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GC-MS/SIM and HPLC method development for monitoring polydimethylsiloxane and its degradation products

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PREFACE

I would like to thank everyone who has been involved in this project and has made it possible for me to work on this topic. Thank you to the team representing the Finnish industry for the informative and interesting discussions, it has been an effortless collaboration. Thank you to both Chunlin Xu and Johan Bobacka for your support. A special thanks to Annika Smeds, Ekaterina Korotkova and Jarl Hemming for your laboratory expertise and help. At last, I would like to thank my supervisor, Patrik Eklund. Thank you for giving me the opportunity to be a part of this project, for your guidance and for giving me a free hand, and therefore, also your trust. Thank you all!

ABSTRACT

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Polydimethylsiloxane (PDMS) is an abundant and highly persistent polymer used in many applications. One of these applications is as an antifoaming agent in the kraft pulping process. This chemical pulping process generates tall oil as a by-product that can be used for producing biodiesel. PDMS has been detected as a contaminant in the biorefineries that is causing challenges in the processes. This work aimed to develop a GC-MS/SIM and HPLC method to detect and monitor the contaminants in different bio-oils. Furthermore, pyrolysis GC-MS was to be utilized for PDMS degradation studies, and an automated normal-phase flash chromatography was to be tested as a potential sample-cleanup procedure. Two GC-MS instruments equipped with different dimensioned columns were used for the detection of the PDMS degradation products hexamethylcyclotrisiloxane (D3), octamehtylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) present in bio-oils. One GC-MS instrument was equipped with an HP-1 column and the other with an HP-5MS column. The condition of the first-mentioned instrument was better maintained compared to the second one and, therefore, lower concentrations were detectable. Additionally, the more sensitive instrument was able to detect contaminants of D3–D5, which were found to originate from the silicone-based inlet septum. The contaminants made the validation of the method more difficult and were taken into consideration in the interpretation of the results. The method's linearity, accuracy and precision were determined by utilizing the HP-5MS instrument. The linearity was found to be good for all three cyclic compounds. The accuracy determination showed that the matrix of the bio-oils somehow affects the response in the detection of D3–D5. Precision was difficult to determine, as too few data points were collected. The HP-1 instrument was utilized for determining the lowest detectable concentration, however, as the contaminants affected the detection, it could only be determined that at least a concentration of 2 ppm D3–D5 in relation to the bio-oil was detectable. GC-MS/SIM analyses of different bio-oils showed that it could be possible to quantify the cyclic compounds directly from the bio-oils. With an RP-HPLC-ELSD, low (5

cSt), medium (50 cSt) and high (1000 cSt) molecular weight PDMS were analyzed. For 5 and 50 cSt PDMS, the separation of components within the molecular weight groups was possible, and each molecular weight group was separable from the others. When spiked in different bio-oils, the matrices interfered completely with the detection of 5 cSt PDMS and slightly with 50 cSt PDMS. The lowest detectable concentration of 1000 cSt PDMS in three different bio-oils, was 1% PDMS in relation to the bio-oil. For lower detectable concentrations, sample cleanup or fractionation should be performed. The normal-phase flash chromatography, equipped with an ELSD, was not suitable for the detection of PDMS in bio-oils. The different molecular weight groups were not separable and detectable when spiked in bio-oils. Automated reverse-phase flash chromatography or preparative HPLC should be tested as potential sample cleanup procedures.

Keywords: PDMS, D3, D4, D5, bio-oil, GC-MS/SIM, RP-HPLC

ABBREVIATIONS

ACN	Acetonitrile
CTO	Crude tall oil
BTO	Bleed and temperature optimized
D3	Hexamethylcyclotrisiloxane
D4	Octamehtylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
DCM	Dichloromethane
ELSD	Evaporative light scattering detector
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography-mass spectrometry
HMW	High molecular weight
HP-SEC	High-performance size exclusion chromatography
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MMW	Medium molecular weight
NMR	Nuclear magnetic resonance
PDMS	Polydimethylsiloxane
RP-HPLC	Reversed-phase high-performance liquid chromatography
SIM	Single ion monitoring
SPE	Solid phase extraction
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
T_m	Melting temperature

TABLE OF CONTENT

1 INTRODUCTION

Crude oil has for a long time been a crucial source of energy, organic chemicals and synthetic materials, all of which we are significantly dependent on. However, due to environmental concerns, decreasing availability and the rising price of crude oil, alternative solutions to the use of fossil-based feedstock are necessary. As a more sustainable alternative, to traditional oil refining, biorefineries utilize renewable materials for producing biochemicals, biogas and biodiesel.[1, 2] From an analytical point of view, these new processes pose new challenges for monitoring product quality and possible contaminations originating from the unconventional raw material. Compared to crude oil, the composition of the biomass varies more significantly, and the development of novel analytical procedures is needed to tackle these challenges.

Tall oil is a by-product from the kraft pulping process that can be used as raw material for biodiesel production. Large amounts of foam are generated during the process, and antifoaming agents are therefore added as prevention. Polysiloxanes are commonly used antifoaming agents and are especially efficient in harsh environments. The polymers consist of alternating units of Si–O, with organic substituents attached to every silicon atom. The most common polysiloxane is polydimethylsiloxane (PDMS), which is a highly stable and persistent polymer. Trace amount of PDMS has been detected in the biorefinery processes as contaminants, originating from the kraft pulping process. To understand the degree of the contaminations, the development of analytical procedures for the identification and monitoring of the contaminants is needed. There are few published articles on different analytical methods for analyzing PDMS, and even less on the detection and determination of PDMS in bio-oil matrices. In literature, the most reported techniques for determining the total silicon content are based on spectroscopic methods, whereas PDMS determination has mostly been conducted using chromatographic systems coupled with a mass detector or element-specific detectors.[3] The focus of this work has been on developing analytical procedures for detecting and determining PDMS and its degradation products present in different bio-oil matrices.

2 BACKGROUND

2.1 Biorefinery

Biorefineries are defined as producers of renewable products such as bioenergy, biofuels and biochemicals from biomass.[1] The raw material of the biorefineries originates from four sectors: agriculture, forestry, industry and aquaculture.[2] Wood-based biomass from the forestry sector is the main feedstock in the pulp and paper industry. Byproducts from these processes can be used for generating heat and power as well as other marketable products, such as tall oil. Tall oil has previously mostly been burned in the pulp and paper mills, however, it could instead be recovered and used in the production of biodiesel. Crude tall oil (CTO) is recovered from the kraft pulping process (sulfate process) and consists of 30%–60% of saponified fatty acids, 40%–60% of resin acids and 5%–10% of unsaponifiables.[4] CTO can be fractionated into tall oil heads, tall oil fatty acids, distilled tall oil (consisting of fatty acids and rosin), tall oil rosin and tall oil pitch.[5]

2.1.1 Kraft pulping process

Kraft pulping is the most common chemical pulping process and a major technology in the paper and pulp industry.[6–8] In an alkaline white liquor, containing NaOH and Na_2S, wood chips are cooked at elevated temperatures with the means of breaking the linkage between lignin and cellulose. Digester systems are used for the physical pulping, and by a pulp washer, the pulp and the spent cooking liquor are separated. By concentrating a combination of the spent cooking liquor and pulp wash water, a black liquor consisting of 65% solids is obtained. The black liquor is further concentrated and is thereafter left to settle. The topmost layer is called tall oil soap and is recovered to be converted into CTO by acidification with sulfuric acid.[5, 9]

2.1.2 Antifoaming agents in the kraft pulping process

In the process of cooking the wood chips in the white liquor, esters of fatty acids, resin acids and sterols hydrolyze as the wood delignifies, which generates surface-active molecules that produce excessive foam. Most of the foaming appears in the process of washing the spent white liquor from the fibers.[8, 10] The foam can be as a topcoat on the surface of the stock as well as bubbles within the stock. The former is easier to control and handle than the latter. The most significant problems caused by foam are the decrease in capacity and effectivity of boilers and tanks, as well as overflow, causing spilling onto floors. Foam can also affect the mechanical pulping and efficiency of the paper machine, causing foam flaws in the final product of the paper. To prevent this, antifoaming agents are added to the washing process.

Silicone-based antifoams are suitable for the process, considering their endurability in harsh environments such as high temperatures (80–90 ºC) and basic conditions (pH: 11–15.5). Most commonly, the silicone-based antifoams are made from the silicone oil polydimethylsiloxane (PDMS). Fibers from Scandinavian softwoods and birch contain high amounts of surface-active compounds, which require harsh environment in the washing process that only silicone antifoams endure. Silicone-based antifoams can act as defoamers that reduce the foam level once added to the process and as antifoaming agents that prevent the formation of foam. In traditional refineries, the addition of the silicone-based antifoams has shown to cause problems in the late-stage processes such as catalyst poisoning.[3, 11]

2.2 Polydimethylsiloxane

PDMS is a homopolymeric polysiloxane, which is characterized by the repeating unit of methyl groups binding to the siloxane backbone, [-Si(CH)$_3$O-], which is illustrated in Figure 1a.[12]

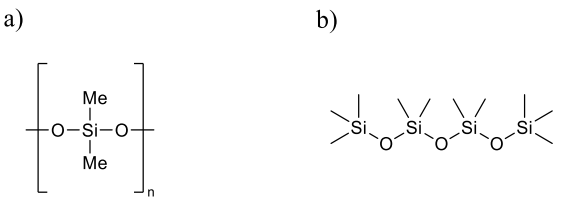


Figure 1 a) The structure of the backbone unit of PDMS and b) the structure of PDMS *trans* state.[12]

Silicon is located under carbon in the periodic table and was believed to show the same characteristic properties as analog compounds of carbon. However, the bond length of silicon and any given element is larger than that of carbon and the element (Table 1), and, therefore, the analog compounds of silicon and carbon behave differently.[8]

Table 1 Bond lengths of silicon and any given element compared to the bond length of carbon and the element.[8]

Element (X)	**Bond length (Å)**	
	Si-X	**C-X**
Si	2.34	1.88
C	1.88	1.54
H	1.47	1.07
O	1.63	1.42

Due to the long bonds, the steric interference of the skeletal backbone is reduced. The Si–O–Si bindings are tetrahedral and compared to the angles of other tetrahedral bindings (ca 110º), the bond angle is larger (ca 143º). This allows it to "flip" through the linear plane of the backbone as well as allowing torsional rotation without any extensive increase in energy.[12, 13] The most favorable conformation of PDMS is the *trans* state (Figure 1b) since it is lower in energy (ca −0.85 kcal mole^{-1}) than that of *cis* state, which is most likely due to the methyl groups having stronger van der Waal interactions in *trans* state.[12, 14]

The synthesis of PDMS can occur by monomer synthesis and polymerization. For monomer synthesis, the "Rochow Process" is mainly utilized, with elemental silicon as the starting point, and is illustrated below.[13, 15]

$$Si + 2\ RCl \rightarrow R_2SiCl_2$$

By hydrolysis of R_2SiCl_2, dihydroxy structures are obtained and condensation of these structures contributes to the repeating [-SiR_2O-] unit. The choice of catalyst indicates which form the polymer will take. The formation of linear high molecular weight polymers is favored by high temperatures and basic catalysts, whereas low molecular weight polymers and small cyclic compounds are favored by acidic catalysts. The most widely used polymerization of PDMS is the chain growth process where cyclic compounds undergo ring-opening polymerization. Due to reversible polymerization reactions occurring in PDMS, the number of main chain monomeric units ([-$Si(CH)_3O$-]) in the molecule varies. This leads to molecular weights of PDMS distributing in a Gaussian pattern at equilibrium.[13] Silicone oils are specified according to their viscosities, which also correlates to the molecular weight of the silicone oil. For example, 5 cSt PDMS has the approximate molecular weight of 800 whereas the approximate molecular weight is 28 000 for 1000 cSt PDMS.[15]

2.2.1 Chemical and physical properties of PDMS

The Si–O bonds of the polymer backbone contribute to several remarkable physical and chemical properties, such as flexibility, high thermal stability and low surface tension. As mentioned earlier, the barrier for torsional rotation about the backbone of PDMS is low, which provides the polymer with high dynamic flexibility and low values of melting temperature (T_m= -40ºC) and glass transition temperature (T_g= -125ºC). These properties are relative to each other. The more flexible the polymer is, the lower the values of T_m and T_g are. Silicon has a lower electronegativity than that of carbon, which makes the bonds with carbon and oxygen less covalent and more ionic. The more polar nature of the Si–O and Si–C bonds combined with the large atom size of silicon contributes to the flexibility and mobility of the

PDMS backbone.[16] PDMS consists of only saturated bonds, which makes the polymer inert and can, therefore, not be attacked by ions or radicals. The inertness is also due to the high bond energy of the Si–O linkage (106.0 kcal/mole).[16] Due to the high oxidation state of PDMS, reduction reactions can only occur in high temperatures, which adds to the stability of the polymer.[13]

2.2.2 Nomenclature

Since there are repeating structural units in polysiloxanes, abbreviations for the different units are used to make the naming of the polymer species easier.[13] The units which make up the polysiloxane can be abbreviated according to the functionalization of SiO (Figure 2). The difunctional main chain unit is abbreviated as "D", the monofunctional terminal unit as "M", the trifunctional units as "T" and the quadrifunctional unit as "Q".

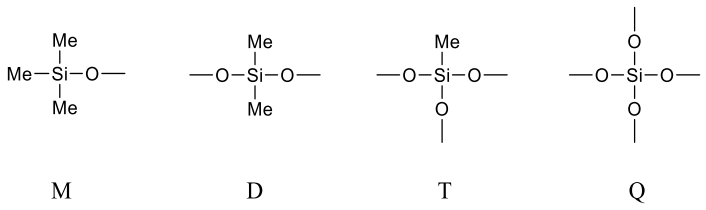


Figure 2 Structures of the abbreviated units of polysiloxanes.[13]

Following this system, the abbreviation of PDMS is "MD_nM". Most commonly, the abbreviations are unprimed, meaning the substituents are methyl groups as in PDMS. For polysiloxanes with other substituents, primed abbreviations are used (Figure 3).

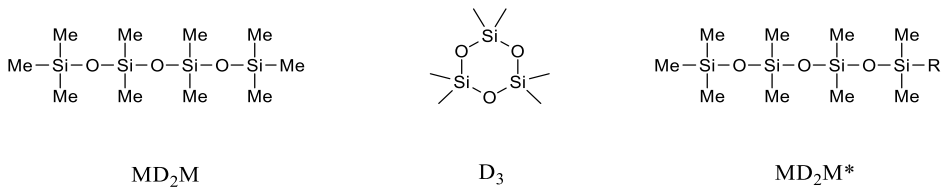
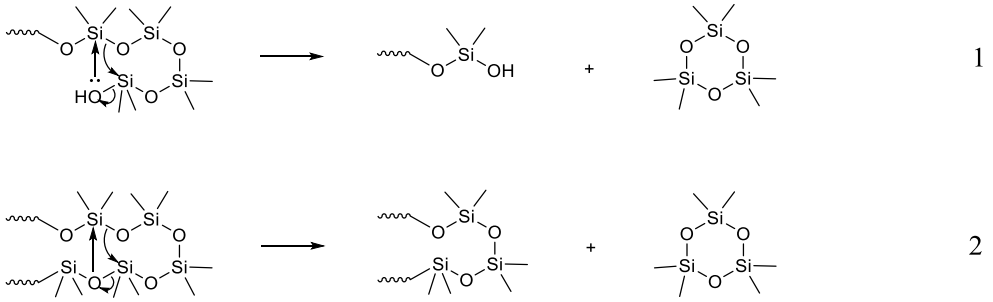


Figure 3 Structure and abbreviation of different PDMS species.[13]

2.2.3 Thermal degradation of PDMS

As mentioned before, PDMS has high thermal stability and, therefore, thermal degradation of the polymer occurs at temperatures higher than its ceiling temperature.[17] The depolymerization of PDMS occurs mostly through terminal and internal backbiting reactions.

This generates an intramolecular cyclic transition state, which requires an activation energy of ca 40 kcal mol^{-1}. Volatile degradation products are formed, with the cyclic trimer hexamethylcyclotrisiloxane (D3) being the most abundant, and with a systematically decreasing number of tetramers, pentamers and oligomers being formed. In Scheme 1, the mechanisms of the terminal (1) and intramolecular (2) backbiting reactions, which give D3, are illustrated.



Scheme 1 The mechanism of depolymerization of PDMS by the terminal (1) and intramolecular (2) backbiting reaction.[17]

These reactions can occur at a slow rate at as low temperatures as 110 ºC, however, in absence of catalysts the reactions are favorable at temperatures above 300 ºC. The depolymerization of PDMS is controlled by the molecular structure rather than by weakest bonds, since the depolymerization occurs by breaking the Si–O bond despite it being a thermally strong bond. The elimination of the cyclic oligomers requires that the empty d-orbital of silicon overlaps with the orbitals of oxygen and carbon in the cyclic transition state of PDMS. The cyclic transition state has been suggested as being the rate-determining step of the reaction. For PDMS terminated with trimethyl groups, the degradation occurs via the intramolecular mechanism. However, it is indicated that when in contact with water, PDMS might be terminated with hydroxyl groups, which accelerates the degradation by terminal backbiting reactions occurring together with the intramolecular reaction. [18, 19]

2.3 Analysis of PDMS

2.3.1 Spectroscopic methods

Fourier transform infrared spectroscopy (FTIR) can be used as a methodology for identification and structural analysis of PDMS.[20] The polymer can be detected by FTIR in very small quantities and information of the analyte being cyclic or linear PDMS is obtained. The relative quantity of the compound present in the sample can also be determined. The bands which can be observed in an IR spectrum of PDMS are listed in Table 2.[21, 22] The most

indicative band of PDMS is the one in the frequency region 1000-1100 cm^{-1}, which is a result of the Si–O–Si stretching. Long-chain siloxanes generate two extensive band frequencies at about 1090 and 1020 cm^{-1}. Cyclic siloxanes have lower stretching frequencies compared to linear ones. The frequency for cyclic siloxanes correlates with the size of the ring, e.g., the frequency for D3 is between 1010 and 1020 cm^{-1}, whereas the region is closer to 1080 cm^{-1} for D4 and 1100 cm^{-1} for D5.[22]

Table 2 Typical frequency regions for PDMS linkages detected by IR.[21]

Linkage	Frequency region (cm^{-1})
Si–C stretching	800
Si–O–Si stretching	**1000-1100**
Si–CH_3 symmetric (strong band)	1260
Si–CH_3 asymmetric (weak band)	1412
C–H stretching	2963

The data of the frequency regions shown in Table 2 were obtained from a Paragon 1000 (Perkin Elmer).[21] The samples analyzed were diluted with dichloromethane and tetrachloromethane. The nominal resolution of the spectrum was 4.0 cm^{-1} and four co-added scans were performed.

Raman spectroscopy is a less commonly used spectroscopic method for the characterization and identification of PDMS. The technique could be used for estimation of the average length of the PDMS chain, the ratio of M–Q PDMS units and the weight-% of PDMS in silicone emulsions.[23] Table 3 shows the frequency region of PDMS analyzed with Raman spectroscopy.

Table 3 Typical frequency region for PDMS linkages detected by Raman.[24]

Linkage	Frequency region (cm^{-1})
Si–O–Si stretching	**488**
Si–CH_3 symmetric rocking	687
Si–C symmetric stretching	708
Si–C asymmetric stretching	787
CH_3 asymmetric rocking	787
CH_3 symmetric rocking	862

CH_3 symmetric bending	1262
CH_3 asymmetric bending	1412
CH_3 symmetric stretching	2907
CH_3 asymmetric bending	2965

Nuclear magnetic resonance (NMR) is a compelling method for analyzing PDMS since the nuclei of the atoms in the polymer can be detected in a magnetic field.[20] With NMR, the total silicon content can be determined, the molecule identified and the ratio and distinction between cyclic and linear PDMS can be made.[20, 25] Additionally, the chain length as well as terminal groups or other modifications of PDMS can be detected.[25] With ^{1}H and ^{13}C NMR, the substituent of the silicone backbone can be determined.[8] In the chemical shift frequency area between 0 and 0.5 ppm, ^{1}H NMR has a high selectivity for various substituents of silicone molecules (Table 4).[25] The selectivity is mostly due to almost no interference of protons from other functional groups in the frequency area.

Table 4 Typical chemical shift of different PDMS species from ^{1}H NMR.[25]

PDMS species	Chemical Shift (ppm)
$Si–(CH_3)_4$	0
Open PDMS chain	0.08
D4	0.1
D3	0.18

^{29}Si NMR has resonances between the frequency area 70 and -300 ppm and PDMS species have been shown to generate chemical shifts around -20 ppm. For example, the chemical shift of D4 is -19.55 ± 0.05 ppm, and -21.9 ppm for 500 cSt PDMS. In cyclic PDMS compounds, all D components within the molecule are equivalent as one and do therefore only generate one signal for the compound. Due to the low abundance of ^{29}Si (4.7%), the NMR signal is weak and, therefore, a low amount of PDMS cannot be detected.[26, 27]

With Atomic absorption spectroscopy (AAS), the total silicon content in a sample can be determined. This method is suitable for quantifying silicone content in samples that have been treated with or contaminated by silicones. PDMS can be detected in concentrations of 1 ppm.[8, 28] Silicon trace analyses can also be achieved by inductively coupled plasma optical

emission spectrometry (ICP-OES). By replacing OES with a mass spectrometer, the detection limit of silicon can be significantly enhanced.[3]

2.3.2 Chromatographic methods

Gas chromatography (GC) is a suitable method for separating volatile and low molecular weight (LMW) compounds, hence, only oligomeric PDMS is suitable for GC analysis.[8] The separation of the analytes is based on different retention times and if coupled with a mass detector (GC-MS), the analytes can be identified based on their mass spectra. The smallest cyclic compound which can be detected by GC-MS is D3. In Table 5, the ionization pattern of D3–D7 can be seen.

Table 5 Information of cyclic oligomers detected by GC-MS.[29]

Cyclic oligomer	Molecular weight	M^+ -15 (*m/z*)	Abundant Ions (*m/z*)
D3	222	207	96, 133, 191
D4	296	281	73, 133, 191, 207, 249, 265
D5	370	355	73, 267, 268
D6	444	429	73, 147, 341
D7	518	503	73, 147, 281, 327, 415

A typical chromatogram of the cyclic oligomers diluted in *n*-hexane detected by GC-MS can be seen in Figure 4.[29] For quantitative analysis, single ion monitoring (SIM) scan mode should be utilized as the GC-MS method.[30]

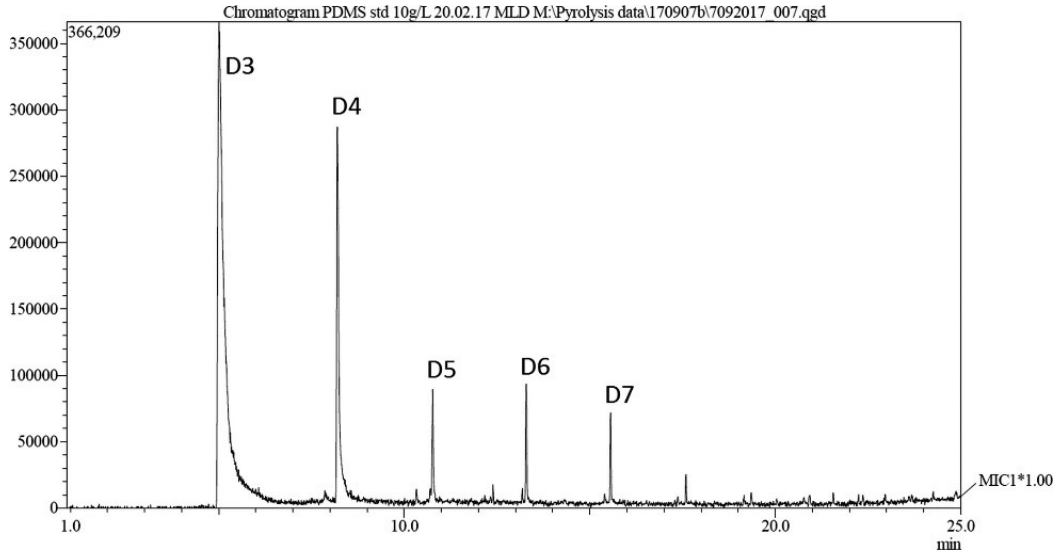


Figure 4 A typical chromatogram of D3–D7 oligomers detected by GC-MS.[29]

One must be aware of what material is used in the instrument system since e.g., the stationary phase in the column and sealants are usually made from PDMS and could interfere with the analytes. Horii and Kannan studied the contamination of D4, D5 and D6 arising from the silicone-based inlet septum used in GC.[31] Different septa and various inlet temperatures (100–250 ºC) were tested by injecting 1 µl of *n*-hexane with a standard mixture of D4–D6. With decreasing inlet temperatures, the signals of D4–D6 decreased. By reducing the inlet temperature from 250 ºC to 200 ºC, the contamination signals decreased by 79% and the standard siloxane signals decreased only by 10%. Five different septa were tested with a Restek bleed and temperature optimized (BTO) septum showing the lowest levels of contaminations. The different tested septa and contamination levels are listed in Table 6. The main source of contamination of the D compounds is from the inlet septum, which can be supported by the studies of Wang *et al*. Two columns with different packings were tested. One was a DB-5MS column with silicone-based packing and the other one was a DB-WAX column with non-silicone-based packing. The two columns gave similar signals of the contaminants when injecting blank samples.[32]

Table 6 Levels of D4-D6 contaminations (pg) from inlet septa in 1 µl *n*-hexane.[31]

Septum	D4	D5	D6
Restek BTO	0.8	0.3	0.2
Restek thermolite	1.6	0.6	0.3
Agilent advanced green	1.7	0.6	0.3
Agilent general purpose red	1.9	1.0	0.8
Agilent general purpose gray	4.1	6.0	2.1

Pyrolysis GC-MS (pyr-GC-MS) is a powerful method for degradation and structural studies of PDMS. Samples are pyrolyzed starting at lower temperatures which then elevate to temperatures as high as 1000 ºC. High molecular weight (HMW) and non-volatile PDMS can be analyzed with pyr-GC-MS, as the PDMS compound degrades into the LMW cyclic and linear compounds in the elevated temperatures.[21, 33, 34] Figure 5 shows a pyrogram of 200 cSt PDMS, where the numbered signals are the degradation products of the polymer. For example, number 1 is a signal from D3 and number 2 from the corresponding linear compound, number 6 is from D4 and number 16 is from D5.[34]

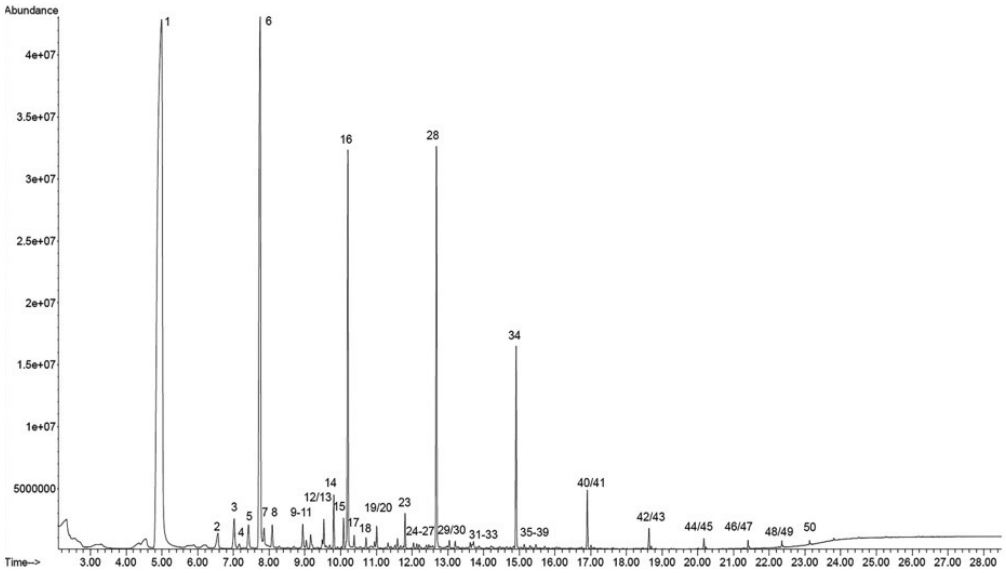


Figure 5 A pyrogram of 200 cSt PDMS.[34]

GC coupled with a flame ionization detector (GC-FID) is suitable for identification studies of PDMS, however, it is not as common and as sensitive as GC-MS/SIM.[3]

For liquid chromatographic studies of PDMS, reversed-phase high-performance liquid chromatography (RP-HPLC) and high-performance size exclusion chromatography (HP-SEC) have mostly been used. LMW compounds, which cannot be separated with GC, have usually been separated using HPLC.[3] Atomic detectors, such as ICP-OES and ICP-MS, coupled to RP-HPLC, have been employed when performing separation and structural identification studies of PDMS. The reported limit of detection (LOD) using ICP-OES is 20–500 µg L^{-1} and 0.1–4 µg L^{-1} when using ICP-MS. LC coupled with a mass detector with an atmospheric pressure chemical ionization (APCI) source is suitable for the characterization of PDMS. In Figure 6, chromatograms of different analyzed PDMS species can be observed. Fraction A corresponds to the cyclic compound D5 with the m/z value of 371 and a retention time of 6 min. Fraction B, C and D correspond to oligomeric PDMS with different terminal groups. The terminal group is dihydroxy for fraction B, hydroxyl for C and methyl for fraction D, with the latter being the most abundant.[21]

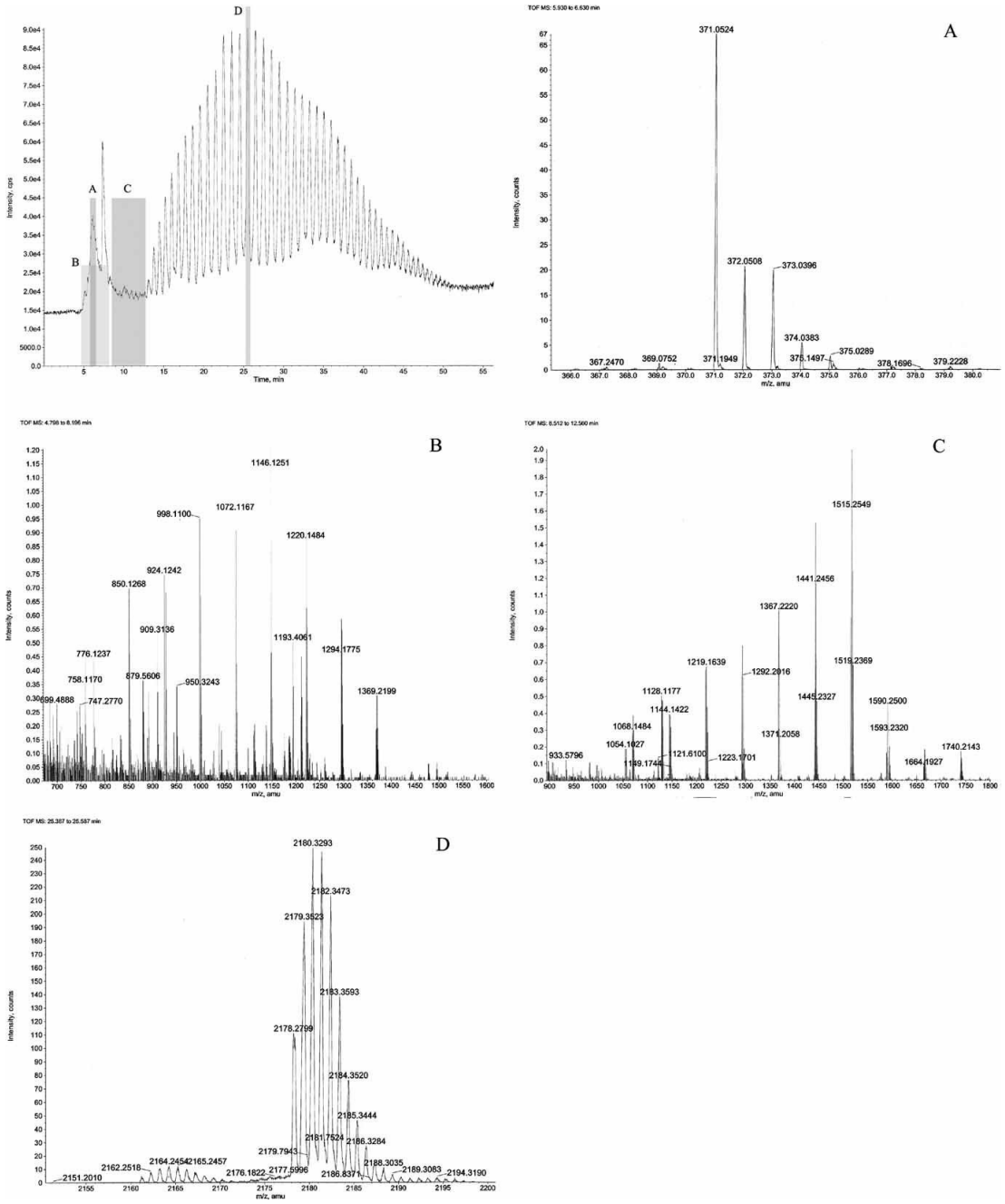


Figure 6 Chromatograms of different PDMS species analyzed with LC-MS(APCI). Fraction A corresponds to D5, fraction B corresponds to oligomeric PDMS with dihydroxy as terminal groups, fraction C corresponds to oligomeric PDMS with hydroxyl as terminal groups and fraction D corresponds to oligomeric PDMS with methyl as terminal groups.[21]

