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Method development for the analysis of polydimethylsiloxane in bio-oils

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Master's thesis carried out under supervision by Chunlin Xu and Patrik C. Eklund

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ABSTRACT

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The chemistry of wood is quite complex. Wood consists of several components, such as cellulose, hemicelluloses, lignin, and extractives, which are all important during pulping. The components are either used as the final product or collected as by-products in the black liquor. Some of the by-products can still be separated from the black liquor and be utilized. One common example of a by-product which is utilized to a much smaller extent than its actual potential is the tall oil. Typically, the tall oil contains small amounts of polydimethylsiloxane (PDMS) in different forms, e.g., high, medium, or low molecular weight (MW), which originate from the antifoaming agents that are added during pulping.

The objective of this work was to develop an improved analytical method for the analysis of PDMS in bio-oil matrices. The experimental procedures utilized during this work were solvent extraction and solid phase extraction (SPE). The analytical techniques for detecting PDMS were high-performance size exclusion chromatography (HP-SEC) and gas chromatography (GC). The focus of this work was

to study the properties of the PDMS as it eluted through SPE cartridges, in order to find an improved method for the fractionation. When using the pure high MW PDMS as model compound, it was concluded that the best choice of eluent was dichloromethane (DCM), and the best choice of cartridge material was florisil. Florisil was chosen due to the high yield of eluted PDMS and the relatively good separation between the PDMS and the bio-oil (BO) 1. For the previously developed SPE method, the eluent was also DCM, however, the cartridge material was silicone hydroxide (SiOH).

Another goal of the experimental work was to separate and concentrate the eluted PDMS from a matrix of bio-oil. In total, there were three different bio-oil matrices utilized. The experiments also comprised utilizing three different PDMS model compounds (i.e., high, medium, and low MW). All three model compounds were spiked separately in all three matrices and fractionated using the florisil cartridge. According to the used solvent combinations during SPE fractionation, the first fractions eluted the low MW PDMS, the middle fractions eluted the medium MW PDMS, and the last fractions eluted the high MW PDMS. The developed florisil SPE method was also successfully applied to separate the highly volatile D_3 and D_4 PDMS degradation products from BO 1. The detection of the volatile D_3 and D_4 PDMS products and the low MW PDMS was possible by GC, while the medium and high MW PDMS were detected by HP-SEC.

The properties and potential of the florisil cartridge were compared with the SiOH cartridge throughout this work. When considering the separation of the PDMS from the bio-oil matrices, the florisil cartridge performed a slightly better separation than the SiOH cartridge. The limit of detection (LOD) and limit of quantitation (LOQ) were determined for the SPE method, using the florisil cartridge, as 37 ppm and 113 ppm, respectively. These values were in the same range as for the previously developed SPE method, which utilized the SiOH cartridge (30 ppm and 92 ppm). The main conclusion from the comparison of the cartridges is that the florisil cartridge resulted in a better separation and isolation of the PDMS from the bio-oil matrices, although based on the LOD and LOQ for the cartridges, the SiOH cartridge was overall slightly better than the florisil cartridge.

ABBREVIATIONS

ATR-FTIR	Attenuated total reflection Fourier transform infrared spectroscopy
BO	Bio-oil
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
CN	Cyanopropyl, *cyanopropyl*
CTO	Crude tall oil, *rå tallolja*
DCM	Dichloromethane
ELSD	Evaporative light scattering detection
ESI	Electrospray ionization
ETAAS	Electrothermal atomic absorption spectrometry
EtAc	Ethyl acetate
FL-SPE	Florisil SPE method, *florisil fastfasextraktionsmetod*
FTIR	Fourier transform infrared spectroscopy, *Fouriertransformerad infraröd spektroskopi*
GC	Gas chromatography, *gaskromatografi*
GC-FID	Gas chromatography-flame ionization detection
GC-MS	Gas chromatography-mass spectroscopy
GPC	Gel permeation chromatography, *gelfiltreringskromatografi*
HPLC	High performance/pressure liquid chromatography
HP-SEC	High performance/pressure-size exclusion chromatography, *exklusionskromatografi*
ICP	Inductively coupled plasma
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LOD	Limit of detection, *detektionsgräns*
LOQ	Limit of quantitation, *kvantifieringsgräns*
MALDI	Matrix assisted laser desorption ionization
MS	Mass spectroscopy
MTBE	Methyl tert-butyl ether
MW	Molecular weight
NH_2	Azanide, *azanid*

NMR	Nuclear magnetic resonance spectroscopy, *kärnmagnetisk resonansspektroskopi*
OH	Hydroxyl group, *hydroxylgrupp*
PDMS	Polydimethylsiloxane(s), *polydimetylsiloxan*
PS	Polystyrene
PTFE	Polytetrafluoroethylene
RI	Refractive index detection
RT	Retention time
SEC	Size exclusion chromatography, *exklusionskromatografi*
SiOH	Silicon hydroxide, *kiselhydroxid*
SI-SPE	SiOH SPE method, *SiOH fastfasextraktionsmetod*
SPE	Solid phase extraction, *fastfasextraktion*
THF	Tetrahydrofuran
TMCS	Trimethylchlorosilane
TOP	Tall oil pitch, *tallbeck*

TABLE OF CONTENTS

1   INTRODUCTION

Tall oil is a by-product from the sulfate process when producing wood pulp in pulp mills.[1] The sulfate process is more commonly known as the kraft process and the produced pulp is called kraft pulp. The tall oil is produced when chemical compounds, such as resin acids and fatty acids, from wood are dissolved in the cooking liquor. Another name for the cooking liquor is black liquor. The chemical compounds from wood form salts in the black liquor and these salts can also be called soaps. However, the black liquor also contains unsaponifiable compounds. The soaps and unsaponifiables are usually suspended in the black liquor. Concentration of the black liquor with the suspension results in a separation of soaps and unsaponifiable compounds from the rest of the black liquor. A common name for the soaps and the unsaponifiables is tall oil soap skimming.

There are several tall oil products that can be obtained by processing tall oil in different ways. Saponification of tall oil results in tall oil soap. Tall oil soap is also formed through neutralization of tall oil. Acidification of the tall oil skimmings results in crude tall oil (CTO). CTO is a dark brown mixture. To remove the dark color, CTO is treated with sulfuric acid. This treatment results in a product called acid-refined tall oil. CTO can also be processed via distillation, which results in distilled tall oil. During distillation of CTO, it can be fractionated into the following fractions: tall oil fatty acids, tall oil rosin, tall oil heads, and tall oil pitch (TOP). The tall oil fatty acids fraction contains more than 90% fatty acids. After removal of the fatty acids, the tall oil rosin fraction remains. The tall oil heads fraction is a result of distillation of CTO under reduced pressure, and it is the low boiling fraction from the distillation. Distillation of CTO results also in an undistilled fraction, which is called TOP. A summary of the treatments of the black liquor and the products is illustrated in Figure 1.
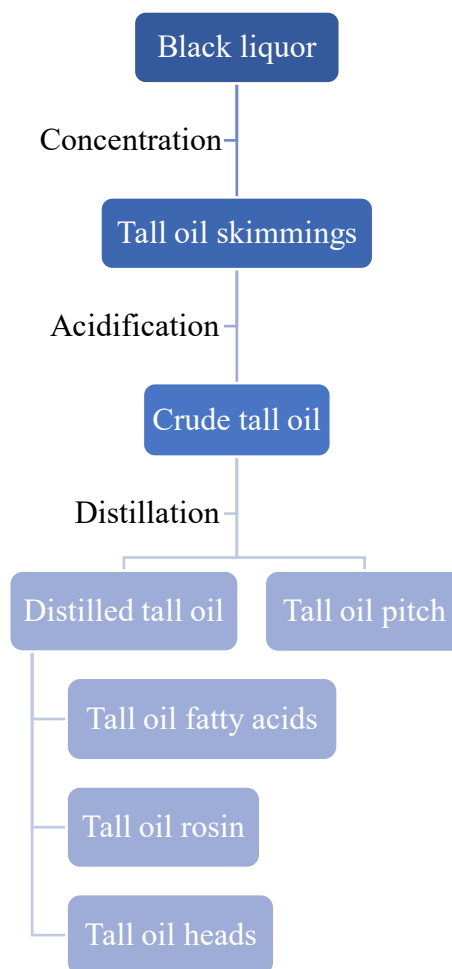
Figure 1. A summary of the different treatments, which result in different products, starting from black liquor.[1]

Since TOP remains in the undistilled fraction, it is considered as a non-volatile compound.[2] The typical yield for obtaining TOP during distillation is between 15% and 40%. This yield is, however, strongly dependent on the quality of the used CTO. Generally, TOP is a mixture of several different components, for example, free acids, esterified acids and unsaponifiable compounds. The ratio between these components is commonly 34–51% free acids, 23–37% esterified acids and 25–34% unsaponifiable compounds. Both the free acids and the esterified acids are composed of fatty acids and resin acids, as well as some other acids. The unsaponifiable compounds are composed of both low-molecular and high-molecular components. Examples of some of the most common acids, or molecules, in the components of TOP are listed in Table 1.

Table 1 Examples of the most common acids found in the TOP components.[2]

TOP component	Common acids/molecules
Free acids	Dehydroabietic acid
	Abietic acid
Esterified acids	Oleic acid
	Linoleic acid
Unsaponifiable compounds	β-sitosterol

CTO is a source of several useful chemicals.[3] These chemicals are in a way renewable through biorefining of CTO. Biorefining of CTO is performed by distillation into distilled fractions and an undistilled fraction (i.e., TOP). The distilled fractions are refined into valuable chemicals, biofuels, or other products, for example, tall oil rosin can be used as components in adhesives or rubbers, while tall oil fatty acids can be produced into soaps or lubricants. The TOP is reused in the pulp mills as fuel for the pulping processes. One problem regarding the biorefining of CTO is that CTO usually contains many impurities, which are residues from the pulping process. The impurities can be, for example, alkali salts and soaps, fibers from cellulose, compounds of lignin[3] or PDMS from antifoaming agents[4], and they cause undesirable problems during distillation of CTO.[3]

This work is divided into two parts: a background and an experimental section. The first part of the background focuses on wood chemistry, pulping methods, and different tall oil products. Some of the tall oil products are already briefly mentioned above. However, the theoretical part of wood chemistry and pulping provides more information to understand tall oil products and how they are formed. The second part of the background discusses PDMS and some of the analytical methods available for the detection of PDMS. One of the main interests of this work was the analysis of PDMS in bio-oil products. The experimental part of this work focused on optimization of the separation between PDMS and bio-oil by using SPE. One important aspect was to achieve a good separation of the eluted PDMS from the bio-oil, to concentrate the PDMS for further analyses. Several factors have been studied during SPE separation, for example, different material of the columns or different combinations of solvents.

2   BACKGROUND

2.1   Wood chemistry

Trees are divided into two types: softwoods and hardwoods.[5] However, it is not only the hardness of the wood that determines which type of tree it is, since some softwood can also be hard, and some hardwoods can be soft. Generally, softwoods produce uncovered seeds in cones, while the seeds of hardwoods are covered and located inside flowers. Softwoods can keep their leaves for several years, while hardwoods typically shed the leaves every fall. Trees consist of roots, branches, leaves and a stem with bark. From a biological point of view, trees consist of cells. The type of cells in a tree differs depending on whether the trees are softwoods or hardwoods. In general, the cells of trees are either structural or non-structural. The structural components of the cells are, for example, cellulose, hemicelluloses, or lignin, while the non-structural components are, for example, extractives or water-soluble compounds. The structural components are located inside the cell walls and the non-structural components are located outside the cell walls.

The main purposes of the wood cells are to provide support, to transport water, to organize metabolism, and to store energy for the tree. The typical structure of the cells in softwoods is a fibrous form, and therefore, the cells of softwoods are often called fibers. The typical structure of the cells in hardwoods is rather a combination of several cell types, for example, fibers and pores. The fibers of the hardwoods are in general shorter and narrower than the fibers of the softwoods. As the cells reach maturity they die, which occurs for both softwoods and hardwoods. The mature dead cells consist of a cell wall, but they are internally empty. The empty and hollow inside of the dead cells is called lumen.

The purpose of the cells differs depending on the location of the cells. The bark of the tree is divided into two layers: inner and outer bark. The inner bark is only a thin layer, which consists of living cells, while the cells of the outer bark are all dead cells. The dead cells of the outer bark act as protection. A cross-section of the stem reveals two different types of mature wood: sapwood and heartwood. The sapwood is the outer part and usually lighter in the color. The cells of the sapwood are mostly dead cells,

and the functions of these cells are to support, to transport water, and to store energy. The inner part of the stem, which is darker in the color, is called heartwood. The cells of the heartwood are also dead, but their function is only to support. Between the mature wood and the inner bark there is a thin layer, which is called cambium. The cells of the cambium are all living, and their function is to produce more mature wood (i.e., tree growth).

2.1.1 Cellulose, hemicelluloses, and lignin

The main components of wood are cellulose, hemicelluloses, and lignin.[5] They are all polymeric, and some other polymers found in smaller quantities are, for example, starch and proteins. Cellulose is the most abundant component, and the content of cellulose varies between 40% and 45% for both softwoods and hardwoods. However, the content of hemicelluloses and lignin differs between softwoods and hardwoods. Generally, softwoods contain 25–30% of both hemicelluloses and lignin, while hardwoods contain between 30% and 35% of hemicelluloses and between 20% and 25% of lignin. Even though the content of hemicelluloses and lignin in softwoods seems to be the same, softwoods normally consist of more lignin than hemicelluloses. The composition of the three major components is not even throughout the whole tree. For the purpose of pulping, non-woody material also works as raw material. Non-woody material is lignocellulosic material other than trees, for example, grass or residues from agriculture. Table 2 shows a brief comparison between the components found in woody material and non-woody material.

Table 2 Comparison of the components of woody and non-woody material.[5]

Chemical component	Woody material	Non-woody material
Carbohydrates	65–80%	50–80%
of which are cellulose	*40–45%*	*30–45%*
of which are hemicelluloses	*25–35%*	*20–35%*
Lignin	20–30%	10–25%
Protein	< 0.5%	5–10%
Inorganic compounds	0.1–1%	0.5–10%

Extractives	2–5%	5–15%

The structure of cellulose is the same for both softwoods and hardwoods. Cellulose consists of linear homopolymers, where the monomer is β-D-glucose in the pyranose ring structure. The linkages between the glucose rings are (1→4)-glycoside bonds. Hemicelluloses are in turn heteropolymers. The monomers of hemicelluloses vary strongly and can be either hexoses, pentoses or deoxyhexoses. The variation of monomer is noticeable when comparing softwoods to hardwoods. The hemicelluloses of softwoods contain more of the hexoses, such as mannose and galactose, while the hemicelluloses of hardwoods are mostly xyloses.

The structure of the lignin is significantly different from the structures of cellulose and hemicelluloses. The main difference is the occurrence of the building units. The building units are irregularly linked to each other with no logical order. The other difference is the type of linkages between the building units. The linkages present in the structure of lignin are either ether bonds or carbon–carbon bonds. The ether bonds are present to a larger extent. The structure of lignin consists of a great variety of functional groups. The variation of functional groups and their occurrence is not only noticeable among different species but also between softwoods and hardwoods. Table 3 shows a brief overview of the occurrence of the different functional groups in softwoods and hardwoods, where the functional groups are calculated per 100 C_6C_3-unit.

Table 3 The occurrence of the functional groups in lignin. The unit is the number of functional groups per 100 C_6C_3-unit.[5]

Functional group	Softwoods	Hardwoods
Phenolic hydroxyl	20–30 groups	10–20 groups
Aliphatic hydroxyl	115–120 groups	110–115 groups
Methoxyl	90–95 groups	140–160 groups
Carbonyl	20 groups	15 groups

2.1.2   Extractives

The term "extractives" is a name for a collection of different chemical compounds.[5] This collection of chemical compounds can consist of several thousand different compounds. In general, woody material for pulping contains between 2% and 5% extractives, while non-woody material contains between 5% and 15% extractives. The extractives are mainly low-molecular compounds, which can be divided into two groups, either lipophilic or hydrophilic. The lipophilic extractives are extracted from the wood by using organic solvents that are non-polar. The hydrophilic extractives are extracted by using water. The extractives work as energy source for the tree or as protection against biological attacks. They can also alter the color or smell of the wood.

Other than the chemical compounds mentioned in the previous section, the extractives include compounds such as terpenes, fatty acids, alcohols, alkenes, lignans, flavonoids, sugars, amino acids, or alkaloids. The amount of the different chemical compounds varies greatly depending on, for example, the growth conditions. The total amount of extractives in wood will naturally decrease during storage of wood. The extractives start to decrease immediately after the felling of the tree in the forest, due to both chemical and biological reactions that occur in the wood. The longer the wood is stored before it is processed, the higher the reduction is in the extractives. Normally, the amount of extractives is much higher in the heartwood and in the bark than in the sapwood.[6] The highest content of extractives is in the stem where the roots of the branches are located.

Extractives are important compounds of the tree and they are considered to have important roles, for example, during the pulping process.[5] Firstly, the extractives are very valuable as starting material for the production of chemicals, especially organic chemicals. Secondly, a higher content of extractives in the wood during pulping results in a significantly higher amount of the by-products, such as raw tall oil.

The lipophilic group of extractives consists mostly of terpenes, terpenoids and fats.[6] They are also called wood resin and by using different methods of extraction, they are isolated in different products. Tar is a black viscous liquid, which is one of the terpenoid-based products. Tar is isolated through pyrolysis of wood. One of the

terpene-based products is, for example, turpentine. One method for the isolation of turpentine is through condensation of the formed gases during kraft pulping. Tall oil is a so-called rosin product, and it is isolated through distillation of the by-product from the kraft cooking (i.e., CTO). The fats are mainly grouped into triglycerides and steryl esters. The triglycerides are isolated as tall oil fatty acids through distillation of CTO. The steryl esters are also isolated through distillation of CTO, however, they remain in the undistilled TOP fraction.

Extractives during pulping

Extractives are greatly affecting the pulping process, for example, in the way they interact with the cooking liquor.[7] Factors that play a role in the effect of extractives during pulping are, for example, the quantity of extractives or the chemical composition of extractives. The quantity of extractives is naturally varying between species and between woody and non-woody materials. Generally, the yield of the pulp is reduced with an increasing quantity of extractives in the raw material. Different chemical compounds of extractives cause different chemical reactions during pulping, for example, discoloration or formation of unwanted deposits. The discoloration is a typical problem for wood storage, and it occurs due to oxidation reactions, which result in dark products. Unwanted deposits can, for example, be accumulated resin that results in blockage in the production line.

The effects of extractives during the pulping process are generally considered as negative. Some more specific and practical effects will now be discussed briefly. During the impregnation of the wood chips in chemical pulping, the penetration rate is reduced. The extractives partly inhibit the penetration of the cooking liquors, however, the intensity of the inhibition is strongly dependent on the quantity of the extractives. During alkaline pulping (i.e., kraft pulping), the used sodium hydroxide (i.e., alkali) reacts more rapidly with the extractives than with the target compound (i.e., lignin). This phenomenon allows less sodium hydroxide to be available for reactions with lignin, and the outcome is a required increase of the consumption of the pulping chemicals. During kraft pulping, the extractives can also react with the lignin due to condensation reactions. The product is a much larger fragment of lignin and the result is a decrease of the solubility of lignin.

2.1.3   Inorganic constituents

The inorganic constituents of trees are considered as everything which is obtained after combustion of all the organic content (i.e., the ash).[5] Normally, the ash consists of several metal oxides, but it can contain whichever element other than carbon, hydrogen, oxygen, and nitrogen. The amount of ash varies between 0.3% and 1.5% for both softwoods and hardwoods. A difference in the ash content is noticeable due to environmental factors, such as the type of available nutrition, the climate, or the growth location. Some of the inorganic constituents are vital for tree growth. In general, however, the inorganic constituents are considered as inconvenient during pulping processes.

2.2   Pulping and biorefining

From an environmental point of view, biorefining is a positive method for the process of converting biomass into energy, biomaterials, and chemicals.[8] Instead of using fossil resources, biorefining produces valuable products using renewable raw materials. The raw material for biorefining varies widely, however, the most commonly used raw material is rich in cellulose (e.g., wood or non-wood materials). The raw material for the process of biorefining can be treated in several ways, resulting in several different products. The three main processing methods for biorefining are mechanical, chemical, and thermal. The compounds that make up wood, as previously mentioned (e.g., cellulose, hemicellulose, lignin, and extractives), all react differently during biorefining processes.

The process of converting cellulose-rich raw material into a mass of fibers is called pulping. The mass of fibers is called, as a product, pulp. For modern pulp mills the pulp is not the only product. During the pulping process, several other by-products are also formed. The main application of the pulp is for papermaking. Other minor applications of the pulp are for cellulose derivatives or for regeneration of cellulose. The by-products also have important applications. Depending on the application of the pulp, the pulp is produced according to different pulping methods. A brief summary

of the different biorefining processes, starting from the raw material and ending with the pulp and black liquor, is illustrated in Figure 2.
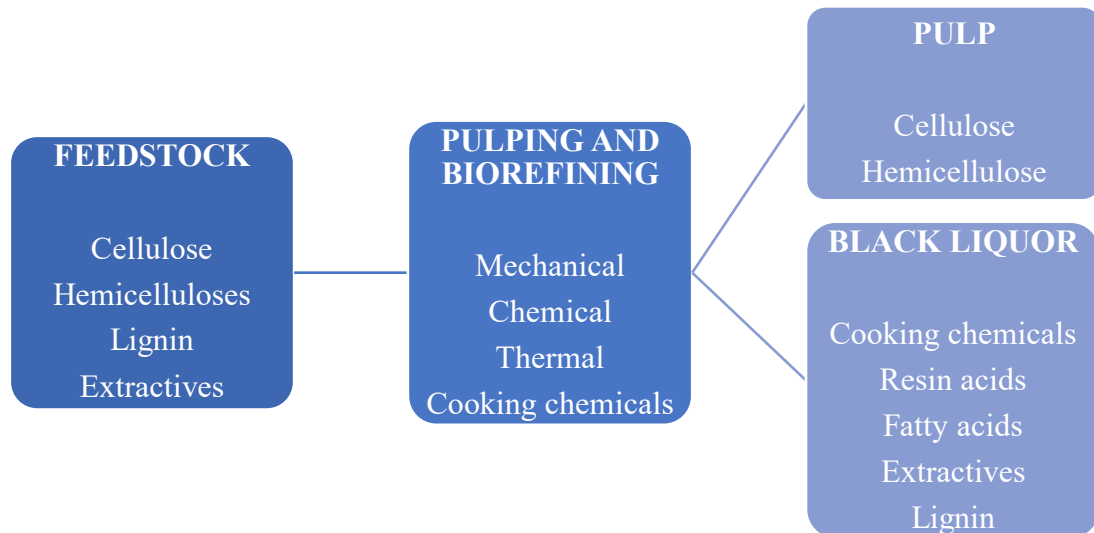
Figure 2. A summary of the biorefining processes, starting from the raw material and ending with the pulp and the black liquor.[8]

2.2.1   Traditional pulping technologies

Mechanical pulping

The treatment of the raw material during mechanical pulping is solely mechanical.[9] The fibers of the raw material are separated from the rest of the material. During mechanical pulping, the lignin is not dissolved, however, it is softened, which enables the separation of the fibers from the lignin. The first step of the process of mechanical pulping is the grinding of the raw material. There are two way for this step: grinding or refining.

