



Review on Quantification of Mineral Oil Aromatic Hydrocarbon (MOAH) from Cosmetics by Analytical Method

Patel Visha Jitendrakumar*, Dr. Alisha Patel*

Department of Pharmaceutical Quality Assurance at ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi, Gujarat, India

*Corresponding author's E-mail: vishapatel119@gmail.com

Received: 10-03-2021; Revised: 15-05-2021; Accepted: 23-05-2021; Published on: 15-06-2021.

ABSTRACT

Mineral oils which consists mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH), which are largely applied in various consumer products like medicines cosmetics etc. MOAH which is potential public health hazard because it include carcinogenic polycyclic compounds. There is a rapid method for quantifying MOAH by proton nuclear magnetic resonance spectroscopy (1H qNMR) in anhydrous cosmetics. The 1H qNMR method is a good complement to the LC-GC-FID method. Another method is a simple and fast developed that uses columns packed with silver-modified silica in supercritical fluid chromatography with flame ionization and UV detection (SFC-FID/UV) for the determination of mineral oil saturated hydrocarbons (MOSH) and also mineral oil aromatic hydrocarbons (MOAH) in purified mineral oil samples. Another method which is based on gas chromatography with vacuum ultraviolet detection (GC-VUV) and relies on the spectral differences between the aliphatic and aromatic compounds in the sample. The detector provides a good selectivity for aromatics, direct quantification of the MOAH content is possible without the need for a laborious pre-separation of the mineral oil. GC-VUV method good sensitive for the analysis of all but gives highest purity mineral oils.

Keywords: Mineral oil, Mineral oil aromatic hydrocarbon, NMR, SFC, GC-VUV.

QUICK RESPONSE CODE →

DOI:

10.47583/ijpsrr.2021.v68i02.003



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2021.v68i02.003>

INTRODUCTION

M INERAL OIL

Definition for mineral oil is 'a liquid mixture of hydrocarbons obtained from petroleum by intensive treatment with sulphuric and oleum, or by hydrogenation, or a combination, and consisting predominantly of saturated C₁₅–C₅₀ hydrocarbons'. Mineral oil are insoluble in water and ethanol, soluble in benzene, ether, petroleum ether, carbon disulphide and volatile oils. Its density is 0.83–0.86 kg/L for the light mineral oil and 0.875–0.905 kg/L for the heavy mineral oil.¹

Medicinally, they are used as laxative or externally as protectant or lubricant; nonmedical uses are formulation aid in foods or in various consumer products as emollient. Other areas in which application for mineral oils like printing inks, cosmetic products, release agents (eg, for the bakery or confectionery industries), packaging materials for food (eg, wax paper, waxed cardboard), and various miscellaneous uses such as in technical products (eg, lubricating oil).² In the cosmetic field, mineral oils and waxes are used in various products which offer broad

viscosity options enabling viscosity regulation of a formulation, they are protective and lubricating properties avoiding dehydration of the skin, and they are stable and dermatologically well tolerated.³

Synonyms like heavy mineral oil, light mineral oil, liquid paraffin, liquid petrolatum, mineral oil mist, paraffin oil, paraffinum liquidum, petrolatum liquid, petroleum oil, white mineral oil and also white oil.¹

People are exposed to mineral oil mist in their workplace by breathing it in, skin contact, or eye contact. In the United States, the Occupational Safety and Health Administration has been set the legal limit for mineral oil mist exposure in the workplace as 5 mg/m³ over an 8-hour workday, the National Institute for Occupational Safety and Health has set an exposure limit of 5 mg/m³ over an 8-hour workday, and 10 mg/m³ short-term exposure has been rescinded according to the 2019 Guide to Occupational Exposure Values which compiled by the ACGIH. Levels of 2500 mg/m³ and higher are indicated as immediately dangerous to life and health of person.⁴

Types of Mineral Oil

- MOSH (mineral oil saturated hydrocarbons)
- MOAH (mineral oil aromatic hydrocarbons)
- MOSH consists of saturated aliphatic and cyclic hydrocarbons where MOAH consist of aromatic partially hydrogenated and highly alkylated compounds.

Highly refined mineral oils and microcrystalline waxes, that comply with the purity requirements for pharmaceuticals,



are used in cosmetic products for dermal application and the MOAH levels in these mineral oils are reduced through the corresponding technical refinement. MOSH are hardly absorbed by the skin, so the dermal application of cosmetic products containing mineral oils does not result in systemic exposure.⁵

Mineral oil hydrocarbons that were important contaminants for the human body, with possible routes of contamination including air inhalation, food intake, and dermal absorption.⁶

Mineral oils, waxes and petrolatum are currently used in cosmetic products which are complex combinations of hydrocarbons obtained through chemical refinement including distillation, extraction, crystallization and hydrogenation from petroleum fractions. The carbon chain length in these mixtures' ranges are C₁₈ to > C₉₀. Depending on the different product type, the mineral oil content in cosmetics may vary from low to nearly 100%.