HP-SEC is the most common LC technique for analyzing HMW PDMS.[3] With an evaporative light scattering detector (ELSD) coupled to HP-SEC, PDMS species can be separated and identified according to the retention times of the compounds. It is a suitable method for the analysis of PDMS in different matrices. In Figure 7, chromatograms of different MW PDMS in chloroform, as well as extracts of medicinal tablets, are shown. The

top layer of the extraction contained the matrix of the tablet, whereas the lower layer contained traces of PDMS.[35]

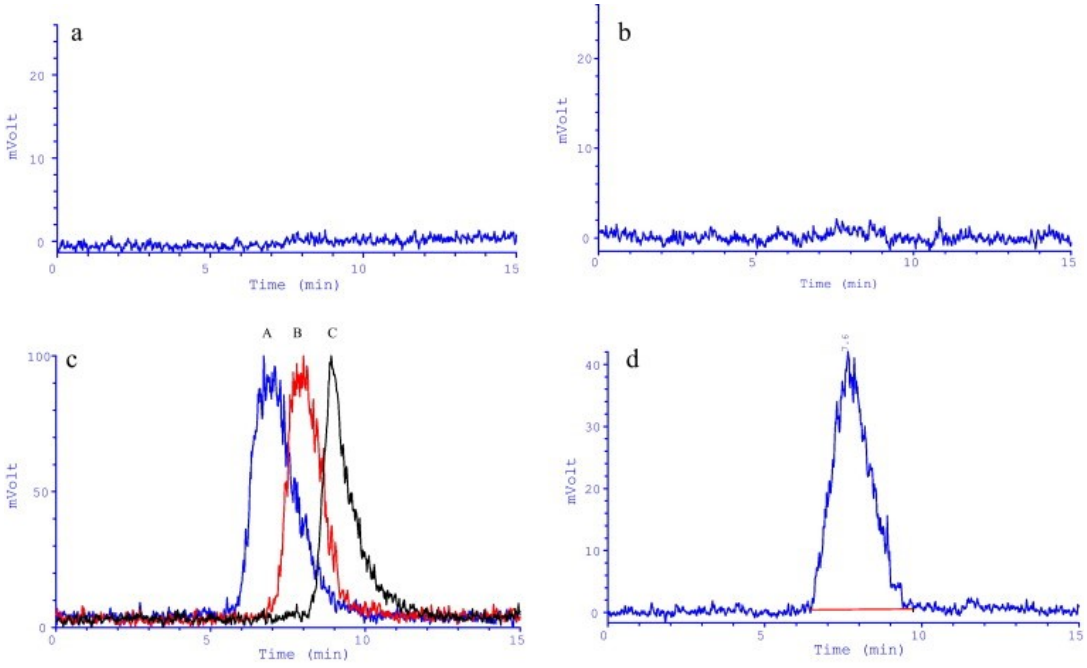


Figure 7 Chromatogram of a) blank, b) top layer extract containing the matrix of Manti gastop tablets, c) 10, 350 and 60 000 cSt PDMS in chloroform and d) lower layer of extract containing traces of PDMS.[35]

2.3.3 Other methodologies for the analysis of PDMS

To avoid the problem of contamination of PDMS species from the chromatographic system, PDMS can be identified and quantified by injecting samples directly into a mass spectrometer. Hunt and George analyzed oligomers from the surface of vulcanized PDMS by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). This high-resolution technique allows the distinction of ions containing different isotopic patterns (Figure 8). The isotopic pattern of each oligomer enables the assignation of accurate molecular structures of the PDMS species in the sample.[36]

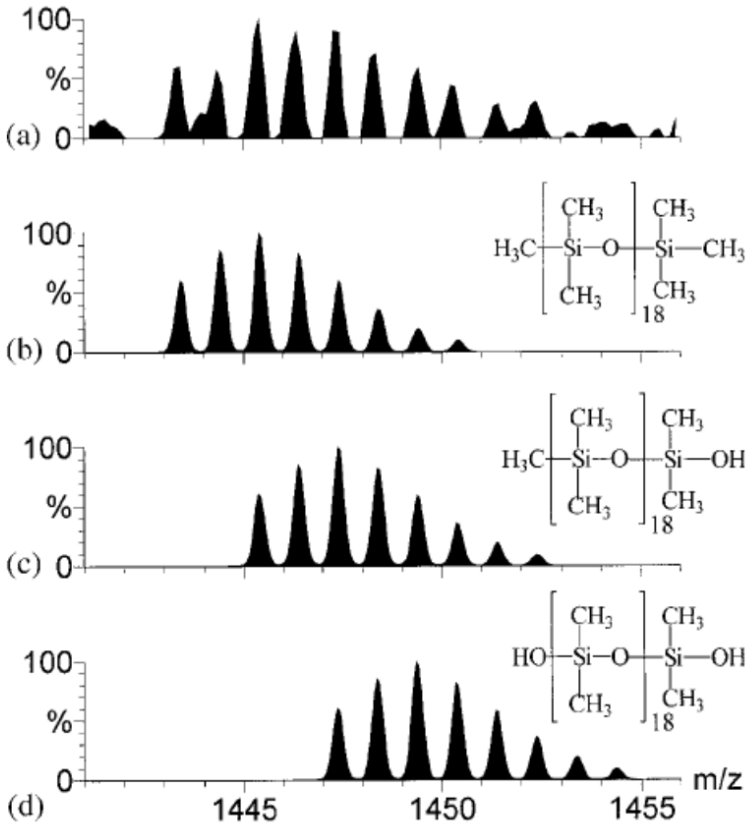


Figure 8 MALDI-TOF mass spectra of the isotopic pattern of a) the detected m/z 1446 peak, b) calculated isotopic pattern for linear 18 unit PDMS with one sodium atom and methyl groups as terminal groups, c) calculated isotopic pattern for linear 18 unit PDMS with one hydroxyl and one methyl as terminal groups and d) calculated isotopic pattern for linear 18 unit PDMS with hydroxyl as terminal groups.[36]

Degradation and thermal stability studies can be achieved by thermogravimetric analysis (TGA). TGA is an appropriate method for analyzing the weight loss of the polymer in both a temperature ramp mode and at a set temperature. In an inert atmosphere, the set temperature could be above 300 ºC. With TGA, the nature of the volatilized compound cannot be determined, only the weight of the volatilized compound. However, TGA-IR could be used for more structure-informative analysis.[37] The behavior of PDMS at low temperatures can be measured by differential scanning calorimetry (DCS).[8]

2.3.4 The choice of method when analyzing PDMS

The choice of method for analyzing PDMS depends on the aim of the analysis as well as the matrix of the sample being analyzed. The type of PDMS being analyzed must be considered, as the properties of LMW PDMS and HMW PDMS differ in e.g., viscosity and volatility. For qualitative analyses, and analyses of higher PDMS concentrations, the usual techniques are FTIR, NMR, Raman, MS and chromatographic methods. Once the desired compounds

have been detected and identified, analytical procedures can be developed for quantitative analysis of low concentrations, which is usually performed with chromatographic systems coupled to mass-spectrometric detectors. The nature of the matrix, from where the PDMS is to be analyzed, affects the approach of the sample preparation. The separation of PDMS from an aqueous matrix is easily done by solvent extraction. By adding an organic solvent into the matrix, the hydrophobic PDMS is separated from the hydrophilic matrix. When PDMS is present in a matrix of lipophilic nature, the separation is more difficult to achieve and requires more demanding separation techniques, such as solid-phase extraction.[38]

2.3.5 Possible sources of PDMS contaminations in laboratories

PDMS is a commonly used material present in laboratory environments as well as in everyday used products. As already stated, silicone is usually used as packing material and sealants in analytical instruments, which can interfere with the analytes. Silicone-based sealants are applied to many other laboratory equipment, such as sealants of test tube caps, which can release contaminants directly into the sample. There are plenty more sources of contamination. In laboratories, PDMS is often used as stopcock grease for glassware, such as seal desiccators. Due to the low surface tension of PDMS, it can creep along surfaces and can be found on surfaces other than the application site. It should also be taken into consideration that the commercial grade of HMW PDMS may contain LMW components to be able to reach the desired viscosity specification.[38]

3 OBJECTIVE

This work is a continuation of previously published theses by Kenneth Arandia, Charlotte Holmberg and Oscar Nyman.[39, 40, 41]

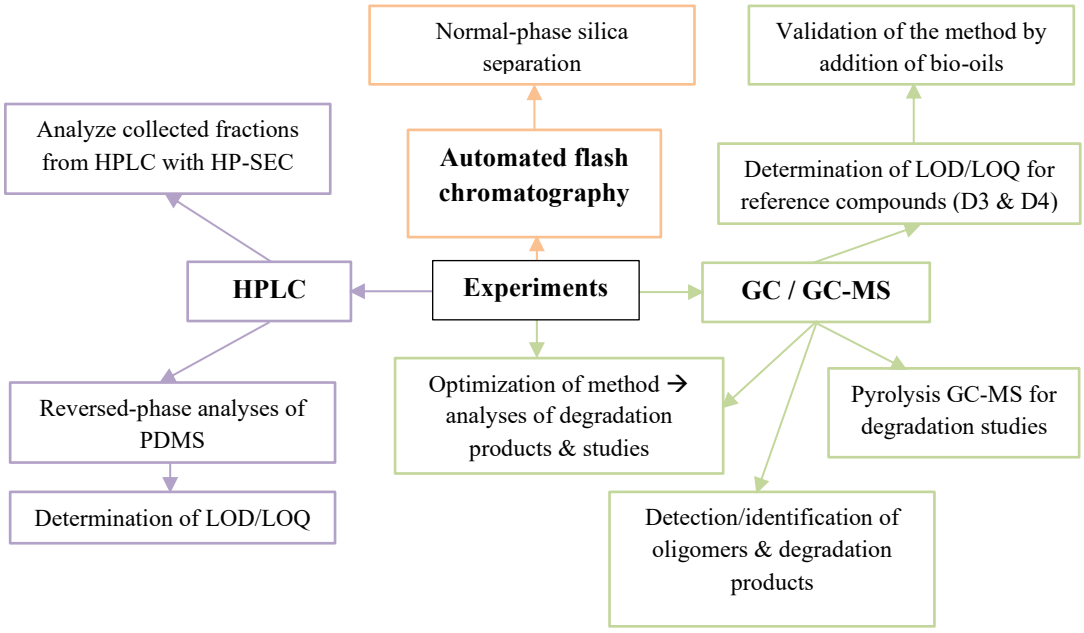
Arandia developed a solvent extraction method by using MeOH and *n*-hexane to fractionate bio-oil matrices spiked with PDMS. By ICP-MS, it was determined that ~90% of the silicon content was collected in the *n*-hexane phase. A solid-phase extraction (SPE) method was tested for the *n*-hexane phase using Strata® Si-1 silica and Thermo Scientific HyperSep SI cartridges. The obtained eluates were analyzed with either HP-SEC, GC-FID or ICP-MS. The SPE gave a recovery of 67% PDMS and was suitable as a basis for concentrating HMW PDMS from bio-oil matrices. For the HP-SEC method, THF with 1% v/v glacial acetic acid was used as the eluent with an isocratic flow. The sensitivity of the ELSD was set to either gain 3 or gain 6. The sensitivity of the method was tested by analyzing HMW PDMS diluted in THF. With gain 3, the lowest detected concentration was 0.012 mg/ml and with gain 6, it was 0.001 mg/ml.

Holmberg continued with testing different cartridges for SPE. There was no significant improvement of the SPE, however, with florisil cartridges, the separation of PDMS from bio-oil matrices was slightly increased with dichloromethane being the most suitable solvent. The early eluting fractions were analyzed with GC-FID and the remaining fractions were analyzed with HP-SEC. When analyzing bio-oil samples spiked with LMW, medium MW (MMW) and HMW PDMS in one sample with HP-SEC, the separation of the MW groups was difficult to achieve. The MMW PDMS could not be detected due to the interference from components in the bio-oil matrix. With the developed SPE procedure, there was a problem with quantifying PDMS after the SPE. With HP-SEC, Holmberg determined the LOD and LOQ of her florisil SPE fractions and compared the results with the LOD and LOQ of Arandia's SiOH SPE fractions. For the florisil SPE method, LOD was calculated to be 37 ppm and LOQ 113 ppm. The values for the SiOH SPE method were calculated to be 30 ppm and 92 ppm, respectively. It should be noted that these values are only theoretical as they were calculated according to the calibration curve established by Holmberg. In a similar fashion as Arandia, Holmberg determined the sensitivity of the HP-SEC method by analyzing samples of HMW PDMS in THF. The lowest detected concentration with the sensitivity value gain 3 was 0.11 mg/ml, whereas, with gain 6 it was 0.0041 mg/ml.

Nyman studied the degradation and stability of PDMS in simulated industrial process environments. The experiments were conducted in an autoclave or sealed tubes in an inert atmosphere. Four different bio-oils were spiked with 20% PDMS and heated at different

temperatures for different periods of time. For bio-oil 4, all HMW PDMS had degraded into oligomers and the LMW cyclic degradation products after 60 min at 250 °C and for bio-oil 1 all PDMS had degraded after 90 min at the same conditions. For bio-oil 2 and 3, only 35% and 10% of the PDMS had degraded. With NMR, GC-MS and GC-FID, the degradation products were analyzed and determined to be the cyclic products D3–D8, with D3–D5 being the most abundant. The presence of the cyclic compounds in the bio-oils supports the claim that PDMS degrades by backbiting reactions.

This work aimed to develop an automated procedure for sample cleanup and to develop and optimize methods of different instrumental techniques for analyzing PDMS and its cyclic degradation products in bio-oil matrices. Ideally, the methods were to be able to lower the detection limit of PDMS and its degradation products. A GC-MS/SIM method was to be developed and optimized, for the detection and identification of the cyclic degradation products (D3–D5) at ppm concentrations in relation to different bio-oil matrices. The developed method was to be compared with the GC-FID method that was used in the previous theses. The comparison was to be made by determining which method would be more sensitive and have the lower LOD. For sample cleanup, automated normal-phase flash chromatographic analysis was to be tested on spiked bio-oil samples in place of the previously tested SPE methods. The collected fractions were to be analyzed with an RP-HPLC method, which was to be compared to the HP-SEC method utilized in the previous theses. Alternatively, sample cleanup was to be done utilizing HPLC, and the collected fractions would be analyzed using the HP-SEC method from previous theses. An overview of the aim of this work is visualized below:



4 EXPERIMENTAL

4.1 Samples and chemicals

Standards of D3, D4, D5, as well as LMW, MMW and HMW PDMS, with the viscosities of 5 cSt, 50 cSt and 1000 cSt, were all obtained from Sigma-Aldrich (USA). Bio-oil samples were all acquired from the Finnish industry. Solvents were of analytical grade and acquired from commercial sources. In Table 7, all solvents used in this work are listed with their quality grade and supplier.

Table 7 Solvents used in this work with their quality grade and supplier.

Solvent	Quality grade	Supplier (country)
Acetonitrile (ACN)	HiPerSolv	VWR Chemicals (France)
Dichloromethane (DCM)	ACS reagent	Sigma-Aldrich (USA)
Ethyl Acetate (EtOAc)	ACS reagent	Honeywell (France)
Methanol (MeOH)	Chromasolv™	Honeywell (France)
n-hexane	LiChromasolv	Merck KGaA (Germany)
Tetrahydrafuran (THF)	For HPLC, ≥ 99.9%	Sigma-Aldrich (France)

4.2 GC-MS/SIM analyses of D3, D4 and D5

A GC-MS/SIM method was developed for analyzing D3–D5 components using two separate GC-MS instruments with different specifications on the columns. One was a GC-MS (Agilent 7890A GC and 5975C MS) equipped with an Agilent 19091S-433 HP-5MS column (30m×0.25mm i.d.×0.25µm film thickness) and the other GC-MS (Hewlett Packard G1530A GC and 5973 MS) was equipped with an Agilent 19091Z-002 HP-1 column (25m×0.2mm i.d.×0.11µm film thickness). Helium was used as the carrier gas with the flow rate 0.9 ml/min and the pressure 6.5 psi. The injection volume was 1 µl with the split ratio 20:1, if not reported otherwise. The first GC instrument utilized auto sampling, and for the other instrument, the injections were done manually. The initial oven temperature for the method was set to 50 ºC with a 6 min hold time. The temperature was set to increase to 300 ºC at 25 ºC / min with a 10 min hold time. At the beginning of the run, there was a 4 min solvent delay for the HP-5MS instrument and a 2 min solvent delay for the HP-1 instrument. The parameters of the method were based on a default method available on the GC-MS instrument with the HP-5MS column, however, with a decreased initial oven temperature, as the volatile cyclic compounds require a lower temperature to be detectable. The parameters

were similar to those parameters used in methods reported in the literature.[29, 31, 42] More detailed information on the instruments' parameters can be seen in Appendix A.

Each compound was analyzed separately with full scan mode to assess the retention times as well as the suitable ions to be selected for the SIM method. The ions monitored in the SIM method were 207 and 190 m/z for D3, 281 and 265 m/z for D4 and 355.1 and 338.9 m/z for D5. For each ion, there was a 100 msec dwell time. For the HP-5MS column, D3 was monitored between 4 and 7 minutes, D4 between 7 and 9.50 minutes and D5 was monitored after 9.5 min. For the HP-1 column, D3 was monitored at 2–6 min, D4 at 6–8 min and D5 was monitored after 8 min. Samples were filtered through 0.2 µm PTFE syringe filters before analysis.

4.2.1 GC-MS/SIM method validation

The validation of the developed GC-MS/SIM method was based on the linearity, LOD, precision and accuracy of the method.

Linearity was decided by plotting peak areas against concentrations of 8 samples of D3–D5 components in *n*-hexane. Concentrations between 0.025 and 1 ppm were analyzed for the calibration curve and the coefficient of determination (R^2) was calculated.

LOD of the method was decided by the minimum concentration that produced signals which were three times greater than the background noise signals. With MS as the detector, the response of D3–D5 is different even though the concentration of the analytes is the same. D3 has the largest response and D5 has the lowest. Therefore, D3 would have the lowest LOD.

Precision was determined by analyzing three samples of three concentrations of bio-oil spiked with D3–D5 (5, 10 and 20 ppm D3–D5 in relation to the bio-oil). The peak areas of the produced signals were plotted against the calibration curve. Accuracy was determined by plotting peak areas of spiked bio-oil samples against the established calibration curve.

4.3 GC-FID analyses of D3, D4 and D5

For GC-FID analyses, a PerkinElmer Clarus 500 instrument equipped with an Agilent J&W HP-1 column (25m×.0.2mm i.d.×0.11µm film thickness) was used. Gaseous hydrogen acted as the carrier gas with a constant pressure of 14 psi. The injections were done automatically with an injection volume of 1 µl and a split ratio of 20:1. The initial oven temperature was 60 °C with a 1 min hold time. The temperature increased by 8 °C / min until reaching 300 °C with a 6 min hold time. The total run time of the method was 37 min. At the beginning of the run, there was a 5 min solvent delay.

4.4 Pyr-GC-MS analyses of PDMS

Pyrolysis GC-MS studies of PDMS were performed by using a Lund Pyrola 2000 MultiMatic interfaced to an Agilent GC-MS system (7890B GC and 5977B MS) equipped with a Zebron ZB-35 column (30m×.0.25mm i.d.×0.25µm film thickness). The pyrolysis chamber temperature was set to 175 °C and the pyrolysis experiments were executed at 650 °C. The analyzed sample amount was approximately 10–100 µg. The temperature of the injector was 280 °C, with a split ratio of 20:1. Helium acted as the carrier gas, with a flow rate of 0.8 ml/min. The initial oven temperature was set to 50 °C for 1 min, followed by an increase of 8 °C / min until reaching 320 °C, with a 5 min hold time. The MS interface temperature was set to 290 °C. The MS operated at full scan mode and the analyzed masses were as follows: 35–350 m/z was scanned from 1.5 to 15 min, 35–500 m/z was scanned between 15 and 25 min and 35–700 m/z was scanned from 25 min onward.

4.5 Normal-phase flash chromatographic analyses of PDMS

For preparative normal-phase chromatographic analyses of PDMS, a Combi*Flash*® EZ Prep system from Teledyne ISCO equipped with an ELSD was used. The eluents were chosen according to the best suitable eluents for the SPE method by Holmberg, i.e., DCM and *n*-hexane. To assess the most suitable ratio of eluents for the preparative analyses, thin layer chromatography (TLC) was implemented on D3, D4, D5 and 50 cSt PDMS using different combinations of the eluents. With DCM, MeOH was used as the polar solvent and EtOAc was used together with *n*-hexane. Two different column systems were used for the combiflash analyses. The first was a combination of Redi*Sep*® Rf Teledyne Isco sample tube and a Redi*Sep*® Rf Teledyne Isco 4 g silica column, and the other was a Redi*Sep*® Rf Teledyne Isco 12 g silica gold column (Figure 9). Separation studies of 5, 50 and 1000 cSt PDMS were performed using both DCM (A) and MeOH (B) as well as *n*-hexane (A) and EtOAc (B) as eluents. Different gradient systems were used between runs, however, eluent B did never exceed 10%.

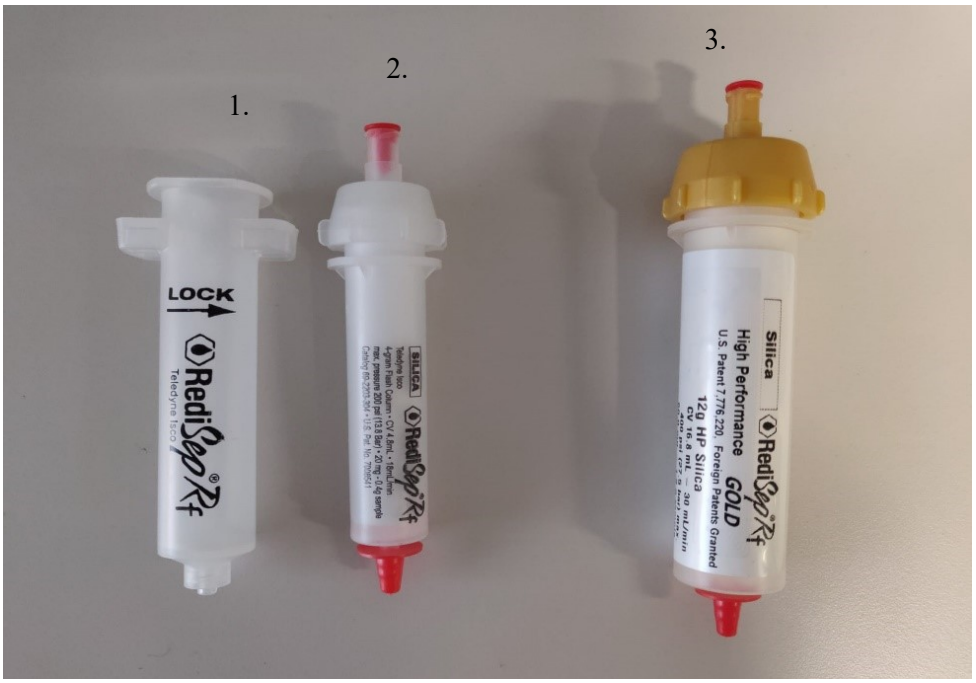


Figure 9 Sample tube (1.), 4 g silica column (2.) and 12 g silica gold column (3.) used for the automized normal-phase flash chromatography.

4.6 Reversed-phase high performance liquid chromatographic analyses of PDMS

A Shimadzu LC system of the Nexera series equipped with a Kinetex RP-18 column (100mm×4.6mm i.d.×2.6µm film thickness) coupled to a Sedere Sedex100 ELSD was used for analyzing 5 cSt, 50 cSt and 1000 cSt PDMS. ACN and EtOAc were used as eluents and the gradient is visualized in Table 8. The injection volume was 50 µl and the flow rate was 1.0 cm^3/min. Some analyses were performed using THF instead of EtOAc as one eluent.

Table 8 Gradient for the RP-HPLC analysis of PDMS.

Eluents	0 min	8 min	12 min	14 min	17 min
ACN	70%	0%	0%	70%	70%
EtOAc	30%	100%	100%	30%	30%

The ELSD operated at 40 °C with a signal filter of 0.5 s. The sensitivity value of the detector was mainly gain 1, however, with lower concentration the sensitivity was set to gain 4. The parameters of the method were chosen according to methods reported in the literature. Cunha and Oliveira developed an HPLC method for analyses of triglycerides, which could be used as a guideline for analyses of PDMS.[43] The higher lipophilicity of PDMS compared to

triglyceride was taken into consideration. Other literature sources were reports from Agilent Technologies and Thermo Fischer Scientific on HPLC analysis of PDMS.[44, 45] Before every analysis, samples were filtered through 0.2 µm PTFE syringe filters.

4.7 Sample preparation

4.7.1 Preparation of stock solutions

Stock solutions of D3, D4 and D5 in *n*-hexane were prepared separately as well as a solution containing all compounds. For the full scan analyses of D3–D5, separate solutions were made for each compound. For further analyses with the SIM method, all compounds were prepared as one solution. Of each compound, 1000 mg was weighed into a 100 ml volumetric flask which was then filled with *n*-hexane, giving a solution of 10 mg/ml.

For HPLC analyses, stock solutions of 5, 50 and 1000 cSt PDMS dissolved in either EtOAc or THF were prepared. In the same way as described above, 10 mg/ml and 1 mg/ml of both separate solutions and solutions of combined viscosities were prepared.

10 mg/ml stock solutions of bio-oils were prepared by weighing 500 mg of bio-oil into a 50 ml volumetric flask which was then filled with either *n*-hexane, EtOAc or THF.

4.7.2 Sample preparation for GC-MS/SIM analyses

A sample set of 8 different concentrations ranging from 0.025 to 1 ppm D3–D4 in *n*-hexane was prepared for the calibration curve. The initial concentrations of the series were 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 7.5 mg/ml and at the end of the sample preparation, concentrations of 0.000025 mg/ml (0.025 ppm), 0.00005 mg/ml (0.05 ppm), 0.000075 mg/ml (0.075 ppm), 0.0001 mg/ml (0.1 ppm), 0.00025 mg/ml (0.25 ppm), 0.0005 mg/ml (0.5 ppm), 0.00075 mg/ml (0.75 ppm) and 0.001 mg/ml (1 ppm) were obtained for the calibration curve.

For spiked bio-oil samples, the desired concentration of bio-oil was 5 mg/ml in *n*-hexane spiked with D3–D5 with the concentrations ranging from 5ppm to 200 ppm in relation to the bio-oil. In total, three different bio-oils were analyzed. In Table 9, the preparation of the concentrations is explained.

Table 9 Explanation of the preparation of 5–200 ppm D3–D5 spiked bio-oil samples diluted in *n*-hexane, with the D3–D5 concentrations being in relation to the bio-oil.

Conc. of D3–D5	Sample preparation
5 ppm	5 ml of 0.00005 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
10 ppm	5 ml of 0.0001 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
15 ppm	5 ml of 0.00015 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
20 ppm	5 ml of 0.0002 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
50 ppm	5 ml of 0.0005 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
100 ppm	5 ml of 0.001 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
150 ppm	5 ml of 0.0015 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil
200 ppm	5 ml of 0.002 mg/ml D3–D5 was added to 5 ml of 10 mg/ml bio-oil

4.7.3 Sample preparation for pyrolysis studies

Samples of PDMS in *n*-hexane were prepared by making solutions of 10 mg/ml of 5, 50 and 1000 cSt PDMS in *n*-hexane. The analyzed sample amount for 50 cSt and 1000 cSt PDMS was ~100 µg, and ~10 µg for 5 cSt PDMS. Spiked bio-oil samples were prepared by making solutions of 80% bio-oil and 20% PDMS in *n*-hexane, with a total sample concentration of 10 mg/ml. Similar to the sample preparation explained in Table 9, solutions of 10 mg/ml spiked bio-oil samples in *n*-hexane with 100 ppm PDMS in relation to the bio-oil were prepared.

4.7.4 Sample preparation for RP-HPLC analyses

Samples of 5, 50 and 1000 cSt PDMS in EtOAc ranging from 0.00015 mg/ml (0.15 ppm) to 1 mg/ml (1000 ppm) were prepared for LOD studies of the RP-HPLC method. In total, there were 9 samples prepared and the concentrations of the samples were always 3 times less than the previous one, starting with 1 mg/ml.

Samples for LOD studies of bio-oil spiked with 1000 cSt PDMS were prepared, where the concentration of bio-oil was 1 mg/ml with the amount of PDMS ranging from 0.1% to 10% in relation to the bio-oil. In Table 10, the preparation of the different samples is explained.

Table 10 Explanation of the preparation of 0.1–10% 1000 cSt PDMS spiked bio-oil samples diluted in EtOAc, with the percentage of PDMS being in relation to the bio-oil.

Percentage of 1000 cSt PDMS	Sample preparation	Abbreviation
	1 ml of 10 mg/ml of 1000 cSt PDMS was added to 9 ml of 10 mg/ml bio-oil solution	A
10%	1 ml of A was added to 9 ml solvent	B
5%	4 ml of B was added to 4 ml 1 mg/ml bio-oil solution	C
2.5%	4 ml of C was added to 4 ml 1 mg/ml bio-oil solution	D
1%	1 ml of B was added to 9 ml 1 mg/ml bio-oil solution	E
0.5%	1 ml of C was added to 9 ml 1 mg/ml bio-oil solution	F
0.25%	1 ml of D was added to 9 ml 1 mg/ml bio-oil solution	G
0.1%	1 ml of E was added to 9 ml 1 mg/ml bio-oil solution	H

4.7.5 Extraction of PDMS spiked bio-oil matrices

The solvent extraction method reported by Arandia was performed on three different bio-oil matrices spiked with PDMS.[39] Three test tubes were taken for each bio-oil, and 250 mg of a bio-oil and 25 mg of PDMS were weighed into a test tube. Thereafter, 4 ml of MeOH and *n*-hexane, as well as 1 µl of 25 % ammonia solution, was added into the tubes. Then, the test tubes were shaken for 1 minute and were left to settle. Next, the samples were centrifuged for 5 minutes at 1450 rpm and the top phase (*n*-hexane phase) was pipetted into 25 ml volumetric flasks. The procedure was repeated twice resulting in a triple extraction. When all top phases had been pipetted into the volumetric flask, they were filled with *n*-hexane, giving a solution of 10 mg/ml. Thereafter, 1 ml of the *n*-hexane solution was transferred into another set of test tubes and evaporated under a stream of N_2. When all *n*-hexane had evaporated, the samples were dissolved with 10 ml EtOAc giving a solution of 1 mg/ml. These solutions were filtered with 0.2 µm PTFE syringe filters and thereafter analyzed with RP-HPLC.