During grinding, the debarked logs are pushed from two sides towards a rotating grindstone.[10] During the process of grinding, water is added to the contact area between the logs and the grindstone, in order to decrease the heat generated from the friction. The surface of the grindstone is covered with grits, which tear away the fibers in the wood. Normally, grinding occurs in atmospheric pressure and this process is called atmospheric groundwood. A variation of the grinding is a pressurized

groundwood process, where the grinding occurs in a pressurized chamber. The pressure allows a higher temperature to be generated on the surface of the grindstone, and because of this the lignin is easier to soften.

The refining process starts with wood chips.[11] The wood chips are fed into the center of a refiner, where there are grooves and gaps. The refiner is rotating and creating a centrifugal force, which forces the wood chips to travel outwards. At the same time as the wood chips travel outwards, they are defibrated and fibrillated due to interactions between the grooves and gaps.

Chemical pulping

The chemical pulping is divided into two main processes: kraft pulping and sulfite pulping.[8] A comparison of the kraft and sulfite pulping processes shows some differences in the properties of the processes. During the kraft pulping, the yield of the process is approximately 50%. The pulp that is produced during kraft pulping is a relatively strong pulp. The kraft pulping process also has low specificity on the raw material. The main advantage of the sulfite pulping process is the high brightness of the produced pulp before bleaching. Other advantages are, for example, a higher yield and a cheaper investment. The difference between the processes is that the sulfite pulping process can be performed at both low and high pH values, while the kraft pulping process is only performed at high pH values.

Kraft pulping

The principle of kraft pulping, or sulfate pulping, is a cooking of the raw material, such as wood chips.[8] The wood chips are treated with cooking liquor, which in this case are the chemicals sodium hydroxide and sodium sulfide. The active ions from the cooking chemicals are the sulfide ion, which mainly reacts with the lignin during cooking, and the hydroxide ion, which mainly reacts with the carbohydrates during cooking. Extractives react through hydrolysis reactions. After completed pulping between the cooking liquor and the feedstock, the pulp is washed in order to remove the cooking liquor. The washed cooking liquor mainly consists of dissolved by-products, such as lignin, hemicelluloses, and extractives.

The aim of the reactions involving the lignin is delignification. The reactions, which results in delignification, are mainly the cleavage of the aryl ether linkages in the lignin structure. Other reactions involving lignin are demethylation and condensation. The delignification reactions result in smaller fragments of lignin, which are soluble in water and become dissolved in the cooking liquor.

Reactions due to the hydroxide ion affect the carbohydrates. Common reactions of carbohydrates during kraft pulping are deacetylation, peeling, stopping and alkaline hydrolysis. The peeling reaction is the removal of an end group in a carbohydrate chain. The stopping reaction converts the end group into an alkali-stable unit and because of that, peeling is prevented. Alkaline hydrolysis is the cleavage of a glycosidic linkage anywhere in the carbohydrate chain. Alkaline hydrolysis results in new end groups that are involved in secondary peeling. The reactions of carbohydrates mainly affect the hemicelluloses because of their low crystallinity and low degree of polymerization.

Sulfite pulping

The sulfite pulping is another chemical conversion of the raw material.[8] The sulfite pulping process is quite similar to the kraft pulping process. The difference between the two processes is the cooking chemical. The cooking chemical for sulfite pulping is sulfur dioxide. The active ions from the cooking chemical for sulfite pulping are hydrogen sulfite ions and sulfite ions. The concentrations of the active ions are highly dependent on the pH of the cooking liquor. The change in the concentrations of the active ions according to the pH is due to a formation of an equilibrium between sulfur dioxide and water.

The reactions that occur during sulfite pulping are somewhat different in comparison to the reactions of the kraft pulping. It is the same chemical compounds of the raw material that react (i.e., lignin, carbohydrates, and extractives), however, the type of reactions differs. The main goal for the reactions of the lignin is delignification. Delignification occurs during sulfite pulping due to sulfonation and hydrolysis. The sulfonation adds sulfonic acid groups to the lignin structure. The hydrolysis cleaves

the aryl ether linkages in the lignin structure. As a result of these reactions, the lignin fragments become more hydrophilic and more soluble in water.

The reactions of the carbohydrates are depending on the pH of the cooking liquor. At low pH values, the main reaction of the carbohydrates is hydrolysis. At high pH values, some peeling of the carbohydrates can also occur. In general, the carbohydrate reactions only affect the hemicelluloses. Reactions of the extractives can be, for example, sulfonation or dehydrogenation.

2.2.2   Unconventional pulping technologies

The aim of the traditional pulping methods is to obtain the fibers of the biomass, while not caring much about the rest of the components of the biomass.[12-14] The components that are dissolved in the cooking liquor, or black liquor, are considered by-products, however, they are usually heavily degraded. There are though good efforts to also utilize the by-products in the black liquor, in order to give a high yield as possible for the utilization of biomass. The unconventional pulping technologies, however, focus on the extraction of the so-called by-products, resulting in non-degraded by-products and a more extensive utilization of the biomass.

BLN-pulping

The BLN-pulping method is used for extraction of biomass.[13,14] The method is still under development, but the main principle is to use hot water to extract the hemicelluloses. The crucial step is in the beginning, when all air must be removed by reducing the pressure. If oxygen is present, the hemicelluloses will degrade. The air-free biomass is then impregnated with hot water. During the extraction, the same extraction water is circulated several times through the biomass. This is positive, as there is a minimal use of water and as the circulation also results in a higher concentration of the extracted components. The BLN-pulping method can also be applied to extract the lignin from the biomass. In that way, the extracted lignin is sulfur-free, in contrast to the sulfur-containing lignin obtained from the traditional pulping method.

Organosolv pulping

Organosolv pulping is also considered an unconventional pulping technology because the method utilizes organic solvents for delignification of biomass.[12] The advantage of using organic solvents over the traditional pulping methods, is that the removed lignin and other by-products are much less degraded and can easily be recovered for further utilization. The process of organosolv pulping is based on using a mixture of organic solvents and water, usually with a ratio ranging between 10 vol% and 50 vol%.[15] The process operates at high temperatures, around 200 °C. Another advantage of using the organosolv pulping method is that more lignin is removed resulting in a higher concentration of cellulose in the pulp.

2.2.3   Other technologies for pulping

The thermal pulping is usually combined with both mechanical and chemical pulping, creating processes called thermomechanical pulping[9] or thermochemical pulping.[16] There are also combinations of mechanical and chemical, called chemimechanical pulping, and even a combination of all three processes, called chemithermomechanical pulping.[9]

After grinding or refining of mechanical pulps and after cooking of chemical pulps, the pulp is further treated according to several steps.[17] The first after-treatments are washing and screening. Washing is performed in order to recover unwanted dissolved material and chemicals. The dissolved materials can be used, for example, as fuel, while the chemicals are reused. Screening is a process that removes impurities from the pulp. The impurities in pulp are usually solid material, such as uncooked wood chips or larger wood pieces. Another after-treatment step is bleaching.[18] The main goal of bleaching is to remove lignin and increase the brightness of the final pulp. There are several different methods that all achieve some bleaching on their own, however, to reach maximized bleaching of a pulp, a sequence of many different bleaching steps is required.

2.2.4   Refining of crude tall oil (CTO)

As previously mentioned, CTO is a by-product from the pulping processes.[19] Although CTO contain several valuable chemical compounds, CTO is almost never used as unrefined. CTO in an unrefined state creates problems for the use of the specific compounds. The use of the fatty acids from unrefined CTO in the soap industry is negatively affected due to the presence of the rosin acids. Likewise, processing of rosin acids from unrefined CTO is disturbed by the presence of the fatty acids. Due to these problems, CTO needs to be refined in order to separate the components from each other. This section will discuss some methods for separating the components of CTO.

Distillation

The components of CTO are very likely to decompose at high temperatures.[19] The rosin acids start to decompose at temperatures below 250 °C. As the temperature rises above 250 °C and towards 300 °C, the decomposition of the components increases. This can cause problems during distillation and to prevent the occurrence of the problems, the distillation is usually performed under vacuum. An increase in the vacuum enables a decrease of the boiling points of the components. With a lower boiling point, the components can be heated until they are boiling without any decomposition.

Normal distillation, or simple distillation, is hardly ever used because the fractionation of the components is highly limited. The simple distillation mostly removes only unpleasantly smelling compounds and dark colored residues, leaving the fatty acids and rosin acids still present in the CTO. Because of this, the distilled CTO by simple distillation has a low quality. Instead, to increase the quality and the fractionation of the components, the distillation can be carried out by using fractionating columns. With the aid of the columns, the distillation enables removal of unwanted products and separation of the fatty acids and rosin acids. However, the distilled separated fractions are never ideal and perfect. The distillation is performed several times. With every new distillation, the temperature of the process is lowered. Decrease of the temperature will also decrease the extent of decomposition.

The vacuum distillation with the fractionating columns enables an almost complete separation of the fatty acids from the rosin acids. However, a separation between the different types of fatty acid components (e.g., stearic acid or oleic acid) is not possible through distillation alone. To enable further separation of the different types of fatty acids, which can also be called saturated or unsaturated, solvent extraction is required. Saturated (e.g., stearic acid) and unsaturated (e.g., oleic acid) fatty acids have different solubility in polar and non-polar solvents. To increase the separation even more, columns are used here in the same way as for the simple distillation.

CTO consists of a low-boiling fraction, which can also be called light oil. The light oil is the first fraction obtained from the distillation. This light oil fraction contains a variety of different compounds, such as unpleasantly smelling compounds, dark colored compounds, unsaponifiables, or decomposition products. Light oil is also being separately refined by distillation. Distillation of light oil is difficult due to the highly similar boiling points of the components. For this reason, fractionating columns with a high fractionation capacity are used for distillation. The distillation residue (i.e., TOP) from CTO also contains valuable chemical compounds. The TOP fraction corresponds to approximately 15–40 % of the CTO. TOP is mainly used as softeners in asphalt or as fuel oil.

Extraction

Extraction of CTO is divided into two types: batch extraction and continuous extraction.[19] The batch extraction is mainly performed to remove some of the unwanted and unimportant components of the CTO, as this is not a method to separate the fatty acids from the rosin acids. Solvents that have been used in successful batch extraction are, for example, petroleum ether or furfural. The batch extraction results in a high yield of a combination of fatty acids and rosin acids. In general, the yield is between 85% and 95%.

The continuous extraction is a refining method that can be used to separate the fatty acids from the rosin acids. The yield of the separated fatty acids and rosin acids is higher for the continuous extraction than for the distillation. This advantage is possibly due to lower temperature used during continuous extraction. However, the purity of

the products from the continuous extraction are not as high as for the products from the distillation.

Refining CTO into fuel

As previously mentioned, CTO can be refined into fuel.[20] One method for this involves a series of different steps. This method results in diesel fuel with a high quality and high yield. The formed diesel from refining CTO is also called second-generation diesel. The problem with refining CTO is the contaminants (e.g., alkali salts, sulfur-containing compounds, lignin, or fibers). When refining for second-generation diesels, the contaminants should first be removed in order to avoid any kind of production problems.

The first step of the method invented by Stigsson and Naydenov[20] is to remove any kind of contaminant which is of non-oil character. This is achieved through specific reactions and separations. The residue from this step is called refined tall oil stream. The second step is to remove a volatile fraction. This volatile fraction is easily removed by treating the refined tall oil stream firstly in a heat-exchanger and secondly in a stripping unit. The residue from the second step is an oil stream containing components with high boiling points (e.g., 170 °C or higher). The third step is performed through a separation of the oil stream. This separation is achieved by using a vacuum distillation column. The oil stream is separated into two fractions, where the first fraction is components with boiling points between 170 °C and 400 °C, and the second fraction is components with boiling points above 400 °C. The last step of the method is treatment of the fraction of components with boiling points between 170 °C and 400 °C. This fraction is forced through a catalytic reactor for deoxygenation. The same fraction is further forced though a second catalytic reactor, which this time is for hydrogenation and desulphurization, before the fuel is formed.

2.3   Tall oil products

2.3.1   Chemical composition

The main components in tall oil products are, as mentioned, fatty acids, resin acids and unsaponifiables.[21] The unsaponifiables are also called neutral compounds, as they are non-acidic. The chemical composition of tall oil products is strongly dependent on several different factors.[1] The two main factors are the geographical location and the species of the trees used as raw material for the pulp.

As an example, when starting from the southeastern areas of United States and moving north all the way to Canada, the chemical composition varies greatly. In the south, the composition of tall oil is 40–50% resin acids, 6–8% unsaponifiables and the rest is fatty acids, which is normally lower than the amount of resin acids. About halfway towards Canada, the amount of resin acids is 30–35%. The decrease in resin acids results in an increase in the amount of fatty acids instead. In the north, the amount of resin acids decreases even further, to about 25%, while the amount of unsaponifiables increases to 12–25%. These differences are also detectable in other parts of the world. In Finland, the composition of tall oil varies between 22% and 49% resin acids, 35% and 71% fatty acids, and 6% and 28% unsaponifiables. Other factors that affect the composition occur during the pulping process, which can be, for example, oxidation or isomerization during chip storage and pulping.

The fatty acids in tall oil products consist mostly of compounds with 18 carbon atoms, but some compounds with 16 or 20 carbon atoms are also found.[21] Common C_{18} fatty acids are, for example, stearic acid, oleic acid, and linoleic acid. Examples of C_{16} and C_{20} fatty acids are palmitic acid and eicosadienoic acid, respectively. The chemical structures of the mentioned fatty acids are illustrated in Figure 3. The resin acids, in tall oil products, have similar carbon backbone, which consists of three fused ring systems.[1] The most common resin acids are, for example, dehydroabietic acid, abietic acid and palustric acid. The chemical structures of the common resin acids are illustrated in Figure 4. The unsaponifiables are mainly alcohols.[21] The most common component is sitosterol, but other alcohols, such as 24 carbon atoms lignoceryl

alcohols are also found among the unsaponifiables. The chemical structures of the common unsaponifiables are illustrated in Figure 5.

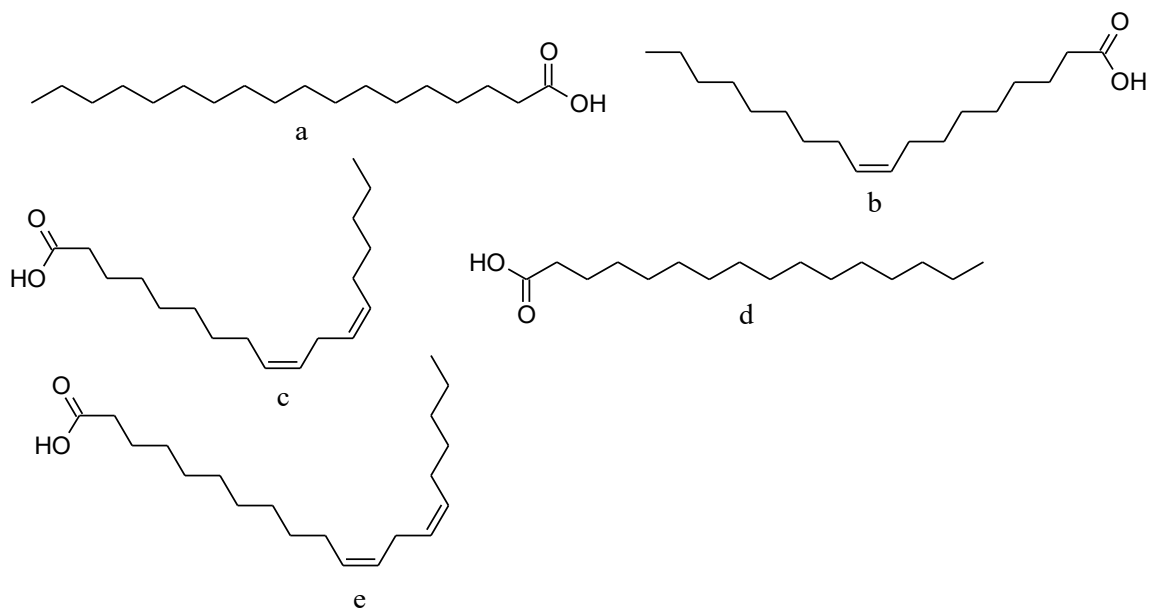***Figure 3. Chemical structures of (a) stearic acid, (b) oleic acid, (c) linoleic acid, (d) palmitic acid, and (e) eicosadienoic acid.***
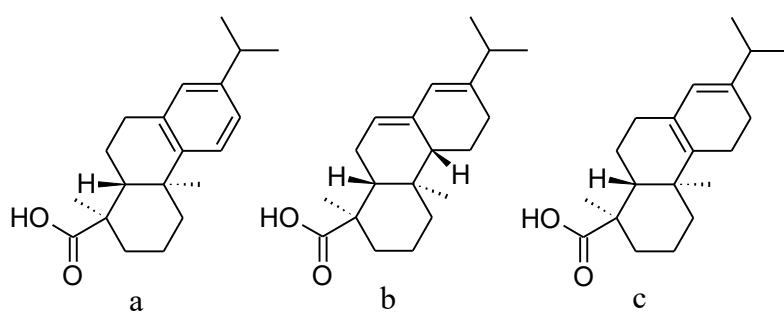

Figure 4. Chemical structures of (a) dehydroabietic acid, (b) abietic acid, and (c) palustric acid.
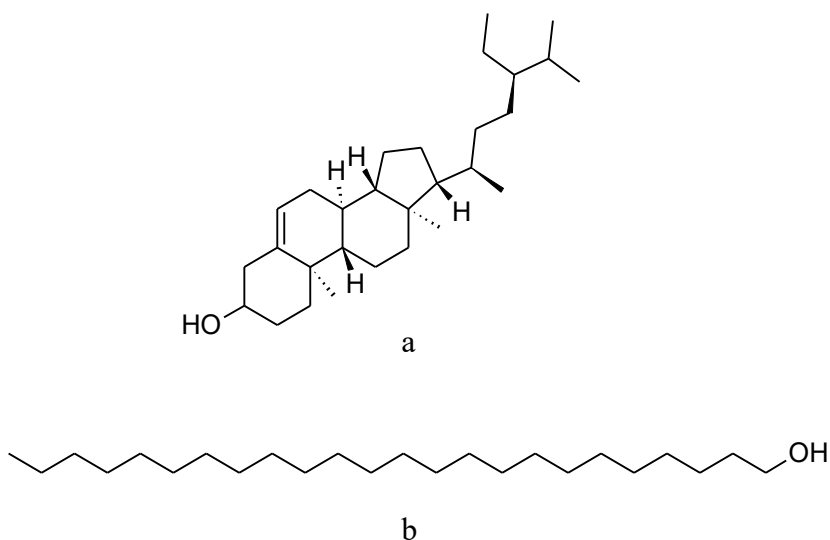
Figure 5. Chemical structures of (a) β-sitosterol and (b) lignoceryl alcohol.

2.3.2   Chemical and physical properties

The components in tall oil products (i.e., the fatty acids and the resin acids) can be modified through several different reactions.[1] The fatty acids are linear with both saturated and unsaturated chains, but they also have a carboxyl group. Based on this, the chemical modifications of fatty acids can be divided into two types. The first type is modification due to a double bond and the second type is modification due to the carboxyl group. Examples of chemical reactions involving the double bond are dimerization, epoxidation, and ozonolysis. Examples of reactions involving the carboxyl group are formation of salt, esterification, and preparation of nitrogen derivatives. The functional groups of resin acids are the same as for fatty acids (i.e., double bond and carboxyl group), though the structure of resin acids is cyclic instead of linear. Modifications of resin acids are divided into the same two types as for fatty acids. Examples of reactions involving the double bond in resin acids are isomerization, disproportionation, hydrogenation, and dimerization. Examples of reactions involving the carboxyl group in resin acids are formation of salt and esterification.

The physical properties of the tall oil products that will now be shortly discussed are vapor pressure (or volatility), solubility, and viscosity. The volatility of TOP is low

(i.e., non-volatile component). Even though TOP is non-volatile it contains volatile components, which have a low vapor pressure. The components of tall oil are insoluble in water. They are, however, soluble in, for example, alcohols, esters, and ketones. The viscosity of CTO is dependent on its temperature. Storing CTO in temperatures below 50 °C results in a high viscosity, due to crystals that forms or that CTO solidifies.

2.3.3   Applications

There are many different applications of tall oil, however, the application is dependent on what kind of tall oil product that is being used.[1] The products that are most widely used are tall oil fatty acids and tall oil rosin. Due to economic reasons, it is, however, crucial to also use all other tall oil products. Now following a brief mentioning of some applications of tall oil products.

The tall oil fatty acids fraction can be divided into two classes: fatty acids with less than 2% resin acids, and fatty acids with between 2% and 10% resin acids. One application of tall oil fatty acids is the manufacturing of dimer acids or polyamine condensates. Other applications of tall oil fatty acids are, for example, as protective coatings, soaps, detergents, disinfectants, lubricants, textiles, and rubber products. The tall oil rosin fraction is also divided into two classes: tall oil rosin with high content of dehydroabietic acid, and tall oil rosin with sulfur content between 400 ppm and 600 ppm. The largest application of tall oil rosin is the production of chemicals, such as resins, emulsifiers, or plasticizers. Tall oil rosin is also widely used in paper making processes, and especially for sizing of paper. Other end uses of tall oil rosin are, for example, inks, adhesives, coatings, floor coverings, and rubber.

The tall oil products, which are not as widely used as tall oil fatty acids and tall oil rosin acids, are CTO, distilled tall oil, tall oil heads, and TOP. CTO is mostly used for distillation. Although, a small part of CTO is used in, for example, ore flotation. The composition of distilled tall oil is 25–40% resin acids and 60–75% fatty acids. Distilled tall oil together with lime results in wall paint. Distilled tall oil is also used to produce surfactants of various types (e.g., water-soluble, oil-soluble, anionic, or cationic). The tall oil heads fraction is used within ore flotation, due to the high content of

unsaponifiables in the fraction. Palmitic acid is concentrated in the tall oil heads fraction, and from there it is produced into pure palmitic acid through solvent crystallization. The TOP fraction has a much higher content of unsaponifiables than the tall oil heads fraction. Even though, TOP is used to produce alkyd-type coating resins. TOP also has a great usage as an asphalt additive. As the TOP fraction contains a low content of sulfur, the TOP fraction is used as a valuable fuel.