Testing of a subset of MOAH containing samples of cosmetic products which indicated compliance with international purity standards (Ph. Eur.). Long-term dermal exposure studies with highly refined mineral oils and waxes are failed to produce any local or any systemic toxicity including tumor formation.⁷

Carcinogenicity of PAHs and MOAH Several PAHs with four to six aromatic rings, including methylated derivatives are carcinogenic in animal studies. Mono and polymethylations convert non-carcinogenic PAHs (e.g. pyrene, anthracene) into genotoxic carcinogens, depending on the methylation position. Conversely, a higher degree of alkylation abrogates carcinogenicity of certain PAHs if the alkylated derivatives become directed to non-toxifying metabolic pathways.⁸

Makeup product which includes pressed powders, mascara, eyeliner, and foundations, all can contain significant amounts of mineral oil, even up to 60%. Eye makeup remover sometimes contains very high levels of mineral oil, or it may be essentially 100% white mineral oil. This is very useful hydrocarbon material can be found in shaving creams and lotions at up to 50%. The scope of mineral oil in personal care products and its route in cosmetic formulation emulsion-type after shave lotions. Most frequently use of white mineral oil in bath products is in bath oils, which contains dose to 100% of mineral oil. Other topical applications of mineral oils including lip products, like lip balm and lipstick. Depending on the method of application, deodorants and antiperspirants may include white mineral oil, generally from 1 to 50% and in sun products, from the very high concentrations is found in tanning oils (nearly 100% mineral oil), to the more moderate amounts are used in sunscreens, sun blocks, and after sun products (about 1 to 40%).⁹

With respect to potential oral exposure, Cosmetics Europe recommends only particular mineral hydrocarbon fractions for which an Acceptable Daily Intake (ADI) has been identified, should be used in cosmetic lip products. In

2009 and 2013, the EFSA established an ADI of 12 mg/kg bw/day for high viscosity and for medium viscosity white mineral oils. In 2002, JECFA issued a new opinion that including the allocation of an ADI at 0-10 mg/kg bw for Class I medium and low viscosity oils in addition for already existing ADI of 0-20 mg/kg bw for high viscosity oils and for high melting point microcrystalline wax. For Class II medium and class III low viscosity oils, the existing ADI of 0-0.01 mg/kg bw that was expanded. In 2013, EFSA confirmed that the JECFA opinion including the ADIs for high-viscosity mineral oils and for Class I medium and low viscosity mineral oils, renamed medium viscosity mineral oils.

Two types of MOAH

- 3-7 ring polycyclic aromatic compounds (PAC; which also contain polycyclic aromatic hydrocarbons, PAHs) that are potentially carcinogenic
- highly alkylated aromatics (that are mostly 1-2 ring compounds) and which do not show carcinogenic effects in highly refined mineral oils

The 3-7 ring aromatics or PAC content is removed by the refining process, which includes the removal of toxic PACs, namely those polycyclic aromatic hydrocarbons (PAHs) which are closely monitored and regulated during their production.¹⁰

Different Analytical Method:

- According to the current state of the art, different analytical methods are used, like,
 - ❖ Online coupled high-performance Liquid Chromatography-Gas Chromatography-Flame Ionization Detection (LC-GC-FID)
 - ❖ Comprehensive two-dimensional Gas Chromatography Mass Spectroscopy (GCxGC-MS)
 - ❖ Nuclear Magnetic Resonance spectroscopy (NMR)
 - ❖ Super Critical Fluid Chromatography (SFC)
 - ❖ Gas Chromatography with Vacuum Ultraviolet detection (GC-VUV)

A. Nuclear Magnetic Resonance Spectroscopy (NMR):

The proton NMR spectroscopy which separates a sample into different signal areas based on the individual atom's electronic environment. For the fraction of MOAH, the spectral aromatic region between δ 9.2 and 6.5 ppm can be integrated and for non-mineral oil compounds the spectral region between δ 6.5 and – 3.0 ppm.

As per review, the quantitative ¹H NMR spectroscopy is a good complement to the LC-GC-FID method. The LC-GC-FID method which elutes firstly MOSH and then MOAH. The large excess of MOSH in cosmetic products (>99.5%) may lead to carryover of the MOSH fraction into the MOAH fraction.



A homogeneity experiment for 12 lipsticks and 12 lip care sticks showed that choosing a specific sample preparation for solid lip products is prescriptive. The procedure which was validated for lip cosmetics (liquid, creamy, and solid), and then robustness of the method was tested on 57 samples from the retail trade.

NMR Method:

All ^1H NMR measurements were performed using a Bruker Ascend 400 spectrometer (Bruker Biospin, Rheinstetten, Germany) which is equipped with a 5mm 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 ml acetone- d_6 /TMS + 480 ml cyclohexane sample solution) were using the Bruker noesygppr1d pulse program with 32 scans, 2 prior dummy scans (DS), and receiver gain of 45 (RG). This pulse program which is used to suppress the H₂O signal and then further optimized for the cyclohexane parameters. For investigations in cyclohexane, cyclohexane suppression was applied. ^1H quantitative NMR has been established using the ERETIC methodology (electronic reference to access in vivo concentrations) which is based on the PULCON principle (pulse length-based concentration determination). The ERETIC factor was established by using a quantification reference (quantref) sample containing ethyl benzene and diethyl phthalate. The quantref sample were prepared by dissolving 54 mg diethyl phthalate in 5ml cyclohexane/ acetone- d_6 . Their recovery had to be $100 \pm 5\%$.

The ERETIC factor which was determined by using the following equation:

$$\text{ERETIC} = I * \text{MW}/C * N$$

Where,

I is absolute integral

MW is molecular weight

N is numbers of protons generating the selected signal

C is concentration.