4.7.6 Sample preparation of normal-phase flash chromatographic analyses

For preparative analyses of D3, D4 and D5, 300 mg of D3 and D4 together with 500 mg D5 was added into 1 ml of DCM. The solution was loaded directly onto a 12 g gold column. For separation studies of 5, 50 and 1000 cSt PDMS, two preparation methods were used. The first was done similarly to the D3–D5 sample preparation. For the other method, 200 mg of each viscosity was weighed into separate round bottom flasks together with 20 ml *n*-hexane and half a sample tube of silica gel. Each solution was stirred and evaporated until a dry powder was obtained. The dry powder was transferred into the sample tube and was manually compressed tightly, and the tube was loaded onto the 4 g column. For a solution containing all viscosities, 200 mg of each viscosity was weighed into a round bottom flask with 40 ml *n*-hexane and half a test tube with silica gel. Further, preparations were done in the same way as for the separate samples. Bio-oil samples were prepared by the same extraction method as described in section 4.7.4. One extraction of only bio-oil was done and another of bio-oil spiked with 5, 50 and 1000 cSt PDMS. From the extracted *n*-hexane phases, 2 ml was loaded directly onto a 12 g gold column.

5 RESULTS AND DISCUSSION

5.1 Analyses with GC-MS/SIM

5.1.1 Comparison of the developed GC-MS/SIM method to previously used GC-FID method

For the comparison of the two methods, samples of 0.1–10 ppm D3–D5 in *n*-hexane were analyzed. With GC-FID, the compounds were detected in 0.5 ppm, however, for 0.1 ppm there were no visible signals in the chromatogram. For GC-MS/SIM, the samples were analyzed with both instruments, and it showed that the instrument equipped with the HP-1 column was able to detect lower concentrations (0.01 ppm) for all compounds than that of the instrument equipped with the HP-5MS column. Only D3 and D4 were detected in concentrations below 0.1 ppm. The difference in the sensitivity is due to the HP-5MS instrument not having as good maintenance as the HP-1 instrument, therefore, not being as clean. The comparison of the methods is shown in Table 11, and the chromatograms of the lowest detected concentrations per instrument are presented in Appendix B.

Table 11 The comparison of detectable concentrations of D3–D5 with GC-MS/SIM and GC-FID.

Concentration of D3–D5 in *n*-hexane	GC-MS/SIM (HP-5MS)	GC-MS/SIM (HP-1)	Detected with GC-FID
10 ppm	X	X	X
5 ppm	X	X	X
3.5 ppm	X	X	X
1.5 ppm	X	X	X
0.75 ppm	X	X	X
0.5 ppm	X	X	X
0.1 ppm	X	X	-
0.05 ppm	X, only D3 and D4 were detected with split ratio 30:1	X	-
0.025 ppm	X, only D3 and D4 were detected with split ratio 30:1	X	-
0.01 ppm	-	X	-

The split ratio for both GC instruments was set to 30:1, and with a lower split ratio or with splitless mode, much lower concentrations could be detected. With the HP-1 instrument, even ppb concentrations of D3–D5 in *n*-hexane could be detected. The GC-FID was operated at split 20:1 and the results of these analyses show that GC-MS/SIM would be a more sensitive method compared to the GC-FID method.

5.1.2 D3, D4 and D5 contamination problem

Similar to the contaminations mentioned in section 2.3.2, contaminations of D3–D5 were detected when analyzing blanks of *n*-hexane with the instrument equipped with the HP-1 column. To study the origin of the contaminants, different blanks were analyzed. Samples of pure *n*-hexane were taken from different, previously unopened, bottles. In all *n*-hexane blanks, signals of D3–D5 were visible in the chromatograms. Therefore, it could be excluded that the contaminants originated from the solvent. Next, injections without solvent or analytes were performed, and the contaminants were still visible in the chromatograms. Only when performing a run without any injections were the contaminants not detected. These observations supported the claim that the contaminants did not originate from the stationary phase of the column, but silicone from the inlet septum. Four different septa were analyzed for comparison: Agilent's Advanced Green, Agilent General Purpose Red, Agilent Long Life Red and Molded Thermogreen™ Low Bleed-2 Green. In Table 12, the peak areas of the produced signals of the septa being used for the first time are given.

Table 12 Peak areas of D3–D5 contaminations of different inlet septa.

Septum type	Peak area of D3	Peak area of D4	Peak area of D5
Advanced Green (Agilent)	5184	2888	315
General Purpose Red (Agilent)	1358	683	130
Long Life Red (Agilent)	2258	824	91
Low Bleed-2 Green (Molded Termogreen™)	1095	798	172

According to Table 12, the Agilent Advanced Green septum generated the largest signals of the contaminants followed by the Agilent Long Life Red septum. The Agilent Advanced Green and Molded Termogreen™ Low Bleed-2 Green septa generated the lowest signals of contaminants. These results are different from those obtained by Horii and Kannan, where

the General Purpose Red septum generated higher levels of contamination than that of the Advanced Green septum.[31] The reason for this difference was not further investigated. As the BTO septum showed the least contamination in the studies of Horii and Kannan, the Low Bleed-2 Green septum was chosen for further analyses with the HP-1 instrument. The septum was left for one week at 300 ºC and afterward, when *n*-hexane was injected and analyzed, the contaminants had decreased by half. On the contrary to the analyses with HP-1, the Long Life Red septum was used as the inlet septum in the HP-5MS, and when analyzing blanks of *n*-hexane with this instrument, there were no detected contaminations of D3–D5. The sensitivity of the instruments could be a reason for this, as the HP-1 instrument was more sensitive. As the septa are usually preconditioned at the manufacturer, high temperatures are presumably not the sole reason for the contamination. The amount of performed injections may also affect the contamination. According to Agilent, the recess in the middle of the septa acts as guidance for the syringe needle of the injector, so that the needle will go through the septum at the same point with every injection.[46] As the HP-5MS instrument utilizes automatic injections, injections done at the same point of the septum could be achieved. With the HP-1 instrument, the injections are done manually, and the insertion point varies at a much larger scale. This could also be a reason for the difference in detecting the contaminants between the two instruments. A solution to the contamination problem could be to use a Merlin Microseal instead of silicone rubber inlet septa. The Merlin Microseal would allow a longer lifetime and would eliminate septum-coring. For all the following reported results of GC-MS/SIM analyses, the contamination should be taken into consideration.

5.1.3 Linearity of the method determined by analyses of D3, D4 and D5 in n-hexane

The linearity of the method was determined from calibration curves, which were obtained by analyzing samples of concentrations between 0.025 and 1 ppm D3–D5 in *n*-hexane (Figure 10). The peak areas of the calibration curves are an average of three parallel analyses. The estimation of the linearity is based on the correlation coefficient (R^2). For all three calibration curves, the R^2 value was greater than 0.993. Therefore, the linearity of all calibration curves can be considered as good.

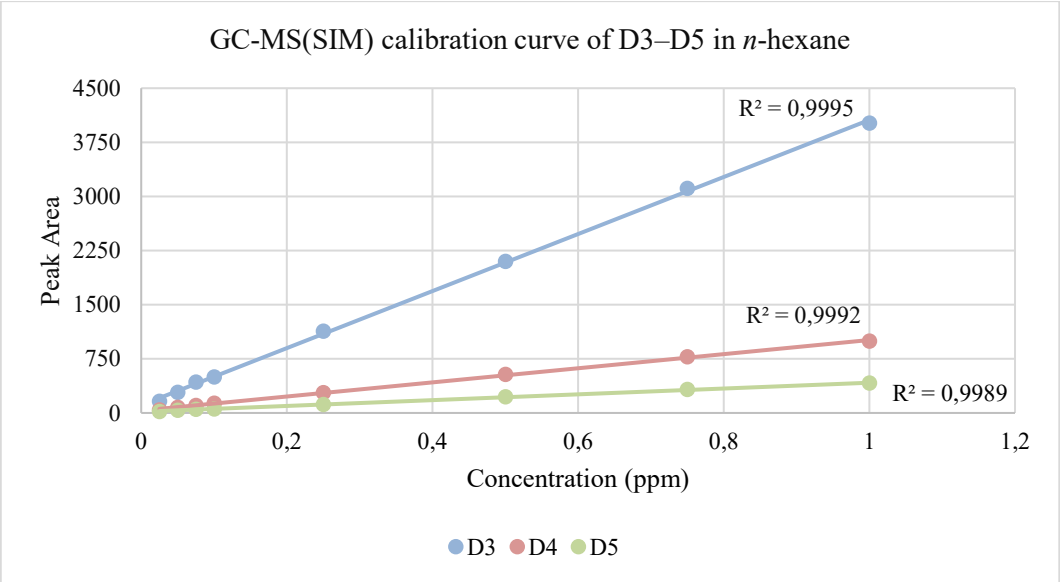


Figure 10 Linearity of the calibration curves of D3, D4 and D5 in *n*-hexane.

However, when examining the lower concentrations of the calibration curve, the linearity is not as good (Figure 11).

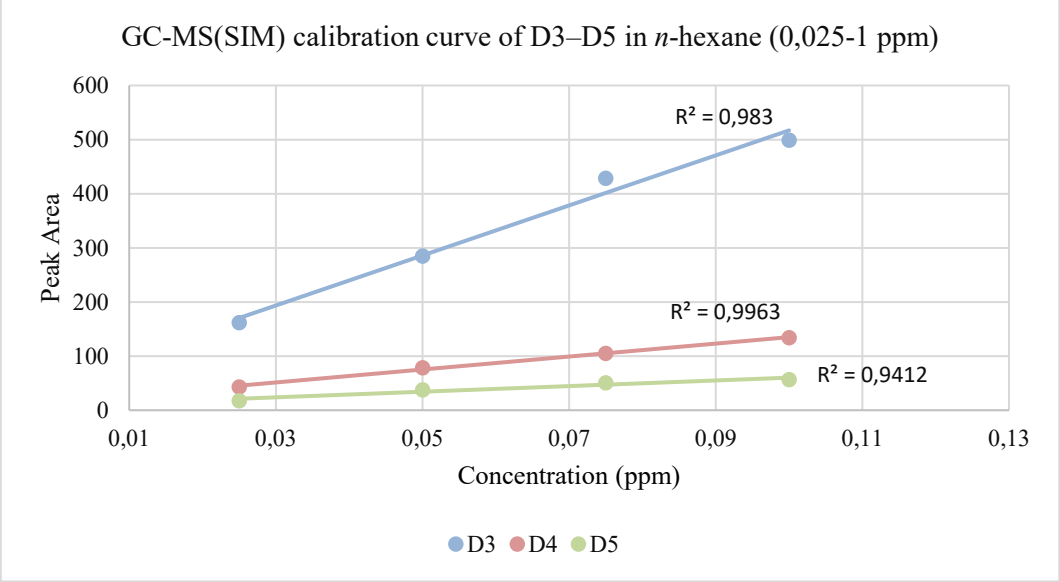


Figure 11 Linearity of the lower concentrations of the calibration curves of D3, D4 and D5 in *n*-hexane.

The R^2 value was below the desired 0.993 for D3 and D5, however, for D4 the linearity remained good. This variation in the lower concentration could be a result of human error when preparing the samples by long pipetting series. A small error has a larger impact on

such low concentrations compared to higher ones. D3, D4 and D5 were analyzed from the same sample and if there is a pipetting error for one of the compounds, the error should be visible for all three compounds. As there is a difference between the compound, this variation could be a result of the contamination of the inlet septum. Overall, the linearity is shown to be good for the developed GC-MS/SIM method.

5.1.4 Limit of detection of D3, D4 and D5 in spiked bio-oil 1 samples

LOD was determined by analyzing samples of low concentrations using the HP-1 instrument. Because trace amounts of D3–D5 can be detected in bio-oil 1, it is difficult to determine the exact LOD. The software used for processing the results did not have an automated LOD calculating feature and, therefore, the LOD had to be calculated manually. This also affects the precision of the determination of the LOD. Three parallel samples, with the concentration of 1 ppm, 2 ppm and 3 ppm D3–D5 in relation to bio-oil 1 were analyzed. The total concentration of the samples was 5 mg/ml in *n*-hexane. The peak areas of D3–D5 present in bio-oil 1, as well as the peak areas of contaminants in *n*-hexane, were subtracted from the peak areas obtained from the spiked bio-oil 1 samples. Between analyses of the samples, blanks of *n*-hexane were analyzed to monitor the peak areas of the contaminants. An average of the contaminants could be determined, however, there was a variation of signals produced for each blank. The variation did cause a difference in peak areas of the parallel samples of each concentration of spiked bio-oil 1 samples, and the average peak areas of the signals generated by each parallel spiked bio-oil 1 sample could be determined and is presented in Figure 12.

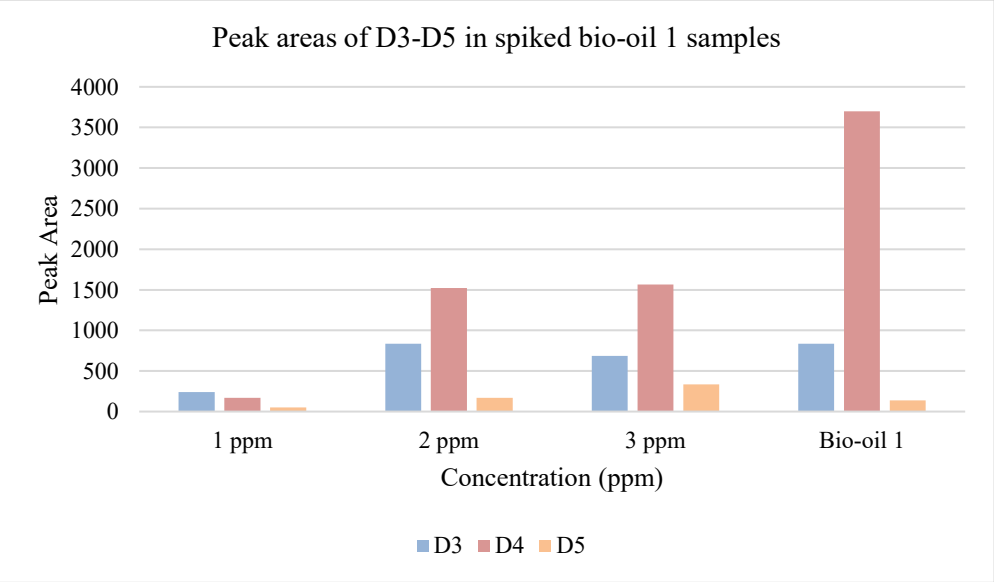


Figure 12 Average peak areas of D3, D4 and D5 in different concentrations of spiked bio-oil 1 samples for LOD studies.

Although Figure 12 shows that 1 ppm D3–D5 in relation to bio-oil 1 would be detectable, the validation of the detection cannot be made. Two out of three samples with the concentration of 1 ppm were below the LOD and as a result, the one sample above the LOD could not be accepted. All parallel samples of the 2 ppm and 3 ppm concentrations were detectable, and it can be reported that the LOD of the GC-MS/SIM method is at least 2 ppm D3–D5 in relation to bio-oil 1. The bar belonging to bio-oil 1 in Figure 12, shows the peak areas of D3–D5 present in the bio-oil without being spiked. The trace amounts of D3–D5 in bio-oil 1 generated peak areas more than twice the size of the lowest detectable concentration of spiked D3–D5. Lower detection limits could be achieved by lowering the split ratio of the method. However, with a lower split ratio, more sample flows through the column, resulting in an increased amount of bio-oil, which could contaminate the column. By lowering the concentration of bio-oil 1 from 5 mg/ml to, e.g., 1 mg/ml, the determination of LOD could be simplified, as the signals of D3–D5 in bio-oil 1 would decrease. By utilizing another inlet septum, such as the earlier mentioned Merlin Microseal, there would possibly be no background levels of D3–D5 and the determination of the LOD of the method would be more precise.

5.1.5 Accuracy and precision of the GC-MS/SIM method

Both accuracy and precision of the method were determined by comparing D3–D5 spiked bio-oil 1 samples with the calibration curves. The samples were analyzed with the HP-5MS instrument. For accuracy, a sample set of concentrations ranging from 5 to 200 ppm D3–D5 in relation to bio-oil 1 was analyzed (Figure 13–15). The curves of the samples are an average of three parallel analyses of the sample set.

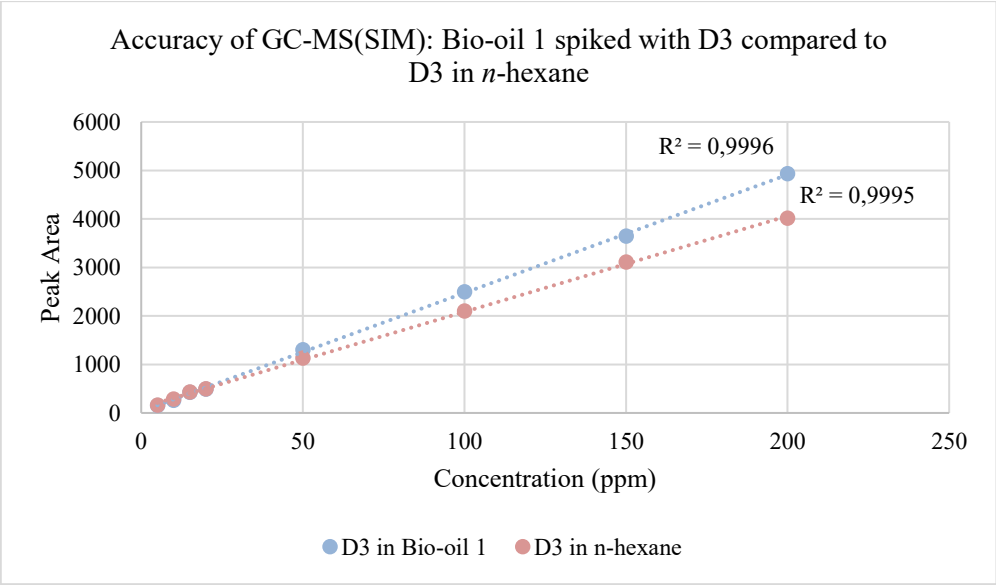


Figure 13 The accuracy of the GC-MS/SIM method determined by comparing D3 spiked in bio-oil 1 to the calibration curve of D3 in *n*-hexane.

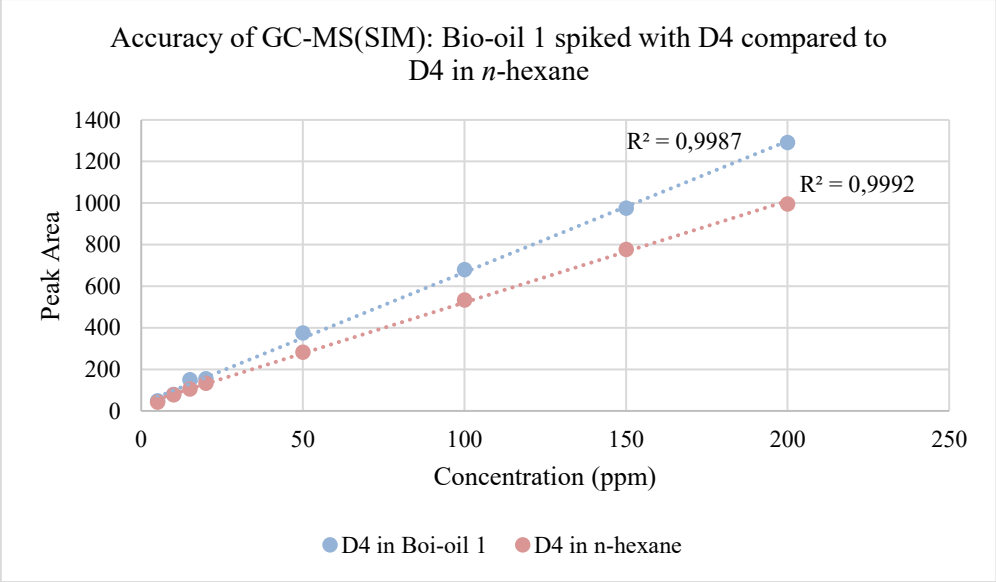


Figure 14 The accuracy of the GC-MS/SIM method determined by comparing D4 spiked in bio-oil 1 to the calibration curve of D4 in *n*-hexane.

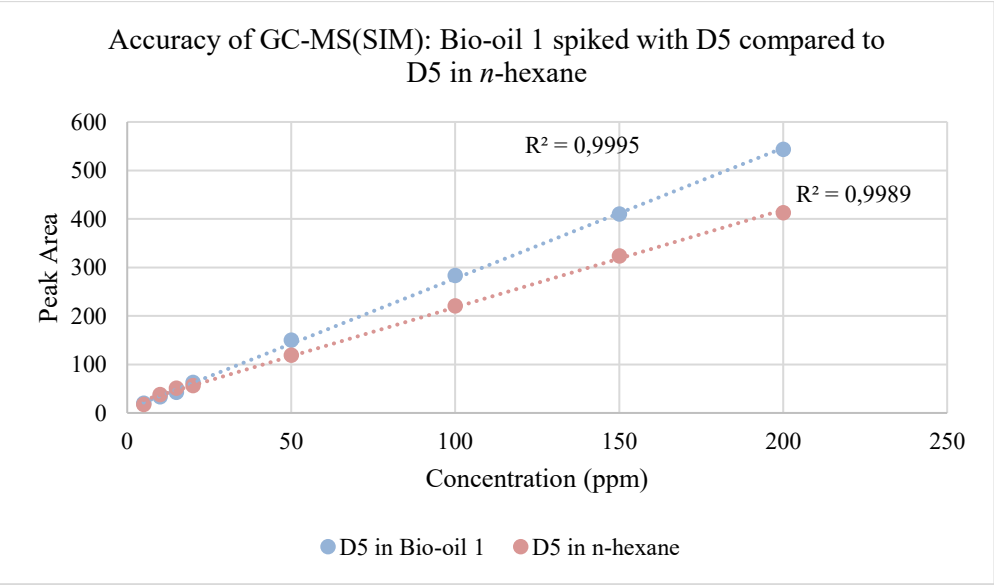


Figure 15 The accuracy of the GC-MS/SIM method determined by comparing D5 spiked in bio-oil 1 to the calibration curve of D5 in n-hexane.

All three compounds showed similar results. The difference between the bio-oil 1 spiked samples and the calibration curves increases with higher concentrations of D3–D5 in bio-oil 1. The reason for this difference was not further investigated, however, the bio-oil matrix may affect the increase in the response since it is not observed when analyzing D3–D5 in *n*-hexane with the same concentration. The analyzed sample set contains a wide range of concentrations and there is a considerate difference between the lowest and the highest concentration (5 ppm vs. 200 ppm D3–D5 in relation to the bio-oil). Therefore, as can be seen in Figure 13–15, the difference in the matrix effect between the lower and the higher concentrations is noticeable.

Precision studies were performed on the four lowest concentrations of D3–D5 spiked bio-oil 1 samples, i.e., 5, 10, 15 and 20 ppm D3–D5 in relation to the bio-oil. Three parallel samples were analyzed and compared to the calibration curve of D3–D5 in *n*-hexane (Figure 16–18).

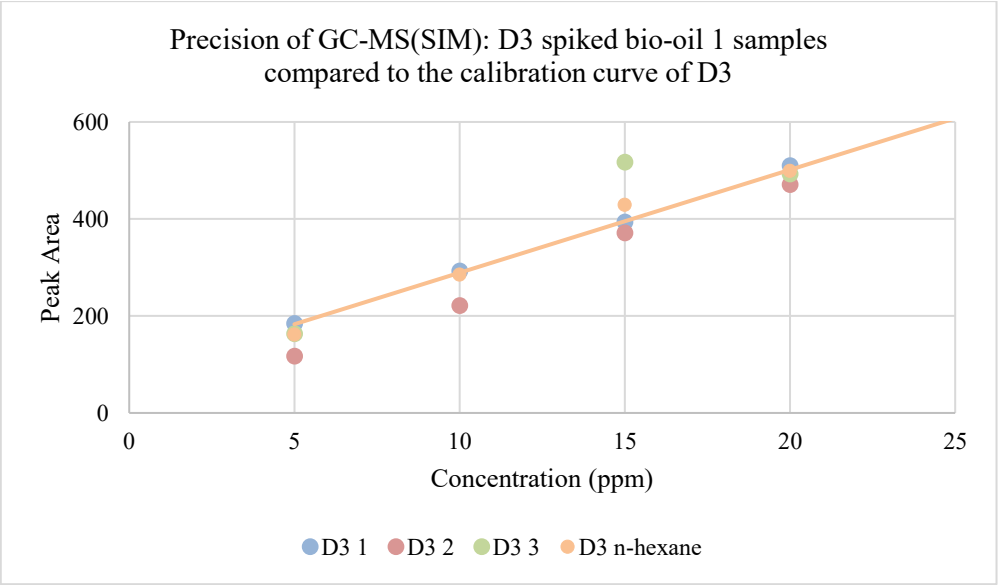


Figure 16 The precision of GC-MS/SIM method determined by comparing four concentrations of D3 spiked bio-oil 1 samples with the calibration curve of D3 in *n*-hexane.

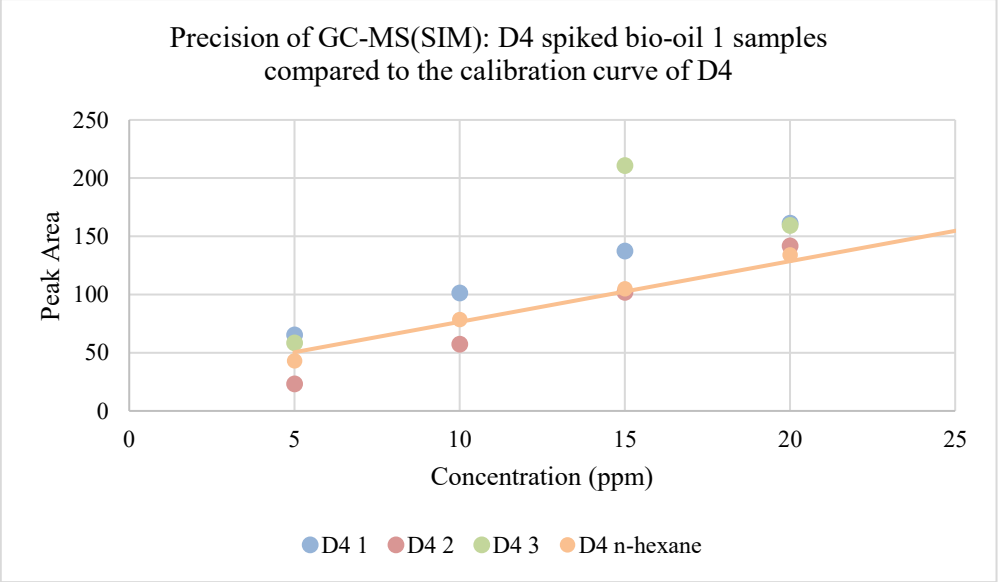


Figure 17 The precision of GC-MS/SIM method determined by comparing four concentrations of D4 spiked bio-oil 1 samples with the calibration curve of D4 in *n*-hexane.

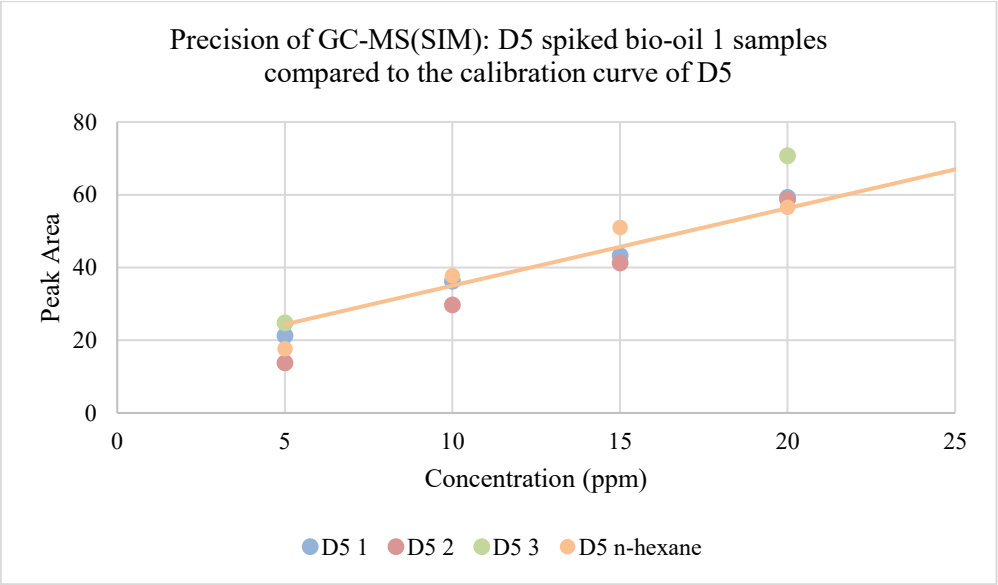


Figure 18 The precision of GC-MS/SIM method determined by comparing four concentrations of D5 spiked bio-oil 1 samples with the calibration curve of D5 in n-hexane.

For all three compounds, there was a slight difference between each parallel analysis. D4 showed the most noticeable difference, whereas, for D3 and D5, the difference was reduced. Some data points of the parallel analyses were deleted due to them being too deviant from the corresponding data points. This deviation was most probably due to human error in the sample preparation. The standard deviation, σ, of the data collected was calculated according to the peak areas of each concentration and can be seen in Table 13.

Table 13 Standard deviation of the parallel concentrations analyzed for precision studies.

Concentration (ppm)	σ for D3	σ for D4	σ for D5
5	28.20461	18.37269	4.588633
10	35.75	22	3.25
15	64.18247	45.39151	1
20	15.79564	8.759122	5.542763

For more precise and accurate determination of the accuracy and precision of the GC-MS/SIM method, more data should be collected. As for now, there are only three parallel runs, that have been executed, and especially the precision is difficult to validate.

5.1.6 Comparison of bio-oil 1 and bio-oil 2 samples

GC-MS/SIM analyses of bio-oil 1 and bio-oil 2 were compared using the HP-5MS instrument. For each bio-oil, 5 mg/ml bio-oil in *n*-hexane was analyzed to compare the trace amount of D3–D5 in the bio-oils. For both bio-oils, only D3 and D4 were detectable, with higher concentrations in bio-oil 1 (Figure 19).

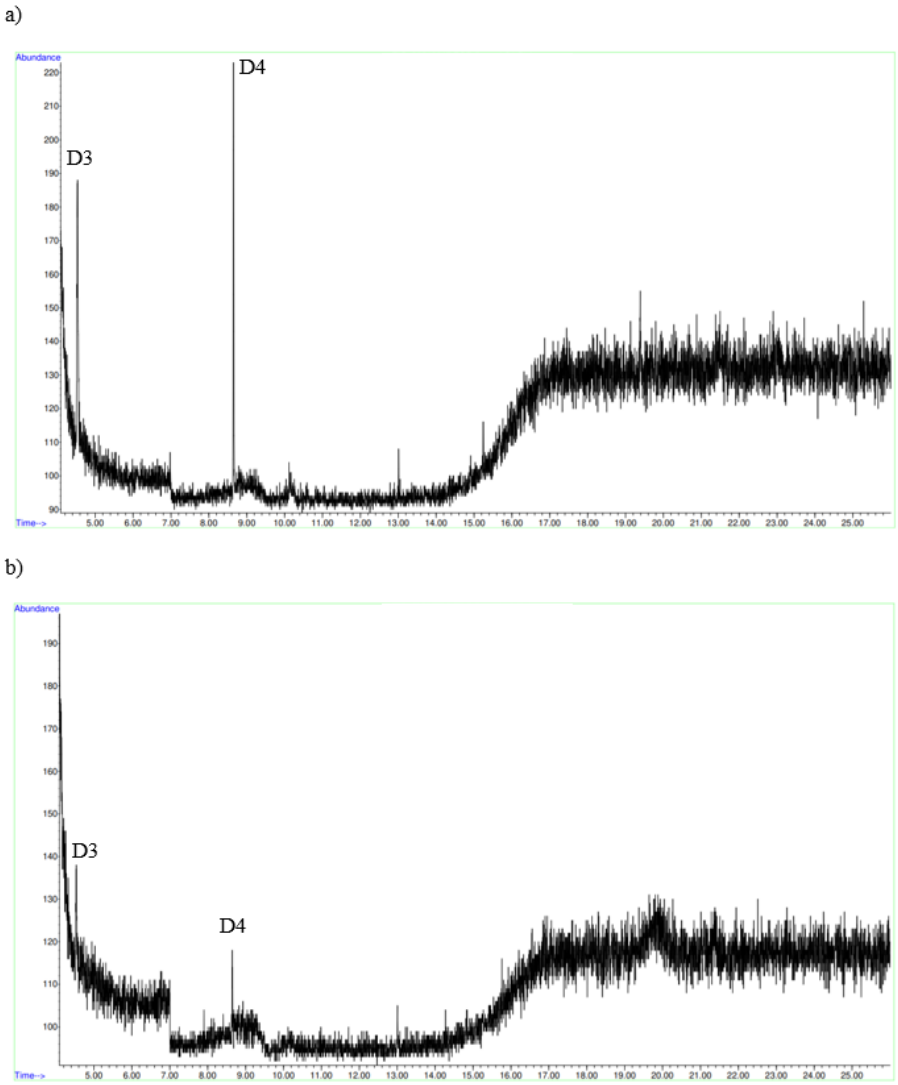


Figure 19 The background levels of D3 and D4 in a) 5 mg/ml bio-oil 1 in *n*-hexane and b) 5 mg/ml bio-oil 2 in *n*-hexane.