2.4   Polydimethylsiloxanes (PDMS)

2.4.1   PDMS in pulp and paper industry

In the pulp and paper industry, antifoaming agents are used.[22] The antifoaming agents are used to decrease the formation of foam during the pulping process. When air is entrained in the pulping system, it causes several production problems, for examples, the effectiveness of the system, the quality of the paper, and the properties of the paper are all decreased. Normally, entrained air also results in generation of air bubbles (i.e., foam). The antifoaming agents are added chemicals that work in one of two ways. The added chemicals either prevent foam formation in the first place or they destroy already formed foam. Strictly speaking, the term antifoaming agent is a chemical used to prevent foam formation and it is usually added before foaming processes, while the term defoamer is a chemical used to break down already existing foam and is added after formation of foam. A couple of criteria for a chemical to work as an antifoaming agent or a defoamer are, for example, that it should be surface active, it must be insoluble in water, and it should have the ability to form dispersed particles.

During the pulp and paper production, several steps cause formation of foam.[23] One example is when washing the kraft pulp, foam is formed in the black liquor. Generally, there are four factors that, if they are present, allow the formation of foam. These factors are as follows: a liquid, chemical agents, air, and mechanical energy. Without defoamers, the formation of foam would be immense. Foam in the pulp and paper industry leads to production problems or even problems with safety or the environment. The created foam fills up the volume of the production tanks and results in a decreased capacity of the tanks, which is considered a production problem.

There are four different types of defoamers available, which are chemically different: oil-based, silicones, surfactants, and emulsions.[22] Oil-based defoamers contain a matrix of oil with dispersed particles of, for example, wax or silica. Silicones, or silicone-based defoamers, contain a matrix of silicone oil with dispersed silica particles. Surfactants are derivatives from fatty alcohols. Emulsions contain a matrix of water with dispersed wax emulsions or additives. The most used type of antifoaming agents or defoamers is silicones, with the silicone oil typically originating from PDMS.[24] The surface of the dispersed silica particles is commonly hydrophobic, due to a chemical treatment.[25] This hydrophobicity allows the silica particles to act as active bubble breakers. The PDMS is used as a source for antifoaming agents and defoamers mostly due to its low surface tension energy.[26]

As previously mentioned, silicones are antifoaming agents or defoamers based on silicone oil and dispersed particles.[24] Both the silicone oil and the hydrophobic particles can act as an antifoaming agent or defoamer by themselves, however, a combination of the oil and the particles is a more effective alternative. The silica particles are generally between 0.1 µm and 10 µm in size, and together with the PDMS oil, the particles are forming a solid–oil droplet of hydrophobic characteristics. These hydrophobic droplets will attack and destroy the foam according to one of two mechanisms: fluid entrainment or film pinch-off. The mechanism of the fluid entrainment works when the droplet spreads out on the foam film between the air–water interface and generating thinning of the film until it ruptures, and the foam is destroyed. The mechanism of the film pinch-off works when the droplet forms a lens on the foam film between the air–water interface and instead of spreading out, the droplet forms a bridging formation between the films. The bridging droplet pinch off the film at the contact points, resulting in rupture and destroying of the foam.

A study has been carried out by Chen et al. for which they studied the effectiveness of the PDMS-based antifoaming agents.[27] The result from the study is that with a higher concentration of antifoaming agent, the more likely the PDMS oil will spread out. With a lower concentration of antifoaming agent, the droplets are more diluted and have a lower possibility to work according to the mechanisms mentioned earlier. Other results were that the effectiveness generally increases with increased viscosity of the PDMS.

There is another study carried out by Patterson, regarding the effect of the antifoaming agent because of different properties of the silica in the antifoaming agent.[25] For this study, the antifoaming agent was prepared by a method called in-situ hydrophobing. The effect, or the performance, of the antifoaming agent was studied for the defoaming of black liquor. The studied parameters were pH, particle size and addition of a promoter. For antifoaming agents with the same pH but different particle size, the study showed that smaller particle sized silicates had a better ability to defoam. When comparing antifoaming agents with the same particle size but different pH, the study showed that a more neutral or acidic pH had better ability to defoam. The studied pH values were 6.3 and 8.6. When comparing the addition of a promoter at different pH values (i.e., 6.3, 8.6, and 9.7), the study showed that the promoter greatly increased the ability to defoam for the acidic pH value (i.e., 6.3). The addition of the promoter at alkaline pH values did not significantly change the ability to defoam.

The silica from the antifoaming agents and the defoamers is a problem in pulp and paper industry especially when considering industrial oil refining.[4] The silica results in several problems during refining, for example, a decrease of the efficiency of the hydrotreating catalysts or a formation of unwanted deposits in the production units.

2.4.2   Silicone polymers

PDMS is one of the most common silicone polymeric compounds.[28] Silicone polymers are characteristic for the repeating oxygen and silicon units in their backbone. The silicon atoms are also bonded to branches, which most commonly are organic groups (e.g., methyl, phenyl, or vinyl). The repeating unit, where silicon is bonded to one oxygen and two side groups, is called siloxane. In the case where the polymer consists of methyl groups as side groups, the structure is called polydimethylsiloxane. The repeating unit of siloxane is illustrated in Figure 6.
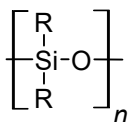
Figure 6. Structure of the repeating unit of siloxane. When R = methyl, it is the repeating unit of PDMS.

Besides the repeating unit in PDMS, the polymer also needs termination at the ends in some way. For PDMS, the ends are terminated with a so called trimethylsilyl unit at each end. In the trimethylsilyl unit, the silicon atom is bonded to one oxygen and three methyl groups. The complete structure of PDMS is illustrated in Figure 7.
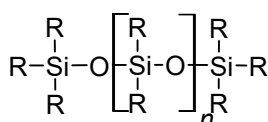
Figure 7. Structure of PDMS, with the repeating unit repeated n (n = 1,2,3…) times and R = methyl.

The units, or building blocks, of silicone polymers can also be abbreviated to only one letter. The siloxane unit is abbreviated with the letter D (i.e., from "di"), because the silicon atom in the unit can expand the chain in two directions through the oxygen atoms. With the same principal, the trimethylsilyl unit is abbreviated with the letter M (i.e., from "mono"), due to the silicon can expand only in one direction. There are also two other units, T (i.e., from "tri") and Q (i.e., from "quad"), where the silicon atom can expand in three or four directions, respectively. The structures of all four units are illustrated in Figure 8. Following this rule of nomenclature, the PDMS can also be referred to as MD_nM.

Figure 8. Structures of the different repeating units, where R = methyl, and their abbreviations.

2.4.3   Properties and applications

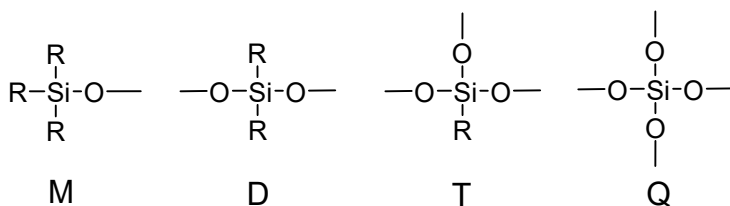The properties of silicone polymers differ distinctly to the properties of carbon polymers, even though the silicon and carbon atoms are in the same group in the periodic table.[29] The bond lengths of silicon are longer than the bond lengths of carbon. Silicon has a much lower electronegativity than carbon. Due to this, silicon together with oxygen results in an extremely polarized bond, in comparison with the polarity of the carbon–oxygen bond. The highly polarized silicon–oxygen bond can without protection result in intermolecular interactions. Luckily, the methyl groups will protect the main chain. Protection of the main chain is also a result of the flexibility of the chain. The rotation energy for the siloxane unit is low, which results in a silicone polymer that can occur in many conformations.

Some specific properties of PDMS are, for example, that they possess a low surface tension. A low surface tension gives the polymers the ability to wet most surfaces. The glass transition temperature of PDMS, and silicone polymers in general, is very low. A cross-linked PDMS polymer will appear as an elastomer in room temperature, even though it is without plasticizers. The polymers also have a high gas permeability, especially to oxygen, nitrogen, or water vapor.

Silicone polymers have lots of useful properties, and they can also easily be modified. This results in a wide range of applications for silicone polymers. The applications of silicone polymers in personal care, pharmaceutical industry, food industry, and pulp and paper industry are very common and will now be briefly discussed.

The type of silicone polymer used in personal care products varies greatly. Many silicone polymers with structural differences are used because they enhance the performance of beauty products. In general, the silicone polymers can, for example, result in good spreading or waterproofing, and enhance volatility or permeability of the beauty product. These structural differences of the silicone polymers are related to the molecular weight, the chemical structure or the substituents present on the backbone. Examples of the performance related to each structural difference are good spreading for silicone polymers with low molecular weight, smooth and soft feel on

the skin for silicone polymers with long linear chains, and more shiny effect for silicone polymers with phenyl substituents.

Differences in the silicone material are also useful in the pharmaceutical industry. Variation of the chemical structure results in different silicone materials, such as fluids, gels, elastomers, and adhesives, which all have specific advantages and specific pharmaceutical applications. Examples of applications are the usage of fluids as lubrication or drug carriers, gels as wound dressing, elastomers as implantation devices (e.g., pacemakers), and adhesives as temporary devices on skin (e.g., catheters) or as transdermal drug delivery systems. One other major advantages of silicone polymers in pharmaceutical application is their good biocompatibility, which allows for a great variety of pharmaceutical applications. The low surface tension and high gas permeability of silicone polymers are also useful properties for pharmaceutical usage.

One of the major process-related problem in both food industry and in pulp and paper industry is foam. Production of foam decreases the efficiency of the process and the important compound to decrease foam is antifoaming agents, or foam control agents. PDMS materials are excellent for the usage as antifoaming agents mainly due to the combination of properties of PDMS, such as low surface tension and flexibility of the backbone. In the food industry it is vital that the additives (i.e., antifoaming agents) do not result in poisonous food products. PDMS-based antifoaming agents have an advantage for this due to the chemical inertness of PDMS. Another application of silicone polymer in food industry is for lubrication of production equipment. Other than antifoaming agents, the silicone polymers can also be used as additives in the pulp and paper industry. The silicone material in the additives works either to enhance the impregnation of the wood chips or to increase the softness of tissues. In the recycling step of paper, silicone polymers will also work as aids for the process of de-inking.

2.5   Analysis of PDMS

2.5.1   Common analytical techniques for analyzing silicone polymers

There are several analytical techniques which allow for determination of silicone present at trace levels.[30] For trace level analysis, sample preparation is critical. Usually, the sample preparation is a form of extraction of silicone from different matrices. The most convenient extraction is by using organic solvents. The following paragraphs will present some of the common analytical techniques for analyzing silicone and silicone polymers: Fourier transform infrared spectroscopy (FTIR), GC, nuclear magnetic resonance spectroscopy (NMR), gel permeation chromatography (GPC), size exclusion chromatography (SEC), inductively coupled plasma optical emission spectroscopy (ICP-OES) and electrothermal atomic absorption spectrometry (ETAAS). All the techniques are available for coupling with different detectors, and the parameters for the techniques vary somewhat. A brief summary of the parameters for PDMS analysis with the mentioned techniques is found in Table 4.

Table 4 Parameters (LOD and LOQ) of PDMS analysis with some of the previously mentioned analytical techniques.

Method	LOD	LOQ
FTIR[31]	0.009% w/v	0.03% w/v
GC-FID[32]	10–50 ppm	-
GC-MS[33]	10 pg/µl	-
NMR[34]	1.7–3.7 mg	-
SEC-ICP-OES[35]	0.60 ppm	2.0 ppm
ETAAS[36]	0.03–0.2 µg/g	-

Analysis of silicone polymers can easily be performed by using FTIR.[29] The principle for the FTIR analytical technique is to identify organic compounds as they are exposed to infrared radiation.[37] FTIR is an extensively available analytical technique, which not only detects the silicone polymers, but also acquires information regarding the chemical structure.[29] However, the FTIR method works best when the sample is composed of isolated analytes, meaning that with a combination of several unknown analytes it is difficult to receive acceptable results.[37] As an analytical technique for

detecting silicone polymers, FTIR usually has a LOD of 1%.[29] Using FTIR, PDMS structures can be separated from, for example, trimethylsilyl groups or copolymers. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) is a possible variation of this analytical technique.[38] The FTIR-spectrum provides information about the wavelengths of the different chemical structure that are present in the sample. A typical FTIR-spectrum of PDMS shows peaks at about 792 cm^{-1}, 1047 cm^{-1}, 1260 cm^{-1}, and 2955 cm^{-1}. The corresponding chemical structures of PDMS is summarized in Table 5.

Table 5 The corresponding chemical structures of PDMS for typical wavelengths in a FTIR-spectrum of PDMS.[38]

Wavelength	Chemical structure of PDMS
792 cm^{-1}	Si-CH$_3$, where the Si–C-stretching is the source of the peak
1047 cm^{-1}	Si–O–Si-stretching is the source of the peak
1260 cm^{-1}	Si-CH$_3$, where the CH$_3$ deformation is the source of the peak
2955 cm^{-1}	Si-CH$_3$, where asymmetric CH$_3$-stretching is the source of the peak

Another technique for analyzing silicone polymers is GC.[29] The principle for the GC analytical technique is to measure volatile components in a sample.[39] The sample is injected and separated in a heated column. GC coupled with mass spectroscopy (GC-MS) allows for detection of low-molecular cyclic siloxanes, which indicate the presence of silicone polymers.[29] However, GC-MS cannot be used for accurate quantification of silicone polymers. Accurate quantification using the GC technique is possible by coupling GC with flame ionization detection (GC-FID). Analytical standards are used for comparison when analyzing unknown analytes with GC.[40] The standards are either internal or external, however, they should be analyzed with the same conditions as the unknown analyte.

NMR is an analytical technique that acquires detailed information regarding the silicone polymer structure.[29] The principle for the NMR analytical technique is to measure the change in energy levels of nuclei, as they are exposed to a strong magnetic field.[41] ^{1}H-NMR and ^{13}C-NMR are used to identify the organic substituents (e.g., methyl) that are bonded to the silicone backbone.[29]

GPC is another widely used analytical technique for analyzing silicone polymers. GPC is also referred to as a SEC technique. The principle for the SEC analytical technique is to separate compounds based on their size.[42] Dissolved compounds are injected into a column filled with a porous material. The smaller compounds move slowly through the column because they are small enough to enter the pores, while the larger compounds move quickly because they are too large to enter the pores. GPC coupled with refractive index (RI) detection acquires information regarding the averages molecular weight and the distribution of the silicone polymers.[29] GPC coupled with laser angle scattering detection acquires information regarding three-dimensional structure of the silicone polymers.

Characterization of PDMS can also be performed using SEC coupled with mass spectroscopy (MS), with either electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI).[43] Both SEC-ESI-MS and SEC-MALDI-MS acquire information regarding the molecular composition of the silicone polymers, which can identify, for example, repeated units, end groups, or impurities. SEC coupled with triple detection (i.e., light scattering detection, viscosity detection, and refractive index detection) can also be used for PDMS characterization. SEC coupled with the triple detection acquires information regarding the absolute molecular weights and distribution of the silicone polymers. SEC can also be coupled with evaporative light scattering detection (ELSD) for the characterization of PDMS.[44-48]

Quantification of Si-compounds in, for example, silicone oils are also possible by using ICP-OES.[30] ICP-OES is a rather simple yet convenient analytical approach for analysis of Si-compounds. The principle for the ICP-OES analytical technique is that the analyte atoms are exposed to a plasma.[49] Due to the plasma, the atoms are excited to a higher energy level and returned to a lower energy level. As a result of the return, the energy is released as emission rays, which are measured and used to identify the

analyte. Other hyphenated methods with inductively coupled plasma (ICP) are, for example, ICP-MS[30] or SEC-ICP-OES.[35] The SEC-ICP-OES method is size exclusion separation coupled with ICP-OES. The result of this combination is a separation of high and low MW compounds and an outstanding determination of silicon distribution in the sample.

Determination of Si-compounds in biological samples by using ETAAS have been studied by Hornung and Krivan.[36] The principle for ETAAS is to introduce the sample into a heated tube of graphite.[50] The detection is based on the amount of absorbed radiation by free atoms.[51]

The ELSD coupled with SEC is the most universal detector available.[44] The principle for the detection is to expose non-volatile with a scattered laser light. A study performed by Mojsiewicz-Pienkowska[44] determines the intensity of the signal when alternating some parameters, while analyzing PDMS. The result from the study states that a change in neither polymerization degree, viscosity, nor the molecular weight of the PDMS will influence the signal intensity.

When operating with ELSD detector, the detection sensitivity can be controlled using different types of "gain".[52] The setting for the gain on a Sedex 85LT detector is available in a range of 1 to 12. An increase of the gain results in an increase of the signal amplification (i.e., sensitivity of the detector). Increasing the gain by one (e.g., from gain 1 to gain 2) correlates in a twofold increase of the sensitivity. Sometimes, the analyte of interest is not detected. In this case, there are some options for trying to increase the sensitivity. One option is to decrease the temperature of the detector. This could help increase the sensitivity as the thermal evaporation is decreased. Another option is to use high quality solvent for mobile phase in order to decrease background noise. Low sensitivity for the detection could also be a sign of dirty nebulizer. ELSD, as a detector in combination with reversed phase liquid chromatography, achieves a LOQ of 88 μg/ml for the detection of silicone oils.[53]

3   OBJECTIVES

This work was based on previously developed analytical procedures for SPE and acted as a continuation of the thesis published by Kenneth Arandia in 2018.[54] Most of the experimental procedures of this work were similar to the procedures of the previously mentioned thesis.

The objective of this work was to develop an improved analytical method for the analysis of PDMS in bio-oil matrices. The goals of the experimental section were to increase the yield of the elution of silicone oil, or PDMS, and to successfully separate the PDMS from all other bio-oil components, during SPE. The reason for separating the PDMS from the bio-oils was to collect and concentrate the PDMS from a spiked bio-oil sample. The concentrated PDMS was analyzed and an attempt to increase the detection (i.e., lower the detection limit) of PDMS by HP-SEC was performed.

The previously developed method for separation by SPE was working well for high MW PDMS. The hypothesis tested in this work was whether the SPE-method developed during this work was able to separate PDMS of different molecular weights (i.e., high, medium, and low MW PDMS). The applicability of the newly developed SPE-method in three different bio-oil matrices was also tested.

4   EXPERIMENTAL SECTION

4.1   Material and instruments

Kimax® borosilicate culture tubes of the size 16 x 100 mm were used for these experiments, together with 15 mm screw caps. The sample vials for HP-SEC and GC-FID analyses were FisherBrand autosampler 1.5 ml short thread vials of the size 32 x 11.6 mm. The caps for the autosampler vials were UltraClean closure 9 mm short thread caps with silicone and polytetrafluoroethylene (PTFE) or with rubber and PTFE.

The Eppendorf Multipipette® M4 together with combitips of the sizes 1–10 ml was used for measuring solvents and samples. VWR Ergonomic High-Performance micropipettes of the sizes 20–200 µl and 100–1 000 µl were used to measure small amounts of solvents or reagents. A 500 µl Gastight® syringe was used to transfer the samples to the autosampler vials. Syringe filters with a 0.2 µm PTFE membrane were used together with the 500 µl syringe in order to remove any small particle. Unplugged 150 mm glass Pasteur pipettes were also used to transfer smaller volumes of samples and SPE fractions.

SPE cartridges produced by different manufacturers were used for fractionation. HyperSep florisil 1 000 mg, HyperSep florisil 2 000 mg, and HyperSep silica 1 000 mg were all from Thermo Scientific (USA). A Chromabond® polares development kit containing cartridges of the size 500 mg were also used. The Chromabond® kit contained cartridges based on different materials: SiOH, florisil, OH (diol), CN, and NH_2. The SPE extraction manifold that was utilized was a Resprep™ 12-port vacuum manifold.

The Pierce Model 18780 Reacti-Vap™ evaporating unit, together with a gas tank of nitrogen (N_2), was utilized to remove any solvent from the samples. A VWR International vortex mixer was used when proper mixing of reagents was needed, such as during silylation. A Memmert oven, set to 70 °C, was used for heating of the samples during silylation. To remove the last residues of solvents, a Heraeus vacuum desiccator

set to 40 °C was used. For the gravimetric measurements, a Mettler Toledo Excellence Plus analytical balance was utilized.

The used instrument for HP-SEC analyses was a Shimadzu LC-10AT VP liquid chromatograph. The liquid chromatograph was connected to a Sedex 85 SEDERE LT-ELSD detector. During analyses, the detector was set to 40 °C. Depending on the concentration of the analyte of interest, the gain of the detector was set to either gain 3 or gain 6. Gain 3 was used for higher concentrations and gain 6 was used for lower concentrations. The instrument was equipped with three columns: one guard column and two actual separation columns. The guard column was a Jordi Gel DVB 500 Å with the dimensions 50 x 7.8 mm. The separation columns were two identical Jordi Gel DVB 500 Å with the dimensions 300 x 7.8 mm. The eluent utilized for HP-SEC analysis was tetrahydrofuran (THF) of high-performance liquid chromatography (HPLC) grade with 1% (v/v) of filtered acetic acid.

For GC-FID analyses, both short column and long column were utilized. The instruments were two separate Clarus® 500 gas chromatographs for each column. The column used as short column was an Agilent J&W Megabore GC column with the dimensions: 6.4 m in length, 0.53 mm as internal diameter, and 0.15 µm thickness of the film. The column used as long column was a HP-1 Agilent Narrowbore GC column with the dimensions: 25 m in length, 0.2 mm as internal diameter, and 0.11 µm thickness of the film. The two rinse solutions used during GC-FID analyses were ethanol and toluene.

The temperature during GC-FID analyses was following different programs whether the short or long column was used. For both columns, there was one program for the injector and one program for the oven. The injector program for the short column was starting at 80 °C, increasing first with 50 °C/min until 110 °C, and then increasing with 15 °C/min until 330 °C. The oven program for the short column was starting at 100 °C increasing with 12 °C/min until 340 °C. The injector program for the long column was starting at 160 °C increasing with 8 °C/min until 260 °C. Two different oven programs were used for the long column. One of the programs was starting at 60 °C increasing with 8 °C/min until 300 °C and the other program was starting at 120 °C increasing with 6 °C/min until 320 °C.