Sample Preparation:

50 mg of cosmetic product was dissolved in 1.5ml CDCl_3 and put in ultrasonic bath. To enhance the solubility of the sample, the ultrasonic bath was heated up to higher temperature. When turbidity occurs, syringe filters with PET membrane or GF/PET membrane which were used for membrane filtration depending on the degree of it. Most cosmetic products contain, in addition to mineral oil aromatic hydrocarbons, other aromatic compounds, like BHT, which interfere with the ^1H QNMR measurement the cleanup can be universally applied to anhydrous cosmetics. For this, 50 mg of cosmetic product was dissolved in 1.5ml cyclohexane in an ultrasonic bath. Subsequently, an SPE cleanup step was carried out. An SPE-glass column (3ml capacity) was provided by PTFE frit, 750 mg \pm 10 mg silica

gel-40 was added. The SPE column was sealed with a second frit and the SPE column was conditioned with 1ml of cyclohexane. Afterwards, 1.5ml of the sample solution was applied to the SPE column and then eluted with cyclohexane into 5ml glass volumetric flask. For NMR measurement, 480 ml of sample solution was taken from volumetric flask and then diluted with 120 ml of acetone- d_6 /TMS.

Validation Concept:

For the validation, 3 lip cosmetics with different consistency were chosen: which are an intensely colored lipstick, a lip balm, and a colored lip gloss with glitter particles. Firstly, all cosmetics were analysed for their MOAH content by ^1H QNMR spectroscopy. For these all 3 products, this was below the detection limit of 0.03g MOAH/100g sample.

Results

The retention behavior of 21 mostly used aromatic ingredients were tested by a TLC experiment. There is not sufficient elution difference in polar compounds and MOAH.

Based on the results of thin-layer chromatography, the SPE cleanup for silica gel/cyclohexane was developed and Most of the investigated polar aromatic compounds are well retained due to their interactions with the hydrophilic groups of the deployed silica gel and that showed no interfering signals in the ^1H NMR spectra between δ 9.3-6.5 ppm after SPE cleanup.

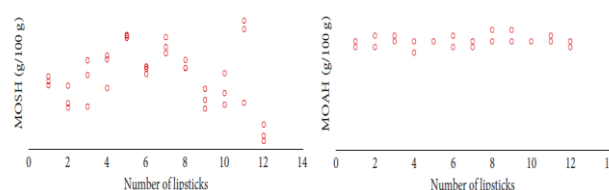


Figure 1: Homogeneity experiment of 36 samples from 12 lipsticks. MOSH (a) test statistic=7.27, $f(0.95) = 2.22$, and the result is highly significant inhomogeneous; MOAH (b) test statistic = 1.09, $f(0.95) = 2.22$, and the result is homogenous.

In this experiment, 12 lipsticks and 12 lip care sticks were examined by regard to the homogeneity of MOSH and MOAH. Figure 1 shows the results of the 36 samples from 12 lipsticks.

Then validation series were measured on two different NMR spectrometers (400 MHz) for include the variation of the devices during validation. Then 3rd robustness criterion takes into account for the sample cleanup by two different technicians. In order to calculate the performance characteristics (recovery, 0.95 prediction band, and critical limits), and all obtained results were regressed on the spiked amounts by weighted least squares regression.



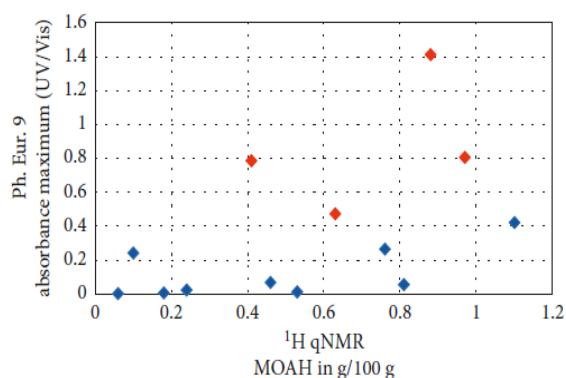


Figure 2: Comparison of the methods Ph. Eur.9 and ^1H qNMR for 13 vaseline samples

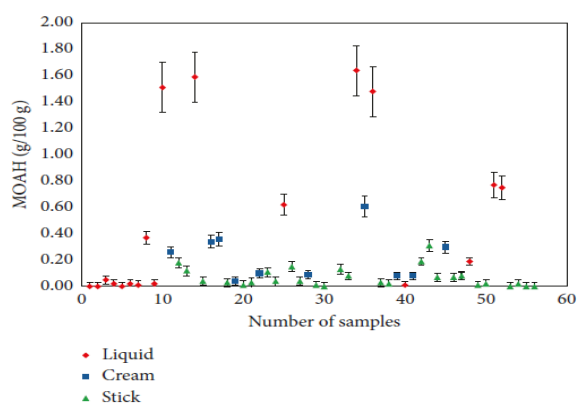


Figure 3: MOAH content in g/100g sample for a selection of retail lip products

Comparison with Reference UV/VIS Methods:

To determine the purity and quality of raw materials from mineral oil origin, photometric methods are still applied. This method is based on UV/Vis measurements of DMSO extracts which need to fulfil some requirements depending on the nature of a mineral oil raw material. Thus, vaseline has been checked for conformity to the established standards. The DMSO extracts are compared with naphthalene solutions. For 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 has been chosen as calculation equivalent for MOAH determination of mineral oil raw materials. Figure 2 shows the comparison of the absorbance maxima in DMSO provided by the Ph. Eur. Method and the MOAH content determined via ^1H qNMR in CDCl_3 for 13 different vaselines. All samples which appeared to be in conformity with the Ph. Eur. standard requirements although the MOAH contents which are varied between 0.06 and 1.10g/100g. Technical-grade products which do not stand out with higher MOAH contents as this is, e.g., also influenced by the degree of alkylation of aromatic rings in NMR measurements.