Spiked samples of bio-oil 1 and bio-oil 2 were also compared (Figure 20). A sample set of 5 mg/ml bio-oil 1 spiked with 5–200 ppm D3–D5 was compared to a similar sample set of spiked bio-oil 2. The peak areas of the trace amount of D3–D5 in the bio-oils were subtracted from the peak areas obtained from the spiked samples.

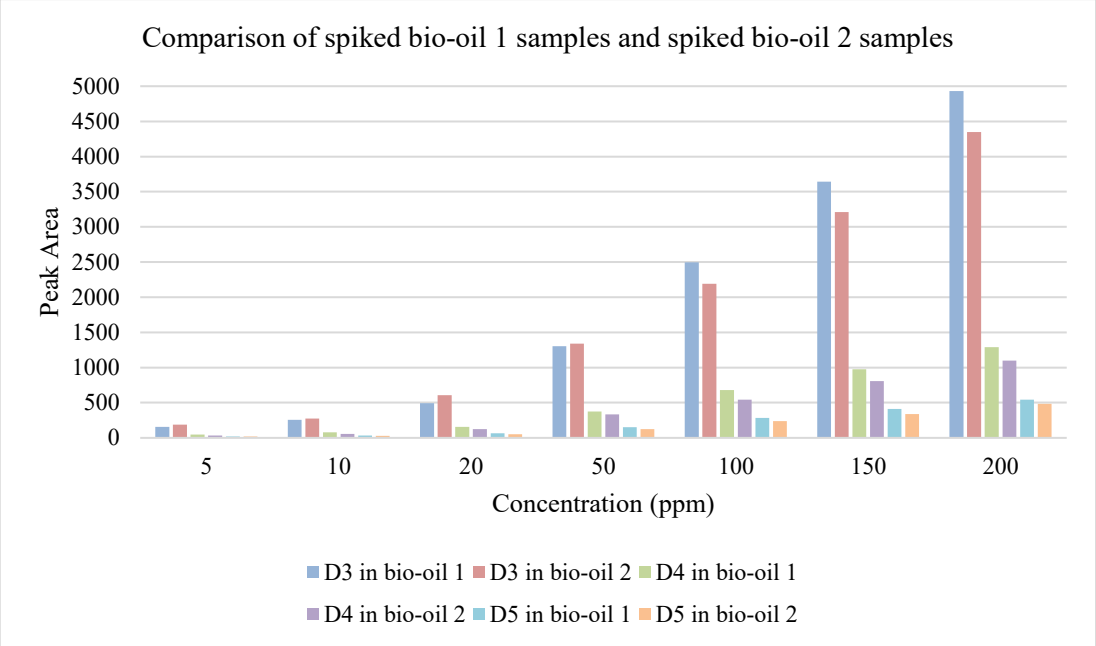


Figure 20 Comparison of spiked bio-oil 1 and bio-oil 2 samples with equal concentrations of D3–D5 in relation to the bio-oil.

The peak areas of the produced signals of each concentration of D3–D5 were similar between bio-oil 1 and bio-oil 2. The smallest difference was in the lower concentrations of D3–D5 with an increasing difference with higher concentrations. It was shown that the matrix affects the response of the D3–D5 detection differently. For the lower concentrations (5–50 ppm), larger signals were generated by D3 in bio-oil 2 than in bio-oil 1, however, D4 and D5 generated larger signals in bio-oil 1.

5.1.7 Determination of the concentration of D3, D4 and D5 in different bio-oils

Eight different bio-oils were analyzed to determine the concentrations of D3–D5 in bio-oils. The concentration of each sample was 5 mg/ml bio-oil in *n*-hexane. A new calibration curve was prepared, and the linearity was good (Appendix D). The concentrations of D3–D5 in each bio-oil sample were calculated according to the calibration curve. The obtained peak areas and concentration of the calibration curve as well as of D3–D5 in the bio-oils are listed in Table 14 and 15.

Table 14 Concentrations and peak areas of D3–D5 in *n*-hexane of the calibration curve.

Concentration (ppm)	Peak area		
	D3	**D4**	**D5**
0.025	180.5	85.5	37
0.05	371	165.5	64.5
0.075	510.5	245.5	97
0.1	602	294	123
0.25	1472.5	731	299
0.5	3033	1484.5	609.5
0.75	4575.5	2213.5	910.5
1	5684	2814.5	1160.5

Table 15 Peak areas and concentrations of D3–D5 present in 5 mg/ml of different bio-oils in *n*-hexane.

Sample	Peak area			Concentration (ppm)		
	D3	**D4**	**D5**	**D3**	**D4**	**D5**
Bio-oil 1	317.5	656.8333	118.5	0.043601	0.221663	0.093084
Bio-oil 3	325.5	1795.167	191.5	0.044984	0.621688	0.155298
Bio-oil 4	306.1667	1244.833	81.66667	0.041641	0.428294	0.061693
Bio-oil 5	80	96.16667	31.16667	0.00254	0.024638	0.018655
Bio-oil 6	616.5	6209	365.8333	0.095293	2.172765	0.303872
Bio-oil 7	99.83333	82.83333	37.16667	0.005969	0.019953	0.023769
Bio-oil 8	246.5	139.6667	35.66667	0.031326	0.039925	0.02249
Bio-oil 9	680.3333	428.1667	57	0.106329	0.141307	0.040671

It should be noted that the concentrations were calculated according to the calibration curve of D3–D5 in *n*-hexane and the matrix effect, discussed in section 5.1.5, should be taken into consideration when interpreting the results. The peak areas of the generated signals of D3–D5 in bio-oils are larger compared to the peak areas of the generated signals of D3–D5 in *n*-hexane with the same concentrations. Therefore, some of the listed concentrations in Table 15 are larger than what is reported. However, the reported concentrations which are below 0.1 ppm should be accurate, as the matrix effect is noticeable for concentrations ≥ 0.1 ppm.

D3–D5 were detectable in all the analyzed bio-oil samples. In all samples, D4 was detected with the highest concentrations, and bio-oil 6 showed to contain the highest amount of D4 and D5. Bio-oil 9 contained the highest amount of D3. These results clearly showed the high response of the detection of D3, which was discussed in section 4.2.1. D3 generated large signals in relation to the concentration, therefore, it should be kept in mind when analyzing these compounds that even if D3 generates the largest peaks, it might not be the compound with the highest concentration in the sample. These results imply that with this GC-MS/SIM method, it could be possible to quantify the amount of D3–D5 present in bio-oils. In Figure 21, the concentrations of D3–D5 in the different bio-oils are compared. Note that the concentration of D4 in bio-oil 6 and bio-oil 3 is cropped, as they were much higher than the rest (ca 2 and 0.6 ppm).

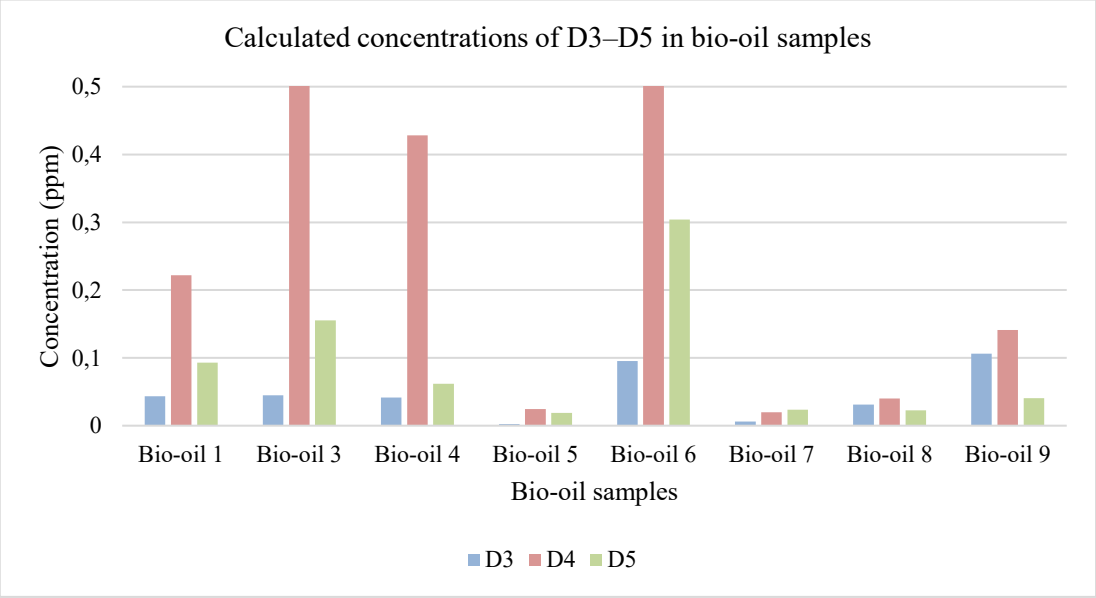


Figure 21 Calculated concentrations of D3–D5 in different bio-oil samples. The calculations were done according to a calibration curve of D3–D5 in *n*-hexane.

5.2 Pyrolysis studies of PDMS

5.2.1 Pyrolysis of pure 5, 50 and 1000 cSt PDMS in n-hexane

For pyrolysis studies, 5, 50 and 1000 cSt PDMS in *n*-hexane were analyzed. As 5 cSt PDMS contains LMW compounds, a full scan GC-MS analysis was performed for comparison purposes. The obtained pyrogram and chromatogram of the 5 cSt PDMS analyses are presented in Figure 22.

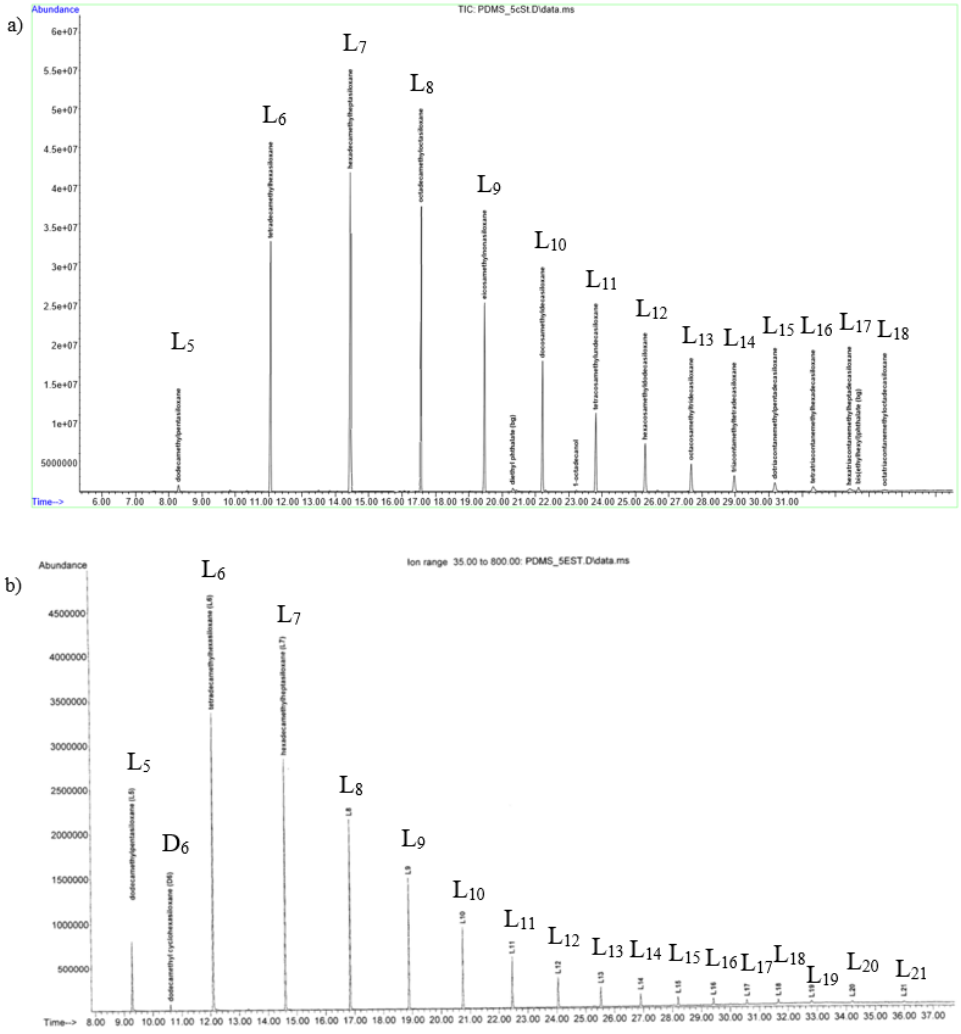


Figure 22 a) Pyrogram and b) GC-MS chromatogram obtained from 10 mg/ml 5 cSt PDMS in *n*-hexane.

In both the pyrogram and the GC-MS chromatogram, it is shown that mostly linear PDMS was detected. The linear compounds with 6, 7 and 8 atoms of silicon (L6, L7 and L8) are the most abundant for both analyses. From the pyrolysis, linear compounds ranging from L6 to L18 were detected, whereas, for the GC-MS, linear compounds up to L21 were detectable. An explanation to why only linear compounds are detected for 5 cSt PDMS could be that for short-chain PDMS, the intermediate which generates the small cyclic compounds is thermodynamically difficult to achieve. The intermediates could be so volatile that they evaporate before the cyclization occurs. In total, pyrolysis of 5 cSt PDMS generated signals from 14 compounds which are of interest.

Pyrolysis performed on 50 cSt PDMS in *n*-hexane generated both cyclic and linear products, with linear compounds being more abundant (Figure 23). Cyclic compounds ranging from

D3 to D19 were detected, and for linear compounds, the detection was between L8 and L18. The signal assigned as L8 is not certain, and if excluded, 50 cSt PDMS generated signals from 21 compounds of interest.

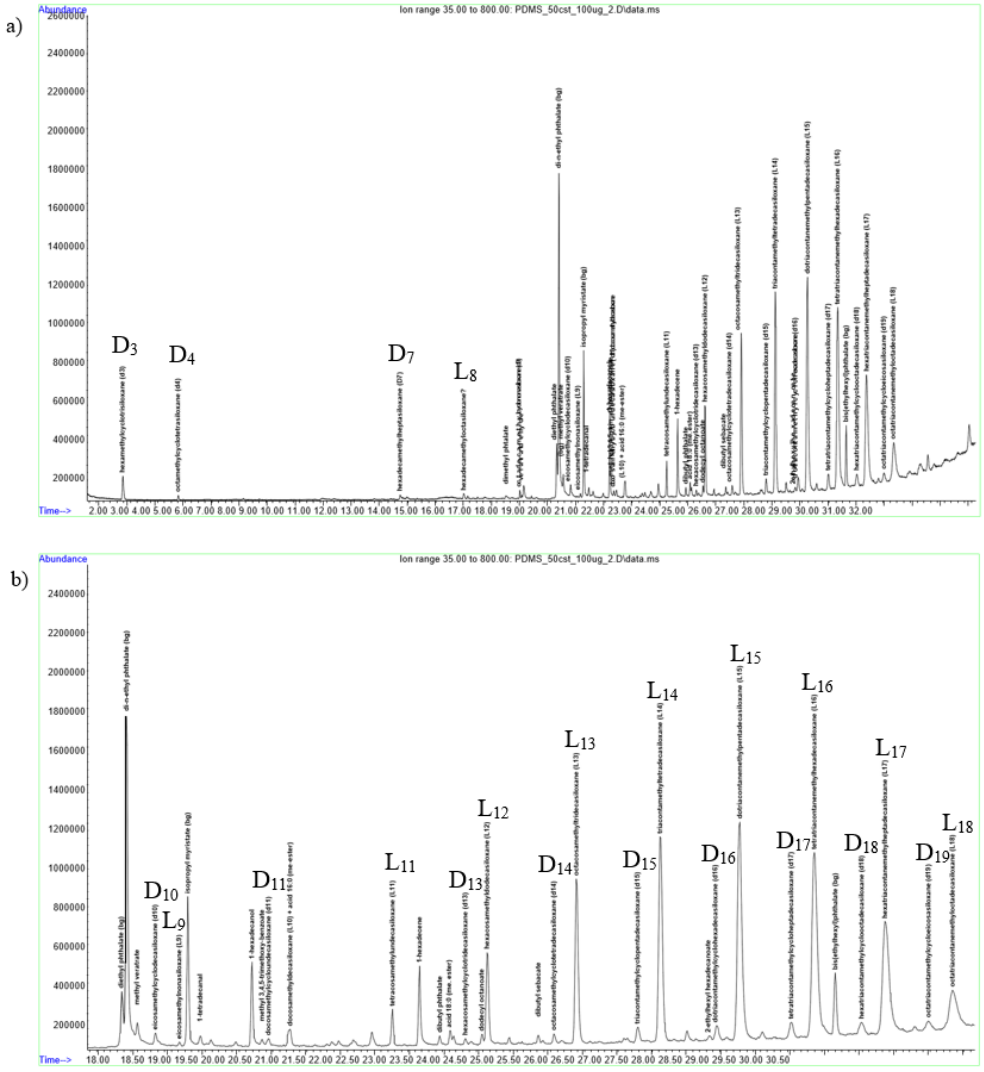


Figure 23 a) Complete and b) zoomed pyrogram obtained from 10 mg/ml 50 cSt PDMS in *n*-hexane. The most significant compounds have been abbreviated.

Pyrolysis performed on 1000 cSt PDMS in *n*-hexane generated signals from only cyclic compounds, ranging from D3 to D18, with D3 being the most abundant (Figure 24). In total, 1000 cSt PDMS generated signals from 16 compounds of interest. The high abundance of cyclic compounds might be due to 1000 cSt PDMS containing long-chain polymers which degrade easily into cyclic compounds, while the remaining long-chain polymers were not detected.

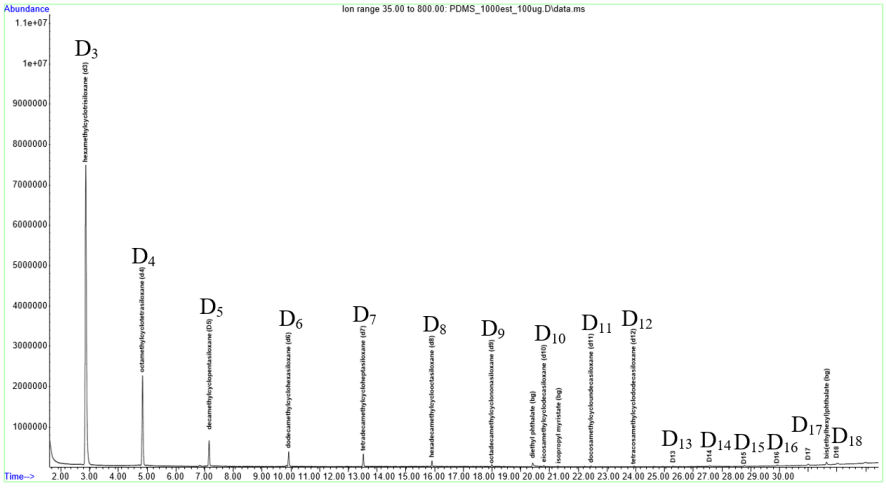


Figure 24 Pyrogram obtained from 10 mg/ml 1000 cSt PDMS in *n*-hexane.

5.2.2 Pyrolysis of bio-oil 1 and bio-oil 10 samples spiked with 5, 50 or 1000 cSt PDMS

Bio-oil samples of 10 mg/ml in *n*-hexane spiked with 20% 5, 50 or 1000 cSt PDMS in relation to the bio-oil were analyzed. Analysis of un-spiked bio-oil 1 in *n*-hexane generated mainly signals of sterols and diterpenoids, whereas analysis of un-spiked bio-oil 10 generated signals from linoleic acid, palmitic acid, squalene and sterols (Appendix C).

Figure 25a visualizes the pyrogram obtained from bio-oil 1 spiked with 20% 5 cSt PDMS. All linear compounds (L5-L18) which were detectable in pyrolysis of 5 cSt PDMS in *n*-hexane were also detectable in the pyrolysis of 5 cSt in bio-oil 1. This indicates that the matrix of bio-oil 1 does not interfere with 5 cSt PDMS. One cyclic compound (D6) was detected from the spiked sample, whereas for pure 5 cSt PDMS, there was no cyclic compound detected with pyrolysis. In Figure 25b, the pyrogram obtained from bio-oil 10 spiked with 20% 5 cSt PDMS is visualized. The pyrogram shows that all but the two largest compounds were detected compared to the 5 cSt in *n*-hexane and in bio-oil 1 (86% of the detectable compounds were detected in bio-oil 10). Bio-oil 10 seems to affect the detection of 5 cSt PDMS more compared to bio-oil 1.

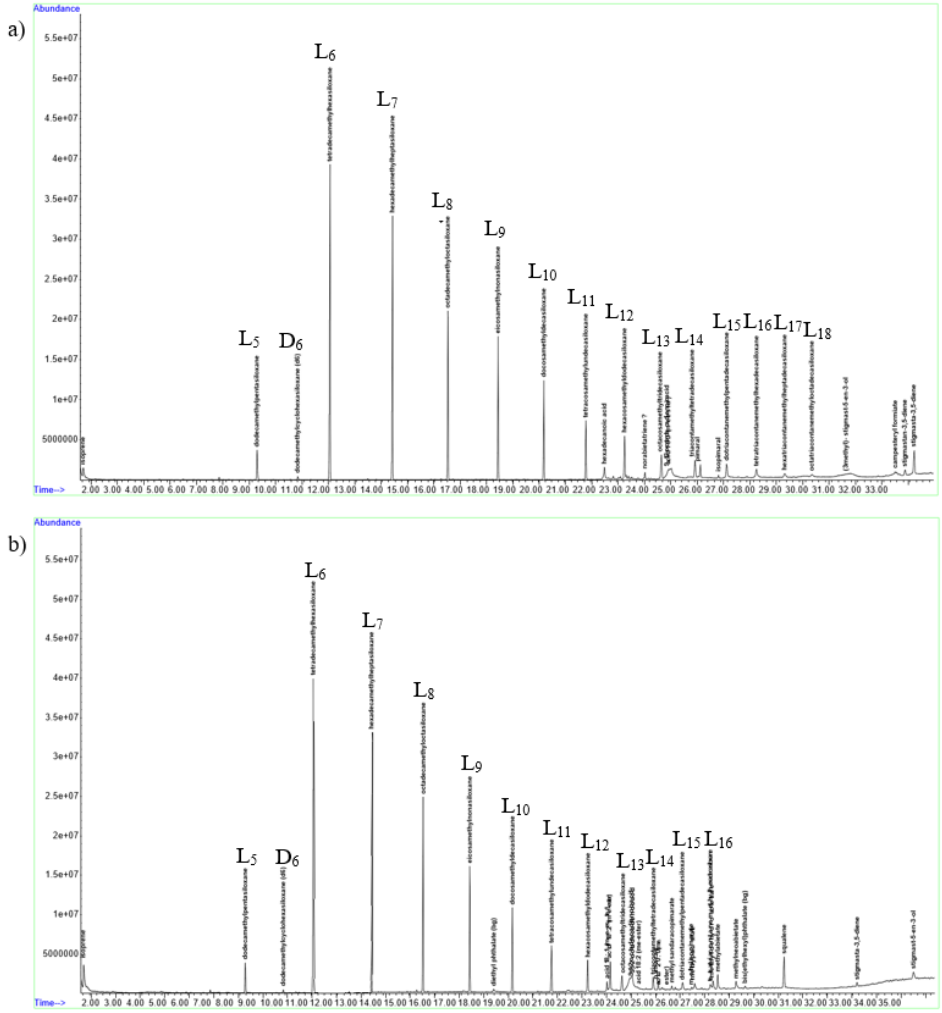


Figure 25 Pyrogram of a) 10 mg/ml bio-oil 1 in *n*-hexane spiked with 20% 5 cSt PDMS in relation to bio-oil 1 and b) 10 mg/ml bio-oil 10 in *n*-hexane spiked with 20% 5 cSt PDMS in relation to bio-oil 10.

The pyrogram obtained from bio-oil 1 spiked with 20% 50 cSt PDMS shows that signals from D3, D4 and L14–L18 were detected (Figure 26a). When comparing this to the detected compounds from 50 cSt in *n*-hexane, only 24% of the compounds were detected in the spiked bio-oil 1 samples (D7–D10, D13–D19 and L9–13 were not detected). The pyrogram implies that the detection of 50 cSt PDMS is significantly affected by the bio-oil 1 matrix. With pyrolysis of bio-oil 10 spiked with 20% 50 cSt PDMS, D3 and D4, as well as L12–L16, were detected (Figure 26b). The same percentage of compounds were detected in both spiked bio-oil samples, however, the bio-oil matrix seems to affect the detection of 50 cSt PDMS slightly differently. The bio-oil 1 matrix affects the middle eluting compounds the most, and all late eluting compounds were detectable. For bio-oil 10 the two largest compounds which elute late (L17–L18) were not detectable, but two more of the middle eluting compounds (L12–L13) were detectable, compared to the bio-oil 1 spiked sample.

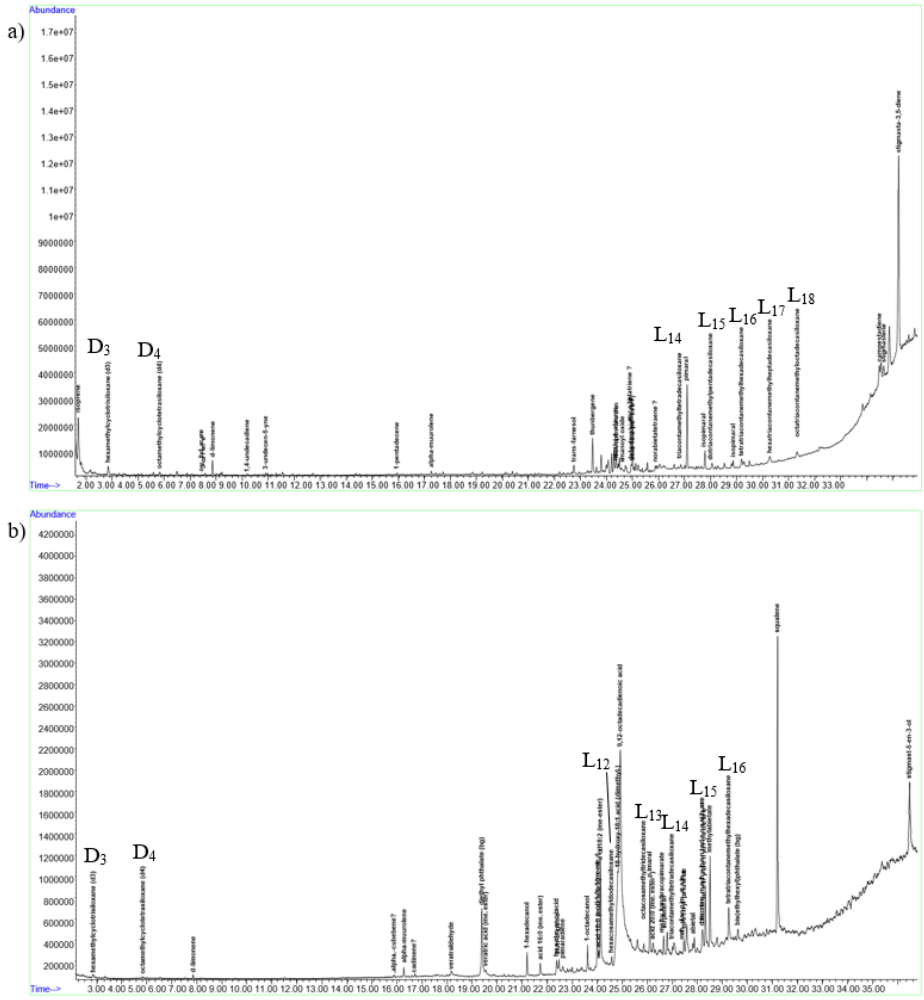


Figure 26 Pyrogram of a) 10 mg/ml bio-oil 1 in n-hexane spiked with 20% 50 cSt PDMS in relation to bio-oil 1 and b) 10 mg/ml bio-oil 10 in n-hexane spiked with 20% 50 cSt PDMS in relation to bio-oil 10**.**

The pyrograms obtained from pyrolysis of bio-oil 1 spiked with 20% 1000 cSt PDMS, and bio-oil 10 spiked with 20% 1000 cSt PDMS are visualized in Figure 27. The detected compounds of the bio-oil 1 spiked sample were D3–D7, which is only 25% of the total detected compounds of 1000 cSt PDMS in *n*-hexane. For the bio-oil 10 spiked sample, cyclic compounds up to D10 were detectable, therefore, the detected compounds were 50% of the compounds detected in 1000 cSt PDMS in *n*-hexane. Both the bio-oil matrices interfere with the detection of the larger cyclic compounds, with bio-oil 1 interfering more.

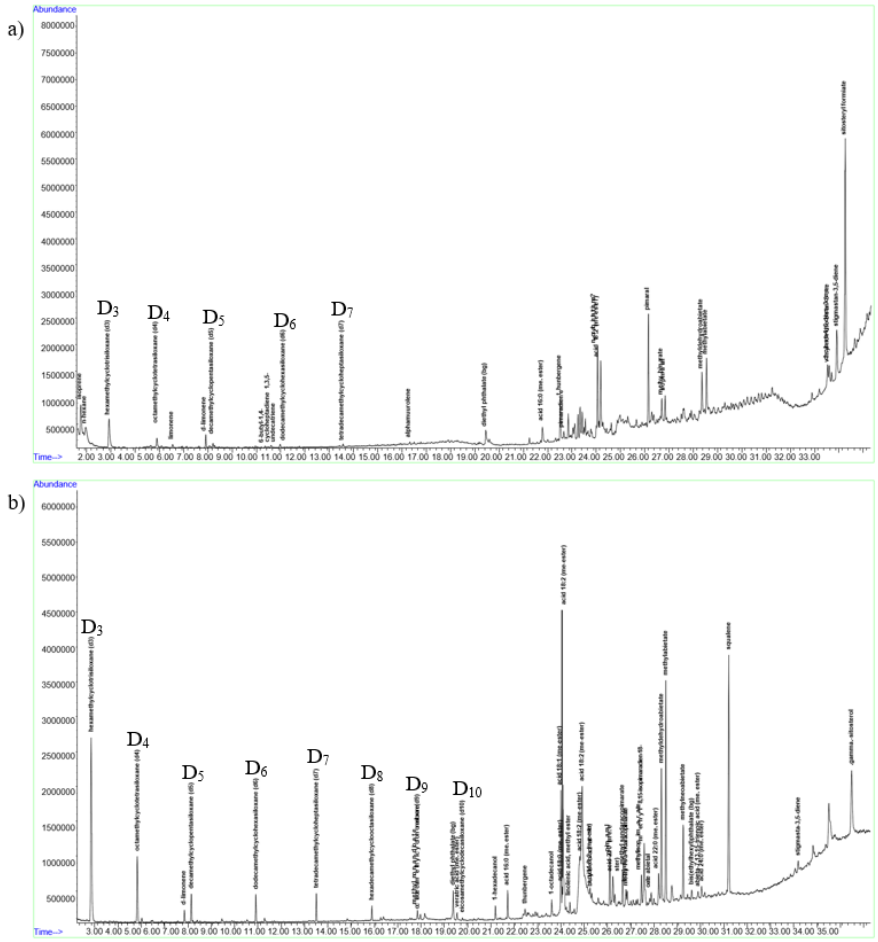


Figure 27 Pyrogram of a) 10 mg/ml bio-oil 1 in n-hexane spiked with 20% 1000 cSt PDMS in relation to bio-oil 1 and b) 10 mg/ml bio-oil 10 in n-hexane spiked with 20% 1000 cSt PDMS in relation to bio-oil 10.