The instrument utilized for GC-MS was of the HP 6890 series GC system. The installed column was a capillary column of type HP-1 methyl siloxane Agilent 19091Z-002. The dimensions of the column were as follows: 25 m in length, 200 μm in diameter, and 0.11 μm of the thickness of the film. The flow in the column was set to be constant. The injection mode was set to splitless and helium was used as a carrier gas. The initial temperature of the oven was 80 °C. The final temperature was 320 °C. The ramp of the temperature was set to 6 °C/min.

4.2   Solvents, reagents, and standards

Listed in Table 6 are the different solvents used during the experimental part of this work. Along with the solvents in Table 6, there is also a list of the quality grades and the manufacturers of the solvents.

Table 6 Solvents used during the experimental part of this work.

Solvent	Quality grade	Manufacturer (country)
Acetic acid	Glacial, analytical reagent grade	Fischer Scientific (UK)
Acetone	Chromasolv™, for HPLC, ≥99.8%	Honeywell (Germany)
Chloroform	Amylene stabilized, for HPLC, ≥99.8%	Sigma-Aldrich (Israel)
DCM	Puriss. p.a., ACS reagent, ≥99.9%	Sigma-Aldrich (France)
Ethanol	ETAX Aa min. 99.5 w-%	Altia Oyj (Finland)
Ethyl acetate (EtAc)	Puriss. p.a., ACS reagent, ≥99.5%	Sigma-Aldrich (France)
Isopropanol	HPLC gradient grade	Fisher Scientific (UK)
Methanol	Chromasolv™, for HPLC, ≥99.9%	Honeywell (Germany)
Methyl *tert*-butyl ether (MTBE)	Chromasolv™, for HPLC, ≥99.8%	Honeywell (Germany)

Hexane	Chromasolv™, for HPLC, ≥97.0%	Honeywell (Germany)
THF	(I) Inhibitor-free, for HPLC, ≥99.9% (II) Chromasolv™ Plus, for HPLC, inhibitor-free, ≥99.9%	(I) Sigma-Aldrich (France) (II) Honeywell (Germany)
Toluene	EMSURE®	Merck KGaA (Germany)

Table 7 is listing the different reagents and standards used during the experimental part of this work. This table is also providing the quality grades and manufacturers of the reagents and standards.

Table 7 Reagents and standards used during the experimental part of this work.

Reagents/standards	Quality grade	Manufacturer (country)
Ammonium hydroxide	EMSURE® 25%	Merck KGaA (Germany)
Betulinol	0.02 mg/ml of TG-standard, C21, CH17	-
Cholesterol	0.02 mg/ml of TG-standard, C21, CH17	-
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)	98+%, nitrogen flushed	Acros Organics (Germany)
Pyridine	ACS reagent	VWR Chemicals (USA)
Silicone oil: (I) high MW (II) medium MW (III) low MW	(I) 1 000 cSt (II) 50 cSt (III) 5 cSt	Sigma-Aldrich (USA)

Trimethylchlorosilane (TMCS)	≥98.0%	Sigma-Aldrich (Germany)

4.3   Experimental procedures

4.3.1   Preparation of stock solutions of PDMS model compounds

A beaker, a 250 ml volumetric flask, and a 250 ml storing flask was cleaned with acetone and dried. The clean and dry beaker was used for silicone oil. Approximately 530 mg (533.65 mg) of high MW silicone oil (i.e., 1 000 cSt) was weighed into the clean beaker and hexane was added to dissolve the silicone oil. The solution was transferred to the clean 250 ml volumetric flask. The beaker was rinsed a couple of times with pure hexane, which was then added to the volumetric flask. The solution in the volumetric flask was diluted to the mark. In order to prepare a stock solution with a concentration of 2 mg/ml, 533.65 mg requires 266.83 ml of hexane. The volumetric flask was only able to hold 264 ml. The solution of 264 ml was therefore transferred to the storing flask before the final 2.83 ml of hexane was added. In order to achieve the most exact concentration, the volumetric flask was rinsed a couple of times with the solution from the storing flask.

An analysis set, which consisted of different concentrations of high MW silicone oil in hexane, was prepared by using the stock solution of silicone oil with a concentration of 2 mg/ml. The stock solution was diluted nine times in total, resulting in concentrations ranging from 1 mg/ml to 0.0015 mg/ml. The highest concentration in the set (i.e., 1 mg/ml) was prepared with 15 ml of hexane and 15 ml of the stock solution. Each new concentration after 1 mg/ml was three times less than the previous. This was easily achieved by adding 10 ml of the higher concentration to 20 ml of hexane. A summary of the concentrations of the analysis set is presented in Table 8. One sample of each concentration was analyzed by HP-SEC, in order to verify the practical detection limit.

Table 8 The different concentrations of silicone oil in hexane for the analysis set.

Vial number	Concentration (mg/ml)
I	1
II	0.33
III	0.11
IV	0.037
V	0.012
VI	0.0041
VII	0.0014
VIII	0.00046
IX	0.00015

Four 30 ml storing vials were cleaned with acetone and dried. The medium MW and the low MW (i.e., 50 cSt and 5 cSt, respectively) model compounds for silicone oil were weighed directly into two of the 30 ml storing vials. Approximately 49.8 mg of the medium MW model compound and 121.3 mg of the low MW model compound were weighed into separated storing vials. Hexane was added to both storing vials. The final concentration was 2 mg/ml for the medium MW stock solution and 5 mg/ml for the low MW stock solution. Stock solutions in hexane were also prepared for D_3 and D_4 PDMS degradation products. D_3 and D_4 are cyclic oligomers containing three or four repeating units of siloxanes, respectively. Approximately 220.6 mg of D_3 PDMS product and 251.1 mg of D_4 PDMS product were weighed into the last two storing vials. Hexane was added and the final concentration of both stock solutions was 10 mg/ml.

4.3.2   High performance-size exclusion chromatography (HP-SEC)

Samples for HP-SEC analysis were first placed in the evaporating unit with N_2 gas purging at 50 ℃ in order to remove any residues of solvents. The dried samples were dissolved in THF and mixed thoroughly. The ideal concentration of the solution for HP-SEC is 1 mg/ml, and the minimum amount of THF that can be used is 0.5 ml.

One ml of each sample dissolved in THF was transferred by using a 500 µl syringe into separate 1.5 ml glass autosampler vials. Before the samples were transferred to the vials, they were filtered through a 0.2 µm PTFE filter. One ml of pure THF was used as the blank sample, which was injected before all the samples were analyzed. The blank sample would verify the noise in the baseline for each run.

The eluent for the HP-SEC run was prepared as a HPLC-grade THF solution with 1% v/v glacial acetic acid (i.e., 1 ml of filtered glacial acetic acid for each 100 ml of THF). The rate of the flow was slowly increased, starting from 0.1 ml/min, and ending at 0.8 ml/min. The parameters for the run were as follows: 50 µl injection volume, 0.8 ml/min isocratic flow, 28 min run-time, temperature of 40 °C, and sensitivity of either gain 3 or gain 6. Gain 3 was used when the concentration of the analyte of interest was around 1 mg/ml. For concentrations lower than 1 mg/ml, gain 6 was used.

4.3.3   Gas chromatography-flame ionization detector (GC-FID)

Samples for GC-FID analysis were taken as 1 ml of the HP-SEC samples dissolved in THF. An internal standard with the concentration of 0.02 mg/ml was added to each GC-FID sample with the volume of 2 ml. The internal standard was pre-prepared betulinol. The pre-prepared 0.02 mg/ml internal standard cholesterol was also used in some cases. Both betulinol and cholesterol contained four individual standards, which were called acid 21:0, betulinol, CH17, and TG-standard. Every test tube with a sample for GC-FID was placed in the evaporating unit with N_2 gas purging at 50 °C. After complete removal of THF and MTBE, the test tubes were placed in the vacuum desiccator at 40 °C for 20 minutes.

A mixture of the silylating reagents was prepared with one part of pyridine, four parts of BSTFA, and one part of TMCS. As the BSTFA and TMCS are sensitive to moisture and normally stored in the refrigerator, they were allowed to cool down to room temperature before preparing the mixture. 150 µl of the mixture of the silylating reagents was added to each sample. The samples were mixed using a vortex mixer before they were placed in the oven at 70 °C, with closed caps. After five minutes, the

samples were mixed a second time using a vortex mixer and placed back in the oven for 40 minutes.

After a total of 45 minutes in the oven, the samples were mixed again by using a vortex mixer and then they were allowed to cool down to room temperature. The samples were transferred into a V-shaped glass insert inside a vial by using a Pasteur pipette. A blank sample was also prepared, which consisted of only the silylating reagents. The samples were analyzed with either short column GC-FID or long column GC-FID. The same sample preparation was also performed for analysis with GC-MS.

4.3.4   Solvent extraction

A separation of the components in BO 1 was performed by solvent extraction between two immiscible solvents: methanol and hexane. One set of borosilicate test tubes was prepared by washing the tubes two times with 3 ml acetone. Two 50 ml volumetric flasks were also prepared by washing them with acetone. The set of test tubes was placed in a vacuum desiccator at 40 °C for 20 minutes. The volumetric flasks were dried by allowing the acetone to evaporate in the oven at 70 °C for a couple of minutes.

Approximately 250 mg of BO 1 was weighed into each test tube. Four ml of both methanol and hexane were added to each test tube. 100 µl of 25% ammonium hydroxide was also added to the test tubes in order to make the separation of the acids more efficient. The test tubes were mixed thoroughly by using a vortex mixer, whereupon the immiscible solvents were allowed to separate naturally.

The hexane phase, which lies on the top, was transferred to one of the 50 ml volumetric flasks by using a Pasteur pipette. The hexane phase from all the test tubes was transferred to the same volumetric flask. Additionally, another 4 ml of hexane was added to the test tubes for a second extraction. The second time, the test tubes were mixed by hand, whereupon the solvents were allowed to separate naturally. The hexane phases from the second extraction were also transferred to the same volumetric flask as before. The hexane phases, in the volumetric flask, were diluted to the mark with hexane. Two borosilicate test tubes were washed with acetone and dried in the vacuum

desiccator for 30 minutes. The clean and empty test tubes were weighed. One of the test tubes was used as a blank, while 1 ml of the diluted hexane phase was added to the other test tube. The hexane was evaporated from the sample by using the evaporating unit with N_2 gas purging at 50 °C. After completed evaporation, the test tubes were placed in the vacuum desiccator at 40 °C for 30 minutes. After 30 minutes, the test tubes were weighed and placed back into the vacuum desiccator. The test tubes were repeatedly weighed with a 15-minute interval.

Two other bio-oils (i.e., BO 2 and BO 3) were also fractionated with the same solvent extraction as BO 1. Three selected real samples with BO 1 as matrix were also fractionated with solvent extraction.

4.3.5   Solid-phase extraction (SPE)

The primary focus of the experimental section was the SPE. Several SPE fractionations were performed, where the parameters were somewhat changed for each analysis. The basics for every SPE fractionation were the same and are described below. Depending on the size of the SPE cartridges, the total volume of one fraction was either 3 ml, 6 ml, or 12 ml, for 500 mg, 1 000 mg, and 2 000 mg cartridges, respectively.

The basic SPE procedure

The solvent combinations were based on the findings of Kenneth Arandia.[54] Table 9 shows the primary solvent combinations used by Kenneth Arandia, which were also used in this work. Each of the solvent combinations were prepared in 500 ml or 250 ml flasks. The used SPE cartridges were placed onto the extraction manifold. Clean test tubes were placed in the extraction manifold, underneath the cartridges for collection of the eluting fractions. A vacuum system was connected to the extraction manifold, as a slightly pressurized manifold is more efficient during the extraction.

Table 9 The primary solvent combinations used for SPE fractionation.

Fraction	Solvent combinations
P1	Pure hexane
P2	12.5% DCM in hexane
P3	12.5% DCM in hexane
P4	12.5% DCM in hexane
P5	12.5% DCM in hexane
P6	15% DCM in hexane
P7	20% DCM in hexane
P8	40% DCM in hexane
P9	Pure DCM
P10	Pure DCM

The cartridges were conditioned with pure DCM followed by pure hexane, one fraction of each solvent. Before the sample was loaded, the DCM and hexane from the conditioning were emptied out from the test tubes. The samples of interest, already dissolved in hexane, were loaded onto the cartridges. The first fraction from the solvent combinations was loaded onto the cartridges and collected into the test tube. After each fraction, the test tubes were changed to new ones, resulting in collection of one fraction per test tube. Each fraction from the solvent combinations was loaded and collected. Every fraction was evaporated by using the evaporating unit with N_2 gas purging at 50 °C. The fractions were evaporated both separately and combined. The combined fractions were usually combined according to the percentage of DCM in the eluent or according to the elution of PDMS (i.e., all the fractions were PDMS was expected to elute were combined into one sample for further analysis). Analysis of the SPE fractions was performed by HP-SEC or GC-FID.

Tests of different solvents and cartridge materials

Since the experimental section of this work was a continuation of the experimental work of Kenneth Arandia, the different SPE tests started where Arandia left off (i.e., using SiOH cartridges for SPE fractionation). The first tests in trying to improve the elution of PDMS on SPE cartridges focused on the effects of different solvents and the effect of different material of the cartridge. The solvents used for testing were DCM,

chloroform, EtAc, acetone, ethanol, THF, MTBE, and toluene. For every different solvent tested, a HyperSep 1 000 mg SiOH cartridge was used. After conditioning with 6 ml of each DCM and hexane, 1.5 ml of pure high MW PDMS with a concentration of 1 mg/ml in hexane was loaded onto the cartridges. One fraction of 6 ml hexane was first eluted before the tested solvents were eluted.

The following SPE cartridges were compared to each other: SiOH, florisil, OH (diol), CN and NH$_2$. All these different cartridges were only 500 mg in size, and the conditioning was performed with 3 ml of both DCM and hexane. For the smaller cartridges, only 1 ml of the pure high MW PDMS sample with a concentration of 1 mg/ml in hexane was loaded onto the cartridges. DCM was used as eluent for every cartridge and 10 ml of DCM was eluted and collected directly after loaded sample.

High MW PDMS in BO 1 matrix

The elution of a pure high MW PDMS and a pure BO 1 sample was tested on separate Chromabond® CN and Chromabond® florisil SPE cartridges. The cartridges were only 500 mg in size and 3 ml of each fraction was used. The cartridges were conditioned with 3 ml DCM and 3 ml hexane, whereupon 1 ml of pure PDMS (with a concentration of 1 mg/ml in hexane) was added to one of the CN cartridges and one of the florisil cartridges. To the other CN and florisil cartridge, 0.5 ml of the BO 1 sample (with a concentration of 16.04 mg/ml in hexane) was added. Identical SPE elution and analysis pre-treatment were performed for each cartridge. The solvent combinations in Table 9 were used. The eluted fractions were combined according to the concentration of DCM, i.e., P2–P5 and P9–P10 were combined while the rest of the fractions were separately analyzed. HP-SEC analysis of the fractions dissolved in THF was performed with gain 3.

The elution of a spiked BO 1 sample was studied using a Chromabond® florisil cartridge. The BO 1 sample was spiked with high MW PDMS and the final concentration of PDMS in the spiked sample was 0.33 mg PDMS per ml hexane-soluble BO 1. One 500 mg florisil cartridge was conditioned and 1 ml of the spiked sample was added to the cartridge. The primary solvent combinations (Table 9) were used. The eluted fractions were combined according to an estimation where the

PDMS would elute, i.e., fractions P1–P6 and P7–P10 were combined. HP-SEC analysis of the fractions dissolved in THF was performed with gain 6.

Comparison between different solvent combinations

A comparison between the primary solvent combinations and an alternative solvent combination scheme was performed for the elution of a spiked BO 1 sample, using two Chromabond® florisil cartridges. The alternative solvent combination scheme also used DCM in hexane, however, it focused more on slowly increasing the polarity between 12.5% DCM and 40% DCM. Table 10 shows the complete scheme of the solvent combinations. A spiked BO 1 sample with a final concentration of 1 mg PDMS per ml hexane-soluble BO 1 was used. The 500 mg florisil cartridges were conditioned and 0.1 ml of the spiked sample was added to both cartridges. One of the cartridges was eluting the sample using the primary solvent combinations (Table 9) and the other cartridge was using the alternative solvent combinations (Table 10). Each fraction was dissolved separately in THF and analyzed with HP-SEC gain 6.

Table 10 The alternative version of the solvent combinations used for SPE fractionation.

Fraction	Solvent combinations
A1	Pure hexane
A2	12.5% DCM in hexane
A3	15% DCM in hexane
A4	15% DCM in hexane
A5	20% DCM in hexane
A6	20% DCM in hexane
A7	30% DCM in hexane
A8	35% DCM in hexane
A9	40% DCM in hexane
A10	Pure DCM

Another comparison between the primary solvent combinations and the alternative solvent combinations was also performed. However, this time the different solvent combinations were also compared between florisil and SiOH cartridges. HyperSep

florisil and HyperSep SiOH, both 1 000 mg, cartridges were used. The cartridges were conditioned and only 0.2 ml of a spiked BO 1 sample with the concentration of 1 mg PDMS per ml spiked sample was added to each cartridge. Each of the eluted fraction was dissolved in 1 ml THF before HP-SEC analysis with gain 6.

Test of cartridge overload

A test of the ability of the cartridge to resist overload was also studied. A comparison was performed between HyperSep florisil cartridge and HyperSep SiOH cartridge. The overload was studied by increasing the sample volume loaded onto the cartridge. The sample used was a spiked BO 1 sample. However, the amount of the PDMS loaded onto the cartridge was kept constant while both the amount of BO 1 components and the sample volume was increased. The constant amount of the PDMS loaded onto the cartridge was 0.2 mg. The tested sample volumes were 0.2 ml, 0.4 ml, 0.8 ml, 1.0 ml, 1.1 ml, and 1.5 ml. The same spiked samples and sample volume were added to both florisil and SiOH cartridges. The solvent combinations used during this test were according to a simplified version of the alternative solvent combinations (Table 11). For each sample volume, the eluted fractions were combined as S1–S4 and S5–S8. The combined fractions were dissolved in 1 ml THF and analyzed with HP-SEC gain 6.

Table 11 The simplified version of the solvent combinations used for SPE fractionation.

Fraction	Solvent combinations
S1	Pure hexane
S2	12.5% DCM in hexane
S3	15% DCM in hexane
S4	15% DCM in hexane
S5	20% DCM in hexane
S6	30% DCM in hexane
S7	35% DCM in hexane
S8	40% DCM in hexane

High MW PDMS in BO 2 and BO 3 matrices

The high MW PDMS model compound was also used to spike other matrices than BO 1. The other matrices tested were BO 2 and BO 3. High MW PDMS was used to spike the hexane-soluble fractions of both BO 2 and BO 3. The final spiked samples consisted of 20% PDMS and 80% BO 2 or BO 3. The separation of PDMS from BO 2 or BO 3 was then tested with HyperSep florisil cartridges. Normal conditioning was used and only 0.2 ml of the spiked sample was added to separate cartridges. The alternative solvent combinations from Table 10 were used. Each eluted fraction was separately dissolved in 1 ml THF and analyzed with HP-SEC gain 6.

Pure medium and low MW PDMS

Pure PDMS model compounds of both medium and low MW were also fractionated through SPE cartridges. The concentration of both PDMS model compounds was 1 mg/ml in hexane. Both PDMS model compounds were fractionated through one HyperSep florisil and one HyperSep SiOH cartridge. Normal conditioning was used and 1 ml of each PDMS model compound was added to each cartridge. The simplified solvent combinations (Table 11) were used for the elution. Each of the medium MW PDMS eluted fractions was dissolved in 1 ml THF and analyzed with HP-SEC gain 3. Two ml of the internal standard betulinol was added to each of the low MW PDMS eluted fractions, which were then evaporated and silylated. The silylated low MW PDMS fractions were analyzed with short column GC-FID.

Medium and low MW PDMS in BO 1, BO 2, and BO 3 matrices

The BO 1, BO 2 and BO 3 matrices were also spiked with both medium MW PDMS and low MW PDMS, separately, before SPE fractionation. The final spiked samples contained 20% medium or low MW PDMS and 80% of the respective matrix material. All the spiked samples were fractionated through HyperSep florisil cartridges. Normal conditioning was used and only 0.2 ml of each spiked sample was added to each cartridge. The simplified solvent combinations in Table 11 were used. Each of the medium MW PDMS eluted fractions in BO 1 matrix was dissolved in 1 ml THF and analyzed with HP-SEC gain 6. The medium MW PDMS eluting fractions in BO matrix were dissolved in 1.9 ml THF, and in the BO 3 matrix the fractions were dissolved in

1.6 ml THF. This was performed in order to obtain quite similar concentrations of PDMS in THF for all three matrices. Two ml of the internal standard cholesterol or betulinol was added to each of the low MW PDMS eluted fractions, which were then evaporated and silylated. The silylated low MW PDMS fractions were analyzed with short column GC-FID.

Pure D_3 and D_4 PDMS degradation products

The SPE fractionation of pure D_3 and D_4 PDMS degradation products was also studied. The pure D_3 and D_4 PDMS products dissolved in hexane were fractionated through both HyperSep florisil and SiOH cartridges. Normal conditioning was used and 2 mg of each PDMS product was added onto each cartridge, which represented a volume of 1 ml as the concentration of the PDMS product samples was 2 mg/ml. The simplified solvent combinations in Table 11 were used. For each of the 6 ml collected fractions, only 150 µl was directly analyzed with long column GC-FID. Since the D_3 and D_4 PDMS products are highly volatile, they were analyzed without any pre-treatment.

D_3 and D_4 in BO 1 matrix

The D_3 and D_4 PDMS product were also spiked in BO 1 matrix and fractionated with SPE. The final spiked samples contained 20% of the D_3 or D_4 product and 80% of the BO 1 matrix. The samples were only fractionated through HyperSep florisil cartridges. Normal conditioning was used, and 0.2 ml of the spiked samples was loaded onto each cartridge. The simplified solvent combinations from Table 11 were used. For each of the 6 ml fractions, only 150 µl was directly analyzed with long column GC-FID. Since the D_3 and D_4 PDMS products are highly volatile, the samples were analyzed without any pre-treatment.

Real unspiked BO 1 samples

Three of the real samples were chosen to be fractionated using the newly developed SPE method, in order to compare it with the previously developed SPE method. The newly developed SPE method consists of florisil cartridge and the simplified solvent combinations (Table 11). The three chosen real samples consisted of a high, medium, and low approximate concentration of PDMS, respectively. The concentrations had

previously been estimated by Arandia based on a calibration curve. The estimated PDMS concentrations were 72 ppm, 44 ppm, and 21 ppm. For each of the samples, three parallel SPE fractionations were performed with HyperSep florisil 2 000 mg cartridge. Normal conditioning was used and only 0.4 ml of the hexane-soluble fraction of each real sample was added to each florisil cartridge. The eluted fractions for each of the samples were combined as S1–S4 and S5–S8 in pre-weighed test tubes. The combined fractions for each sample were weighed and dissolved in only 0.5 ml THF before HP-SEC analysis with gain 6. Table 12 shows the weights of the different PDMS-eluting fractions (i.e., S5–S8) and the concentration of the fractions dissolved in THF.