RESULTS OF LIP COSMETICS

Different lip products obtained from the retail trade as test products, and their MOAH content was analysed. The

products were classified by their consistency into liquid, creamy, and solid lip products. The wide range in the magnitude of MOAH content among lip cosmetics (from 0.0g/100g to 1.6g/100g). Figure 3 The majority of the products tested had an MOAH content of less than or equal to 0.1g/100g sample. Higher MOAH values (MOAH in the range of 0.1g/100g–0.4g/100g) were determined for some liquid and creamy lip products. For lip gloss products, very high MOAH levels were found (0.4g/100g–1.6g/100g). Such high MOAH contents were also obtained for mineral oil raw materials and vaseline of technical quality, which are shown in the method comparison Ph. Eur. ^1H qNMR. This product should be further scrutinized if they are in compliance with the purity criteria of the European cosmetics regulation.¹¹⁻¹³

B. Gas Chromatography with Vacuum Ultraviolet Detection (GC–VUV):

Biedermann et al. who developed a fully automated on-line normal phase liquid chromatography (NPLC) - gas chromatography - flame ionization detection (GC-FID) method by using a silica column for the quantitative analysis of low levels of MOAH in foods and cosmetics. In this method, the NPLC step was carried out to perform a pre-separation of mineral oil saturated hydrocarbons (MOSH) and MOAH. A method which used Solid Phase Extraction (SPE) for the pre-separation of MOSH and MOAH was developed by Moret et al. in 2011. For improving the MOSH/MOAH separation, the silica sorbent was replaced by silver-loaded silica making the determination of the cut point of MOSH and MOAH less critical. Still, for samples with MOAH contents less than 1% interferences of MOSH readily occur.

Nowadays a novel detector for GC has been introduced that's name is Vacuum Ultraviolet (VUV) detector. This detector which measures the absorbance of gas phase compounds in the far UV wavelength range from 120 to 430 nm. Essentially most of compound absorbs strongly in this wavelength ranges. At low wavelengths all compounds, aliphatic and aromatic can be detected, and at higher wavelengths only the aromatics and unsaturated compounds can absorb. Which is extremely attractive feature for MOSH/MOAH analysis.

AgNPLC fractionation

LC fractionation of the samples was carried out on a Waters Alliance 2695 LC instrument with a Waters 996 DAD detector (Waters, Etten-Leur, The Netherlands). This method was based on that described by Biedermann and Grob with some new modifications for increase the separation gap between the MOSH and MOAH fractions. Instead of a 250x2mm ID column packed with silica, use two serially connected 100mm x 4.6mm ID x 5 μm AgNO₃ loaded silica columns. The MOSH/MOAH separation that was performed by using a gradient starting with hexane held for 12 min.

SPE fractionation

Empty glass SPE cartridges which were packed with 0.5g of silver impregnated silica gel. Conditioning was performed by apply heating the cartridges at 120°C for 2 h, washing with 10 mL of DCM and with 4 mL of hexane. The volume of sample which is applied to the silver-silica SPE was 0.5 ml. Elution of the MOSH was carried out with 5 ml of hexane.

GC-FID

GC analysis of the MOSH and MOAH fractions which obtained from SPE or LC was carried out by using an Agilent 6890N C instrument system with a Focus-PAL auto sampler (GL Sciences, Eindhoven, The Netherlands). The sample (1 µL) was injected with splitless mode (2 min splitless time) at 350°C in a 4 mm ID liner packed with glass wool. The capillary column, 15m x 0.32mm x 0.1µm DB5-HT (Agilent), was used with constant flow of 2 mL/min using helium as carrier gas. The temperature program has been ran from 60°C (3 min) to 350°C (3 min) at 15°C/min. 350°C temperature was set for FID and the data collection rate was 200 Hz.

GC-VUV

A VGA-101 VUV detector (VUV Analytics, Cedar Park, TX, USA) was connected to an Agilent G1530 A GC system equipped with the Optic 3 injector and auto sampler is Focus-PAL (GL Sciences). The chromatographic conditions were identical to the GC-FID analyses. The temperature for transfer line and the flow cell of the VUV detector were set at 350°C. Nitrogen was used for make-up gas and pressure is 0.35 psi. The data collection rate was 100 Hz.

RESULTS AND DISCUSSION

VUV spectroscopic differences of MOSH and MOAH:

The VUV spectra of compounds which can differ significantly depending on the different functional groups which are present in the molecules. For the saturated compound, that representing the MOSH fraction of the mineral oil, and the spectrum monotonously decreases moving from the lowest wavelength of 125 nm to longer wavelengths. For these compounds there is no absorbance is observed at wavelengths above 180 nm. The aromatic compounds show a different behaviour. Molecules with one ring that shows an absorbance maximum around 185 nm.

For study of the spectral differences between real MOSH and MOAH fractions, eight different mineral oil samples were separated using SPE. Fig. 4. shows the overall averaged VUV absorbance spectra obtained for the MOSH and MOAH fractions from these 8 oils.