Pyrolysis of bio-oil 1 spiked with 100 ppm PDMS was also carried out. The obtained pyrogram was similar to that of the pyrogram obtained when only analyzing bio-oil 1 in *n*-hexane. This indicates that low concentrations of PDMS, when spiked in bio-oil 1, are not detectable. The pyrolysis method used for these analyses seems to be most suitable for analyses of samples containing higher concentrations of PDMS.

5.3 RP-HPLC analyses of PDMS

5.3.1 Retention times and responses of 5, 50 and 1000 cSt PDMS

Separate samples of 5, 50 and 1000 cSt PDMS in THF (1mg/ml) were analyzed with RP-HPLC using ACN and THF as eluents. A sample of 1 mg/ml bio-oil 1 in THF was also analyzed to determine the elution of components in the matrix. The generated chromatograms of each analysis were superimposed for comparison (Figure 28).

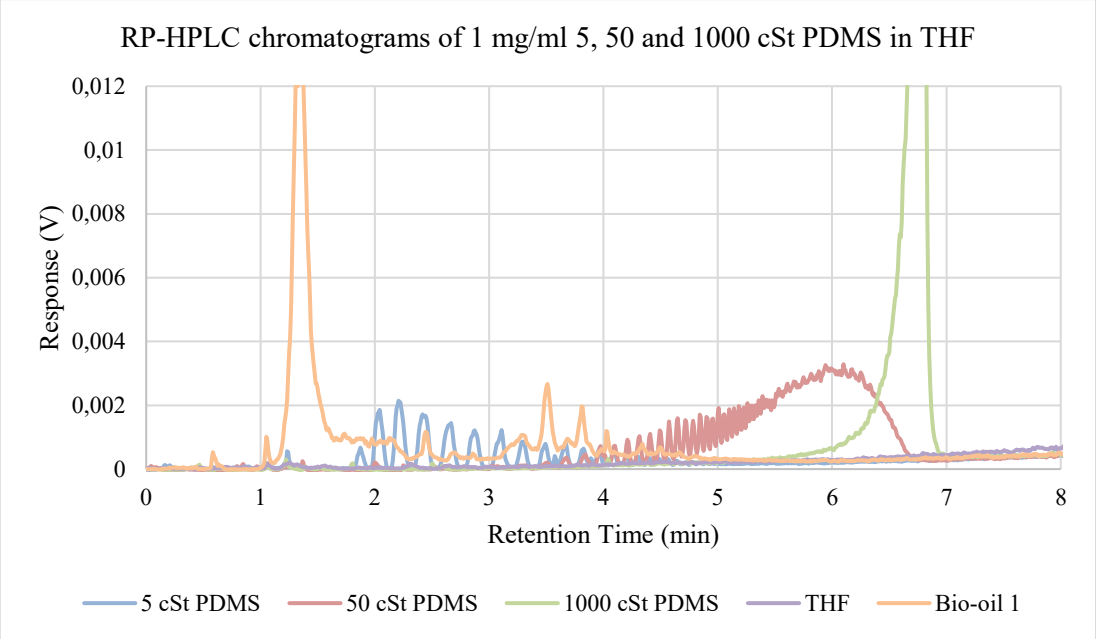


Figure 28 RP-HPLC chromatograms of 5, 50 and 1000 cSt PDMS (diluted in THF), bio-oil 1 and THF.

All three MW groups of PDMS were detectable with the RP-HPLC method, with 1000 cSt PDMS being the most abundant. The detection of 5 cSt PDMS occurred at a retention time between ca 2 min and 4 min, between ca 3.5 min and 6.5 min for 50 cSt and 1000 cSt, the retention time was between ca 5.5 and 7 min. For the 5 and 50 cSt PDMS, a clear separation of components within the MW groups was detected. These noticeable separations show that RP-HPLC would be a promising technique for separation studies of PDMS. Components of the bio-oil 1 matrix eluted between 1 min and 5 min. The largest peak in the bio-oil 1 chromatogram was generated by the early eluting polar components. As 5 cSt PDMS eluted between 2 and 4 minutes, it would be most affected by the bio-oil matrix. Bio-oil 1 components also overlapped with the early eluting 50 cSt components and no overlapping of the components for 1000 cSt PDMS occurred.

Samples of 1 mg/ml 5, 50 and 1000 cSt PDMS in EtOAc were also analyzed, with ACN and EtOAc as eluents. One sample of all MW groups in the same sample was analyzed as well as one of 50 cSt PDMS and one of 1000 cSt PDMS. All obtained chromatograms were superimposed and are illustrated in Figure 29.

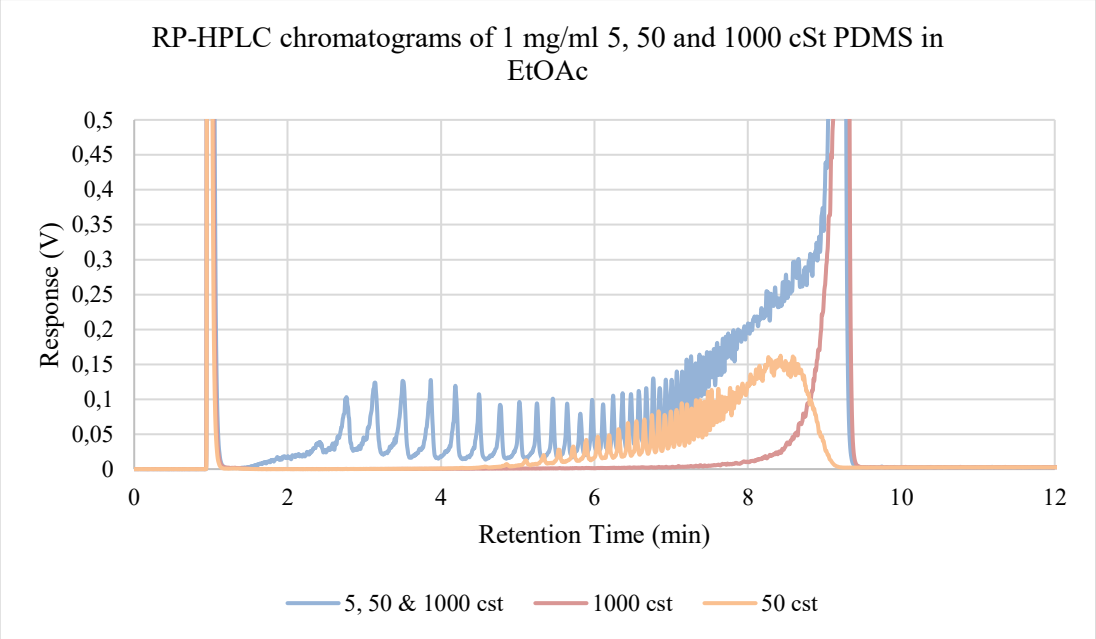


Figure 29 RP-HPLC chromatograms of 5, 50 and 1000 cSt PDMS diluted in EtOAc as well as separate samples of 50 and 1000 cSt PDMS.

With ACN and EtOAc as eluents, and EtOAc as sample solvent, the analytes eluted later than with THF instead of EtOAc. When comparing the sample of all MW groups of PDMS in the same sample and with separate analyses of 50 and 1000 cSt PDMS, the retention times seem not to differ. There was no separation of each MW group when analyzing them from the same sample, however, a clear separation of components within 5 cSt was still visible and with higher MW, the separation of the component decreased. If the retention time of each MW PDMS is known beforehand, the compounds could be distinguished when analyzing them in the same sample. As THF produced more background noise, EtOAc was chosen as the eluent for the rest of the analyses.

5.3.2 Limit of detection of pure 50 cSt and 1000 cSt PDMS in EtOAc

Concentrations of 1000 cSt PDMS in EtOAc between 0.00015 mg/ml and 1 mg/ml were analyzed for the limit of detection studies. Concentrations above 0.012 mg/ml were detectable with the sensitivity of gain 1, and for the lower concentrations, gain 4 was used for the detection. Figure 30 shows the chromatograms of the samples analyzed with gain 1 and Figure 31 shows the chromatograms of the samples analyzed with gain 4.

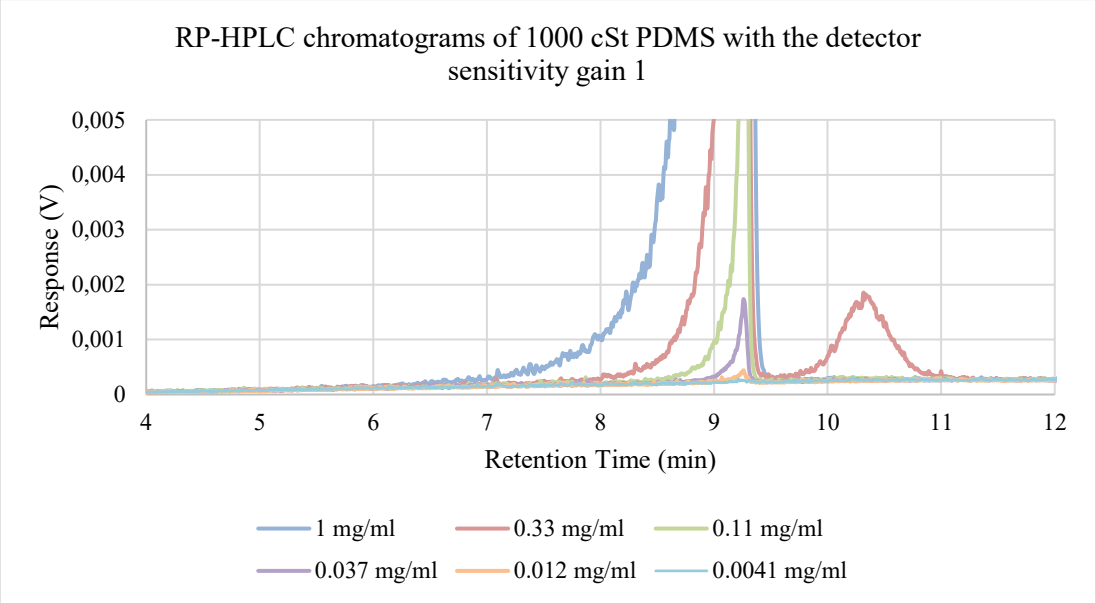


Figure 30 RP-HPLC-ELSD chromatograms of 1 mg/ml to 0.0041 mg/ml 1000 cSt PDMS in EtOAc analyzed with detector sensitivity gain 1.

With the sensitivity gain 1, even the concentration of 0.012 mg/ml 1000 cSt PDMS was detectable. The red peak between 9.5 min and 11 min belongs to a contaminnt. It was reported by Holmberg that the practical LOD for HP-SEC analyses of 1000 cSt PDMS was 0.11 mg/ml with the detection sensitivity gain 3, which corresponds to gain 1 of the ELSD utilized in this work. However, Arandia was able to detect concentrations down to 0.012 mg/ml with the same sensitivity as Holmberg. These results show that the sensitivity of RP-HPLC-ELSD and HP-SEC-ELSD is similar.

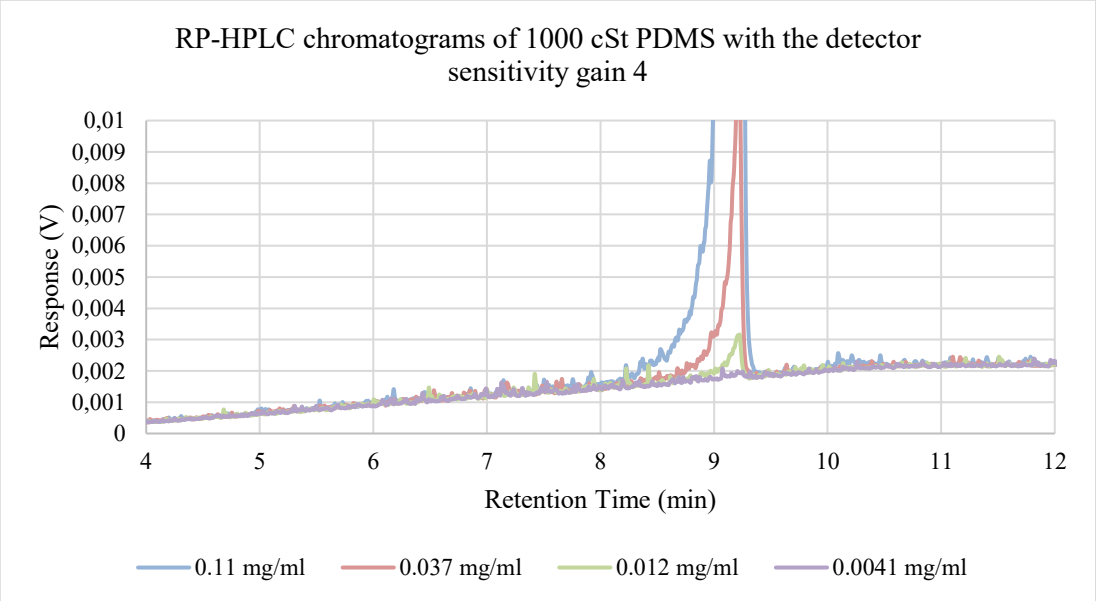


Figure 31 RP-HPLC-ELSD chromatograms of 0.11 mg/ml to 0.0041 mg/ml 1000 cSt PDMS in EtOAc analyzed with detector sensitivity gain 4.

With the sensitivity gain 4, the lowest detectable concentration was 0.012 mg/ml, which is the same as the LOD with the sensitivity of gain 1. The reason why lower concentration were no detected could be that as the detection sensitivity was increased the background noise levels became higher. When comparing the chromatograms of Figure 30 and Figure 31, the difference in the response of the background noise is noticeable. At the detection sensitivity gain 1, the background noise has the response of below 0.0005 V and for gain 4 the response is between 0.003 and 0.0005 V. With HP-SEC, Holmberg was able to detect concentrations as low as 0.0041 mg/ml with the sensitivity gain 6, which correspond to gain 4 of this work. Arandia was able to detect the concentration of 0.001 mg/ml with gain 6. These results show that with higher detector sensitivity, the HP-SEC-ELSD instrument that Arandia and Holmberg used could detect lower concentrations compared to the RP-HPLC-ELSD instrument used in this work.

The same set of concentrations was analyzed for LOD studies of 50 cSt PDMS in EtOAc. For these analyses, only detection sensitivity gain 1 was utilized. The obtained chromatograms of the concentrations are visualized in Figure 32.

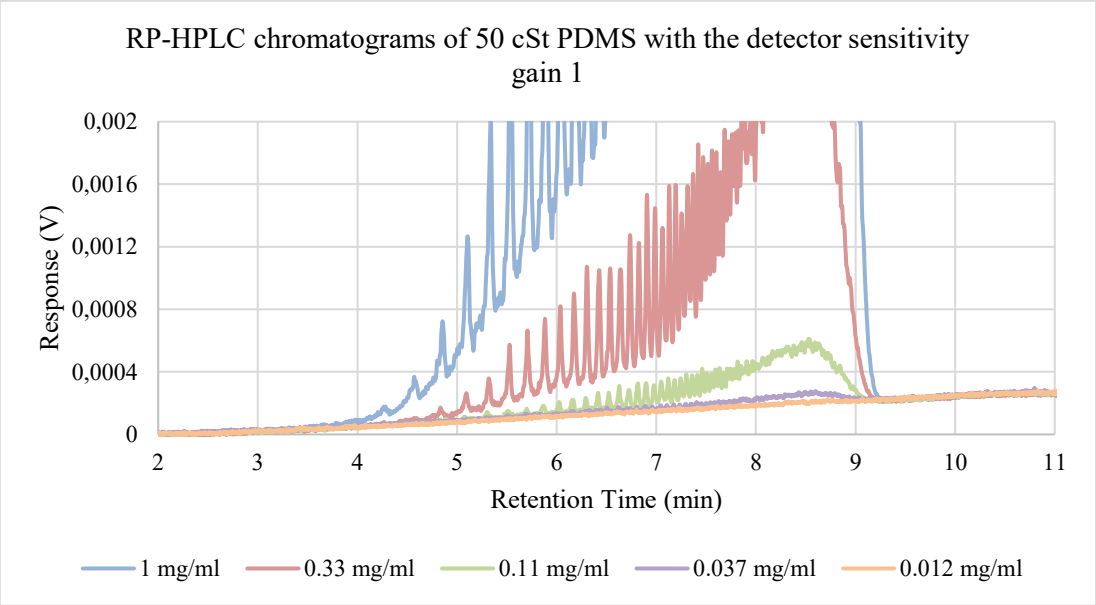


Figure 32 RP-HPLC-ELSD chromatograms of 1 mg/ml to 0.012 mg/ml 50 cSt PDMS in EtOAc analyzed with detector sensitivity gain 1.

With the detection sensitivity gain 1, 0.037 mg/ml 50 cSt PDMS in EtOAc was detectable with a low response. As expected, the LOD for 50 cSt PDMS is higher than for 1000 cSt PDMS, since the detector response is lower for 50 cSt PDMS.

5.3.3 Bio-oil 1 spiked with 5, 50 and 1000 cSt PDMS

Bio-oil 1 samples with the concentration of 1 mg/ml diluted in EtOAc, spiked with 10% PDMS in relation to bio-oil 1 were analyzed with RP-HPLC. A sample of 1 mg/ml bio-oil 1 diluted in EtOAc spiked with 10% of each MW PDMS in one sample was also analyzed.

The chromatograms in Figure 33 show that the determination of 5 cSt PDMS is not possible when spiked in bio-oil 1 samples. As stated in section 5.3.1., components of the bio-oil 1 matrix and 5 cSt PDMS elutes at the same time, and the interference of these analytes makes it impossible to identify 5 cSt PDMS when spiked in bio-oil 1 at this concentration and lower. The peak with the retention time at ca 9.5 min in Figure 33, is generated from a contaminant and should not be taken into consideration, as the contaminant is not visible in other chromatograms.

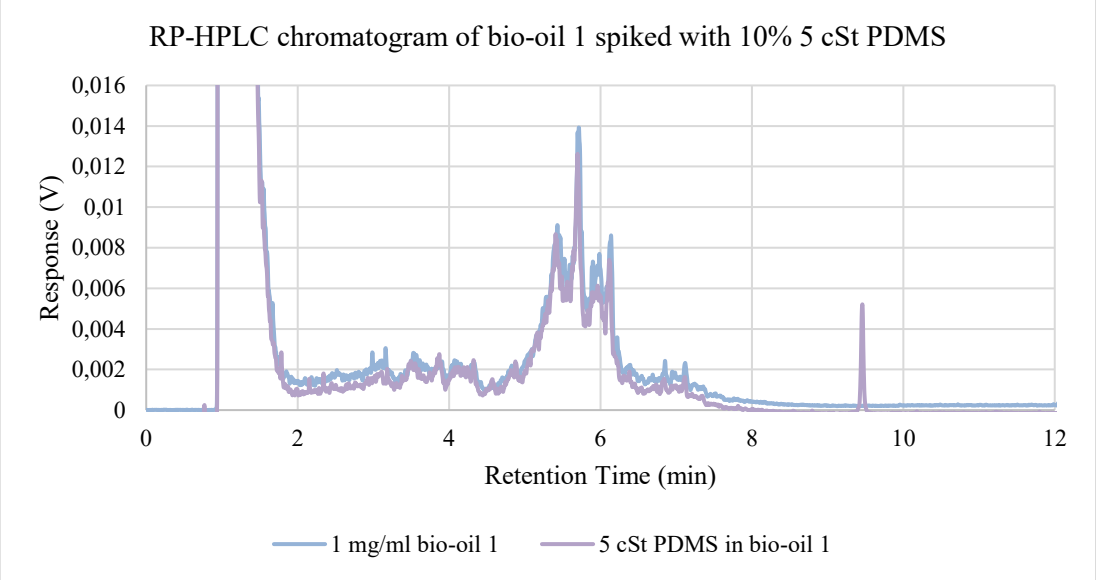


Figure 33 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 1 and 1 mg/ml bio-oil 1 spiked with 10% 5 cSt PDMS in relation to bio-oil 1.

The two superimposed chromatograms of 1 mg/ml bio-oil 1, and bio-oil 1 spiked with 10% 50 cSt PDMS are similar, however, with a slight difference in the response between 7 and 9 min (Figure 34). The chromatogram of spiked bio-oil 1 has a response in this area, whereas the response of only bio-oil 1 is the same as the baseline. This indicates that the 50 cSt PDMS is partly separatable from the bio-oil 1 matrix.

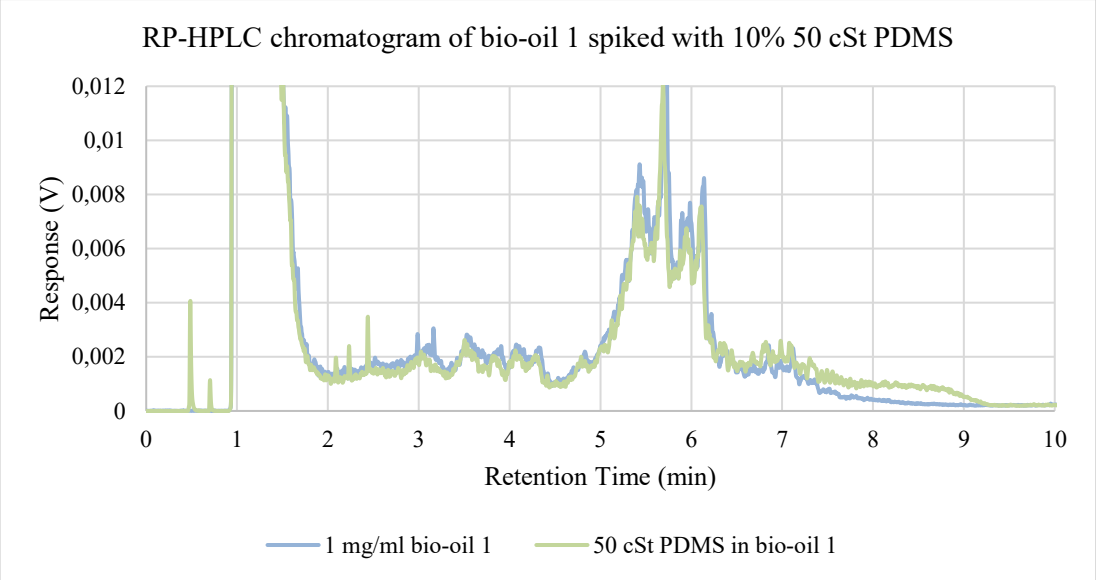


Figure 34 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 1 and 1 mg/ml bio-oil 1 spiked with 10% 50 cSt PDMS in relation to bio-oil 1.

From the superimposed chromatograms of 1 mg/ml bio-oil 1 and the bio-oil spiked with 10% 1000 cSt PDMS, a clear separation of 1000 cSt PDMS and the bio-oil matrix can be seen (Figure 35). It can be stated that there is no significant interference of the bio-oil 1 components and 1000 cSt PDMS.

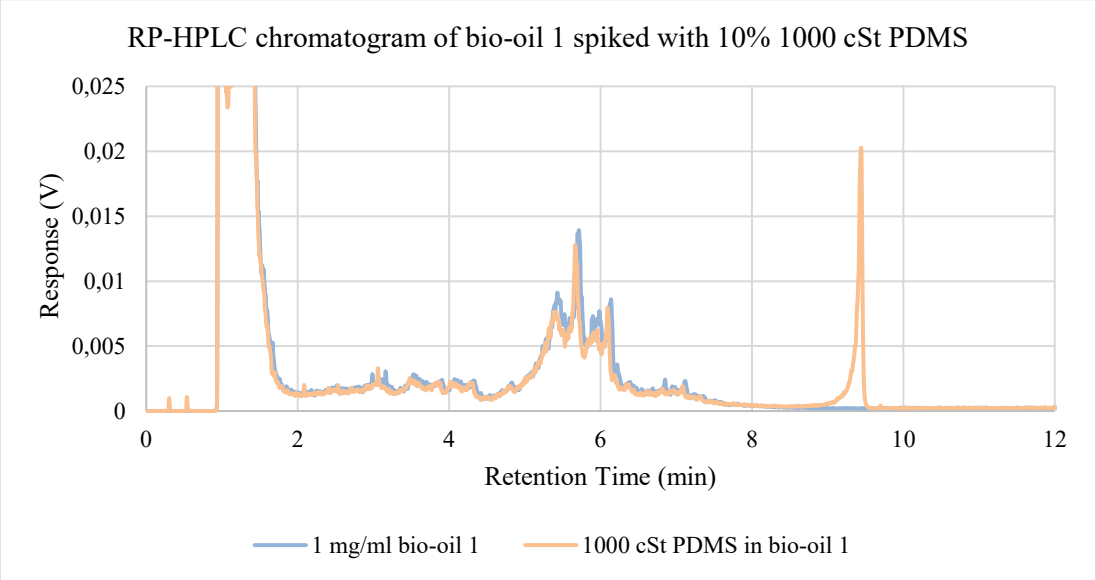


Figure 35 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 1 and 1 mg/ml bio-oil 1 spiked with 10% 1000 cSt PDMS in relation to bio-oil 1.

When superimposing chromatograms of 1 mg/ml bio-oil 1 and 1 mg/ml bio-oil 1 spiked with 5, 50 and 1000 cSt PDMS, a clear separation of the 1000 cSt PDMS from the bio-oil 1 matrix can be observed, with decreasing separation of the 50 cSt and no separation of the 5 cSt (Figure 36).

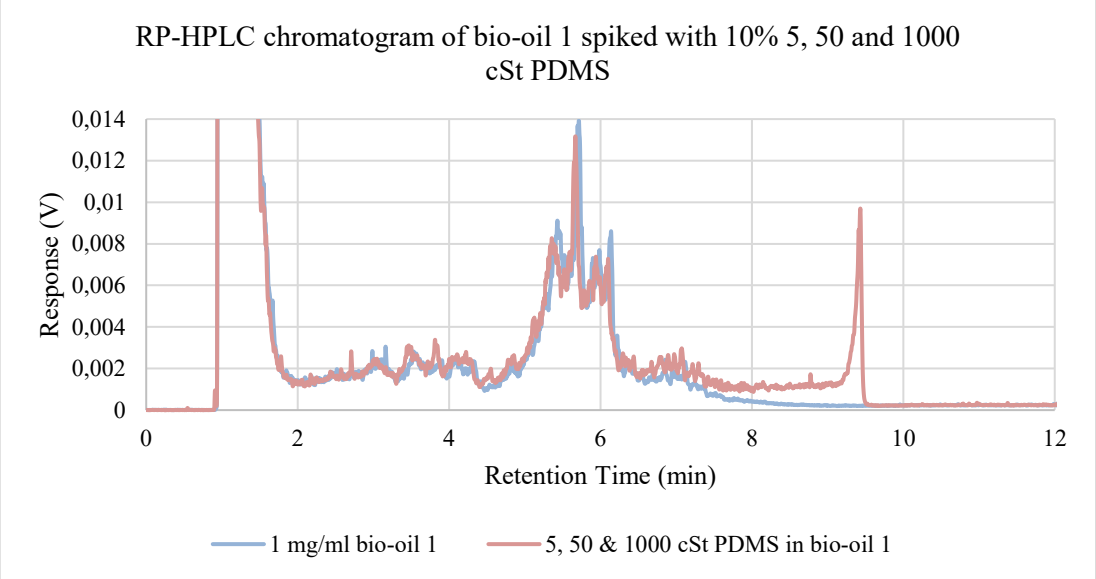


Figure 36 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 1 and 1 mg/ml bio-oil 1 spiked with 10% 5, 50 and 1000 cSt PDMS in relation to bio-oil 1.

5.3.4 Bio-oil 10 spiked with 5, 50 and 1000 cSt PDMS

Analyses of 1 mg/ml bio-oil 10 in EtOAc and bio-oil 10 spiked with 10% 5, 50 and 1000 cSt PDMS in relation to the bio-oil, were performed in the same fashion as for spiked bio-oil 1 samples (section 5.3.3). Figure 37–40 shows chromatograms obtained from analyses of both individual analyses of each MW group, as well as analyses of all MW groups in the same sample.

When comparing chromatograms obtained from 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 5 cSt PDMS, there is no significant difference between the chromatograms (Figure 37). As for bio-oil 1, the interference of bio-oil 10 components was total with 5 cSt PDMS, showing that there was no separation of 5 cSt PDMS from the bio-oil 10 matrix. One difference between the bio-oil 1 matrix (Figure 33) and bio-oil 10 was that the interfering components of bio-oil 10 (retention time ca 4–6 min) eluted later than that of the interfering components of bio-oil 1 (retention time ca 4.5–7 min).

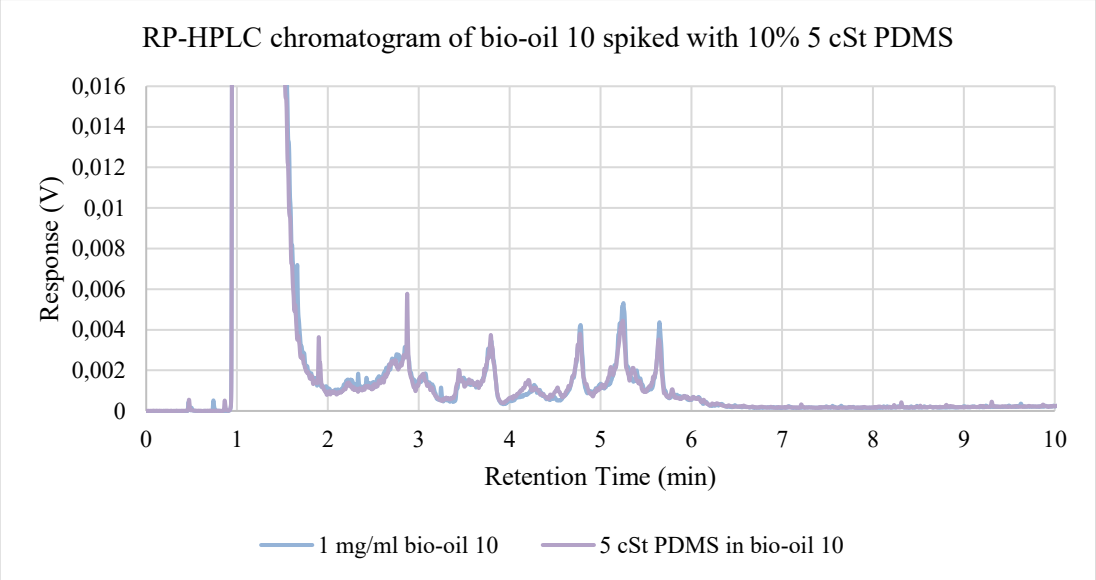


Figure 37 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 5 cSt PDMS in relation to bio-oil 10.

The comparison of chromatograms obtained from 1 mg/ml bio-oil 10 to 1 mg/ml bio-oil 10 spiked with 10% 50 cSt PDMS, indicated that there is a separation of 50 cSt PDMS from bio-oil 10 (Figure 38). Between 6 min and 9.5 min, the response of the analytes differed. The response of 50 cSt PDMS in bio-oil 10 was lower than the response of 50 cSt PDMS in bio-oil 1 (Figure 34). There was a slight difference in the interference of bio-oil 10 and bio-oil 1 with 50 cSt PDMS, which was due to bio-oil 10 components eluting earlier compared to interfering components of bio-oil 1. For bio-oil 1, the separation of 50 cSt PDMS from bio-oil 1 occurs after 7 min, whereas the separation occurs after 6 min from bio-oil 10. The sharp signal after 9 min was generated from contamination and should not be considered.