Table 12 Weights of PDMS eluting fractions and their concentration in THF.

	BO 1 (3)	**BO 1 (4)**	**BO 1 (5)**
S5–S8	1.94 mg	1.50 mg	1.65 mg
Conc. in THF	3.89 mg/ml	3.0 mg/ml	3.31 mg/ml

Realistically spiked BO 1 samples

The BO 1 was also spiked with more realistic amount of high MW PDMS. This meant spiked samples with the concentration of PDMS as follows: 700 ppm, 450 ppm, 300 ppm, 150 ppm, 75 ppm, 50 ppm, and 25 ppm. Each of the spiked samples was fractionated through three parallel HyperSep florisil 2 000 mg cartridges. Normal conditioning was used and the sample load for each of the cartridges was 0.4 ml. The simplified solvent combinations and 12 ml of each fraction were used during the fractionation. The eluted fractions were combined as S5–S8 as they were evaporated. The combined fractions were dissolved in 0.5 ml THF and HP-SEC analysis with gain 6 was performed.

5   RESULTS AND DISCUSSION

5.1   High performance-size exclusion chromatography (HP-SEC)

The detection of pure high MW PDMS occurred at a retention time (RT) between 13 min and 17 min. Evidently, the detection was strongly dependent on the concentration of the analyte in the sample. The analysis set with different concentrations (Table 8) was analyzed with HP-SEC-ELSD using both gain 3 and gain 6. Detection with gain 3 was possible for concentrations above 0.11 mg/ml, which is illustrated in Figure 9. However, for concentrations below 0.11 mg/ml, gain 3 was not sensitive enough and gain 6 was rather used in those cases. Figure 10 illustrates the detection of the PDMS concentrations below 0.11 mg/ml. With gain 6, the lowest detectable concentration of PDMS was 0.0041 mg/ml in THF. For concentrations below 0.0041 mg/ml see appendix A Figure A1. The practical detection limit for this analysis was established to be 0.004 mg/ml of pure high MW PDMS in THF.
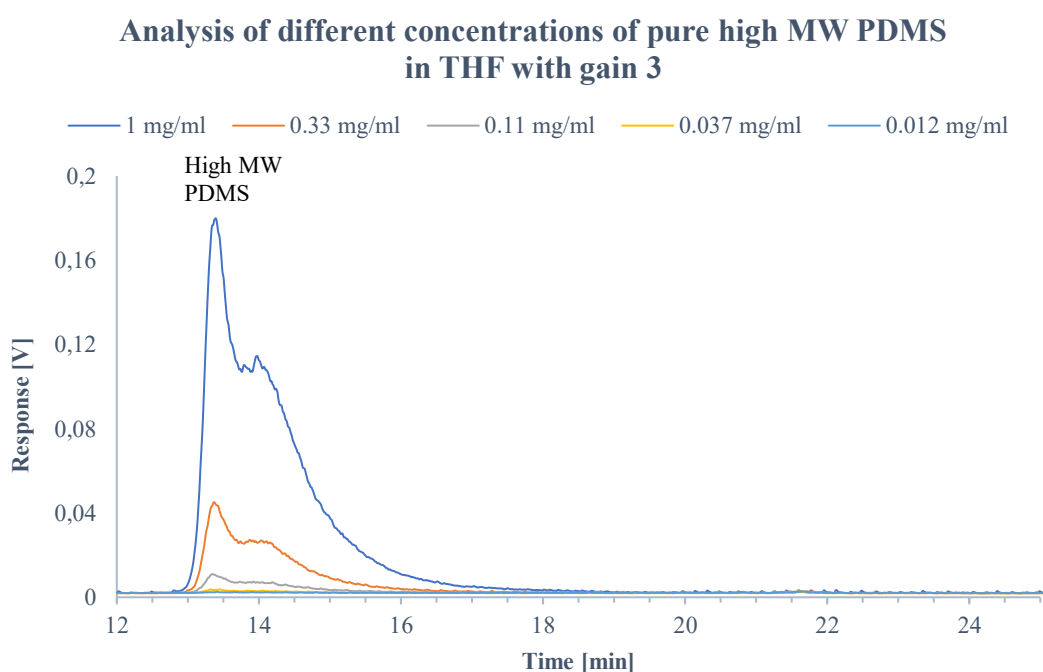
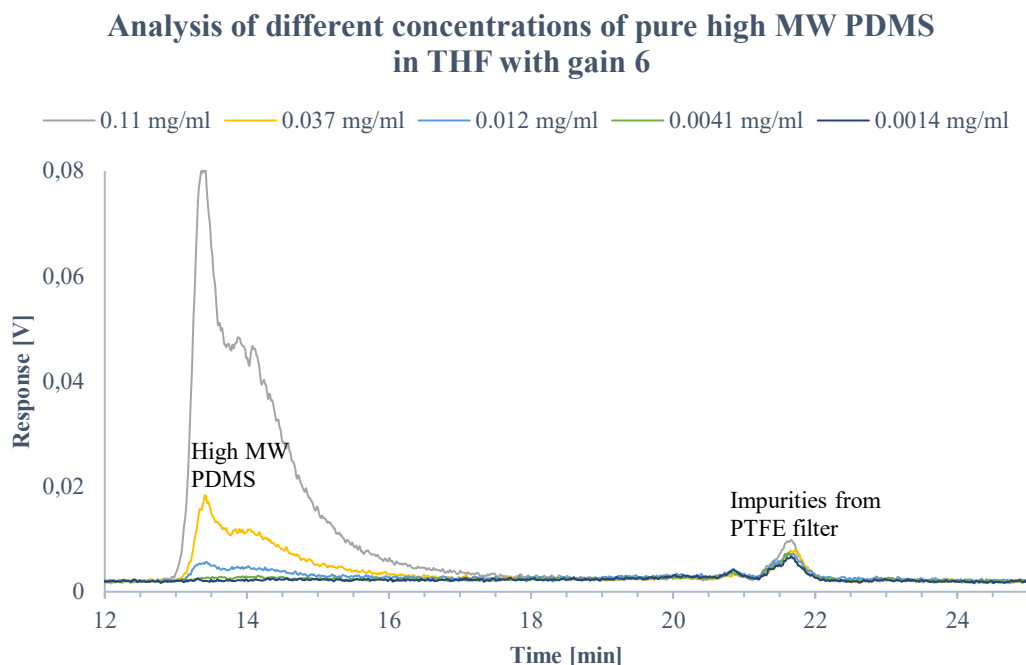*Figure 9. HP-SEC chromatogram of the PDMS concentrations above 0.11 mg/ml.*

Analysis of different concentrations of pure high MW PDMS in THF with gain 6

Figure 10. HP-SEC chromatogram of the PDMS concentrations below 0.11 mg/ml.

The detection of the pure medium MW PDMS model compound is illustrated in Figure 11. A typical separation of the different molecular sizes in the model compound was obtained. The detection of pure medium MW PDMS occurred at RT 14–19 min. The higher retention time, compared to the retention time of high MW PDMS, confirmed the sample consisted of smaller molecules. The detection of pure medium MW PDMS with HP-SEC works as a reference material of the retention time of the material.

Analysis of pure medium MW PDMS in THF with gain 3
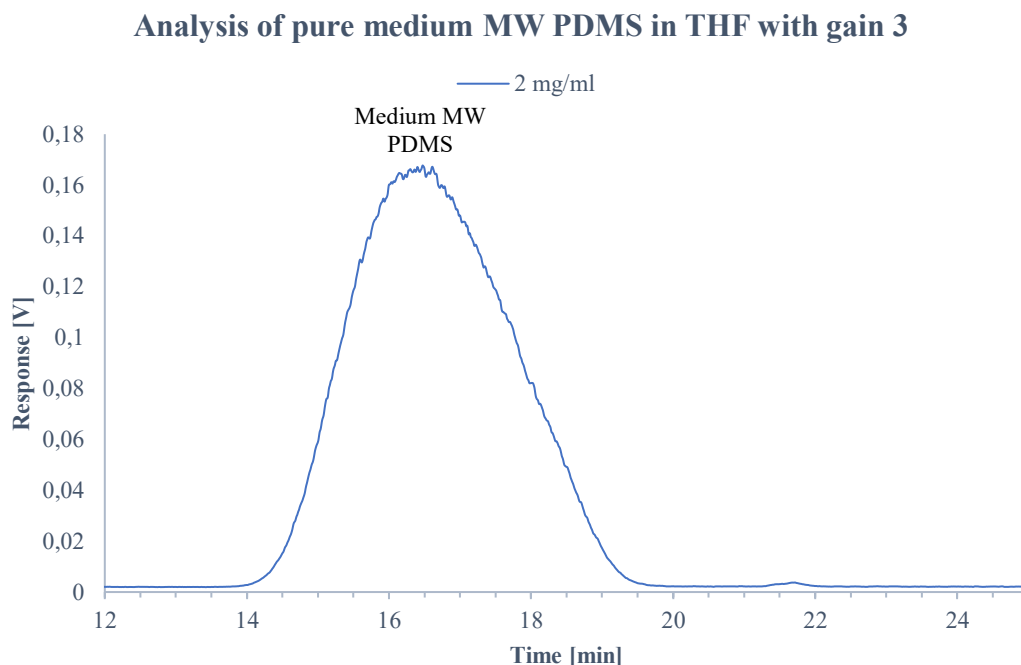
Figure 11. HP-SEC chromatogram of the pure medium MW PDMS.

5.2   Gas chromatography-flame ionization detector (GC-FID)

The GC-FID was used as an analytical method for the more volatile components (i.e., low MW PDMS, D_3 and D_4 PDMS degradation products). Pure samples dissolved in hexane of each of the volatile component were analyzed in order to receive reference material of the retention times of the components. Figure 12 illustrates the GC-FID chromatogram of the pure low MW PDMS model compound. The low MW PDMS model compound consisted of differently sized small and short siloxanes. In the chromatogram (Figure 12), these siloxanes are numbered as 1, 2, 3, 4, etc. Only the five first PDMS peaks are numbered, though the numbering continues for every continuously repeated peak. The peak areas are summarized in appendix B (Table B1), where both the separate peak areas and the total peak area of the low MW PDMS are available.

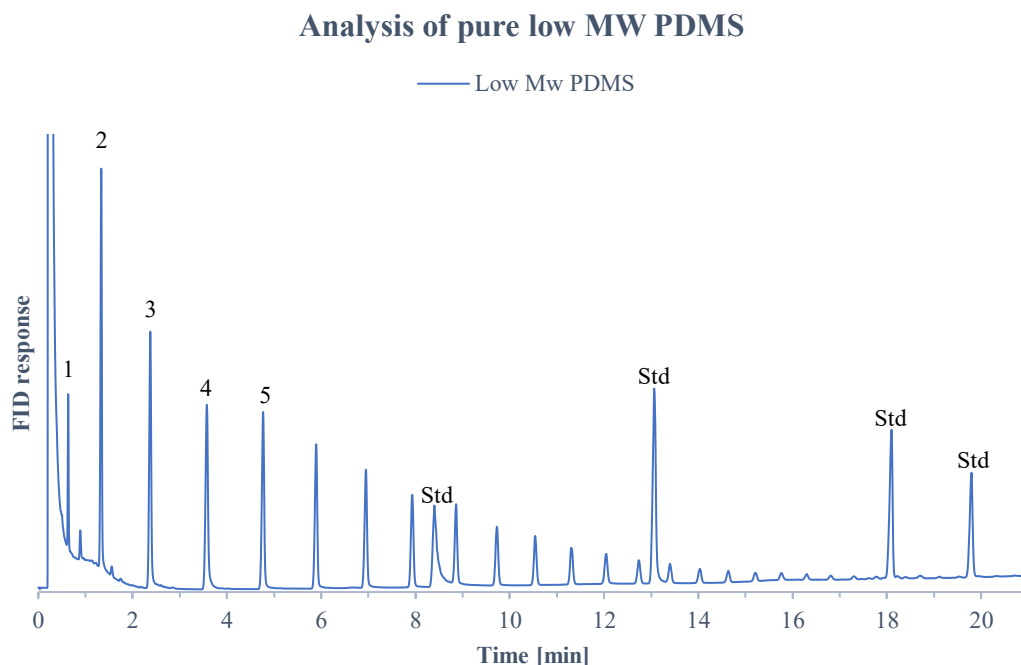## Analysis of pure low MW PDMS

Figure 12. GC-FID chromatogram of the pure low MW PDMS, together with internal standards (std). Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column was used.

Figures 13 and 14 illustrate the detection of the highly volatile D_3 and D_4 PDMS degradation products, respectively. The solvent peaks originated from ethanol, hexane, and toluene. Hexane was the solvent in which the analyte was dissolved. The impurities were most likely from one of the solvents. The RT for each of the solvents was at 0.8 min for ethanol, at 1.0 min for hexane, and at 1.3 min for toluene. The RT for D_3 was at about 1.6 min and for D_4 at about 3.25 min. The peak areas of D_3 and D_4 are available in appendix B (Table B2), where also the concentration of the used samples is mentioned.
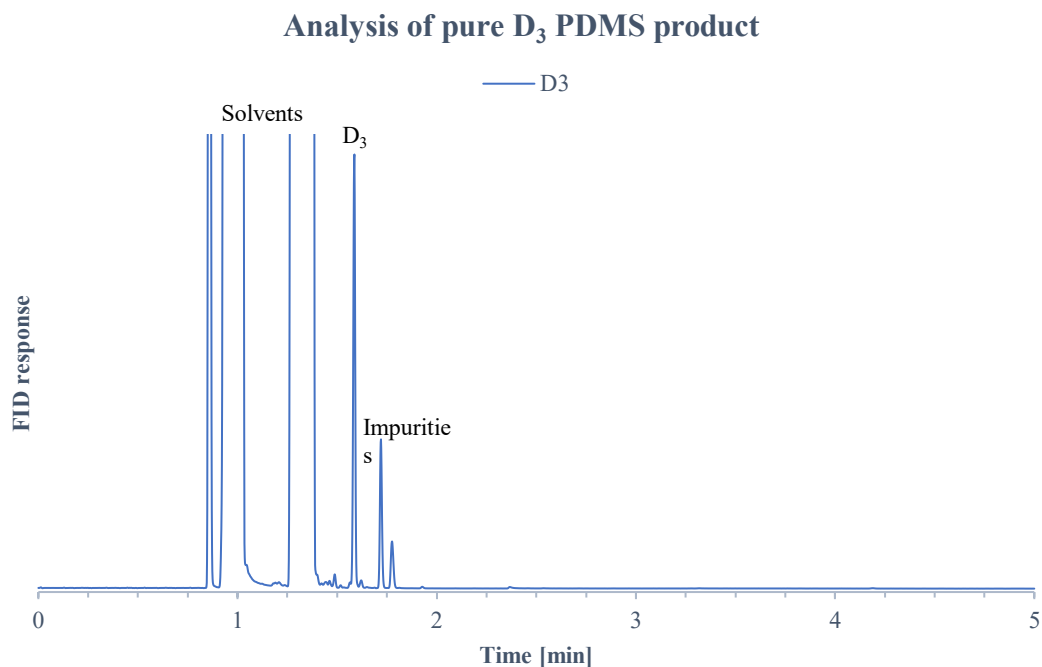
Analysis of pure D$_3$ PDMS product

Figure 13. GC-FID chromatogram of the pure D$_3$ PDMS product. Long column was used.

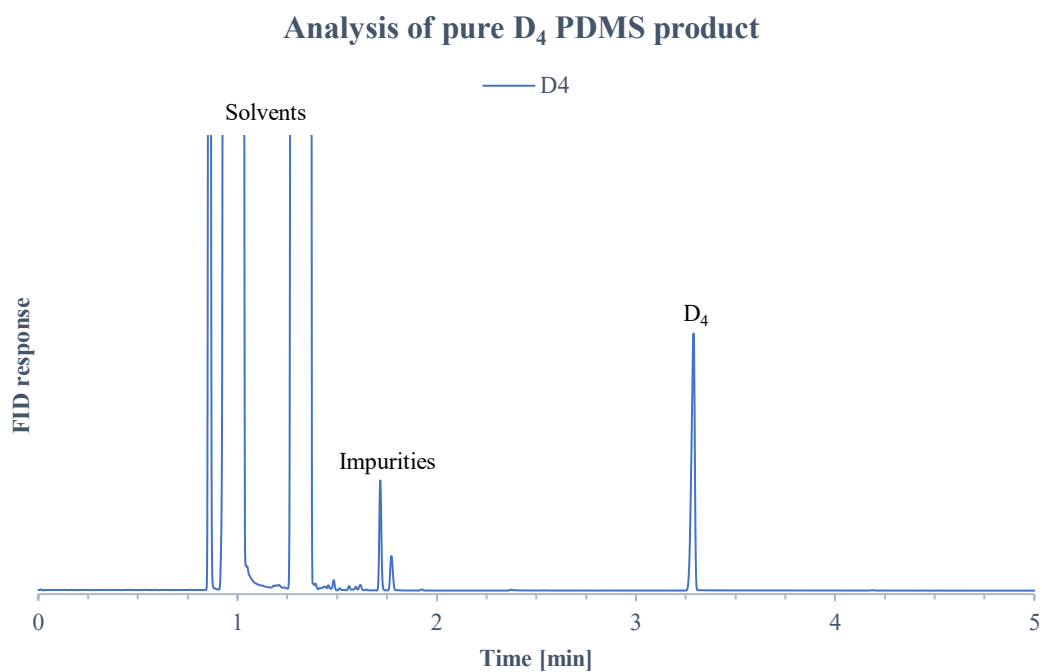## Analysis of pure D$_4$ PDMS product

Figure 14. GC-FID chromatogram of the pure D$_4$ PDMS product. Long column was used.

5.3   Solvent extraction

Five different BO 1 samples were fractionated by solvent extraction, of which two were fractionated two times. Solvent extraction was also performed one time on a BO 2 sample and a BO 3 sample. After each performed solvent extraction, the concentration of the hexane phase was determined gravimetrically. The gravimetrically determined concentration gave a relative mass distribution of the matrix material between the two phases. The amount of extracted BO 1 material in the hexane phase varied between 53.5% and 79.4% and the extracted BO 1 material in the methanol phase varied between 20.6% and 46.1%. The same mass distribution percentages for the BO 2 and the BO 3 samples varied slightly from the BO 1 samples. This was mainly due to the difference in the chemical composition between the three different samples. Figure 15 illustrates the mass distribution of the extracted phases. The gravimetrically determined concentrations of the extracted phases are summarized in Table 13.
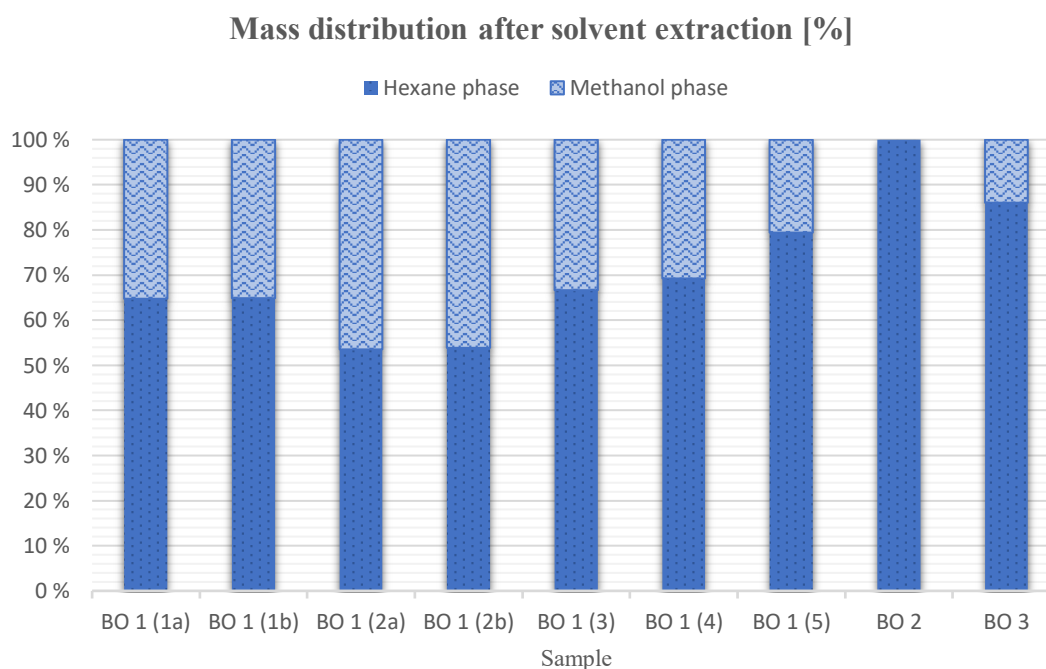

Figure 15. Mass distribution of the bio-oil samples between the hexane and the methanol phases after solvent extraction.

Table 13 Summary of the gravimetrically determined concentrations of the extracted phases.