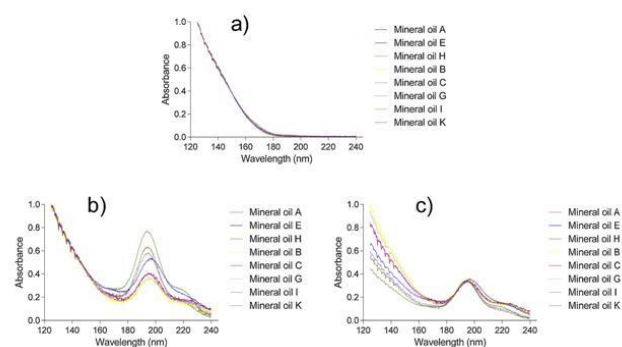


Figure 4: GC-VUV absorbance spectra of a) MOSH normalized at 125 nm, b) MOAH normalized at 125 nm and c) MOAH normalized at 195 nm from eight different mineral oils.

Direct determination of the MOAH content by GC-VUV:

The presence of absorbance bands are specific for aromatics in the VUV spectra in principle enables direct quantitative assessment of MOAH levels in mineral oils without the any MOSH/MOAH pre separation. For obtaining of the MOAH content from the GC-VUV data, for that two spectral filters were applied. The first filter covers the 190 to 240 nm region and detects only the MOAH species and the second filter covers the entire range of wavelengths recorded (125–240 nm). This second filter is applied to be able to correct the data for differences in total mineral oil responses due to sample discrimination in the GC injection and that was evaluated by comparing the response of different mineral oils at the same concentration. To be able to calculate the MOAH percentage directly from the GC-VUV chromatogram, for that MOSH amount first needs to be calculated.

$$\text{MOAH}_{125-240} = 1.38 \times \text{MOAH}_{190-240} \quad (1)$$

Because VUV spectroscopy that follows the additivity principles of the Beer-Lambert law, the MOSH response can be calculated from:

$$\text{MOSH}_{125-240} = \text{TR}_{125-240} - \text{MOAH}_{125-240} \quad (2)$$

In these equations $\text{MOSH}_{125-240}$, $\text{MOAH}_{125-240}$ and $\text{TR}_{125-240}$ are the peak areas in the chromatogram of the MOSH, MOAH and total mineral oil, respectively, all calculated from the 125-240 nm signal. $\text{MOAH}_{190-240}$ is the corresponding area that is calculated from the 190-240 nm signal. The above areas can be converted into mass percentages of MOSH and MOAH in the mineral oil using the Eq. (3) and (4):

$$\text{MOAH}\% = 100 \times \text{MOAH}_{125-240} \times \text{RRF}_{\text{MOAH}} / (\text{MOSH}_{125-240} \times \text{RRF}_{\text{MOSH}}) + (\text{MOAH}_{125-240} \times \text{RRF}_{\text{MOAH}}) \quad (3)$$

$$\text{MOSH}\% = 100 - \text{MOAH}\% \quad (4)$$

Where RRFs for the relative response factors for MOSH and MOAH relative to methane. In this work, an RRF_{MOSH} of 0.775 was used based on previous data. For MOAH, value an RRF_{MOAH} of 0.425 ± 0.055 was calculated. This value which was obtained as an average value of the RRF values for the eight different MOAH fractions described

previously. Each RRF was calculated following Eq. (5), where A_{MOSH}/A_{MOAH} is the ratio of the areas of the two fractions:

$$RRF_{MOAH} = [(M_{MOAH} \times A_{MOSH} (125-240 \text{ nm})) / (M_{MOSH} \times A_{MOAH} (125-240 \text{ nm}))] \times RRF_{MOSH} \quad (5)$$

Once these RRF are established, it can be used for the direct GC-VUV MOAH quantitation in unknown mineral oil samples without the necessity of any MOSH/MOAH pre-separation, i.e. the laborious LC or SPE step that can be avoided. This is reflected in the analysis time. For conventional methods a run takes 46 min, and the proposed GC-VUV method is only 25 min.

Analysis of different samples:

For determine the practical application limits of the newly proposed rapid GC-VUV method for MOAH analysis, a series of starting samples and intermediates of white oil production of different origins and different MOAH levels were analysed. The GC-VUV results which are not statistically significantly different from those obtained by the two standard methods, that was evaluated by applying an analysis of variance (ANOVA) to each sample. On this point, it is important to mention that the repeatability of the MOAH measurements which is much better for the GC-VUV method as compared to the standard methods. It is due to the absence of the complex manual sample pre separation step. The samples analyzed in the comparison that have MOAH contents from less than 0.13% to almost 50%. Their successful analysis clearly shows that applicability of the method to oils containing both high and low aromatics content and fractions from different stages in the purification process. Oil is free of aromatics, but still highly selective silver-loaded silica stationary phase was used, still there is some MOSH eluted in the time window where the MOAH would elute. This problem is related with the complexity of the sample. Fig. 5 shows the MOAH content obtained for 18 different mineral oil samples using GC-VUV.¹⁴

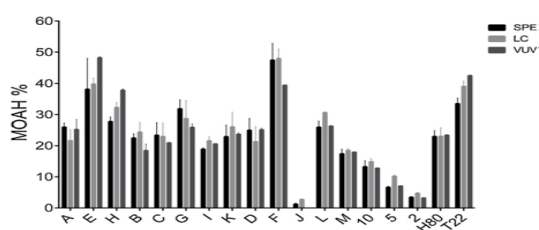


Figure 5: Comparison of the aromatic percentage using GC-VUV and the two standard methods.

