range in the magnitude of MOAH content among lip cosmetics (from 0.0 g/100 g to 1.6 g/100 g). The majority of the products tested had an MOAH content of less than or equal to 0.1 g/100 g sample (Table 10). Higher MOAH values (MOAH in the range of 0.1 g/100 g–0.4 g/100 g) were determined for some liquid and creamy lip products. For some lip gloss products, very high

TABLE 10: Categorization of MOAH content among lip cosmetics.

MOAH (g/100 g)	Number of lip products	Percentage	Percent solid/creamy/liquid
≤0.1	36	63	58/14/28
0.1–0.4	13	23	54/31/15
≥0.4	8	14	0.0/13/88

MOAH levels were found (0.4 g/100 g–1.6 g/100 g). Such high MOAH contents were also obtained for mineral oil raw materials and vaseline of technical quality, as shown in the method comparison Ph. Eur./^{1}H qNMR (Figure 9). Such products should be further scrutinized if they are in compliance with the purity criteria of the European cosmetics regulation.

4. Conclusions

As demonstrated, most of the polar aromatic compounds such as aldehydes, carboxylic acids, and their esters, as well as alcohols differ significantly from MOAH in their chemical structure or polarity and hence are well retained during SPE due to their interactions with the hydrophilic groups of the deployed silica gel and can be removed by a matrix-based cleanup. Only for sterically very demanding compounds, a breakthrough rate is recorded. The comparative investigation of BHT and BHA shows that BHA retards well despite a sterically demanding tri-tert-butyl group. Only the strongly shielded BHT with two tri-tert-butyl groups shows poor elution behaviour and cannot be separated from MOAH by the SPE cleanup.

In conclusion, the proposed SPE cleanup enables the determination of MOAH for anhydrous cosmetic agents (e.g., lip care products) by ^{1}H qNMR spectroscopy as an important element amongst others such as LC-GC-FID to quantify MOAH-equivalents. In future, it will be essential to characterize MOAH fractions using those elements and hopefully gaining more information about the composition of those fractions by more specific methodologies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

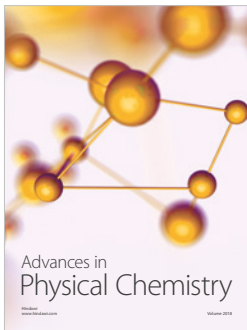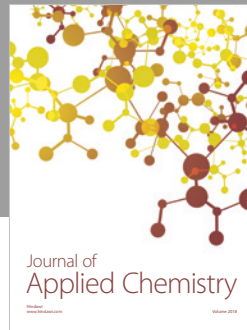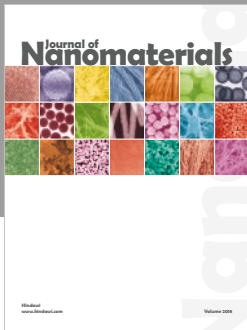The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

References

[1] S. Weber, K. Schrag, G. Mildau, T. Kuballa, S. G. Walch, and D. W. Lachenmeier, "Analytical methods for the

determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH)-a short review," *Analytical Chemistry Insights*, vol. 13, article 1177390118777757, 2018.

[2] M. Biedermann, C. Munoz, and K. Grob, "Update of on-line coupled liquid chromatography-gas chromatography for the analysis of mineral oil hydrocarbons in foods and cosmetics," *Journal of Chromatography A*, vol. 1521, pp. 140–149, 2017.

[3] D. W. Lachenmeier, G. Mildau, A. Rullmann et al., "Evaluation of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in pure mineral hydrocarbon-based cosmetics and cosmetic raw materials using ^{1}H NMR spectroscopy," *F1000Research*, vol. 6, p. 682, 2017.

[4] M. Niederer, T. Stebler, and K. Grob, "Mineral oil and synthetic hydrocarbons in cosmetic lip products," *International Journal of Cosmetic Science*, vol. 38, no. 2, pp. 194–200, 2015.

[5] N. Concin, G. Hofstetter, B. Plattner et al., "Evidence for cosmetics as a source of mineral oil contamination in women," *Journal of Women's Health*, vol. 20, no. 11, pp. 1713–1719, 2011.

[6] European Pharmacopoeia, "Vaselinum album," *European Pharmacopoeia*, vol. 8, pp. 5201-5202, 2014.

[7] M. Biedermann, K. Fiselier, and K. Grob, "Aromatic hydrocarbons of mineral oil origin in foods: method for determining the total concentration and first results," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 19, pp. 8711–8721, 2009.

[8] M. Biedermann and K. Grob, "Comprehensive two-dimensional GC after HPLC preseparation for the characterization of aromatic hydrocarbons of mineral oil origin in contaminated sunflower oil," *Journal of Separation Science*, vol. 32, no. 21, pp. 3726–3737, 2009.

[9] Y. B. Monakhova, M. Kohl-Himmelseher, T. Kuballa, and D. W. Lachenmeier, "Determination of the purity of pharmaceutical reference materials by ^{1}H NMR using the standardless PULCON methodology," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 100, pp. 381–386, 2014.

[10] P. Steliopoulos, E. Stickel, H. Haas, and S. Kranz, "Method validation approach on the basis of a quadratic regression model," *Analytica Chimica Acta*, vol. 572, no. 1, pp. 121–124, 2006.