Sample	Concentration in hexane [mg/ml]	Concentration in methanol [mg/ml]
BO 1 (1a)	16.0	8.6
BO 1 (1b)	16.4	9.0
BO 1 (2a)	13.7	11.9
BO 1 (2b)	13.5	11.5
BO 1 (3)	15.9	8.0
BO 1 (4)	17.5	7.8
BO 1 (5)	20.1	5.2
BO 2	26.1	-
BO 3	21.7	3.6

HP-SEC-ELSD analysis of the extracted phases (i.e., hexane and methanol) was performed in order to confirm the composition of each fraction. An unfractionated BO 1 sample contained steryl ester, fatty acids, and resin acids, which is illustrated in Figure 16. During solvent extraction, the steryl esters rather stayed in the hexane phase, while the fatty acids and the resin acids stayed in the methanol phase. Figures 17 and 18 illustrate the HP-SEC chromatograms of each extracted phase. When comparing the different BO 1 samples, both unfractionated and the extracted fractions, it is clearly visible how all the samples contained different amounts of each component. However, the separation of the steryl esters from the fatty and resin acids was successfully performed for all the samples.
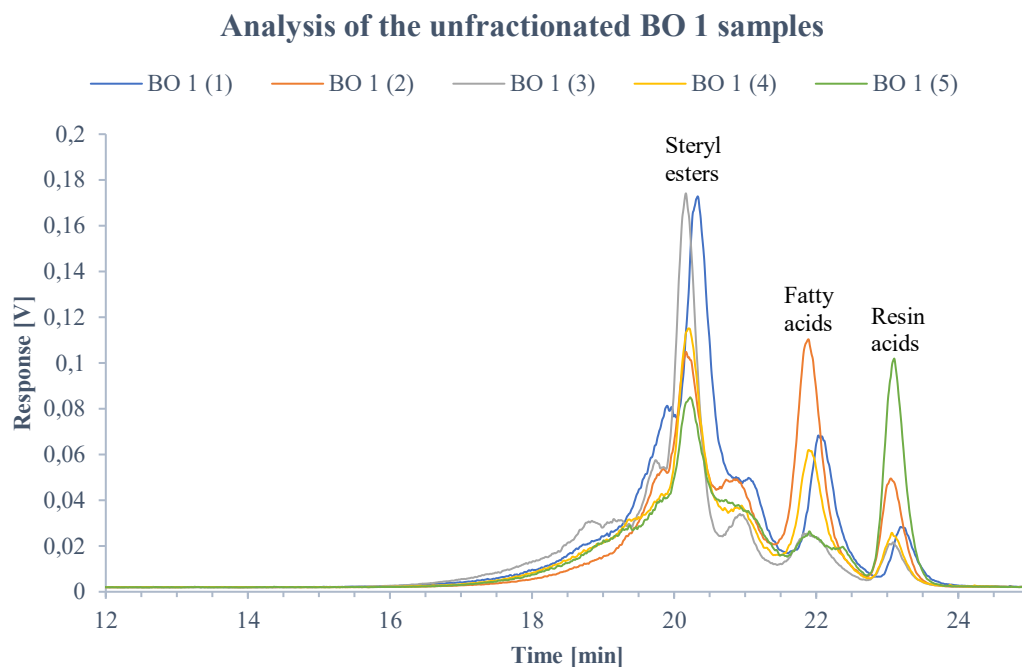
Figure 16. HP-SEC chromatogram of the composition of the BO 1 samples without any fractionation. Gain 3 was used.
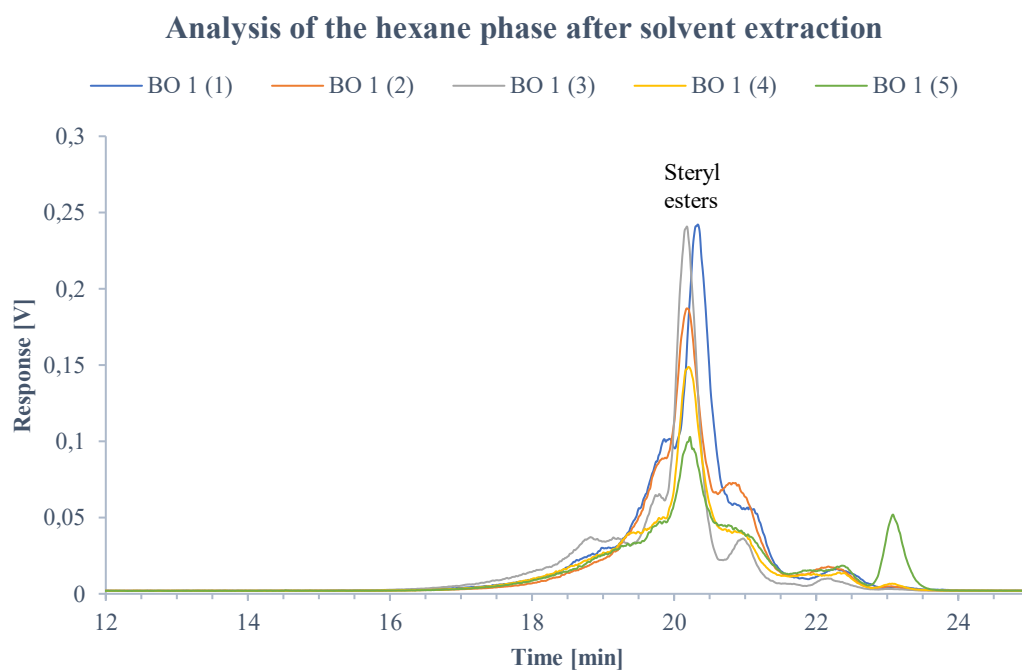

Figure 17. HP-SEC chromatogram of the composition of the hexane phase after solvent extraction of the BO 1 samples. Gain 3 was used.

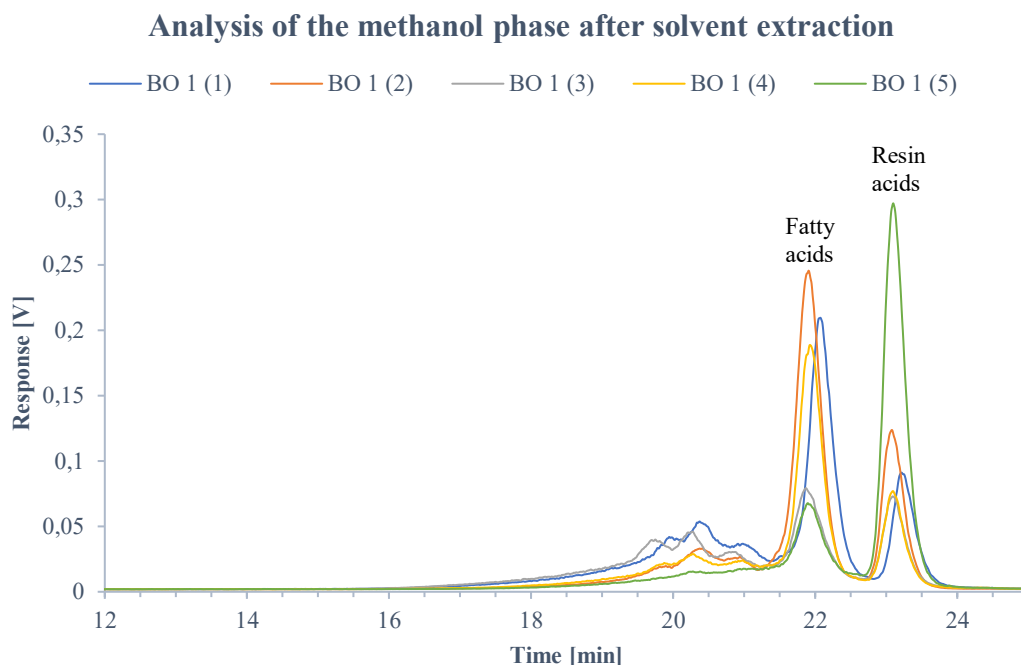**Analysis of the methanol phase after solvent extraction**

Figure 18. HP-SEC chromatogram of the composition of the methanol phase after solvent extraction of the BO 1 samples. Gain 3 was used.

Since the BO 2 and the BO 3 samples did not contain much of the same components as BO 1, close to 100% of the material was more likely to be soluble in the hexane phase. The BO 2 and the BO 3 unfractionated samples contained mostly of glycerides together with a small amount of fatty acids. During solvent extraction, the glycerides stayed in the hexane phase, while the fatty acids stayed in the methanol phase. Figures 19 and 20 illustrate the HP-SEC chromatograms of the different solvent extraction phases for the BO 2 sample and the BO 3 sample, respectively.
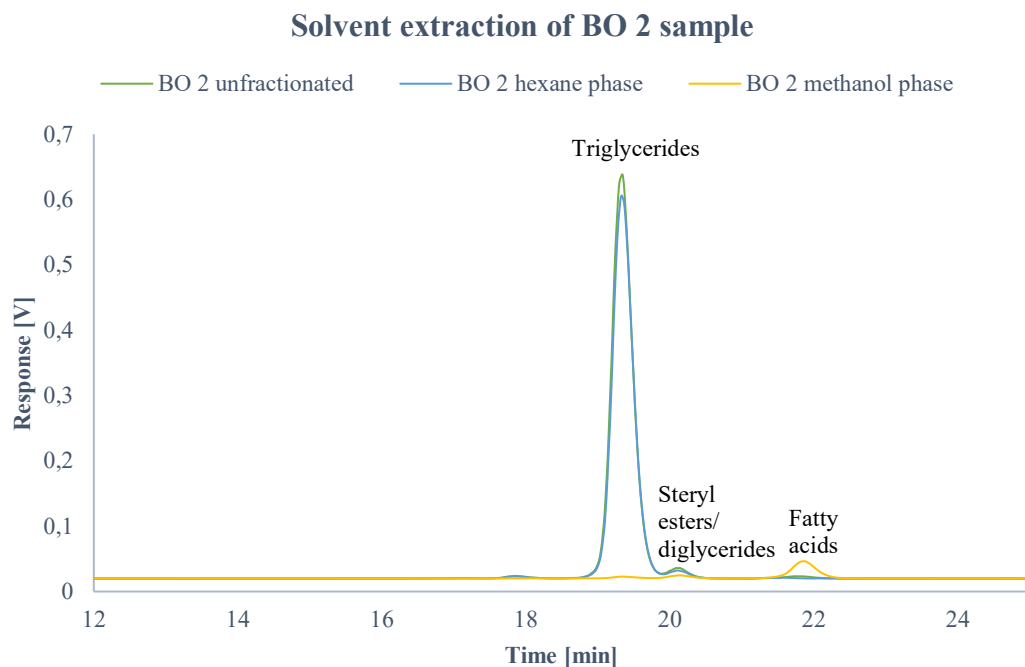
Solvent extraction of BO 2 sample

Figure 19. HP-SEC chromatogram of the composition of the different phases of solvent extraction for the BO 2 sample. Gain 3 was used.

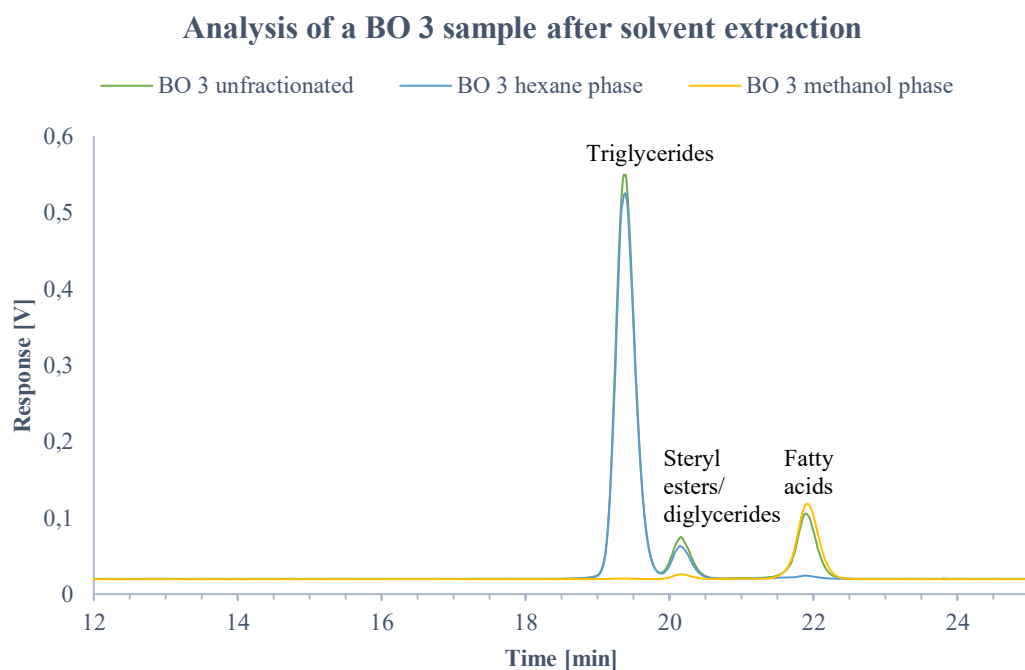## Analysis of a BO 3 sample after solvent extraction

Figure 20. HP-SEC chromatogram of the composition of the different phases of solvent extraction for the BO 3 sample. Gain 3 was used.

5.4   Solid-phase extraction (SPE)

5.4.1   Tests of different solvents and cartridge materials

Each of the tested solvents was eluted with a 10 ml volume of the solvent. Only some selected solvents were eluted a second time with a 20 ml volume of the solvent. The comparison between a 10 ml and 20 ml volume of the solvent is illustrated in Figure 21, where toluene was used. It is clearly shown that a higher volume of the eluent solvent eluted slightly more of the pure high MW PDMS and the conclusion that all the tested solvents would have behaved in that similar way was made.
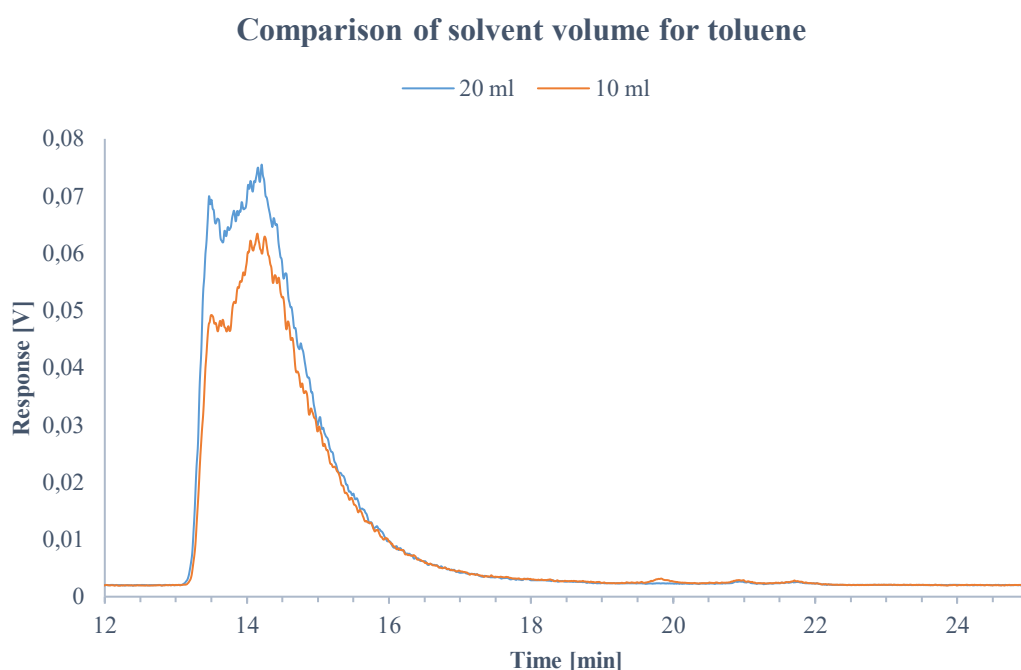

Figure 21. Comparison of the elution of pure silicone oil using different volumes of the eluent solvent. Gain 3.

Among all the tested solvents, DCM, toluene, and chloroform resulted in the best elution of pure high MW PDMS. However, the yield of the eluted high MW PDMS was not exceeding 50% for any of the solvents. The second-best elution of the high MW PDMS was achieved with MTBE, EtAc, and THF. Acetone and ethanol did not elute high MW PDMS very well. Figure 22 illustrates the elution of high MW PDMS

for all the tested solvents. The conclusion from this test is that DCM was the best option and DCM was then used in further tests.
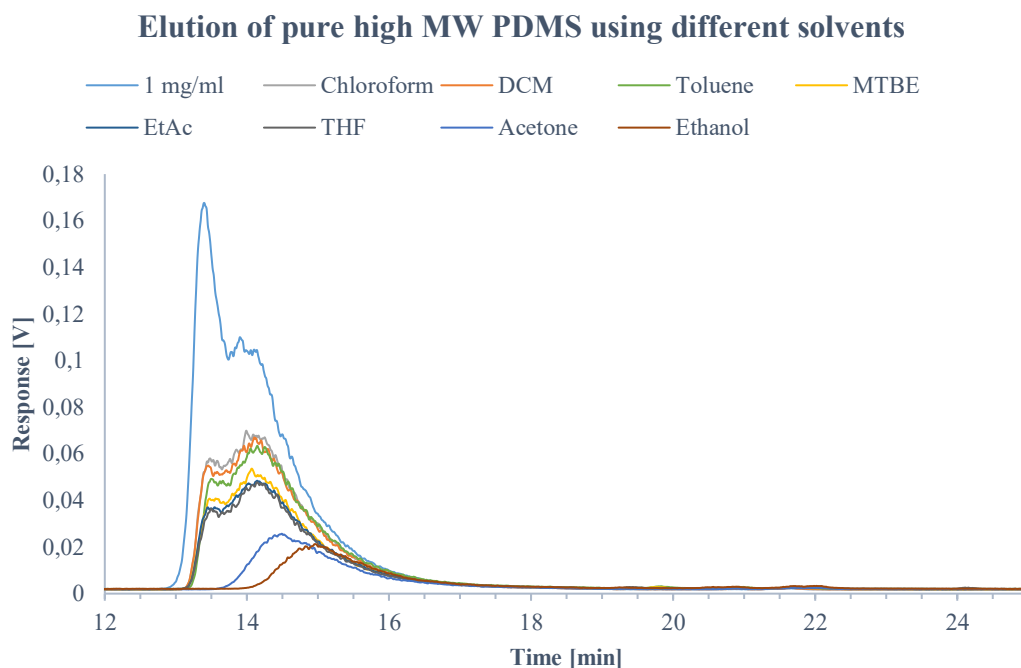

Figure 22. HP-SEC chromatogram of the capacity to elute pure high MW PDMS on SiOH cartridges for the different solvents, where 1 mg/ml works as a reference for pure silicone oil without SPE. Gain 3 was used.

The elution of pure high MW PDMS on SPE cartridges with different materials was one of the next tests, which resulted in various outcomes. The cartridge with CN material was the only cartridge resulting in 100% yield in the elution of pure high MW PDMS. The cartridge with florisil material showed second-best elution, about 80% yield. SiOH cartridge compared to CN or florisil cartridges was eluting the high MW PDMS in a much lower yield, almost about 50%. Figure 23 illustrates the elution of the pure high MW PDMS on the different SPE cartridges tested. The conclusion from the different cartridge material test is that CN cartridge was a good option, and it was used in further tests.
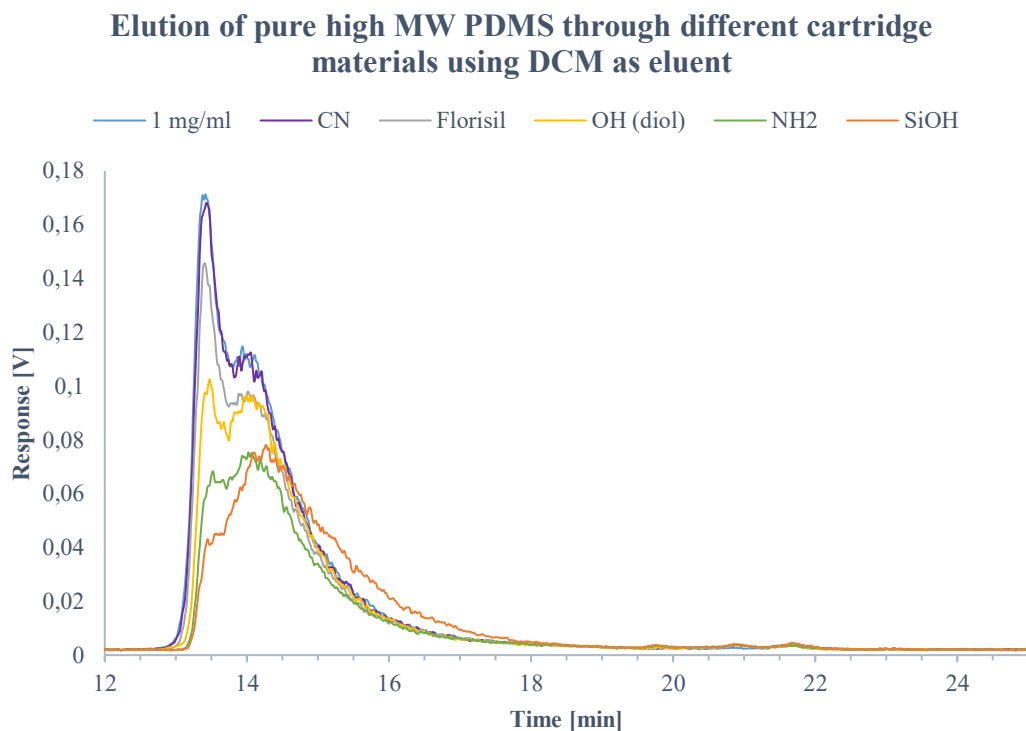
Elution of pure high MW PDMS through different cartridge materials using DCM as eluent

Figure 23. HP-SEC chromatogram of the elution of pure high MW PDMS on the SPE cartridges with different materials, where 1 mg/ml works as a reference for pure silicone oil without SPE. Gain 3 was used.

5.4.2   High MW PDMS in BO 1 matrix

As the CN cartridge was shown to be the best option regarding the yield of the eluted high MW PDMS, the elution of both pure high MW PDMS and pure BO 1 material was studied on separate cartridges. With the primary solvent combinations used (Table 9), the pure high MW PDMS eluted already in the first fraction. For the same solvent combinations, most of the BO 1 components eluted also in the first fraction. Some of the BO 1 material was also present in the next fractions, the combined P2–P5. Figure 24 illustrates the separate elution of the two studied materials. The conclusion drawn from these results is that a BO 1 sample spiked with high MW PDMS would also give the same results (i.e., elution of the high MW PDMS and the BO 1 material in the same fractions). As a good separation of the high MW PDMS from the BO 1 material was the goal for the SPE, the CN cartridge would not work

sufficiently enough and tests using the CN cartridges were therefore discontinued. Further tests were focusing on using the second-best SPE cartridge (i.e., florisil).
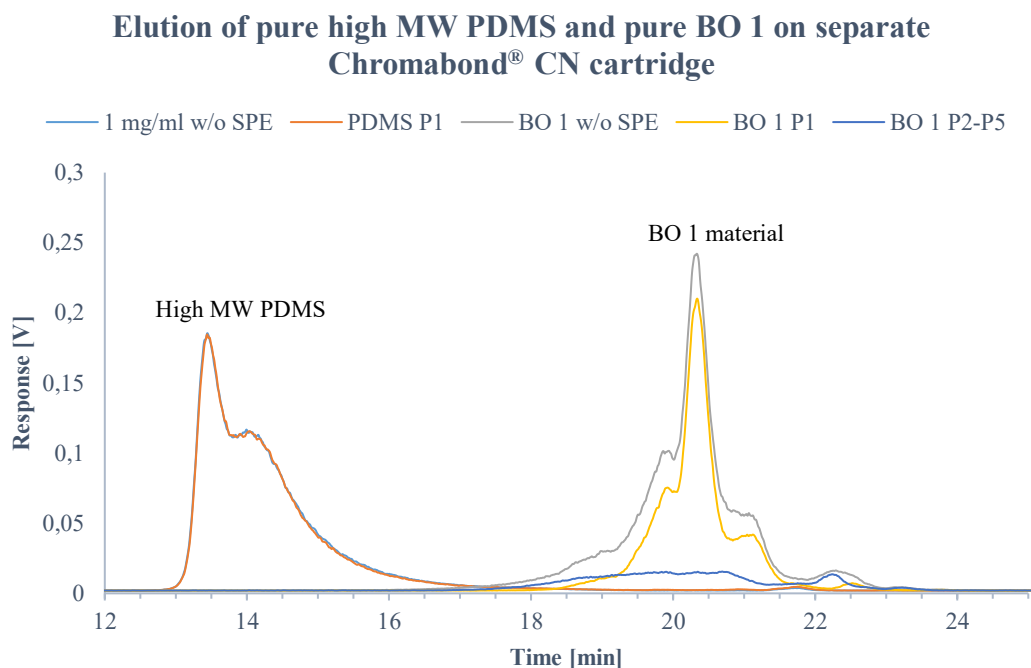
Elution of pure high MW PDMS and pure BO 1 on separate Chromabond® CN cartridge

Figure 24. HP-SEC chromatogram of the elution of pure high MW PDMS and pure BO 1 material on separate CN cartridges, where 1 mg/ml and BO 1 work as references for the materials without SPE. Gain 3 and primary solvent combinations were used.