C. Super Critical Fluid Chromatography (SFC):

Separation of complex hydrocarbon mixtures that is based on aromaticity (polarity) is difficult because it requires a very high selectivity for the aromatic ring system of the molecules. Gas chromatography (GC) that can provide this selectivity for very small molecules, and also for the very high molecular weight compounds encountered in typical MOSH/MOAH analyses is insufficient. In application of

supercritical fluid chromatography (SFC) which shows the advantages of LC and GC. In SFC, all LC stationary phases is use. Silica columns have been extensively applied for this, and silver-loaded silica columns are also used. If these columns would be capable of separating the heavier mineral oils into MOSH and MOAH by using pure carbon dioxide as the mobile phase. MOSH and MOAH levels could be determined directly by use of just one single chromatographic method, SFC-FID. Additionally, SFC also solve other issues which are related to the online LC-GC method, and that is incomplete elution of the high molecular weight compounds in the GC-FID quantification step.

SFC-FID/UV selectivity:

For the experiments to map the SFC selectivity, mixtures of aliphatic marker compounds (C11, C13, Cycy and Cho) and selected aromatic standards (1MN, BP, TBB, D12B and Ant) were prepared in hexane and its levels around 300 µg/ml per compound. Separation of the compounds was carried out on an Acquity UPC 2 SFC instrument with PDA detection (Waters, Etten-Leur, the Netherlands) coupled to an external FID. For FID detection, part of the CO₂ mobile phase flow was diverted to the FID. To do so, a low dead-volume T- piece (Valco Instrument Company Inc., Schenkon, Switzerland) was connected to the exit of the column just prior by UV detector. A 90 cm long, 50 µm internal diameter fused-silica capillary was used to transfer the mobile phase flow to the FID of an adjacent Agilent G1530 GC system (Agilent, Amstelveen, the Netherlands). The outlet of the transfer capillary which was positioned just below the flame jet of the FID. The temperatures of the GC and FID were set at 60 and 300°C, respectively. Hydrogen and air flows for the FID were found 36 and 400 mL/min, respectively.

The injection volume for SFC experiments was 1 µl. For the separation of the compounds a column set consisting of either a bare silica column, 150 × 4.6 mm Polaris 5 Si-A 5 µm, or two serially connected 100 × 4.6 mm silver-loaded silica Chromsep SS 5 µm columns (Agilent) was used. Pure CO₂ which was used as the mobile phase and flow rate of 1 ml/min. Temperature was 60°C and pressure was set at 110 bar. Data acquisition was carried out at 20 Hz for the FID and 25 Hz for the UV detector. UV chromatograms that were acquired and processed by using Empower 3 (Waters) either in the MaxPlot mode (190–400 nm) for deconvolution, at 254 nm for maximum selectivity towards aromatic species. Compounds that are eluted by using hexane as the mobile phase at 0.5 ml/min.

Optimization for the SFC-FID/UV method for mineral oil analysis:

Optimization of the SFC parameters, i.e. temperature, pressure and flow rate, was done by using a two-level factorial experimental design. The response for optimize was the MOSH/MOAH resolution.

Validation of the SFC-FID/UV method:

To evaluation of the performance of the proposed SFC-FID/UV methods, like linearity, repeatability, limit of detection (LOD) and limit of quantification (LOQ) were assessed following the Eurachem guideline for method validation. Linearity was performed at the concentration range of 2.5-100 mg/ml. Repeatability was determined at three concentration levels, 7.5, 15 and 30 mg/ml.

Analysis of real samples:

Solutions of mineral oils 1-7 at concentrations of 10 mg/ml in hexane were analyzed by using SFC-FID/UV. The injection volume was taken 1 μ l. CO₂ temperature was 60°C, pressure was 138 bar and flow rate set at 1 ml/min. The GC-FID analysis of the isolated MOSH and MOAH fractions was performed using an Agilent 6890 N instrument equipped with a Focus-PAL autosampler (GL Sciences, Eindhoven, the Netherlands). The sample (1 μ l) was injected with splitless mode (2 min splitless time) at 350°C using a liner packed with glass wool. The capillary column was a 15m \times 0.32mm \times 0.1 μ m DB5-HT column (Agilent) and it was operated at a constant flow of 2 ml/min using helium as carrier gas. The temperature program ran from 60°C (3 min) to 350°C (3 min) at 15°C/min. The FID temperature that was set at 350°C, hydrogen and air flows were 36 and 400 ml/min, respectively and then data collection rate was 200 Hz. The MOSH/MOAH composition of the mineral oils was calculated by using the total area and the areas of the respective fractions.

Result

SFC-FID/UV selectivity:

To understand the interaction of mineral oil constituents with the stationary phase under SFC conditions, for that mixtures of standard compounds were injected first and all compounds elute in less than 20 min from both columns. The alkanes C11 and C13 co-elute with the solvent peak (hexane) for the bare silica column, which indicate the absence of retention for these compounds. Unfortunately, the bare silica column and the silver-loaded silica both can not given a full separation of the compounds by aromaticity nature. The stronger retention for the aromatics on the silver loaded phase that is most likely due to the strong interaction of the aromatic π electrons with that vacant orbitals of silver ions. Fig. 6 shows the chromatograms obtained for the NPLC-UV/RID and the SFC-FID separation of the aliphatic and aromatic standards on the silver-loaded silica column.