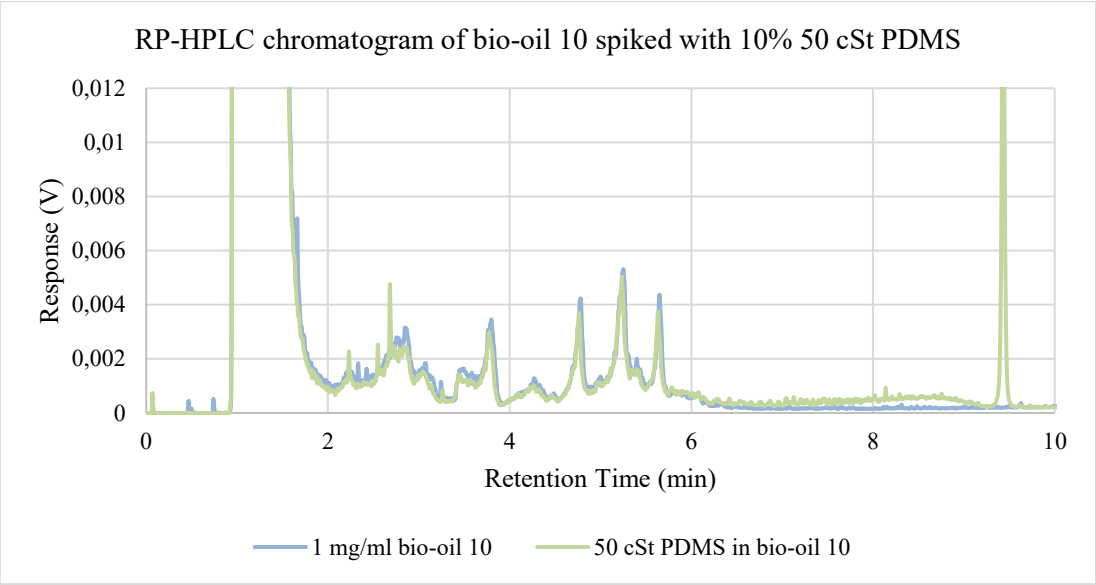


Figure 38 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 50 cSt PDMS in relation to bio-oil 10.

There is a clear separation of 1000 cSt PDMS from the bio-oil 10 matrix, which can be seen when comparing the chromatograms of 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 1000 cSt (Figure 39). This result is very similar to that of the separation of 1000 cSt PDMS from bio-oil 1 (Figure 35).

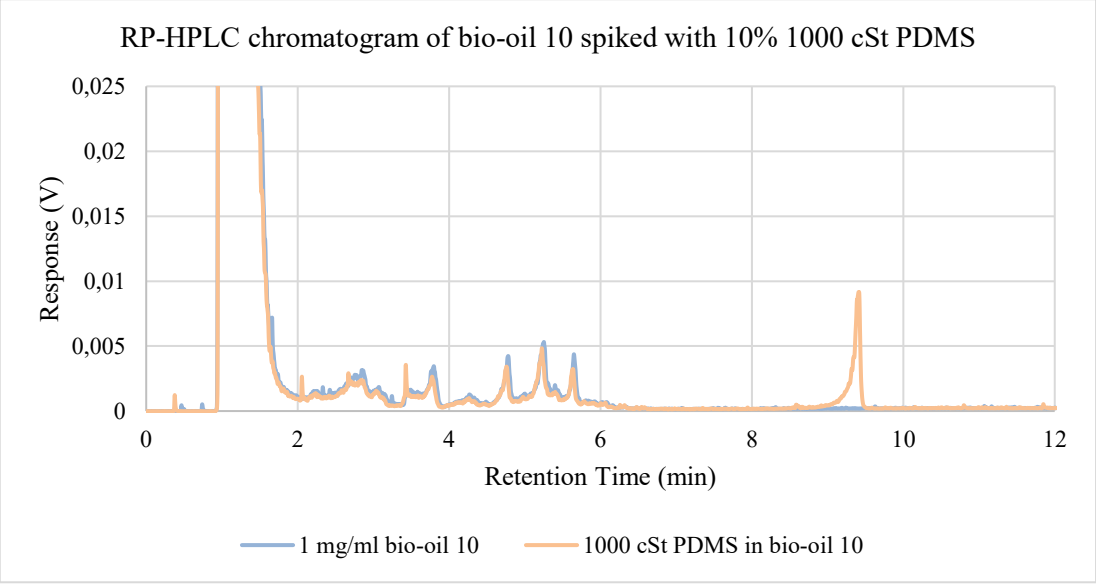


Figure 39 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 1000 cSt PDMS in relation to bio-oil 10.

The chromatogram obtained from the analysis of 1 mg/ml bio-oil 10 spiked with 10% 5, 50 and 1000 cSt PDMS shows the same result as the chromatograms obtained from each separate analysis of the MW groups (Figure 40). The interference of the bio-oil 10 matrix and 5 cSt PDMS is total, with a decreasing interference for 50 cSt PDMS and no interference with 1000 cSt PDMS.

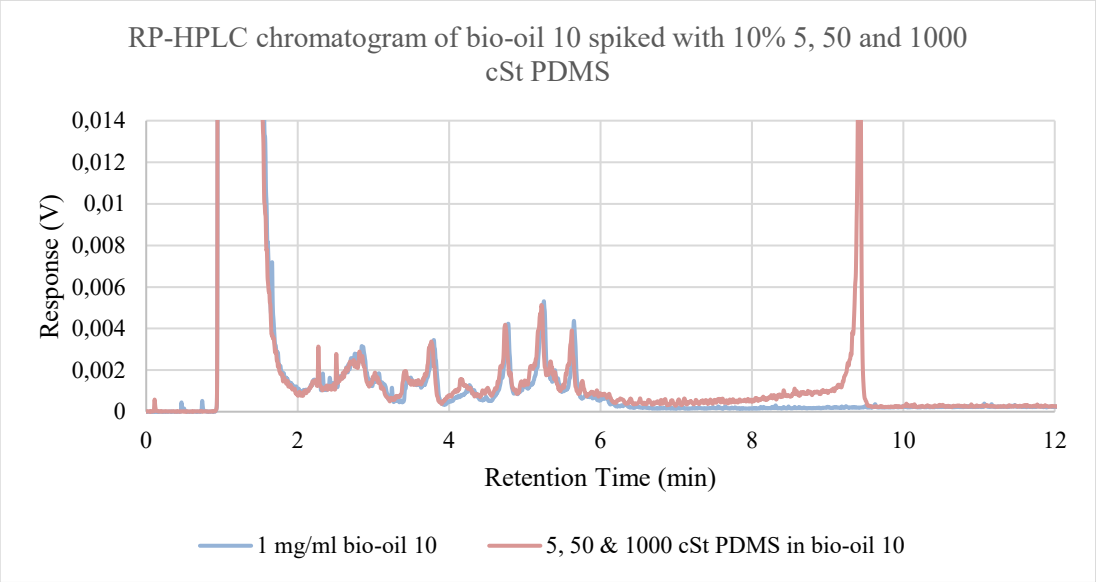


Figure 40 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 10 and 1 mg/ml bio-oil 10 spiked with 10% 5, 50 and 1000 cSt PDMS in relation to bio-oil 10.

5.3.5 Bio-oil 2 spiked with 5, 50 and 1000 cSt PDMS

RP-HPLC analyses of bio-oil 2 spiked with 10% 5, 50 and 1000 cSt PDMS gave similar results as for the same analyses of spiked bio-oil 1 and bio-oil 10 samples. The bio-oil 2 matrix interfered with 5 cSt PDMS, with a decreasing interference with 50 cSt and no interference with 1000 cSt. Similar to the bio-oil 10 matrix, the interfering components of bio-oil 2 eluted earlier than that of the bio-oil 1 components and did, therefore, interfere less with the 50 cSt PDMS. Obtained chromatograms of bio-oil 2 spiked with 10 % 5, 50 and 1000 cSt PDMS in the same sample and 1 mg/ml bio-oil 2 in EtOAc are illustrated in Figure 41.

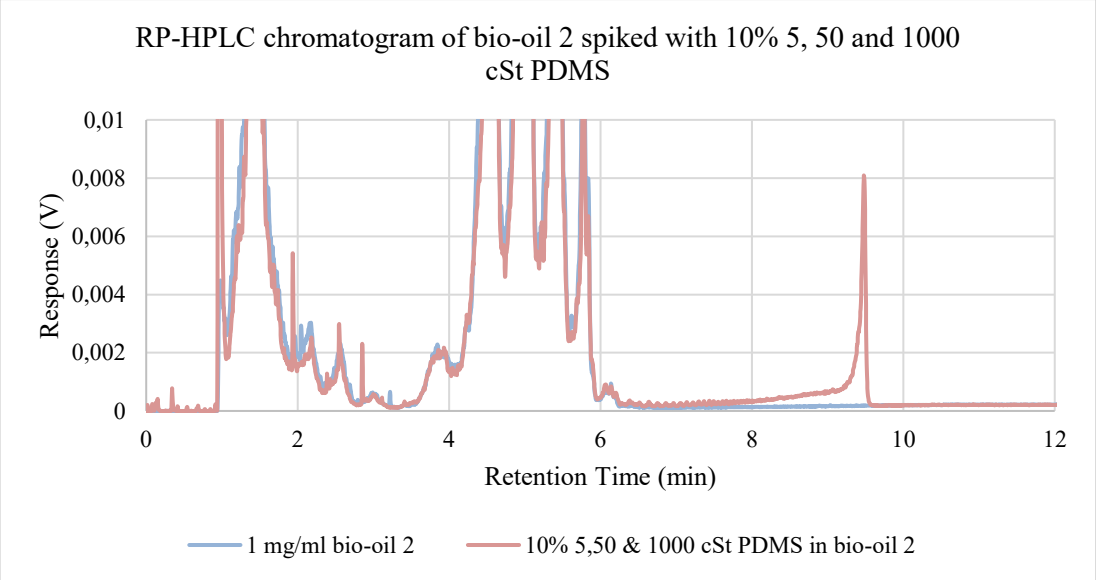


Figure 41 Comparison of RP-HPLC chromatograms of 1 mg/ml bio-oil 2 and 1 mg/ml bio-oil 2 spiked with 10% 5, 50 and 1000 cSt PDMS in relation to the bio-oil.

5.3.6 LOD studies of 1000 cSt PDMS when spiked in bio-oil matrices

Due to 1000 cSt PDMS having a significant separation from the bio-oil matrices and having the best response out of the three different PDMS MW groups, further LOD studies of spiked bio-oil samples with 1000 cSt PDMS were performed. The concentration of each sample was 1 mg/ml bio-oil spiked with 0.25–10% 1000 cSt PDMS.

The chromatograms obtained from the LOD studies of 1000 cSt PDMS spiked in bio-oil 1, are shown in Figure 42. The lowest detected concentration was shown to be 0.5% 1000 cSt PDMS in relation to bio-oil 1. There is a slight increase in the detected signal of 0.5% 1000 cSt PDMS, whereas for 0.25% 1000 cSt PDMS, there is no signal in the same area. It should be noted that the detector was cleaned prior to the bio-oil 1 analyses and, therefore, the baseline is much cleaner compared to the following LOD analyses of bio-oil 2 and bio-oil 10.

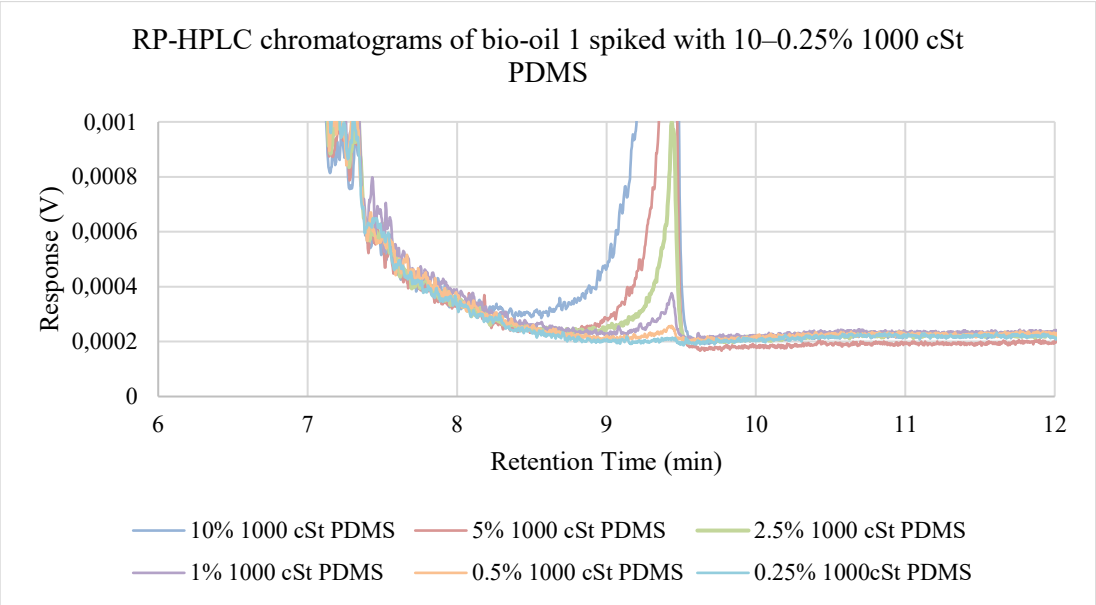


Figure 42 Chromatograms of LOD studies of bio-oil 1 spiked with 0.25–10% 1000 cSt PDMS.

The chromatograms of the LOD studies of bio-oil 10 spiked with 1000 cSt PDMS, show the noisier background level compared to the bio-oil 1 analyses (Figure 43). With certainty, it can be determined that 1% 1000 cSt PDMS is detectable. If examining the chromatogram obtained from 0.5% PDMS, there is a weak signal in the area where 1000 cSt PDMS elutes. However, the chromatogram has a noisier background and, therefore, it cannot be excluded that the peak where 1000 cSt elutes could be background noise rather than a response of the detection of 1000 cSt PDMS.

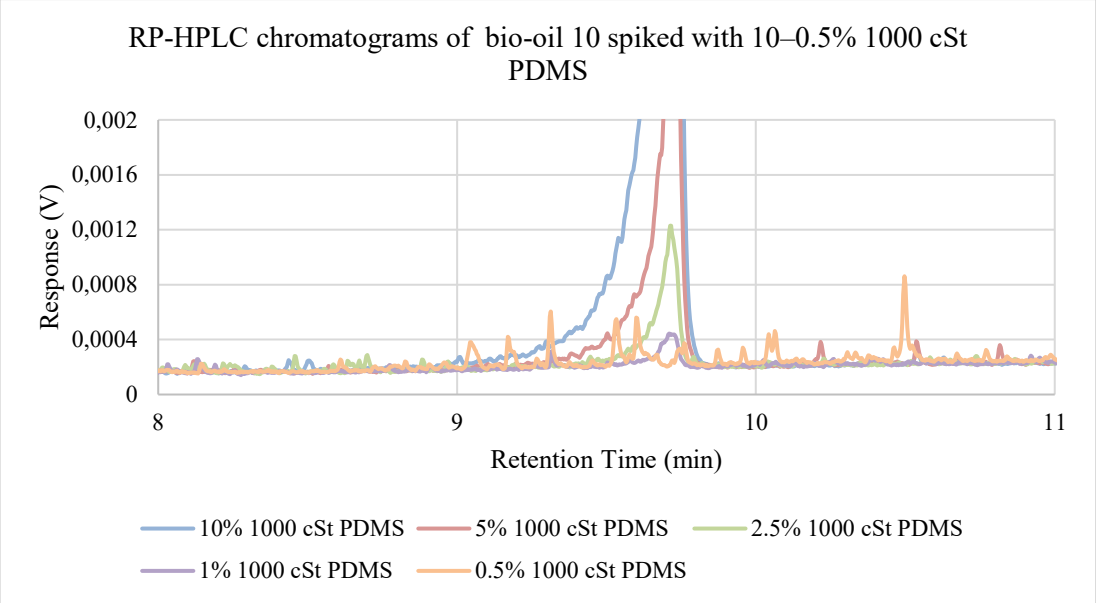


Figure 43 Chromatograms of LOD studies of bio-oil 10 spiked with 0.5–10% 1000 cSt PDMS.

The chromatograms obtained from LOD studies of bio-oil 2 spiked with 1000 cSt PDMS show similar results as for bio-oil 10 analyses (Figure 44). For 1% 1000 cSt PDMS, a clear signal was detected where 1000 cSt PDMS elutes, and for 0.5% 1000 cSt there was no response. This indicated that the LOD for bio-oil 2 spiked samples, was at 1% 1000 cSt PDMS in relation to bio-oil 2.

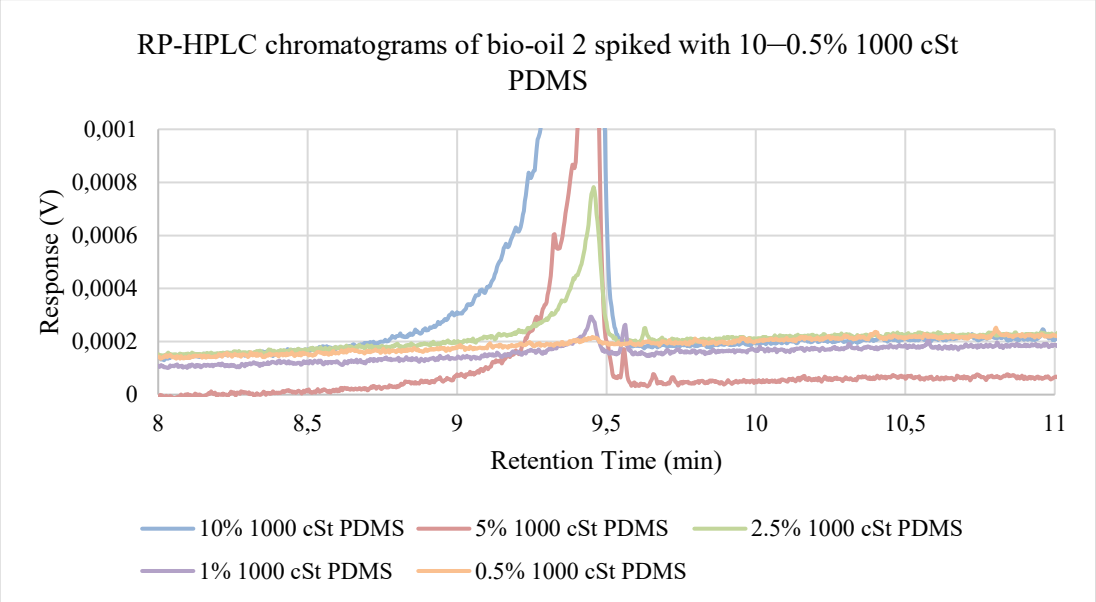


Figure 44 Chromatograms of LOD studies of bio-oil 2 spiked with 0.5–10% 1000 cSt PDMS.

5.3.7 Analyses of spiked bio-oil samples after solvent extraction

Solvent extraction was performed on each bio-oil spiked with 10% 1000 cSt PDMS, to examine any changes in the detected components of bio-oil 1 and PDMS. EtOAc was used as the polar solvent and *n*-hexane as the non-polar solvent for the extraction. As PDMS is non-polar, the *n*-hexane phase was analyzed. The concentration of the sample after the extraction was 1 mg/ml bio-oil spiked with 10% 1000 cSt PDMS. Naturally, as the interfering components of the bio-oil matrices elute close to PDMS, non-polar components were collected in the *n*-hexane phase. Therefore, the only difference between extracted bio-oil samples spiked with PDMS and non-extracted is the polar component of the bio-oil matrices, which elutes at early retention times. Superimposed chromatograms of extracted and non-extracted bio-oil samples are visualized in Figure 45–47. The injected concentration of each sample was the 1 mg/ml bio-oil in EtOAc spiked with 10% 1000 cSt PDMS.

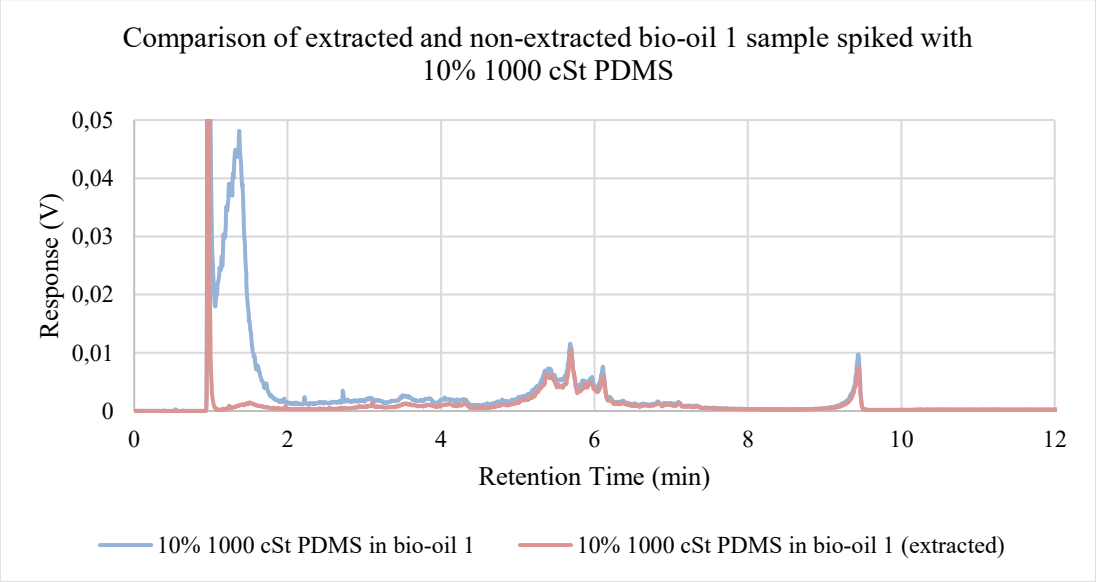


Figure 45 Chromatograms obtained from RP-HPLC analyses of extracted and non-extracted bio-oil 1 samples spiked with 10% 1000 cSt PDMS in relation to bio-oil 1.

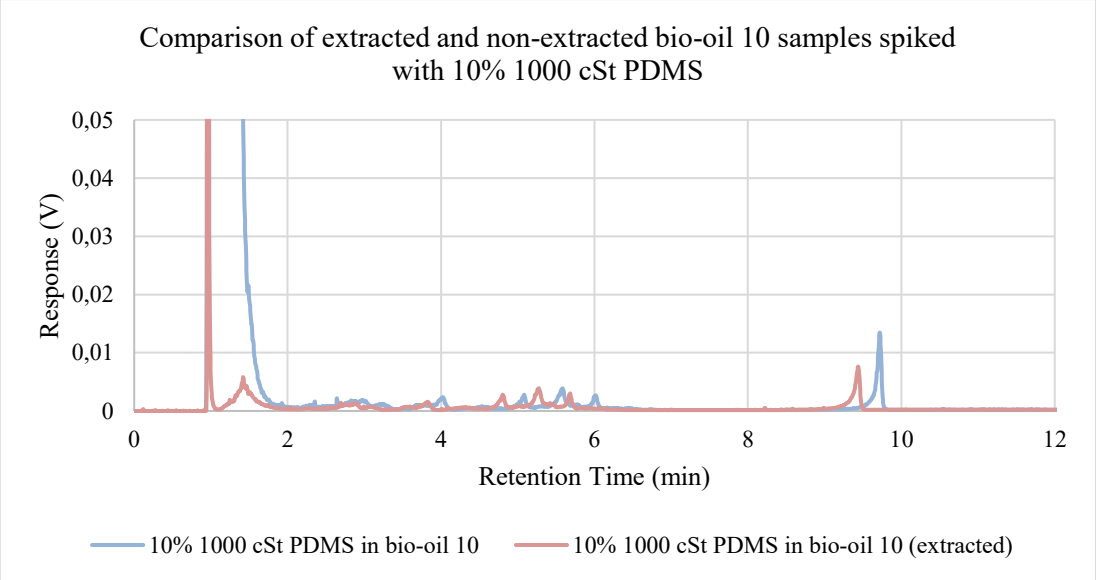


Figure 46 Chromatograms obtained from RP-HPLC analyses of extracted and non-extracted bio-oil 10 samples spiked with 10% 1000 cSt PDMS in relation to bio-oil 10.

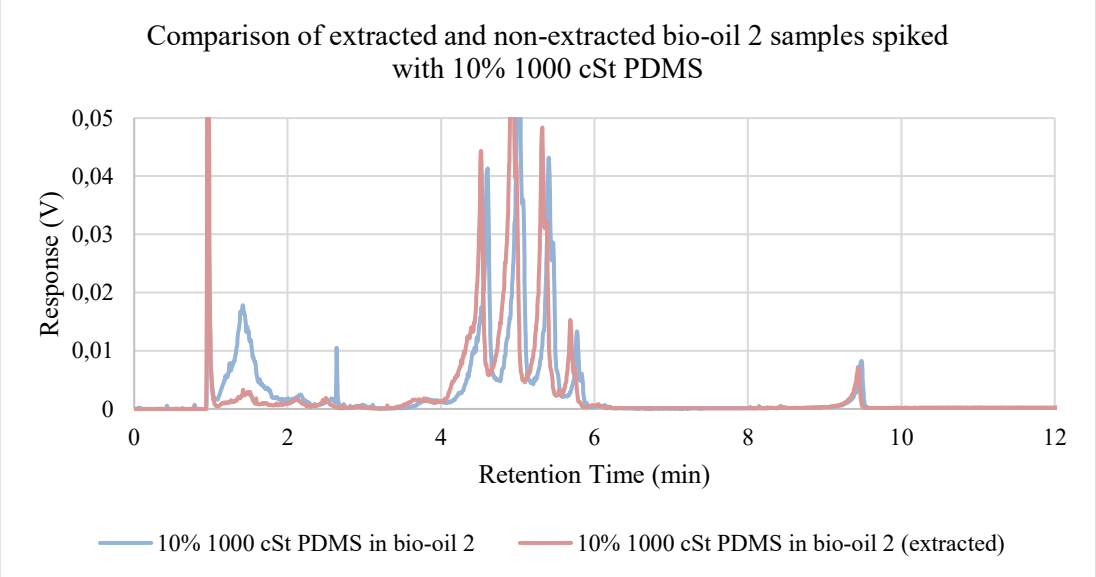


Figure 47 Chromatograms obtained from RP-HPLC analyses of extracted and non-extracted bio-oil 2 samples spiked with 10% 1000 cSt PDMS in relation to bio-oil 2.

As already described, the only difference in the extracted and non-extracted samples are the polar components which elute before 2 min for each bio-oil. In Figure 46, there is a difference in the retention times of the analytes, which is not a result of the extraction, but a drift in the retention times. If the chromatograms would be completely superimposed, the chromatograms would be almost identical to one another after 2 min. This indicates that

solvent extraction is not sufficient to improve the limit of quantification of PDMS when spiked in bio-oil matrices. Proper sample cleanup, such as SPE or preparative HPLC should be tested.

5.4 Normal-phase flash chromatographic analyses of PDMS

5.4.1 TLC studies as eluent combination reference

TLC was performed on D3, D4, D5 and 50 cSt PDMS with different combinations of MeOH, DCM, EtOAc and *n*-hexane, to evaluate suitable eluents for automated normal-phase flash chromatography. Figure 48 shows a typical obtained TLC result, as all solvent combinations gave similar results.

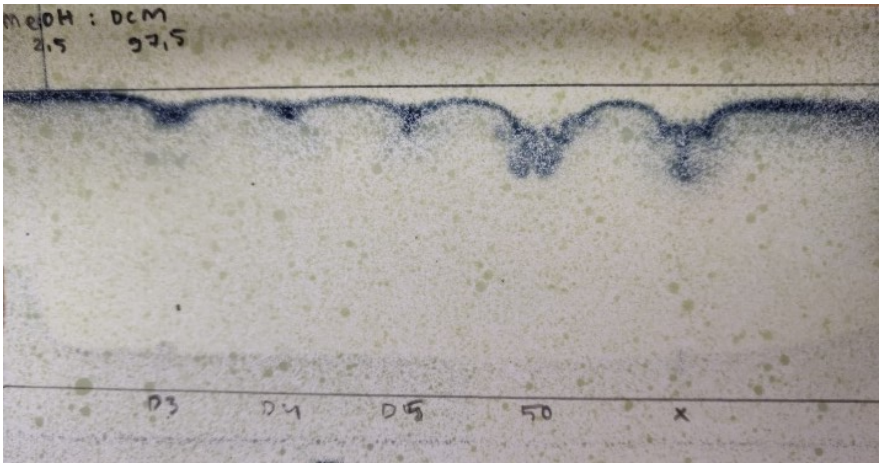


Figure 48 TLC plate of D3, D4, D5 and 50 cSt PDMS with 2.5% MeOH and 97.5% DCM as eluents.

For all compounds, there was no interference with the silica stationary phase, except for slight retardation of the 50 cSt PDMS. The same result was given when using e.g., 100% DCM or 100% EtOAc as eluents. It was decided that all analyses with normal-phase chromatography were to be carried out using a combination of a non-polar and polar solvent, where the polar solvent never exceeded 10%.

5.4.2 Analyses of PDMS

High concentrations of D3, D4 and D5 were analyzed with the automated normal-phase flash chromatographic system, with DCM (A) and MeOH (B) as eluents. The detection of the compounds was extremely low or none (Figure 49). The low signal with the retention time 0.5 min is generated by D5. Signals for the other cyclic compounds could not be assigned. This indicated that ELSD was not a suitable detector for analyses of the cyclic compounds.

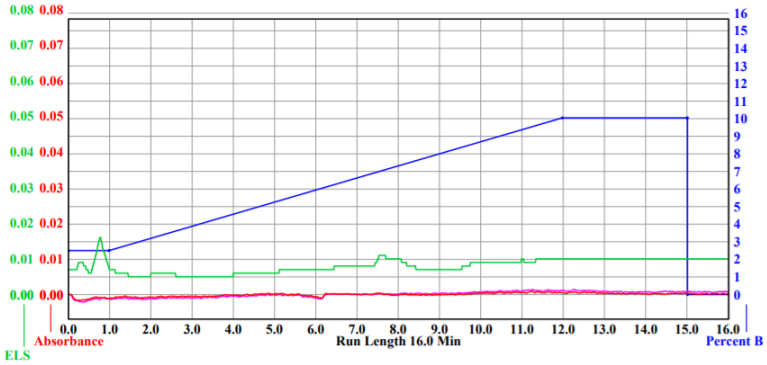


Figure 49 Chromatogram of normal-phase flash chromatography analysis of D3–D5 in DCM, with DCM (A) and MeOH (B) as eluents.

Analyses of separate samples of 5, 50 and 1000 cSt PDMS in EtOAc were carried out, and for each MW group, a signal was produced (Figure 50).