The elution of pure high MW PDMS and the elution of pure BO 1 on separate cartridges were repeated, using the florisil cartridge instead of the CN cartridge. With the florisil cartridge, the elution of the pure high MW PDMS was more spread out between several fractions. However, the eluted pure high MW PDMS was concentrated to the last fractions, P8–P10, which was a promising improvement compared to the CN cartridge. The elution of the pure BO 1 material was, also for the florisil cartridge, concentrated to the first fractions, P1–P5. Figure 25 illustrates a comparison between the fractions, where most of the pure high MW PDMS and the pure BO 1 were eluting.
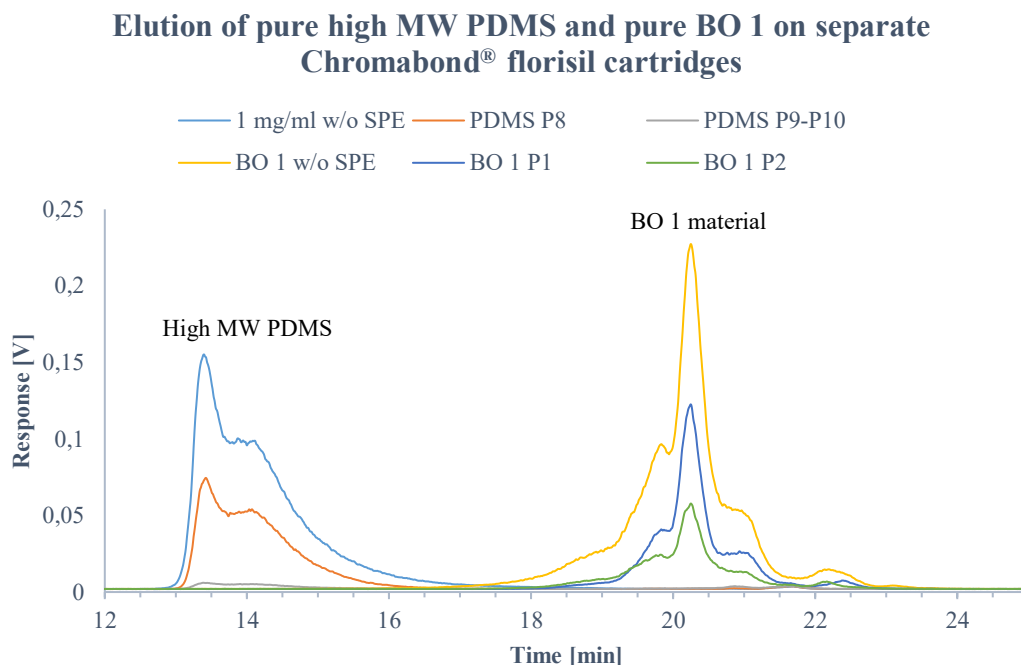
Elution of pure high MW PDMS and pure BO 1 on separate Chromabond® florisil cartridges

Figure 25. HP-SEC chromatogram of the elution of pure high MW PDMS and pure BO 1 material on separate florisil cartridges, where 1 mg/ml and BO 1 work as references for the materials without SPE. Gain 3 and primary solvent combinations were used.

Comparing the elution of a BO 1 sample spiked with high MW PDMS with the previous results (Figure 25) provided some unexpected information. The elution of high MW PDMS, together with BO 1 on the same cartridge, shifted from the later fractions to the earlier fractions. All the high MW PDMS and the BO 1 eluted in the same fractions, which was not an improvement. These results were insinuating there was an overload of the cartridge occurring. Figure 26 illustrates the eluted fractions for the spiked BO 1 sample.
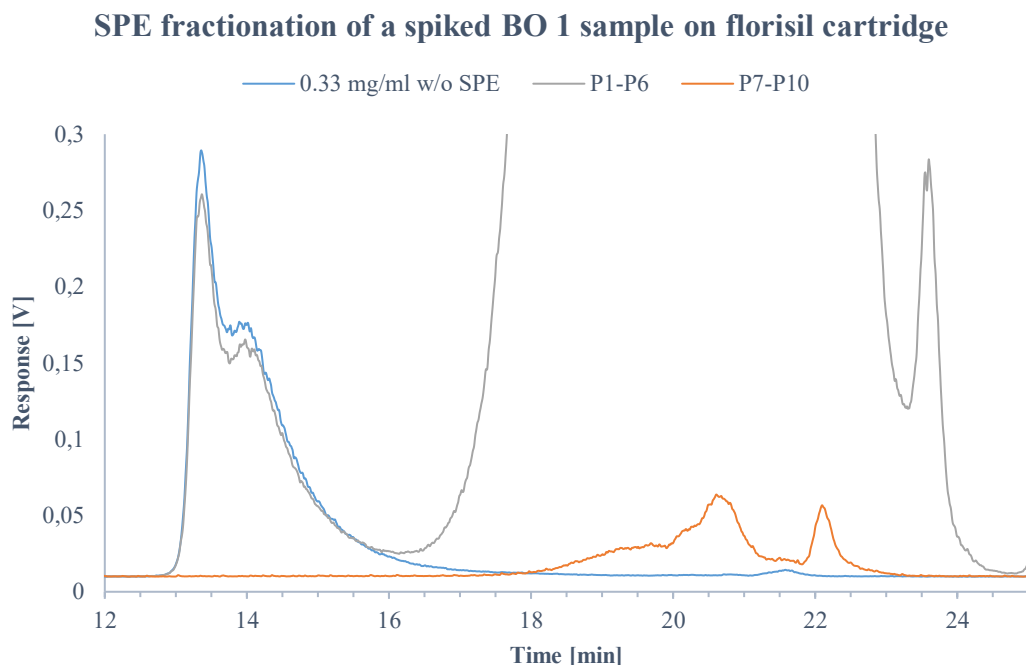
SPE fractionation of a spiked BO 1 sample on florisil cartridge

Figure 26. HP-SEC chromatogram of the elution of a spiked BO 1 sample, where 0.33 mg/ml works as a reference for the high MW PDMS without SPE. Gain 6 and primary solvent combinations were used.

5.4.3   Comparison between different solvent combinations

A decrease in the loaded sample volume was studied along with a change in the used solvent combinations, in order to avoid overload. A comparison was then performed, where the primary solvent combinations in Table 9 were compared with an alternative version (Table 10). Figures 27 and 28 illustrate the results from the SPE fractionation on florisil cartridges. The fractions shown in Figures 27 and 28 are the same (i.e., E7–E9) in order to show how different the elution of both the high MW PDMS and the BO 1 were for the two different solvent combinations.

A better picture of which solvent combination that resulted in an improved separation was achieved by comparing the peak areas of all the fractions where some high MW PDMS was eluting. The peak areas of eluted high MW PDMS and BO 1 (Table C1 and C2 in appendix C) revealed that there was more of the high MW PDMS in total eluting with the primary solvent combinations. However, the total amount of eluted

BO 1 material was less for the alternative solvent combinations. This showed that even though the elution of high MW PDMS was more spread out for the alternative solvent combinations, it was possible to concentrate the high MW PDMS since less BO 1 was eluting in the same fractions.

Figure 27. HP-SEC chromatogram of a spiked BO 1 sample on a florisil cartridge, where 1 mg/ml and spiked BO 1 work as references for the materials without SPE. Sample volume of 0.1 ml loaded onto cartridge. Gain 6 and primary solvent combinations were used.
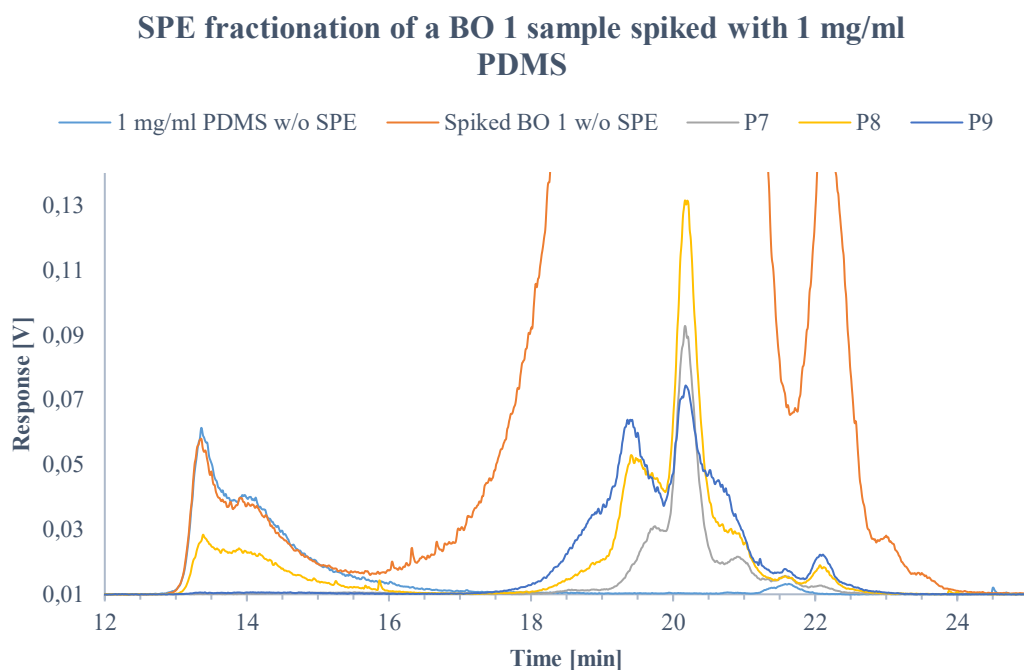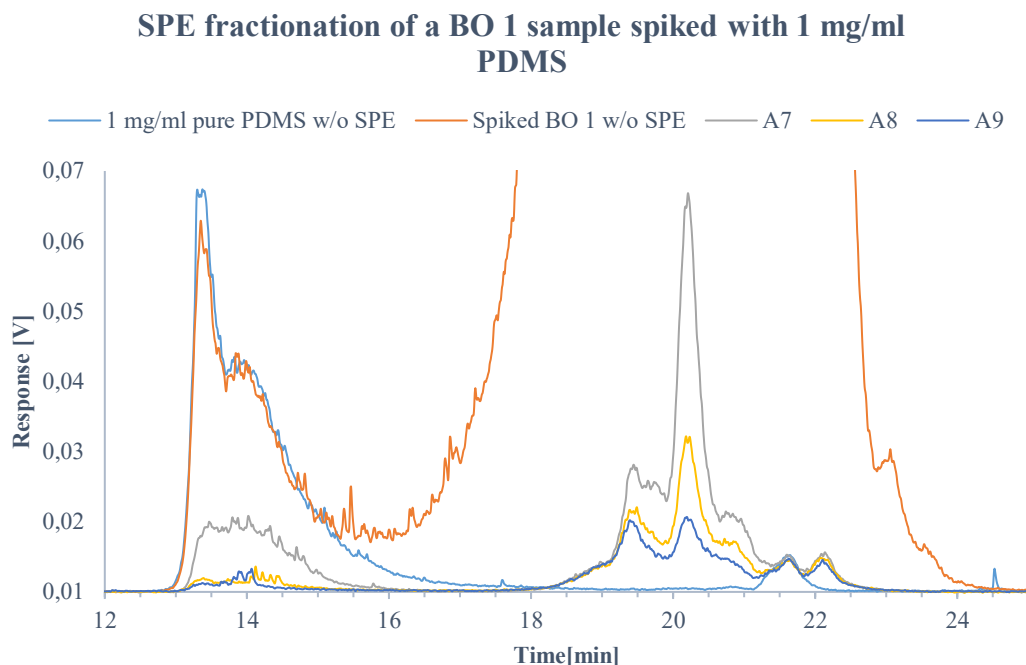
Figure 28. HP-SEC chromatogram of a spiked BO 1 sample on a florisil cartridge, where 1 mg/ml and spiked BO 1 work as references for the material without SPE. Sample volume of 0.1 ml loaded onto cartridge. Gain 6 and alternative solvent combinations were used.

5.4.4   Spiked BO 1 sample

The elution of a spiked BO 1 sample when comparing the SPE florisil cartridge and the SiOH cartridge is illustrated in Figure 29. Figure 29 illustrates the fraction where most of the high MW PDMS was eluting (i.e., fraction A7 for both cartridges). From the chromatogram, it is clearly visible that both cartridges had quite similar ability to elute the high MW PDMS. However, the florisil cartridge seemed to be adsorbing the BO 1 material better than the SiOH cartridge.

A comparison can also be made between the peak areas for the different fractions from the florisil and the SiOH cartridge. When comparing the peak areas of the eluted high MW PDMS with the peak areas of the BO 1 in the same fractions where high MW PDMS was eluting, there was clearly less BO 1 together with the high MW PDMS for

the florisil cartridge. In this sense, the florisil cartridge resulted in a slightly better separation. The peak areas are available in appendix C, Table C3 and C4.

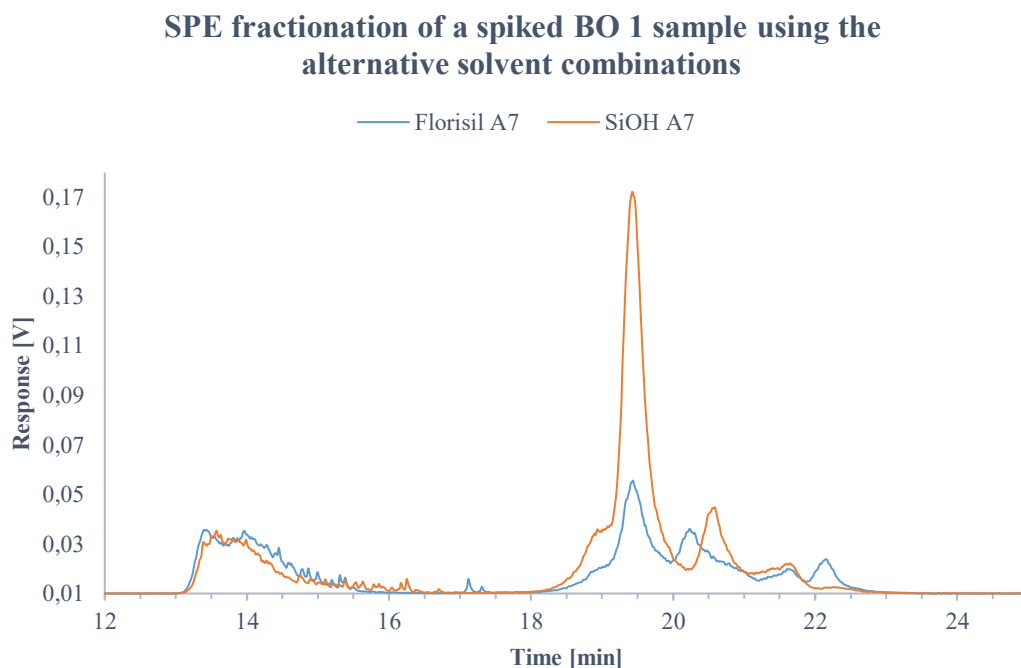**SPE fractionation of a spiked BO 1 sample using the alternative solvent combinations**

Figure 29. HP-SEC chromatogram of a spiked BO 1 sample, comparing the florisil and SiOH cartridges. Sample volume of 0.2 ml loaded onto each cartridge. Gain 6 and alternative solvent combinations were used.

5.4.5   Test of cartridge overload

A summary of the concentration of high MW PDMS in each spiked sample is available in appendix D (Table D1). The HyperSep florisil cartridge started showing overload already with 0.4 ml sample loaded onto the cartridge. For each volume between 0.4 ml and 1.5 ml, the elution of high MW PDMS was completely shifted from the later fractions to the earlier fractions, showing clear overload. Figure 30 illustrates the shifted elution of high MW PDMS for the highest sample volume loaded (i.e., 1.5 ml).

Florisil SPE fractionation of a spiked BO 1 sample using the simplified solvent combinations

Figure 30. HP-SEC chromatogram of the elution of a spiked BO 1 sample, using florisil cartridge, where overload is clearly visible. Gain 6 and simplified solvent combinations were used.

The HyperSep SiOH cartridge, however, had a higher ability to resist overload. For all the loaded sample volumes, the high MW PDMS elution was kept in the later fractions. Only for the highest sample volume (i.e., 1.5 ml), there were some signs of a small overload, when some high MW PDMS also eluted in the earlier fractions. Figure 31 illustrates the eluted fractions for the highest sample volume (i.e., 1.5 ml) loaded onto the SiOH cartridge.

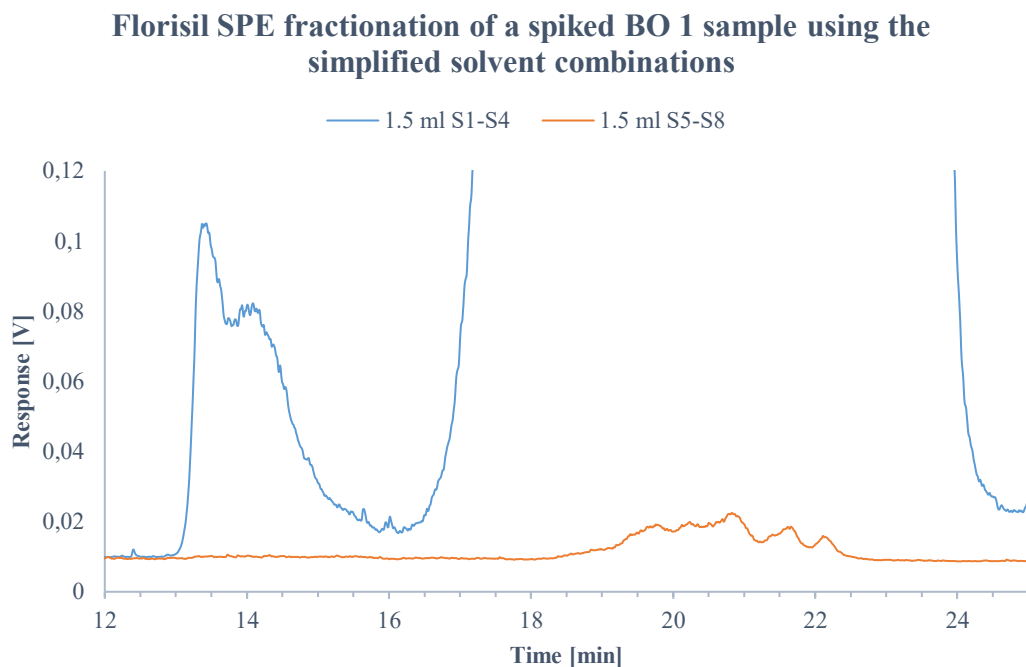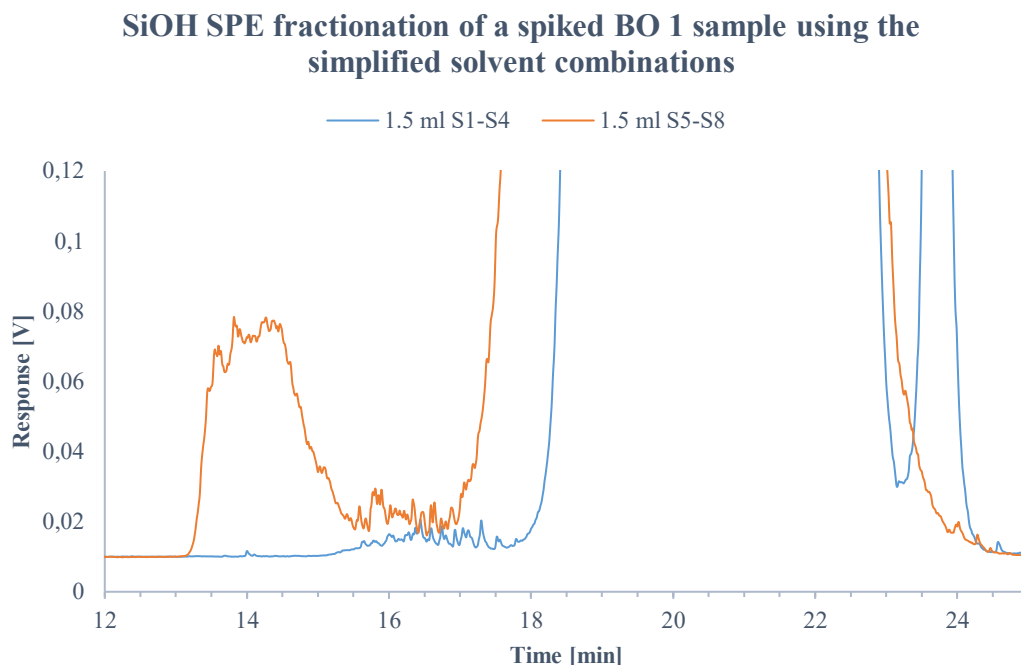**SiOH SPE fractionation of a spiked BO 1 sample using the simplified solvent combinations**

Figure 31. HP-SEC chromatogram of the elution of a spiked BO 1 sample, using SiOH cartridge, where overload is not as clearly visible as for the florisil cartridge. Gain 6 and simplified solvent combinations were used.

5.4.6   High MW PDMS in BO 2 and BO 3 matrices

Tests using BO 2 and BO 3 as matrices were also performed, in order to compare the behavior of the elution of the high MW PDMS in the three different matrices. The elution of high MW PDMS in a matrix of BO 2 seemed to begin much earlier than in a matrix of BO 1. This earlier elution was also the result in a matrix of BO 3. Though the elution of high MW PDMS was occurring much sooner, the elution for the BO 2/BO 3 components was occurring much later than for the BO 1 components. Figure 32 illustrates the fractions for the BO 2 sample where most of the high MW PDMS was eluting and where most of the BO 2 components were eluting (i.e., A5–A10). Figure 33 illustrates the same fractions but for the BO 3 sample. In Figures 32 and 33, it is visible how the elution of the matrix components was increasing in the later fractions, starting from fraction A7.