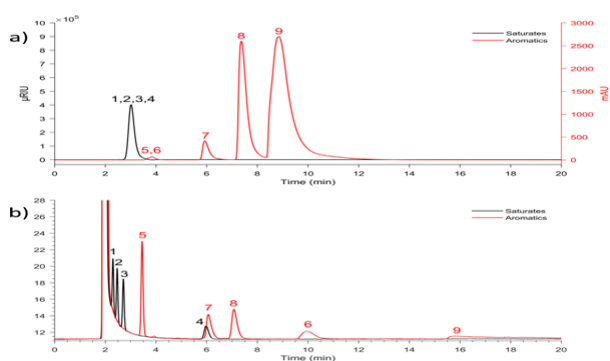


Figure 6: Chromatograms of the separation of the aliphatic (black) and aromatic (red) standard compounds on a silver-loaded silica column by (a) NPLC-UV/RID and (b) SFC-FID. Standards: C11 (1), C13 (2), Cycy (3), Cho (4), TBB (5), D12B (6), 1MN (7), BP (8) and Ant (9). Hexane was used as mobile phase in the NPLC-UV/RID separation. CO₂ at 60°C, 110 bar and 1 mL/min was used as mobile phase in the SFC-FID separation.

Retention and selectivity in packed column SFC have been studied. In these studies, there are three main effects are identified:

- (i) Solubility in the mobile phase
- (ii) Interaction with the stationary phase-mobile phase complex (the mobile phase, CO₂, is absorbed on the stationary phase forming a mono or multilayer)
- (iii) Displacement of the analytes from their stationary phase by the molecules of mobile phase

Figure 7 shows the overlay of the SFC-FID and SFC-UV chromatograms of mineral oil 1 at the optimal temperature and pressure conditions after normalization of the two signals.

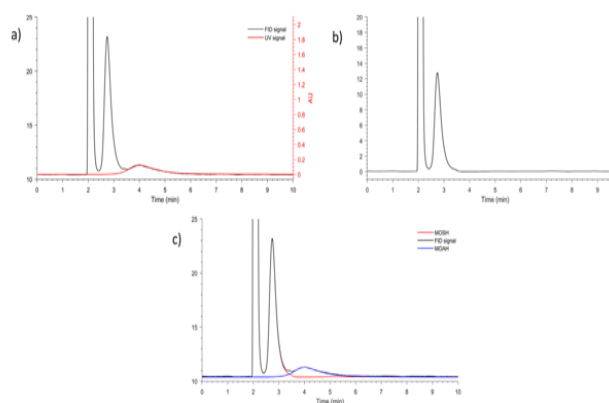


Figure 7: Deconvolution process of the MOSH and MOAH peaks in the FID chromatogram using the UV signal, (a) normalized SFC-FID and SFC-UV chromatograms, (b) SFC-FID chromatogram after subtraction of the normalized UV signal, in which MOAH peak is removed, and (c) deconvoluted SFC-FID chromatogram of mineral oil 1. MOSH/MOAH separation was carried out on a silver-loaded silica column and CO₂ at 60°C, 138 bar and 1 mL/min was used as mobile phase

SFC-FID/UV method optimization for mineral oil analysis:

For identify the optimal chromatographic conditions for the MOSH/MOAH separation, a two-level factorial experimental design optimization was performed by using temperature, pressure and flow as factors.

Normalization of the response was done by using the following equation:

$$FID_{new(t)} = [UV(t) (\Delta FID) / UV_{max}] + FID_{min}$$

Where $FID_{new(t)}$ is the MOAH FID signal at time t, $UV(t)$ represents the UV signal at time t, ΔFID is the difference between the FID response at the time of the maximum UV response and the baseline FID response along the chromatogram, UV_{max} is the maximum UV response along the UV chromatogram and FID_{min} is the minimum, i.e. baseline, FID response.

Analysis of real samples:

If viscosity is higher or the molecular weight of the sample, the broader the MOSH and MOAH humps. A broader MOSH hump results in more severe co-elution of the MOSH and MOAH humps complicating during deconvolution process. Statistical analysis by using ANOVA showed that the results are not statistically significantly different between the two methods. However, the results which is obtained by the newly proposed method show lower RSD values. Fig. 8 compares the MOAH composition of five different mineral oils that meet the viscosity/molecular weight requirements which obtained by using the new SFC-FID/UV method with data obtained by the classical off-line SPE-GC-FID method.¹⁵

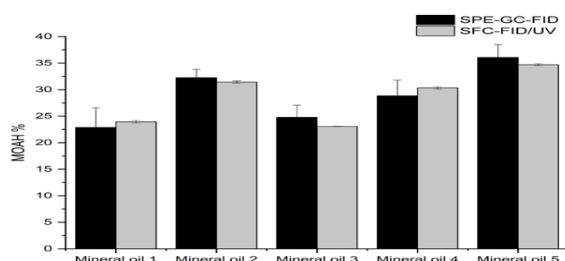


Figure 8: Comparison of the aromatic percentage of five different mineral oil samples using the SFC-FID/UV and the off-line SPE-GC-FID standard method.

CONCLUSION

In conclusion, the proposed SPE cleanup enables for the determination of MOAH for anhydrous cosmetic agents (e.g., lip care products) by using ¹H qNMR spectroscopy to quantify MOAH equivalents. The proton NMR spectroscopy that separates a sample into different signal areas which are 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. MOAH content in lip cosmetics from 0.0g/100g to 1.6g/100g was found.