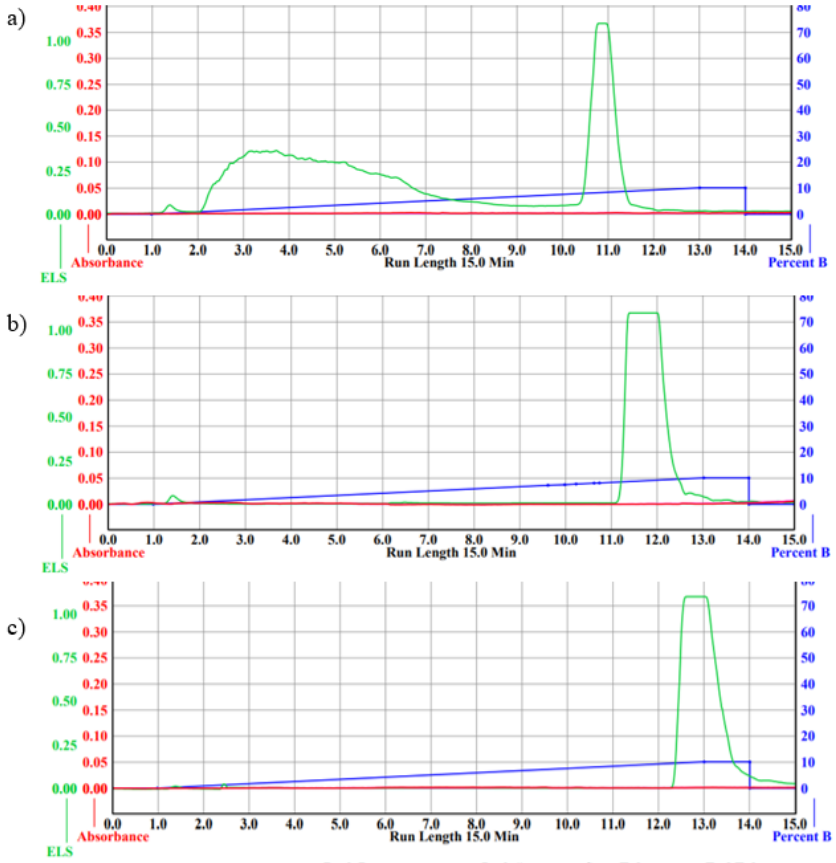


Figure 50 Chromatograms of normal-phase flash chromatographic analyses of a) 5 cSt PDMS, b) 50 cSt and c) 1000 cSt PDMS, with *n*-hexane as eluent A and EtOAc as eluent B.

The retention times of each MW group were close to each other, with 5 cSt PDMS eluting between ca 10.5 and 11.5 min, 50 cSt PDMS eluting between ca 11 and 13 min and 1000 cSt PDMS eluting between ca 12.5 and 15 min. The close retention times are a problem when wanting a separation of the compounds when they are present in the same sample. This is visualized in Figure 51, which shows the chromatogram obtained when analyzing a sample containing all PDMS MW groups.

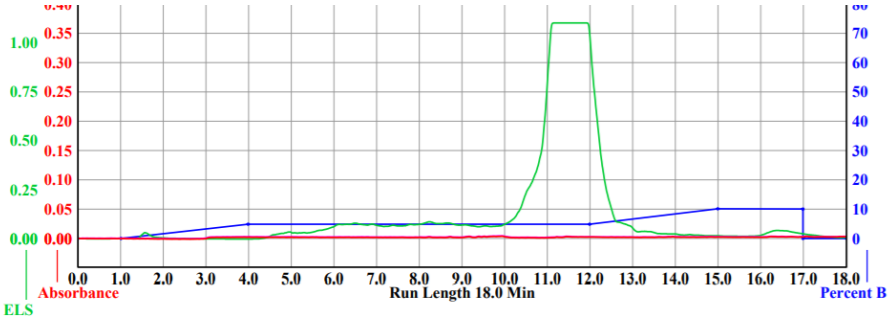


Figure 51 Chromatogram of normal-phase flash chromatographic analyses of a sample containing 5, 50 and 1000 cSt PDMS, with *n*-hexane as eluent A and EtOAc as eluent B.

To examine the effect that the eluents have on the separation of 5, 50 and 1000 cSt PDMS, a sample containing all MW groups was analyzed with DCM and MeOH as eluents (Figure 52).

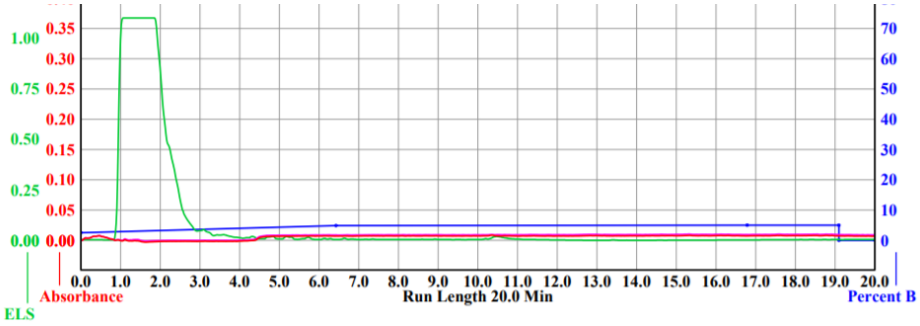


Figure 52 Chromatogram of normal-phase flash chromatographic analyses of a sample containing 5, 50 and 1000 cSt PDMS, with DCM as eluent A and MeOH as eluent B.

Figure 51 and 52 show that there is only one peak generated from each MW group, meaning there was no separation occurring. The only difference to the analysis utilizing *n*-hexane and EtOAc as eluents was that with DCM and MeOH the compounds elute early.

Although there was no separation of each MW PDMS, normal-phase flash chromatography as a sample cleanup method was tested on bio-oil 1 samples spiked with PDMS. The idea

was that PDMS could be fractionated from the bio-oil matrix, and the collected fractions could then be analyzed with the HPLC method, for quantitative analysis. Extracted samples of un-spiked bio-oil 1 and spiked bio-oil 1 with PDMS, were analyzed. *n*-hexane (A) and EtOAc (B) were used as eluents. The obtained chromatograms are presented in Figure 53.

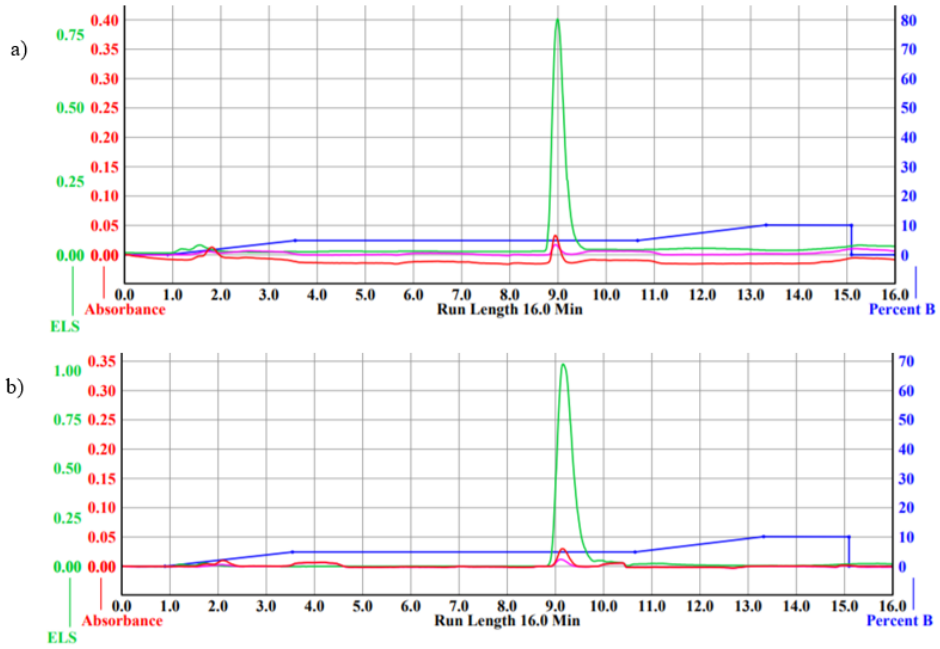


Figure 53 Chromatograms of normal-phase flash chromatography of a) bio-oil 1 in *n*-hexane and b) bio-oil 1 spiked with 14% 5, 50 and 1000 cSt PDMS. *n*-hexane (A) and EtOAc (B) were used as eluents.

Both chromatograms obtained from analysis of bio-oil 1 in *n*-hexane, as well as bio-oil 1 in n-hexane, spiked with 14% 5, 50 and 1000 cSt PDMS, are almost identical. As there is only one signal with the same retention time in both chromatograms, it can be concluded that the signals are generated from components present in the bio-oil matrix. There is no other peak visible in the chromatogram obtained from analysis of bio-oil 1 spiked with PDMS, which indicates that the detection of PDMS when spiked in bio-oil 1 is not possible. With these results, it was decided that normal-phase flash chromatography was not a suitable technique for the fractionation of bio-oil samples spiked with PDMS. For continued studies utilizing the automated flash chromatographic system, it would be advisable to test reversed-phase columns with similar method parameters as for the RP-HPLC method developed in this work (section 4.6.).

6 SUMMARY AND CONCLUSIONS

Methods for analyzing different MW groups of PDMS and its degradation products have been developed and optimized. A GC-MS/SIM method was developed to detect low concentrations of D3–D5. The compounds were successfully detected and identified with the method using two GC-MS instruments with different specifications on the columns. One instrument utilized an HP-5MS column and the other an HP-1 column. The method was compared to the previously used GC-FID method, by analyzing D3–D5 diluted in *n*-hexane with concentrations ranging from 0.01 ppm to 10 ppm. The GC-FID instrument operated with the split ratio 20:1, and both GC-MS instruments operated with the split ratio of 30:1. With GC-FID, the lowest detectable concentration was 0.5 ppm. The instrument with the HP-5MS column was able to detect D5 in 0.1 ppm, and D3 and D4 were detectable in 0.025 ppm. The HP-1 instrument was able to detect all three compounds with a concentration of 0.01 ppm. The second instrument was shown to be more sensitive, and with a lower split ratio or even splitless mode, even ppb concentrations could be detected. However, contaminations of D3–D5 from the inlet septum of the GC were detected in concentrations that interfered with the results. When analyzing with the HP-1 instrument, the contaminations needed to be constantly monitored and taken into consideration when interpreting the obtained results. It could be a possibility to avoid the contaminations if utilizing a silicone-free inlet septum, such as a Merlin Microseal. However, this type of inlet seal was not tested in this work.

The GC-MS/SIM method was validated by determining the method's linearity, accuracy and precision with the HP-5MS instrument, and the LOD was determined with the HP-1 instrument. Three parallel sample sets containing concentrations ranging from 0.025 to 1 ppm D3–D5 in *n*-hexane were analyzed for the calibration curves. The correlation coefficient for the calibration curves of each compound was above 0.993, which is desired for good linearity. However, the linearity was not as good for the lowest concentrations of the calibration curves. Only D4 provided good linearity for these concentrations. A reason for the worse linearity could be the interference of D3–D5 contaminants, which have a greater effect on lower concentrations compared to the higher ones. Another reason could be a human error when preparing samples of such low concentrations. Accuracy and precision were determined by comparing bio-oil 1 samples spiked with D3–D5 with the calibration curve. Bio-oil 1 samples spiked with 5–200 ppm D3–D5 in relation to the bio-oil were analyzed for the determination of accuracy. For each D-compound, the linearity of the spiked bio-oil 1 samples was good, and for the lowest concentrations, the obtained signals were comparable with the calibration curve. However, with higher concentrations, the difference

between the bio-oil 1 spiked samples and the calibration curve increased. The matrix of the bio-oil somehow affected the detection response of D3–D5. For precision studies, three parallel analyses of four concentrations (5, 10, 15 and 20 ppm D3–D5 in relation to bio-oil 1) were analyzed and compared to the calibration curve. The determination of precision was difficult achieve, as too few data points were collected for the interpretation. However, the obtained results showed a slight variation of each parallel sample, with the variation being largest for D4. For accurate validation, more parallel samples should be analyzed. The HP-1 instrument was used for the LOD studies of D3–D5 spiked in bio-oil 1. The determination of LOD was difficult to achieve, as the contamination of D3–D5 interfered. Parallel samples of 1 ppm, 2 ppm and 3 ppm D3–D5 in relation to bio-oil 1 were analyzed. For the 1 ppm samples, two out of three samples were below the LOD, whereas all parallel samples of the 2 ppm and 3 ppm samples were above. Therefore, it can be concluded that at least 2 ppm D3–D5 spiked in bio-oil 1 can be detected. It would be advisable that more reliable LOD studies would be performed with the instrument utilizing the Merlin Microseal in place of a silicone-based septum. Concentrations of D3–D5 in different bio-oils were calculated according to the calibration curve. With the developed GC-MS/SIM method, the trace amount of D3–D5 found in bio-oils were detectable and for some bio-oils, the concentrations of D3–D5 were even quantifiable.

Pyrolysis was performed on samples of 5, 50 and 1000 cSt PDMS prepared in *n*-hexane, as well as spiked in bio-oil samples with the ratio of 20% PDMS and 80% bio-oil. For 5 cSt PDMS, linear compounds ranging from L5 to L21 were detected, with L6–L8 being the most abundant. Because the 5 cSt PDMS contains short-chained PDMS, the cyclic transition state required for forming the cyclic compounds might be thermodynamically unfavorable. Pyrolysis of 50 cSt PDMS generated signals of cyclic compounds between D3 and D19 and of linear compounds between L8 and L18, with the linear compounds being the most abundant. For 1000 cSt PDMS, only cyclic compounds of D3–D18 were detected, with D3 being the most abundant. As 1000 cSt PDMS contains long-chained PDMS, it degrades easily into small cyclic compounds. Pyrolysis of bio-oil 1 spiked with 5 cSt PDMS, generated signals of all compounds which were detected in pyrolysis of 5 cSt PDMS in *n*-hexane. For bio-oil 10 spiked with 5 cSt PDMS, 86% of these compounds were detected. The bio-oil 10 matrix seems to interfere more with 5 cSt PDMS compared to bio-oil 1. For both bio-oil 1 and bio-oil 10 samples spiked with 50 cSt PDMS, only 24% of the compounds were detected when analyzing 50 cSt PDMS in *n*-hexane. However, bio-oil 1 interfered more with the middle eluting components, whereas bio-oil 10 interfered more with the late eluting components. Results obtained from the pyrolysis of bio-oil 1 and bio-oil 10 spiked with 1000

cSt, support the claim that bio-oil 1 components interfere more with the middle eluting compounds than bio-oil 10. For 1000 cSt PDMS in bio-oil 1, only D3–D7 were detected, whereas, for 1000 cSt PDMS in bio-oil 10, D3–D10 were detected. bio-oil 1 spiked with 100 ppm PDMS was analyzed, however, no signal from PDMS was generated. Pyrolysis is helpful in thermal degradation studies of different MW PDMS, as they tend to act differently during pyrolysis. However, when analyzing bio-oils spiked with PDMS, only high concentrations of PDMS were detectable with the method utilized in this work.

An RP-HPLC-ELSD method was tested for the detection of 5, 50 and 1000 cSt PDMS in a solvent as well as spiked in bio-oils. LOD studies were performed on 50 and 1000 cSt PDMS, with concentrations ranging from 0.00015 mg/ml to 1 mg/ml in EtOAc. With the detector sensitivity gain 1, the lowest detectable concentration was 0.012 mg/ml 1000 cSt, and 0.037 mg/ml 50 cSt PDMS. With the sensitivity gain 4, the lowest detectable concentration was 0.012 mg/ml 1000 cSt PDMS. The LOD studies of 1000 cSt PDMS are comparable with similar studies performed in previous theses utilizing HP-SEC-ELSD. With the HP-SEC-ELSD, the LOD with sensitivity gain 3 (comparable with gain 1 of these analyses) was 0.012 mg/ml, and with the sensitivity gain 6, the LOD was 0.001 mg/ml. This indicates that with higher sensitivity, the HP-SEC-ELSD instrument can detect lower concentrations. However, the separation of components within the 5 and 50 cSt PDMS is significantly better with the HPLC-ELSD instrument. With HP-SEC, only one peak is obtained for the 50 cSt PDMS, whereas, for the HPLC, separate peaks of components within 5 and 50 cSt PDMS are obtained.

The detection of 5, 50 and 1000 cSt PDMS, when spiked in bio-oil 1, bio-oil 2 and bio-oil 10, was also investigated. For all bio-oils, the matrix interfered with 5 cSt PDMS, and it could not be detected. The interference was less for 50 cSt PDMS, with the bio-oil 1 matrix interfering the most. The response of 50 cSt PDMS, when spiked in the bio-oils, was low compared to that of the response of 1000 cSt PDMS. For all bio-oils, there was no interference of the matrix with the detection of 1000 cSt PDMS. Therefore, 1000 cSt PDMS was chosen for LOD studies of PDMS spiked bio-oil samples. The lowest detectable concentration for spiked bio-oil 2 and bio-oil 10 samples was 1% 1000 cSt PDMS in relation to the bio-oil. For bio-oil 1, the lowest detectable concentration was 0.5% 1000 cSt PDMS. The ELSD had prior to the bio-oil 1 analyses been cleaned, which could be a reason for the detection of the lower concentration. For future studies with the HPLC, sample clean-up or fractionation of bio-oil samples spiked with PDMS should be performed to be able to lower the LOD. As proposed in the objectives (section 3), the HPLC could be used as a preparative

method for fractionation, and the collected fractions could either be analyzed with the RP-HPLC-ELSD or HP-SEC-ELSD instrument.

Normal-phase flash chromatography was tested as an alternative fractionation method to the previously developed SPE methods. Analyses of high concentrations of D3–D5 in DCM, generated extremely low responses or no response at all, indicating that ELSD is not suitable for the detection of the cyclic compounds. Analyses of 5, 50 and 1000 cSt PDMS when prepared in EtOAc, generated strong signals, however, with retention times overlapping. When analyzing the different MW groups in one sample, one peak for each MW group was obtained. Two different combinations of eluents (EtOAc:*n*-hexane and MeOH:DCM) were tested to see if the separation could become better, however, without any success. Thereafter, samples of bio-oil 1 spiked with PDMS were analyzed, to investigate the separation of PDMS from the bio-oil 1 matrix. There was no difference in the chromatogram obtained from the analysis of plain bio-oil 1 compared to the chromatogram obtained from the PDMS spiked bio-oil 1 samples, which indicates that there is a total interference of the matrix and PDMS. It could be continued with testing if the eluent combination of *n*-hexane and DCM could improve both the separation of each PDMS viscosity and PDMS from the bio-oil matrix. Overall, the results imply that normal-phase flash chromatography is not suitable for the purposes aimed in this work. Fractionation, performed by the automated flash chromatography, of bio-oil samples spiked with PDMS might be achieved by using a reverse-phase column with the same parameters as the HPLC method developed in this work.

7 SVENSKA SAMMANFATTNING

7.1 Utveckling av GC-MS/SIM- och HPLC-metoder för analysering av polydimetylsiloxan och dess nedbrytningsprodukter

Polydimetylsiloxan (PDMS) är en homopolymer som karakteriseras av en grundkedja bestående av upprepade enheter av Si–O med två metylgrupper bundna till varje kiselatom (Si). Si–O bindningarna förser PDMS med flera anmärkningsvärda kemiska och fysikaliska egenskaper, såsom flexibilitet, hög termostabilitet och låg ytspänning. Dessa egenskaper ger PDMS dess mångsidiga tillämpningar inom olika områden. En av dessa tillämpningar är som skumdämpningsmedel i sulfatprocessen (eng. kraft pulping process) i massa- och pappersindustrin. Sulfatprocessen är en kemisk behandlingsprocess där pappersmassa framställs. Flis av ved upphettas i höga temperaturer i vitlut, en alkalisk lösning av NaOH och Na_2S, vilket bryter bindningar mellan lignin och hemicellulosa. I detta skede genereras stora mängder skum, vilket kräver en tillsats av skumdämpningsmedel. Därefter separeras vitluten och pappersmassan genom en tvättningsprocess. En kombination av vitluten och tvättvattnet ger svartlut, som har en torrhalt på ca 65%. Svartluten koncentreras och en såpa bestående av främst fettsyror erhålls. Genom att neutralisera såpan med svavelsyra fås tallolja, som sedan kan användas som råmaterial i tillverkningen av biodiesel i bioraffinaderier. Spår av PDMS och dess nedbrytningsprodukter från skumdämpningsmedel har detekterats i talloljan och kan orsaka problem i bioraffinaderi processer.

Det är viktigt att det finns analytiska metoder för att identifiera och kontrollera kontaminationer i processer. Kvalitativa metoder som har rapporteras för att analysera PDMS är främst FTIR, NMR, Ramanspektroskopi och masspektrometri. För kvantitativa analyser av PDMS har främst separationsmetoder kopplade till olika detektorer använts, såsom HP-SEC-ELSD och GC-MS. Syftet med denna avhandling var att utveckla metoder för att analysera PDMS och dess nedbrytningsprodukter i olika biooljor, som en fortsättning på avhandlingar skrivna av Kenneth Arandia, Charlotte Holmberg och Oscar Nyman. En GC-MS/SIM-metod för att analysera nedbrytningsprodukter av PDMS, d.v.s. D3, D4 och D5, samt en HPLC-ELSD-metod för att analysera främst mellan- och högmolekylär PDMS skulle utvecklas. Därtill skulle en automatisk normal fas-kolonnkromatografisk metod prövas i provförberedningssyfte.

För utvecklingen av GC-MS/SIM-metoden användes två instrument med olika kolonndimensioner. Det ena instrumentet var utrustat med en HP-5MS kolonn (30m×0.25mm i.d.×0.25µm) och det andra med en HP-1 kolonn (25m×.0.2mm i.d.×0.11µm). En väsentlig skillnad mellan instrumenten var att det förstnämnda instrumentet var utrustat

med automatisk injicering, medan injektionerna gjordes manuellt med det andra instrumentet. HP-5MS-instrumentet användes för att validera linjäritet, noggrannhet och precision för den utvecklade metoden, medan det andra instrumentet användes för att fastställa den lägsta detekterbara koncentrationen. Känsligheten av instrumenten jämfördes och därtill jämfördes även den nya metoden med GC-FID-instrumentet, som användes i de tidigare arbetena. Koncentrationer mellan 0,01 och 10 ppm D3–D5 i hexan analyserades. HP-5MS-instrumentet kunde detektera D5 i koncentrationen 0,1 ppm, och endast D3 och D4 i 0,025 ppm. HP-1-instrumentet detekterade D3–D5 i 0,01 ppm, och storleken på signalerna i den lägsta koncentrationen visade att även lägre koncentrationer kunde detekteras. Det är främst instrumentens olika tillstånd som bidrar till skillnaden och inte de olika kolonnerna. Med GC-FID var den lägst detekterbara koncentration 0,5 ppm. Detta visade att HP-1-instrumentet var det känsligaste och att GC-MS/SIM-metoden detekterar lägre koncentrationer än vad som var möjligt med GC-FID. Med det känsliga HP-1-instrumentet kunde kontaminationer av D3–D5 i prover av ren hexan detekteras. Olika källor till kontaminationerna undersöktes och det visade sig att kontaminationerna härstammade från ett s.k. ”inlet septum” i instrumentet. Flera olika septa studerades och kontaminationerna kunde detekteras från dem alla. Detta är på grund av att de flesta septa är gjorde av silikon, vilket är ett problem då man analyserar prov av silikon med ett hög känsligt instrument. Ett icke-silikon-baserat septum, såsom Merlin Microseal, kunde i framtida arbeten testas för att undvika kontaminationerna. Vid tolkning av all data togs kontaminationerna i beaktande.

Linjäriteten bestämdes genom att analysera åtta prov med olika koncentrationer av D3–D5 i hexan (0,025–1 ppm). Toppareorna från de genererade signalerna plottades mot koncentrationerna, för att få kalibreringskurvor enskilt för D3, D4 och D5. Determinationskoefficient (R^2) var över 0,993 för alla kalibreringskurvor, vilket krävs för att kunna godkänna linjäriteten för en metod. Däremot var linjäriteten för de fyra lägsta koncentrationerna sämre och endast för D4 var R^2 över 0,993. Denna skillnad kan bero på mänskliga fel vid provförberedelserna av dessa låga koncentrationer men kan också orsakas av de tidigare nämnda kontaminationsproblemen, som främst påverkar de lägsta koncentrationerna. Noggrannheten bestämdes genom att jämföra kurvor av D3–D5 som har tillsatts i biooljeprov med de tidigare erhållna kalibreringskurvorna. Linjäriteten för kurvorna av D-föreningarna som erhölls från de spikade biooljeproven var god och för de lägsta koncentrationerna överlappade kurvorna med kalibreringskurvorna. Däremot ökade skillnaden mellan biooljeproven och kalibreringskurvorna med högre koncentrationer. Denna skillnad orsakas troligtvis av biooljematrisen, eftersom skillnaden i detektionsresponsen inte kan ses då D3–D5 i endast hexan analyseras. Precisionen undersöktes genom att tre

parallella analyser av fyra koncentrationer av D3–D5 tillsatta i biooljeprov jämfördes med kalibreringskurvan. För alla D föreningar kunde ändå en liten avvikelse mellan de enskilda koncentrationerna noteras, varav D4 visade den största skillnaden. Men på grund av att för få datapunkter erhölls, var det svårt att bekräfta precisionen. Instrumentet utrustad med HP-1 kolonnen användes för att bestämma detektionsgränsen för metoden, men detta var svårt att åstadkomma på grund av kontaminationerna av inlet septumet. Parallella prov med koncentrationerna 1, 2 och 3 ppm D3–D5 i förhållande till bioolja analyserades. För koncentrationerna 2 och 3 ppm var alla prov ovanför detektionsgränsen, men endast ett av de tre parallella proven med koncentrationen 1 ppm D3–D5 var ovanför gränsen. Därmed kunde det konstateras att åtminstone koncentrationer av 2 ppm och högre av D3–D5 i förhållande till biooljan är detekterbara. För en mer exakt bestämmelse av detektionsgränsen skulle det vara rekommenderat att pröva Merlin Microseal i stället för ett silikon-baserat septum. Koncentrationen av D3–D5 i olika biooljeprov uträknades genom att jämföra de erhållna signalerna med kalibreringskurvan. Koncentrationen var så hög för de flesta biooljorna, att kvantifiering av D3–D5 direkt ur biooljan skulle vara möjligt.

Analyser med pyrolys-GC-MS utfördes för att studera nedbrytningsprodukter av låg- (5 cSt), mellan- (50 cSt) och högmolekylär (1000 cSt) PDMS (20%) i olika biooljor (80%). Pyrolys av 5 cSt PDMS i hexan genererade endast linjära nedbrytningsprodukter (L5-L21), vilket kan bero på att det inte är termodynamiskt fördelaktigt för de korta kedjorna i lågmolekylär-PDMS att omvandlas till den cykliska intermediären som krävs för att bilda de cykliska nedbrytningsprodukterna. Vid pyrolys av 50 cSt PDMS kunde både cykliska (D3–D19) och lineära föreningar detekteras (L8-L18), och för 1000 cSt PDMS detekterades endast cykliska föreningar (D3–D18). Pyrolys av 5 cSt PDMS tillsatt i bioolja 1 genererade alla föreningar som var detekterbara i pyrolys av 5 cSt i hexan, medan 86% av dessa föreningar detekterades av 5 cSt PDMS i bioolja 10. För både bioolja 1 och 10 tillsatta med 50 cSt PDMS detekterades 24% av de föreningar som kunde detekteras i pyrolys av 50 cSt PDMS i hexan. Komponenter i bioolja 1 påverkade detektionen av medeleluerande föreningar, medan bioolja 10 påverkade de föreningar med högre retentionstider. Detta stöder även resultaten erhållna från pyrolys av 1000 cSt PDMS tillsatta i de båda biooljorna. Endast D3–D7 kunde detekteras i bioolje 1 prov och D3–D10 var detekterbara i prov av bioolja 10. Pyrolys av 100 ppm 1000 cSt PDMS i bioolja 1 utfördes, men inga nedbrytningsprodukter kunde detekteras. Pyrolys är en bra metod för att studera nedbrytningen för olika molekylvikter av PDMS. Den metod som användes i detta arbete visade sig vara mer lämplig för analys av högre koncentrationer av PDMS i biooljeprov.

En HPLC-ELSD-metod testades för analyser av 5, 50 och 1000 cSt PDMS i lösningsmedel och i biooljor. Detektionsgränsen av 50 och 1000 cSt PDMS undersöktes genom att analysera prov med koncentrationer mellan 0,00015 mg/ml och 1 mg/ml i etylacetat. Den lägst detekterbara koncentrationen av 50 cSt PDMS var 0,037 mg/ml och för 1000 cSt PDMS kunde även 0,012 mg/ml detekteras. Detta tyder på att detektorn har en bättre respons till 1000 cSt PDMS än 50 cSt PDMS. Dessa analyser kunde jämföras med liknande analyser utförda med HP-SEC-ELSD i de tidigare arbetena. Med högre känslighet på detektorn kunde lägre koncentrationer detekteras med HP-SEC, men en tydlig separation av komponenter i 5 och 50 cSt PDMS kunde erhållas av HPLC medan HP-SEC gav endast en topp för alla komponenter. Därefter undersöktes detektionen av 5, 50 och 1000 cSt PDMS efter att de hade tillsatts i tre olika biooljor. Komponenter av alla biooljor eluerade samtidigt som 5 cSt PDMS och den lågmolekylära PDMS kunde inte detekteras. Överlappningen minskade för 50 cSt PDMS och var som lägst för bioolja 2. För alla biooljor skedde ingen överlappning med 1000 cSt PDMS och därmed användes 1000 cSt PDMS för att fastställa detektionsgränsen för de tre olika biooljorna spikade med PDMS. För bioolja 2 och 10 var 1% 1000 cSt PDMS i förhållande till biooljan den lägsta detekterbara koncentrationen, och för bioolja 1 var denna koncentration 0,5%. Den lägre koncentrationen kan bero på att detektorn hade rengjorts före analysen utfördes. För att minska på överlappningen av biooljekomponenter och PDMS samt kunna detektera lägre koncentrationer rekommenderas rening av proven eller fraktionering av proven. Preparativ HPLC kunde användas för fraktionering av proven och de samlade fraktionerna kunde därefter analyseras med antingen HPLC-ELSD eller HP-SEC-ELSD.

Automatisk normal fas-kolonnkromatografi testades för att användas som fraktioneringsmetod i stället för den tidigare utvecklade fastfasextraktionen. Höga koncentrationer av D3–D5 i dikolormetan analyserades, men endast små eller inga signaler genererades. Detta tyder på att ELSD inte är en lämplig detektor för de lågmolekylära cykliska föreningarna. Därefter analyserades 5, 50 och 1000 cSt PDMS utspätt i etylacetat. Alla tre molekylvikter genererade skarpa toppar, men med en aning överlappande retentionstider. Detta blev tydligare då alla molekylvikter var närvarande i samma prov och det genererades endast en gemensam topp för molekylvikterna. Olika kombinationer och elueringsmedel undersöktes (etylacetat:hexan och metanol:diklormetan), men separationen av molekylvikterna blev inte bättre. Analyser av bioolja jämfördes med analyser av PDMS tillsatt i bioolja 1. De erhållna kromatogrammen var likadana, vilket indikerar att biooljematrisen överlappar totalt med PDMS. Dessa resultat tyder på att normal fas-kolonnkromatografi inte är en lämplig metod för att analysera PDMS i biooljeprov. Däremot

kunde en omvänd fas-kolonnkromatografi med liknande parametrar som den testade HPLC-metoden prövas för eventuell fraktionering av PDMS i biooljeprov.

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9 APPENDICES

Appendix A.

```
                          INSTRUMENT CONTROL PARAMETERS:    GCMS online
                          ----------------------------------------------

   C:\MSDCHEM\2\DATA\MARIE\SIM METHOD\D3-D5 SIM.M
      Fri Dec 03 17:24:47 2021

Control Information
------- -----------

Sample Inlet      :  GC
Injection Source  :  GC ALS
Mass Spectrometer :  Enabled


 No Sample Prep method has been assigned to this method.

GC
Oven
Temperature
Setpoint                                    On
(Initial)                                   50 °C
Hold Time                                   6 min
Post Run                                    70 °C
Program
#1 Rate                                     25 °C/min
#1 Value                                    300 °C
#1 Hold Time                                10 min


Equilibration Time                          0 min
Max Temperature                             350 °C
Slow Fan                                    Disabled
Cryo                                        Off

ALS
Front Injector
Syringe Size                                10 µL
Injection Volume                            1 µL
Injection Repetitions                       1
Injection Delay                             0 sec
Solvent A Washes (PreInj)                   2
Solvent A Washes (PostInj)                  3
Solvent A Volume                            5 µL
Solvent B Washes (PreInj)                   2
Solvent B Washes (PostInj)                  2
Solvent B Volume                            5 µL
Sample Washes                               3
Sample Wash Volume                          5 µL
Sample Pumps                                3
Dwell Time (PreInj)                         0 min
Dwell Time (PostInj)                        0 min
Solvent Wash Draw Speed                     300 µL/min
Solvent Wash Dispense Speed                 6000 µL/min
Sample Wash Draw Speed                      300 µL/min
Sample Wash Dispense Speed                  6000 µL/min
Injection Dispense Speed                    6000 µL/min
Viscosity Delay                             7 sec
Sample Depth                                Disabled

Sample Overlap
Mode                                        Sample overlap is not enabled

ALS Errors                                  Pause for user interaction

Front SS Inlet He
Mode                                        Split
Heater                                      On    250 °C
Pressure                                    On    8,1004 psi
Total Flow                                  On    22,472 mL/min
Septum Purge Flow                           On    3 mL/min
Gas Saver                                   On    20 After 2 min mL/min
Split Ratio                                 20 :1
Split Flow                                  18,545 mL/min

Thermal Aux 2 (MSD Transfer Line)
```

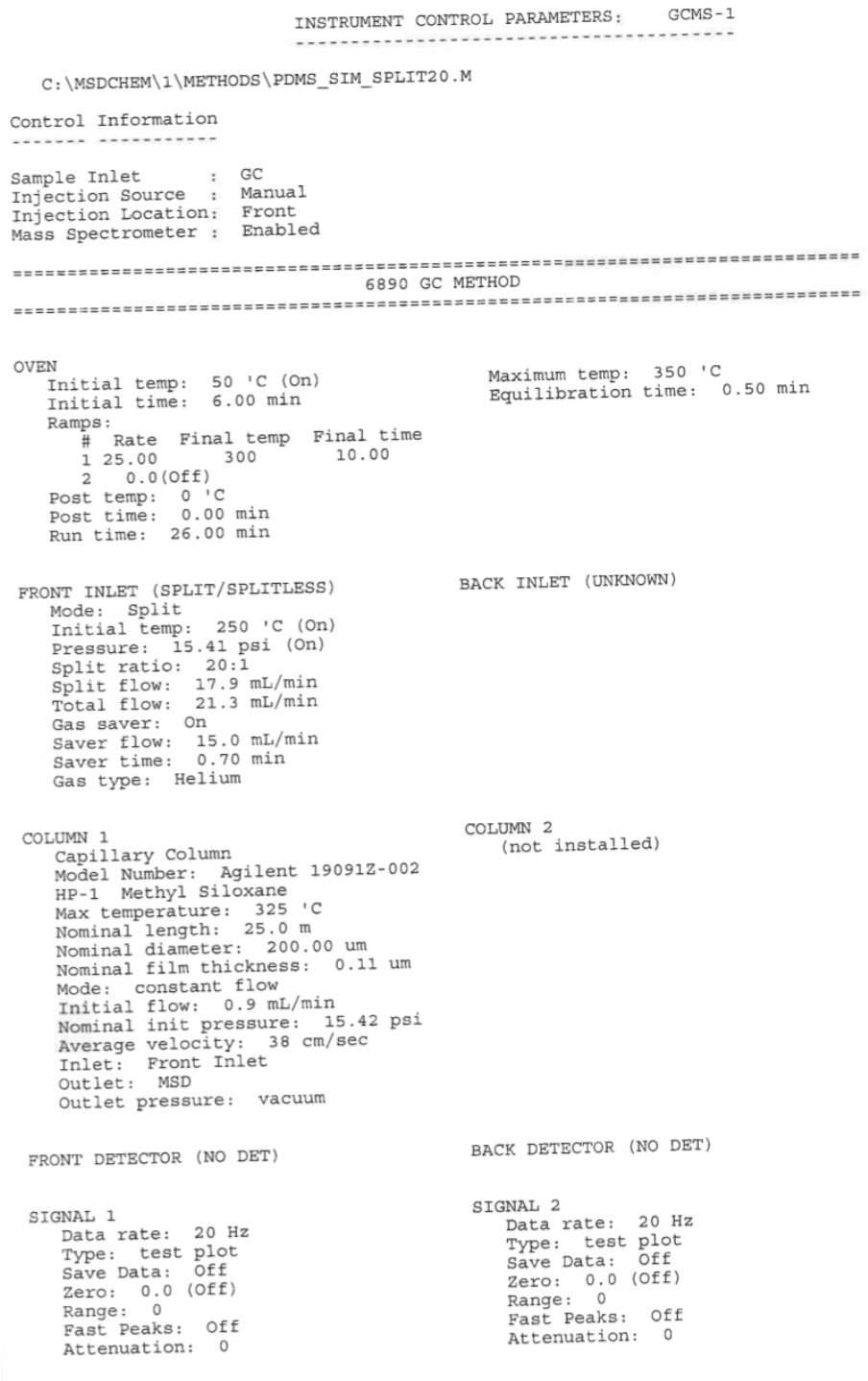
Figure A1 GC-MS/SIM parameters of GC instrument utilizing HP-5MS instrument (Figure 1/3).

```
Temperature
Setpoint                                    On
(Initial)                                   250 °C
Hold Time                                   0 min
Post Run                                    0 °C


Column
Column #1
Flow
Setpoint                                    On
(Initial)                                   0,92725 mL/min
Hold Time                                   0 min
Post Run                                    0,57353 mL/min


-60 °C–450 °C (450 °C): 30 m x 250 µm x 0,25 µm
In                                          Front SS Inlet He
Out                                         MSD
(Initial)                                   50 °C
Pressure                                    6,4623 psi
Flow                                        0,92725 mL/min
Average Velocity                            35,694 cm/sec
Holdup Time                                 1,4008 min


Signals
Signal #1:  Test Plot
Description                                 Test Plot
Details
Save                                        Off
Data Rate                                   50 Hz
Dual Injection Assignment                   Front Sample

Signal #2:  Test Plot
Description                                 Test Plot
Details
Save                                        Off
Data Rate                                   50 Hz
Dual Injection Assignment                   Back Sample

Signal #3:  Test Plot
Description                                 Test Plot
Details
Save                                        Off
Data Rate                                   50 Hz
Dual Injection Assignment                   Back Sample

Signal #4:  Test Plot
Description                                 Test Plot
Details
Save                                        Off
Data Rate                                   50 Hz
Dual Injection Assignment                   Back Sample

                              MS ACQUISITION PARAMETERS


General Information
------- -----------

Tune File               : atune.u
Acquistion Mode         : SIM


MS Information
-- -----------

Solvent Delay           : 4.00 min

EMV Mode                : Relative
Relative Voltage        : 0
Resulting EM Voltage    : 1541
```

Figure A1 GC-MS/SIM parameters of GC instrument utilizing HP-5MS instrument (Figure 2/3).

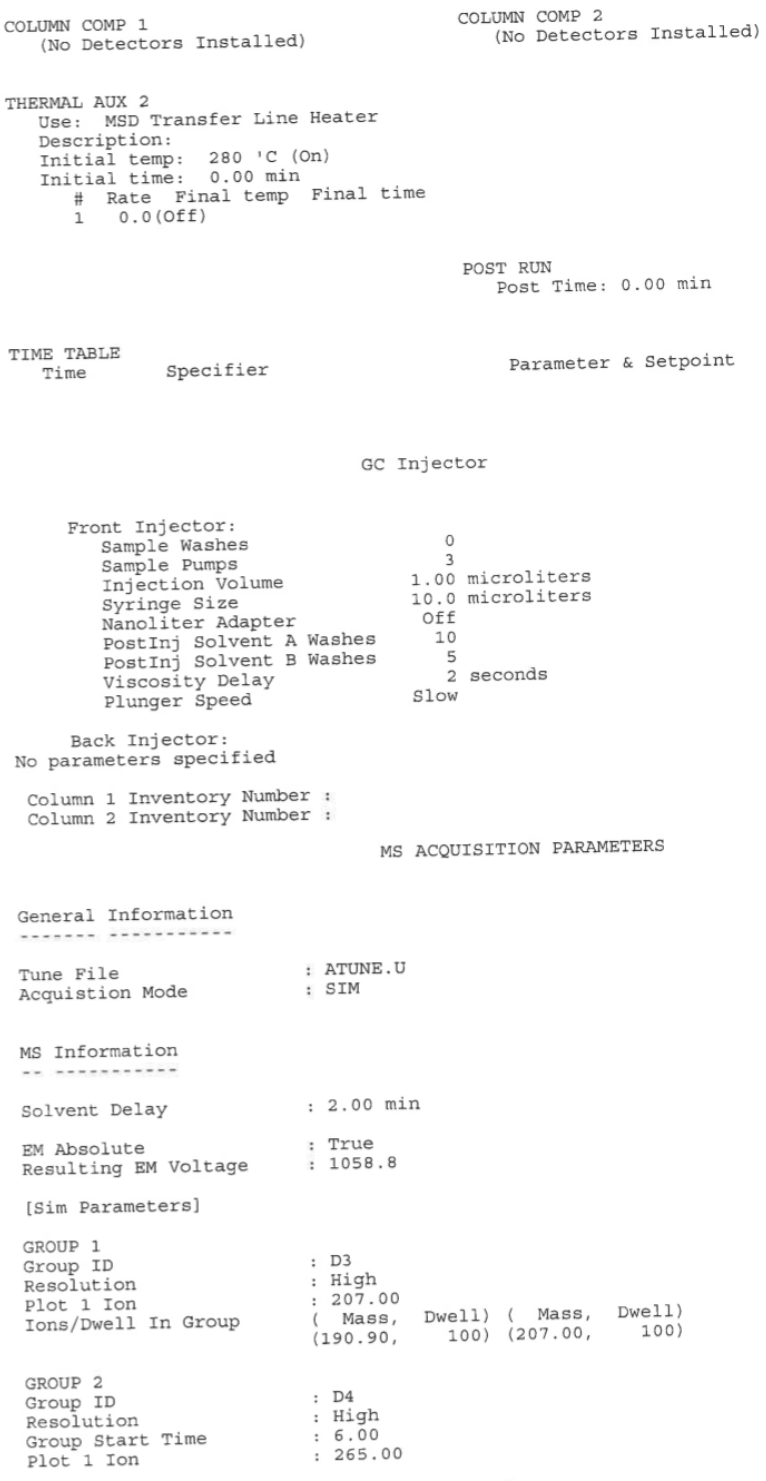
```
[Sim Parameters]

GROUP 1
Group ID                : D3
Resolution              : High
Plot 1 Ion              : 190.90
Ions/Dwell In Group     (  Mass,  Dwell) (  Mass,  Dwell)
                        (190.90,    100) (207.00,    100)

GROUP 2
Group ID                : D4
Resolution              : High
Group Start Time        : 7.00
Plot 1 Ion              : 265.00
Ions/Dwell In Group     (  Mass,  Dwell) (  Mass,  Dwell)
                        (265.00,    100) (281.00,    100)

GROUP 3
Group ID                : D5
Resolution              : High
Group Start Time        : 9.50
Plot 1 Ion              : 338.90
Ions/Dwell In Group     (  Mass,  Dwell) (  Mass,  Dwell)
                        (338.90,    100) (355.10,    100)

[MSZones]

MS Source               : 230 C   maximum 250 C
MS Quad                 : 150 C   maximum 200 C

                          END OF MS ACQUISITION PARAMETERS

                           TUNE PARAMETERS for SN: US83141676
                        -----------------------------

 Trace Ion Detection is OFF.

 EMISSION    :      34.610
 ENERGY      :      69.922
 REPELLER    :      34.814
 IONFOCUS    :      90.157
 ENTRANCE_LE :      32.000
 EMVOLTS     :    1541.176
                              Actual EMV  :    1541.18
                              GAIN FACTOR :       1.00
 AMUGAIN     :    2483.000
 AMUOFFSET   :     121.188
 FILAMENT    :       1.000
 DCPOLARITY  :       0.000
 ENTLENSOFFS :      20.580
 MASSGAIN    :    -404.000
 MASSOFFSET  :     -38.000

                         END OF TUNE PARAMETERS
                       ----------------------------------


                              END OF INSTRUMENT CONTROL PARAMETERS
                              ----------------------------------
```

Figure A1 GC-MS/SIM parameters of GC instrument utilizing HP-5MS instrument (Figure 3/3).



```
                    INSTRUMENT CONTROL PARAMETERS:    GCMS-1
                    --------------------------------------

  C:\MSDCHEM\1\METHODS\PDMS_SIM_SPLIT20.M

Control Information
------- -----------

Sample Inlet      : GC
Injection Source  : Manual
Injection Location: Front
Mass Spectrometer : Enabled

================================================================================
                              6890 GC METHOD
================================================================================

OVEN
   Initial temp:  50 'C (On)                  Maximum temp:  350 'C
   Initial time:  6.00 min                    Equilibration time:  0.50 min
   Ramps:
      #  Rate  Final temp  Final time
      1 25.00      300       10.00
      2   0.0(Off)
   Post temp:  0 'C
   Post time:  0.00 min
   Run time:  26.00 min

FRONT INLET (SPLIT/SPLITLESS)              BACK INLET (UNKNOWN)
   Mode:  Split
   Initial temp:  250 'C (On)
   Pressure:  15.41 psi (On)
   Split ratio:  20:1
   Split flow:  17.9 mL/min
   Total flow:  21.3 mL/min
   Gas saver:  On
   Saver flow:  15.0 mL/min
   Saver time:  0.70 min
   Gas type:  Helium

COLUMN 1                                   COLUMN 2
   Capillary Column                           (not installed)
   Model Number:  Agilent 19091Z-002
   HP-1  Methyl Siloxane
   Max temperature:  325 'C
   Nominal length:  25.0 m
   Nominal diameter:  200.00 um
   Nominal film thickness:  0.11 um
   Mode:  constant flow
   Initial flow:  0.9 mL/min
   Nominal init pressure:  15.42 psi
   Average velocity:  38 cm/sec
   Inlet:  Front Inlet
   Outlet:  MSD
   Outlet pressure:  vacuum

FRONT DETECTOR (NO DET)                    BACK DETECTOR (NO DET)

SIGNAL 1                                   SIGNAL 2
   Data rate:  20 Hz                          Data rate:  20 Hz
   Type:  test plot                           Type:  test plot
   Save Data:  Off                            Save Data:  Off
   Zero:  0.0 (Off)                           Zero:  0.0 (Off)
   Range:  0                                  Range:  0
   Fast Peaks:  Off                           Fast Peaks:  Off
   Attenuation:  0                            Attenuation:  0
```

Figure A2 GC-MS/SIM parameters of GC instrument utilizing HP-1 instrument (Figure 1/3).



```
COLUMN COMP 1                                  COLUMN COMP 2
   (No Detectors Installed)                       (No Detectors Installed)


THERMAL AUX 2
   Use:  MSD Transfer Line Heater
   Description:
   Initial temp:  280 'C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

                                               POST RUN
                                                  Post Time: 0.00 min


TIME TABLE
   Time       Specifier                            Parameter & Setpoint


                                GC Injector


     Front Injector:
        Sample Washes                    0
        Sample Pumps                     3
        Injection Volume              1.00 microliters
        Syringe Size                  10.0 microliters
        Nanoliter Adapter              Off
        PostInj Solvent A Washes        10
        PostInj Solvent B Washes         5
        Viscosity Delay                  2 seconds
        Plunger Speed                 Slow

     Back Injector:
No parameters specified

 Column 1 Inventory Number :
 Column 2 Inventory Number :

                                MS ACQUISITION PARAMETERS


General Information
------- -----------

Tune File                : ATUNE.U
Acquistion Mode          : SIM


MS Information
-- -----------

Solvent Delay            : 2.00 min

EM Absolute              : True
Resulting EM Voltage     : 1058.8

[Sim Parameters]

GROUP 1
Group ID                 : D3
Resolution               : High
Plot 1 Ion               : 207.00
Ions/Dwell In Group      (  Mass,  Dwell) (  Mass,  Dwell)
                         (190.90,    100) (207.00,    100)

GROUP 2
Group ID                 : D4
Resolution               : High
Group Start Time         : 6.00
Plot 1 Ion               : 265.00
```

Figure A2 GC-MS/SIM parameters of GC instrument utilizing HP-1 instrument (Figure 2/3).

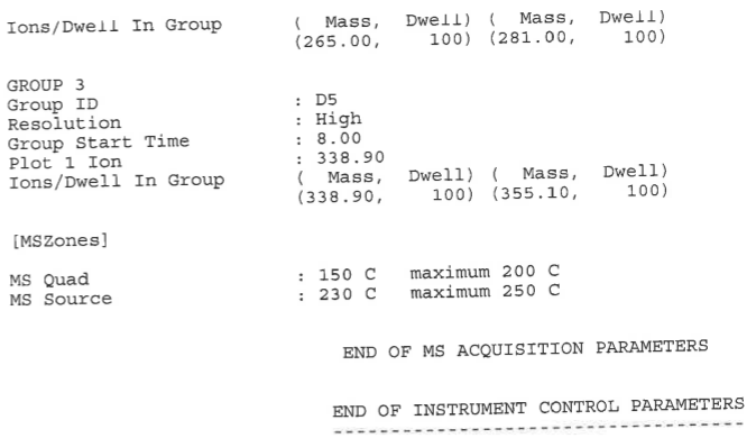


Figure A2 GC-MS/SIM parameters of GC instrument utilizing HP-1 instrument (Figure 3/3).

Appendix B.

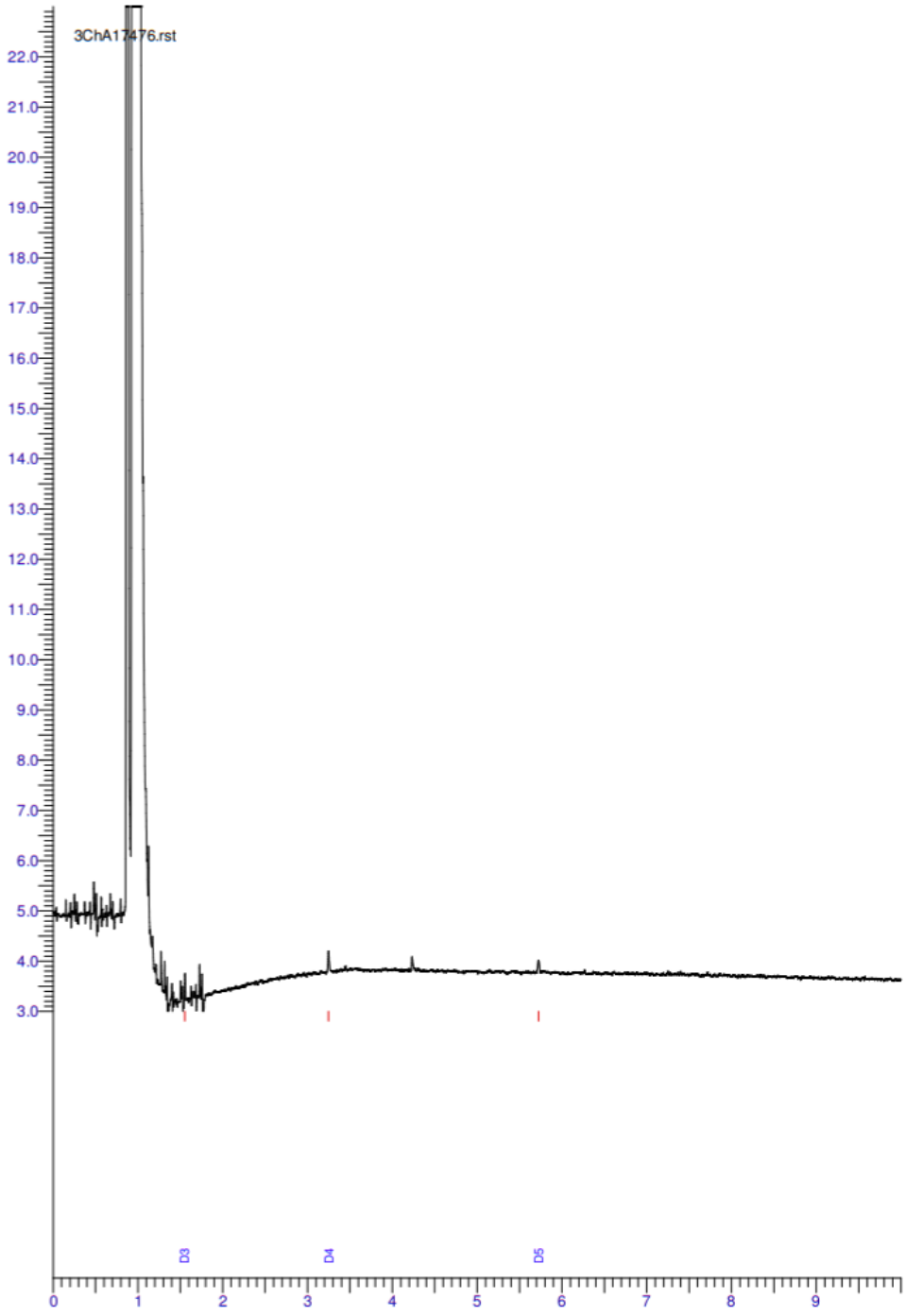


Figure B1 Chromatogram of the lowest detected concentration of D3–D5 with GC-FID. The concentration of the samples is 0.5 ppm D3–D5 in n-hexane.

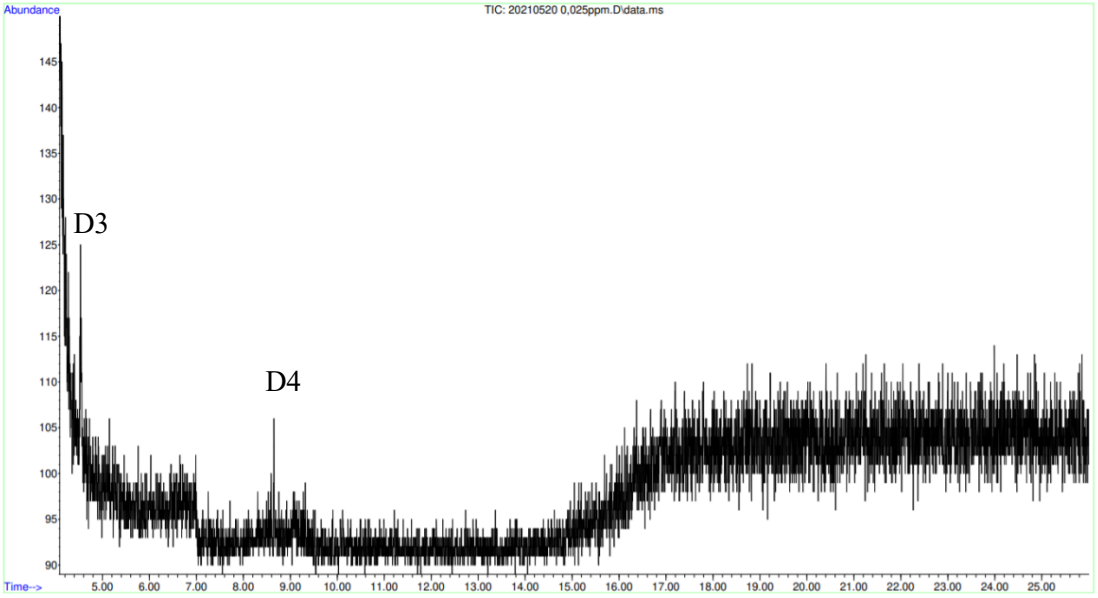


Figure B2 Chromatogram of the lowest detected concentration of D3 and D4 with GC-MS/SIM equipped with an HP-5MS column. The concentration of the samples is 0.025 ppm D3 and D4 in n-hexane.

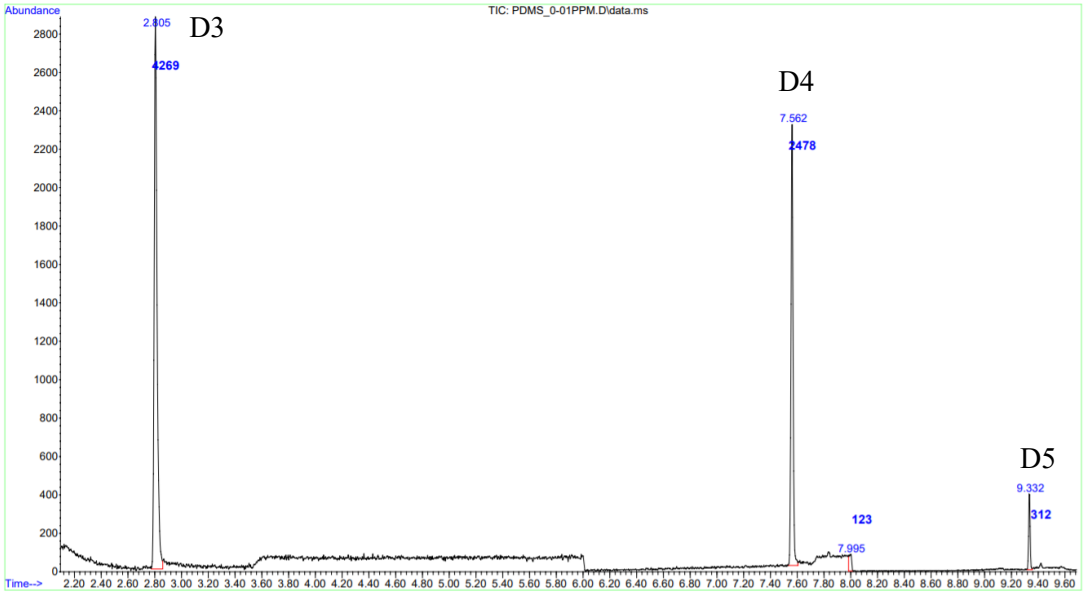


Figure B3 Chromatogram of the lowest detected concentration of D3–D5 with GC-MS/SIM equipped with an HP-1 column. The concentration of the samples is 0.025 ppm D3–D5 in n-hexane.

Appendix C.

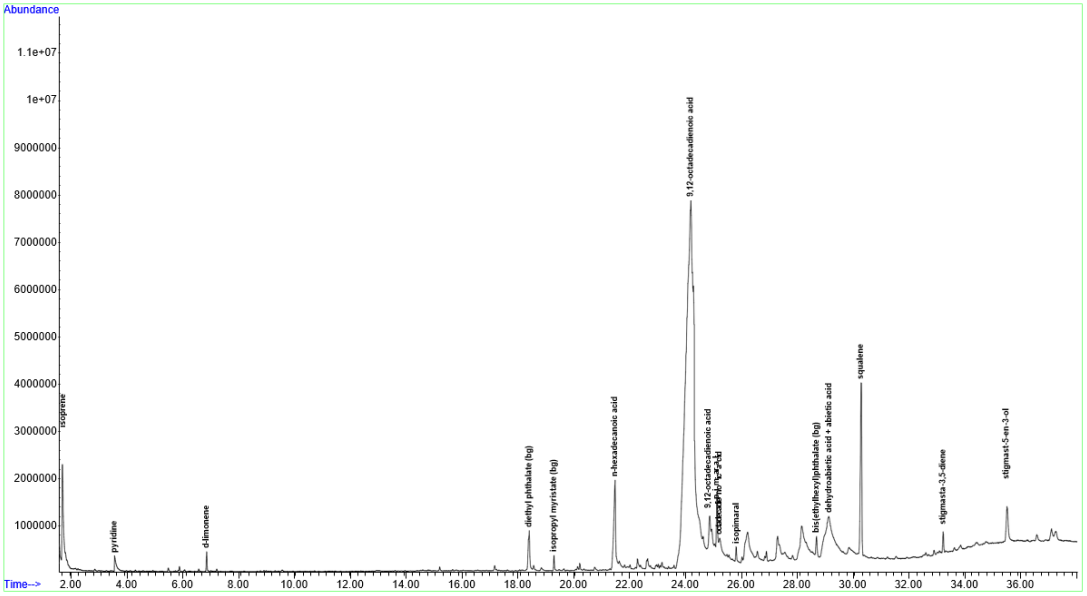


Figure C1 Pyrogram of 10 mg/ml bio-oil 10 in n-hexane.

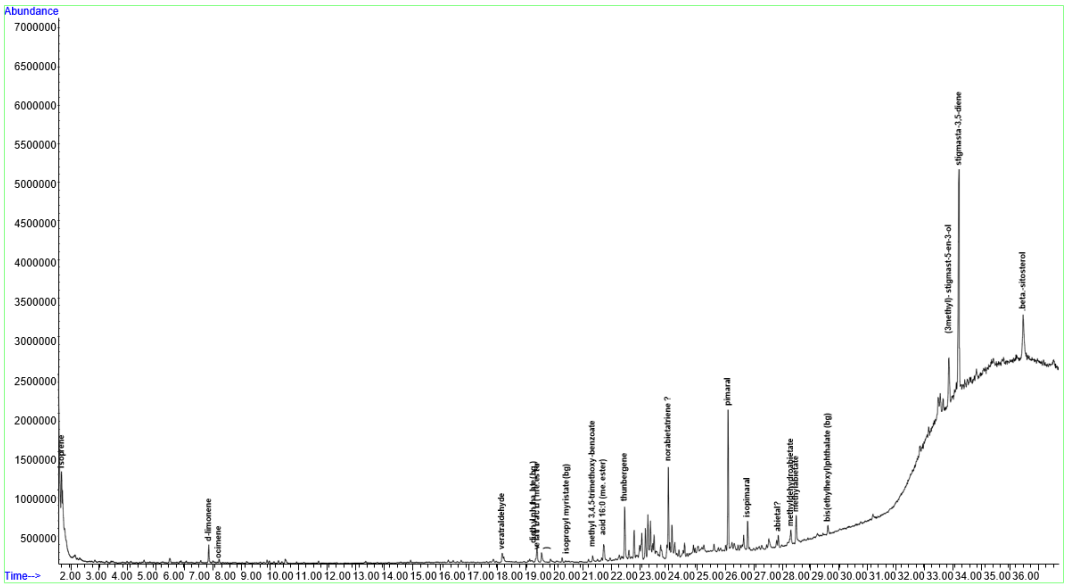


Figure C2 Pyrogram of 10 mg/ml bio-oil 1 in n-hexane.

Appendix D.

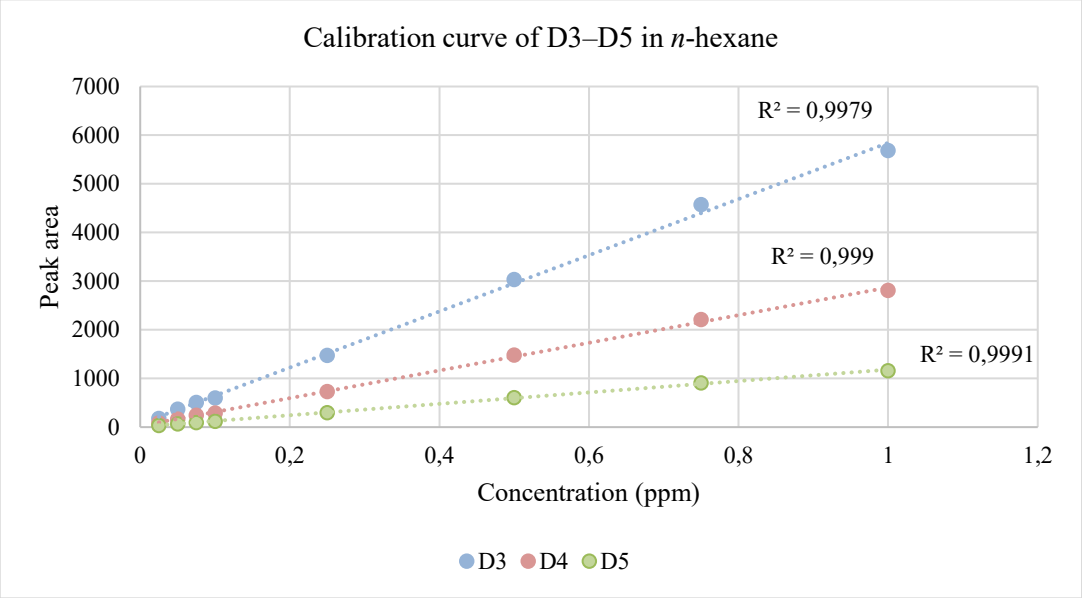


Figure D Calibration curve of D3–D5 in n-hexane analyzed with the HP-5MS instrument after it was cleaned. The calibration curve was used for the calculation of the concentrations of D3–D5 in bio-oils.