A direct GC-VUV method which was developed for the determination of the MOAH content of purified mineral oil samples. Detection limits were found around 0.13%, making the method sufficiently sensitive for all highest purity samples. Selectivity provided by the VUV detector in which no sample pre-separation is needed, which take short time for the analysis significantly and eliminates difficult experimental setups and/or manual sample handling steps.

SFC-FID/UV method for the determination of the MOSH and MOAH content of purified mineral oil samples was developed that is fast and simple. The optimal CO₂ temperature, pressure and flow rate were determined by using a two-level factorial experimental design resulting in optimum settings of 60°C, 138 bar and 1 ml/min, respectively. Two-step conventional methods for mineral oil analysis, MOSH and MOAH content could be determined in a single run in this method. The proposed method showed it is linear ($R^2 > 0.9995$) in the range of concentrations selected (2.5-100 mg/ml) and has a better repeatability than the standard methods (< 5%). MOAH detection limits which was better than 0.36%, which makes this method sufficiently sensitive for analysis of all the highly purified mineral oils.

REFERENCES

1. A.V.Rawlings, K.J.Lombard, A review on the extensive skin benefits of mineral oil. *International journal of cosmetic science*, 2012; 34: 511-518. DOI: [10.1111/j.1468-2494.2012.00752.x](https://doi.org/10.1111/j.1468-2494.2012.00752.x) PMID: 22994201
2. Sandra Weber, karola Scharg, *Analytical Methods for the Determination of Mineral oil saturated Hydrocarbons (MOSH) and Mineral oil Aromatic Hydrocarbons (MOAH) - A short review*. *Analytical Chemistry Insights*, 2018; 13: 1-16. DOI: [10.1177/1177390118777757](https://doi.org/10.1177/1177390118777757) PMID: 29887729
3. B.Chuberre, E. Aravisiakala, *Mineral oils and waxes in cosmetics : an overview mainly based on the Current European regulation and the safety profile of these compounds*. *European Academy of Dermatology and Venereology*, 2019; 33(7): 5-14. DOI: [10.1111/jdv.15946](https://doi.org/10.1111/jdv.15946) PMID: 31588613
4. *Mineral oils, untreated or mildly treated*. IARC monographs –100F, 179-196.
5. *Highly refined mineral oils in cosmetics: Health risks are not to be expected according to current knowledge*. *Bundesinstitut fur Risikobewertung*, 2018; 1-27. DOI: [10.17590/20180702-124741-0](https://doi.org/10.17590/20180702-124741-0)
6. Nicole Concin, Barbara Plattner, *Evidence for cosmetics as a source of Mineral oil contamination in women*. *Journal of women's Health*, 2011; 20. DOI: [10.1089/jwh.2011.2829](https://doi.org/10.1089/jwh.2011.2829) PMID: 21970597
7. David S. Morrison, Jurgen Schmidt, Ricardo Pauli, *The Scope of Mineral oil and its role in cosmetic*



- formulation. *Journal of Application Cosmetology*, 1996; 14: 111-118.
8. Ralph Pirow, Annegret Blume, Nicole Hellwig, Mineral oil in food, cosmetic products and in products regulated by other legislation. *critical review in toxicology*, 2019; 49(9): 742-789.
 9. T.petry, D.Bury, R.Fautz, Review of data on the dermal penetration of mineral oils and waxes used in cosmetic applications. *Toxicology letters*, 2017; 280: 70-78. DOI: [10.1016/j.toxlet.2017.07.899](https://doi.org/10.1016/j.toxlet.2017.07.899) PMID: 28789996
 10. "Mineral Hydrocarbons in Cosmetic lip care products", *cosmetic Europe Recommendation*, 2018.
 11. Sandra Weber, Tamina Schmidt, Paul Schumacher, Quantification of Mineral oil Aromatic Hydroarbons (MOAH) in Anhydrous Cosmetics using ¹HNMR. *Journal of chemistry*, 2019.
 12. Drik W. Lachenmeier, Gerd Mildau, Anke Rullmann, Evaluation of Mineral oil Saturated Hydrocarbon (MOSH) and Mineral oil Aromatic Hydrocarbon (MOAH) in pure Mineral Hydrocarbon based cosmetics and cosmetics raw materials using ¹HNMR spectroscopy. *F1000 Research*, 2017; 6:682: 1-19. DOI: [10.12688/f1000research.11534.2](https://doi.org/10.12688/f1000research.11534.2) PMID: 28721203
 13. Sandra Weber, Karola Schrag, Gerd Mildau, Analytical Method for the Determination of Mineral oil Saturated Hydrocarbons (MOSH) and Mineral oil Aromatic Hydrocarbon (MOAH) – A short review. *Analytical Chemistry Insights*, 2018; 13: 1-16. DOI: [10.1177/1177390118777757](https://doi.org/10.1177/1177390118777757) PMID: [29887729](https://pubmed.ncbi.nlm.nih.gov/29887729/)
 14. Alan Rodrigo, Garcia – cicourel, Hans Gerd Janssen, Direct Analysis of Aromatic hydrocarbons in purified mineral oils for foods and cosmetics applications using Gas Chromatography with Vacuum Ultraviolet detection. *Journal of Chromatography A*, 2019; 1590: 119-120.
 15. Alan Rodrigo, Garcia - Cicourel, Bas Van de velde, Supercritical Fluid Chromatography as a rapid single step method for the determination of mineral oil saturated and aromatic hydrocarbons in purified mineral oils for food and cosmetic applications. *Journal of Chromatography A*, 2020; 1614: 1-11.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: editor@globalresearchonline.net

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