SPE fractionation of a spiked BO 2 sample on the florisil cartridge

Figure 32. HP-SEC chromatogram of the fractionation of high MW PDMS from a BO 2 matrix, where spiked BO 2 works as a reference for the material without SPE. Gain 6 and alternative solvent combinations were used.

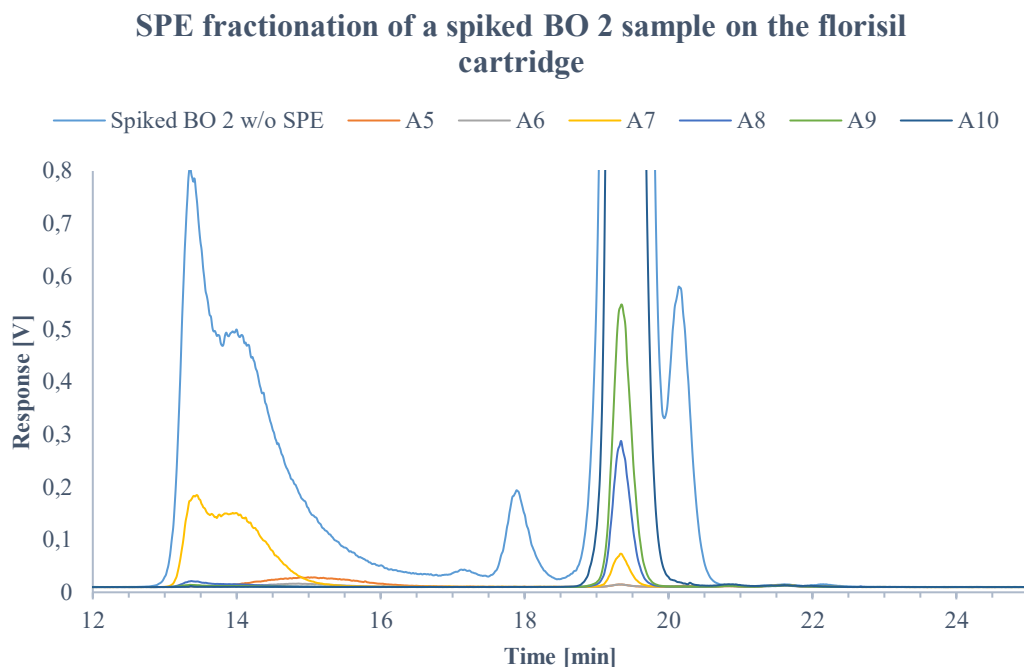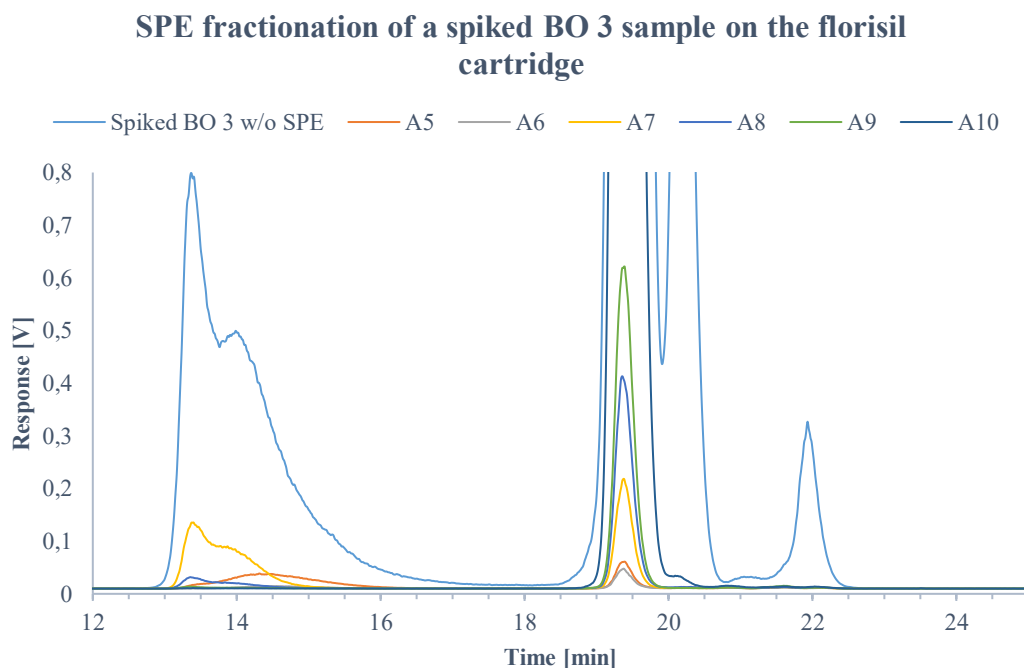**SPE fractionation of a spiked BO 3 sample on the florisil cartridge**

Figure 33. HP-SEC chromatogram of the fractionation of high MW PDMS from a BO 3 matrix, where spiked BO 3 works as a reference for the material without SPE. Gain 6 and alternative solvent combinations were used.

5.4.7   Pure medium MW PDMS

The developed florisil SPE method was also applied to the medium MW PDMS, to record the compatibility of the SPE method. The pure medium MW PDMS model compound was separated by SPE in order to receive a reference material. The SPE fractionation was performed as a comparison between the florisil cartridge and the SiOH cartridge, and the simplified solvent combinations (Table 11) were used for both cartridges. The results from the HP-SEC analysis of the eluted fractions showed a clear separation by molecular size between the different fractions. Figures 34 and 35 illustrate the HP-SEC chromatograms of the florisil and SiOH cartridge, respectively. Both cartridges behaved in the same way, which was that the non-polar fractions were eluting smaller molecules compared to the more polar fractions, which were eluting larger molecules. The difference between the medium and high MW PDMS was that the medium MW PDMS eluted already in the earliest fractions (i.e., S2–S6), while the high MW PDMS was eluting in the later fractions.
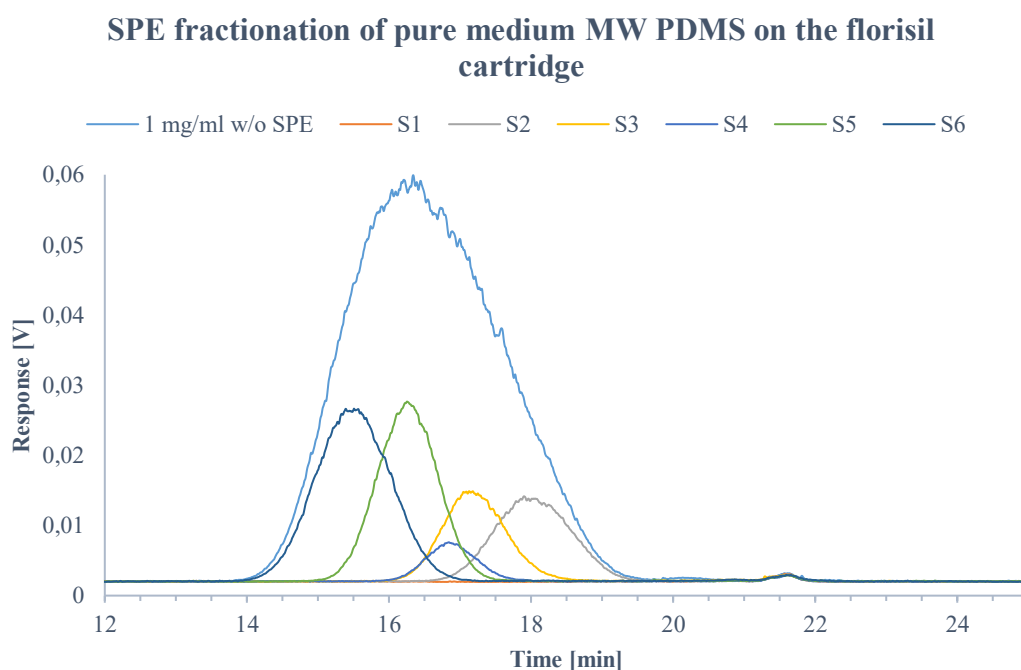

Figure 34. HP-SEC chromatogram of the separation of pure medium MW PDMS, where 1 mg/ml works as a reference for the material without SPE. Gain 3 and simplified solvent combinations were used.

SPE fractionation of pure medium MW PDMS on the SiOH cartridge

Figure 35. HP-SEC chromatogram of the separation of pure medium MW PDMS, where 1 mg/ml works as a reference for the material without SPE. Gain 3 and simplified solvent combinations were used.
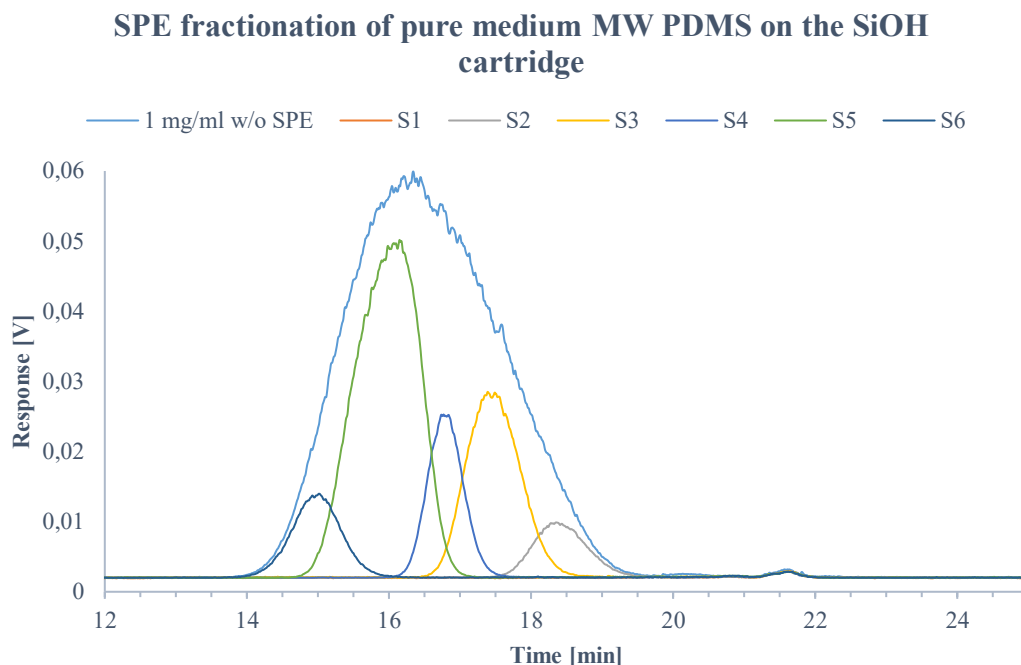
The molecular weight distribution of the eluted fractions in Figures 34 and 35 was also calculated. Based on the calculations of the molecular weight distribution, the average molecular weight of the pure medium MW model compound before SPE was determined to be 6 500. According to the manufacturer, this value should be 3 780. The reason for the difference in the molecular weight was due to the calibration used for the calculations. In this case, the molecular weight of the eluted medium MW PDMS was compared against a calibration of polystyrene (PS), and the results were therefore more general. Although the results were not completely accurate, it is still seen from Figures 36 and 37 that the average molecular weight of the eluted fractions was increasing with increasing polarity of the solvent. The average molecular weight of these fractions is also illustrated in appendix E (Figures E1 and E2) as an integral molecular weight curve.

Molecular weight distribution after SPE fractionation on the florisil cartridge

Figure 36. The molecular weight distribution of pure medium MW PDMS post-SPE, where 1 mg/ml represents the material without SPE.

Molecular weight distribution after SPE fractionation on the SiOH cartridge

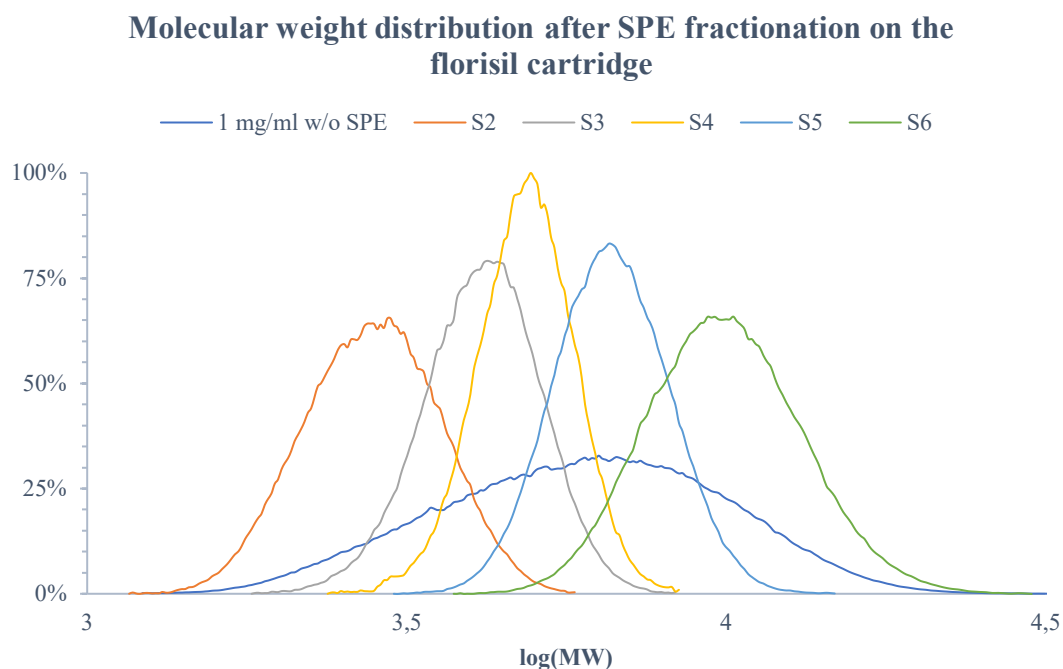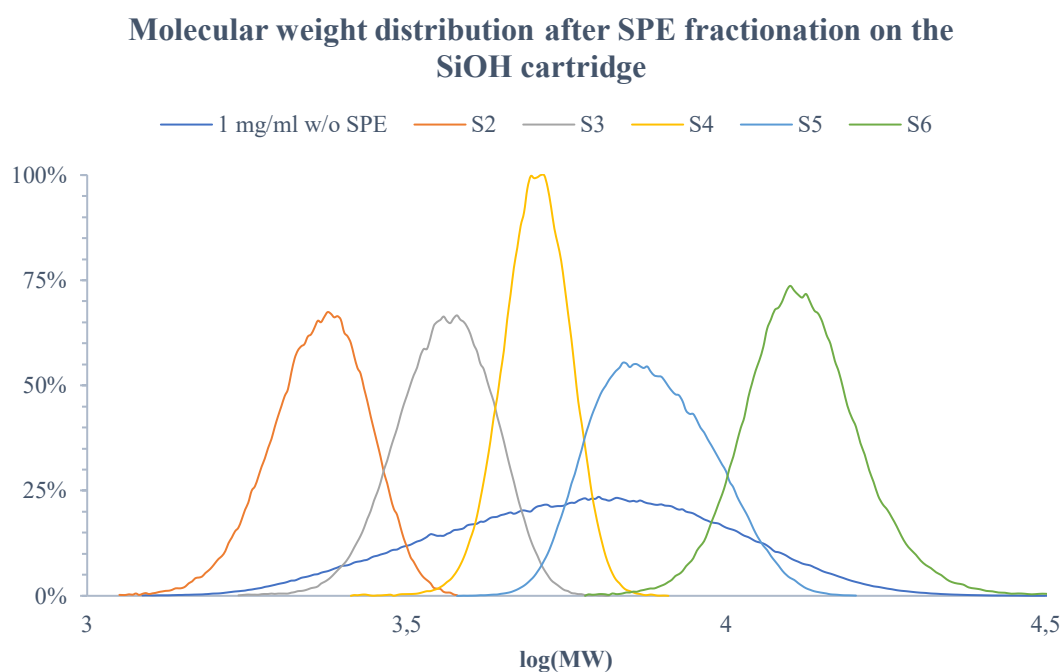*Figure 37. The molecular weight distribution of pure medium MW PDMS post-SPE, where 1 mg/ml represents the material without SPE.*

5.4.8   Medium MW PDMS in BO 1 matrix

The same behavior of the medium MW PDMS was also visible together with BO 1. That is, the fractions were eluting different sized molecules, and that the molecular size was increasing with increasing polarity. Figure 38 illustrates the chromatogram of the eluted fractions. Since the average molecular size of the medium MW PDMS was smaller than for the high MW PDMS, there was some overlapping of the PDMS peak with the BO 1 peak. The elution of the medium MW PDMS was still in the earliest fractions even together with BO 1 as matrix. The tendency of the increasing molecular size with increasing polarity was seen for the fractions S2–S6.

Figure 38. HP-SEC chromatogram of the fractionation of a spiked BO 1 sample with medium MW PDMS, where spiked BO 1 works as a reference for the material without SPE. Gain 6 and simplified solvent combinations were used.

The molecular weights of the medium MW PDMS in the eluted fractions of the spiked sample were also calculated. PS was used as calibration also this time. The same tendency was seen with BO 1 as matrix as was seen with only pure medium MW PDMS, which is illustrated in Figure 39. An integral molecular weight curve of these same fractions is illustrated in appendix E (Figure E3).

Molecular weight distribution of a spiked BO 1 sample after SPE fractionation on the florisil cartridge
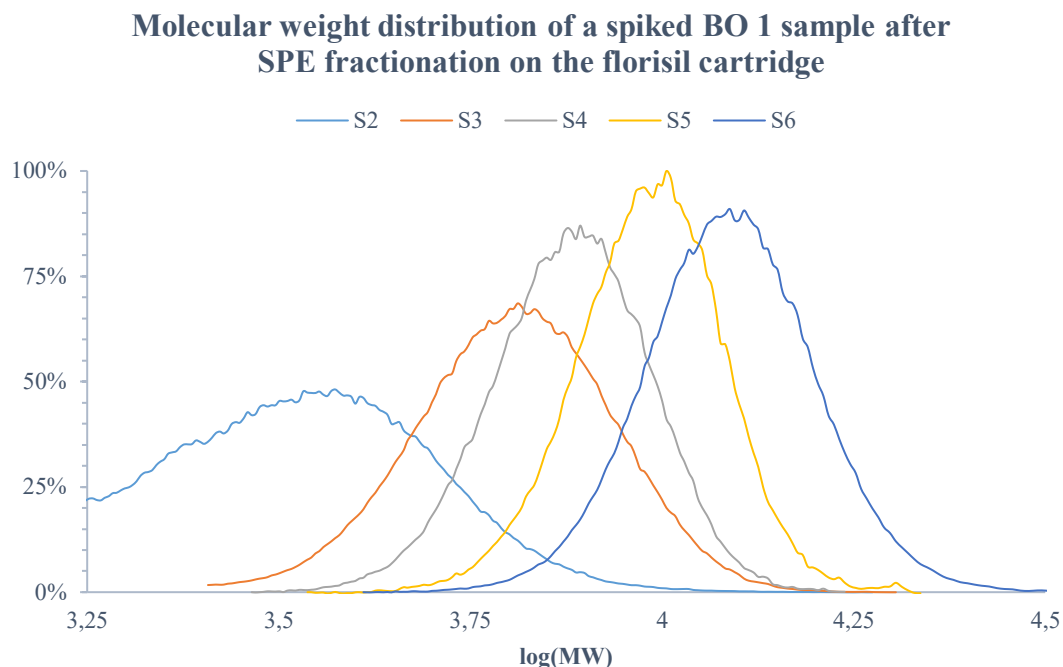
Figure 39. The molecular weight distribution of the medium MW PDMS in a spiked BO 1 sample post-SPE.

5.4.9   Medium MW PDMS in BO 2 and BO 3 matrices

The elution of medium MW PDMS was also studied in the BO 2 and the BO 3 matrices in order to compare the results with the BO 1 matrix. The same tendency of the fractionation was also clearly visible for both the BO 2 and BO 3 matrices. The clear difference between the BO 2 or the BO 3 matrix and the BO 1 matrix was that the components of BO 2 and BO 3 were eluting mainly in the later fractions. There was not much of the components of the matrix present in the PDMS eluting fractions. The chromatograms of the spiked BO 2 sample and the spiked BO 3 sample are illustrated in Figures 40 and 41, respectively.

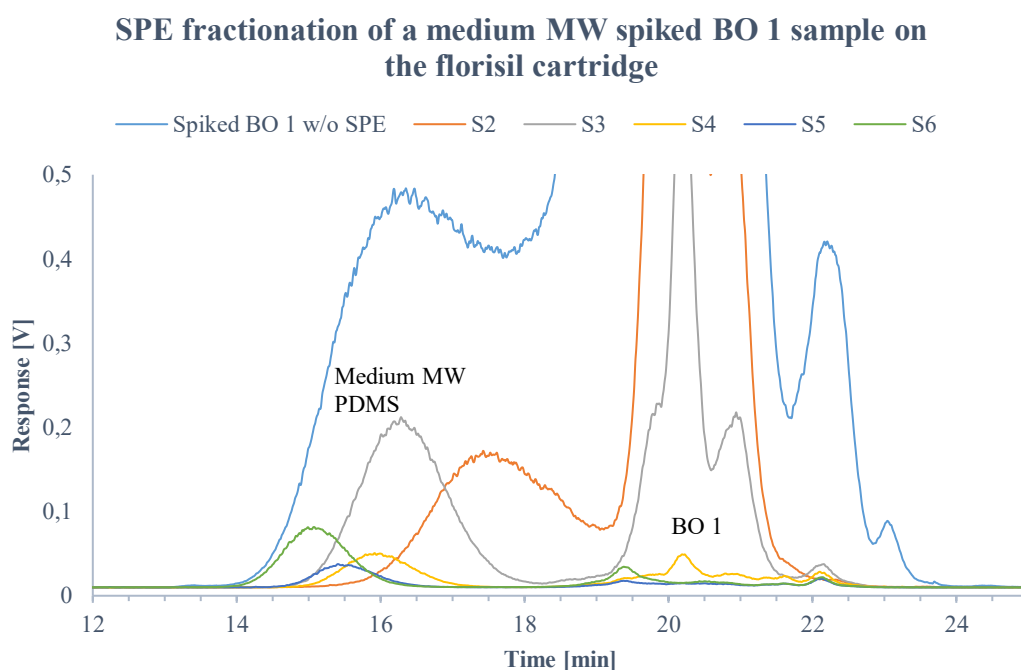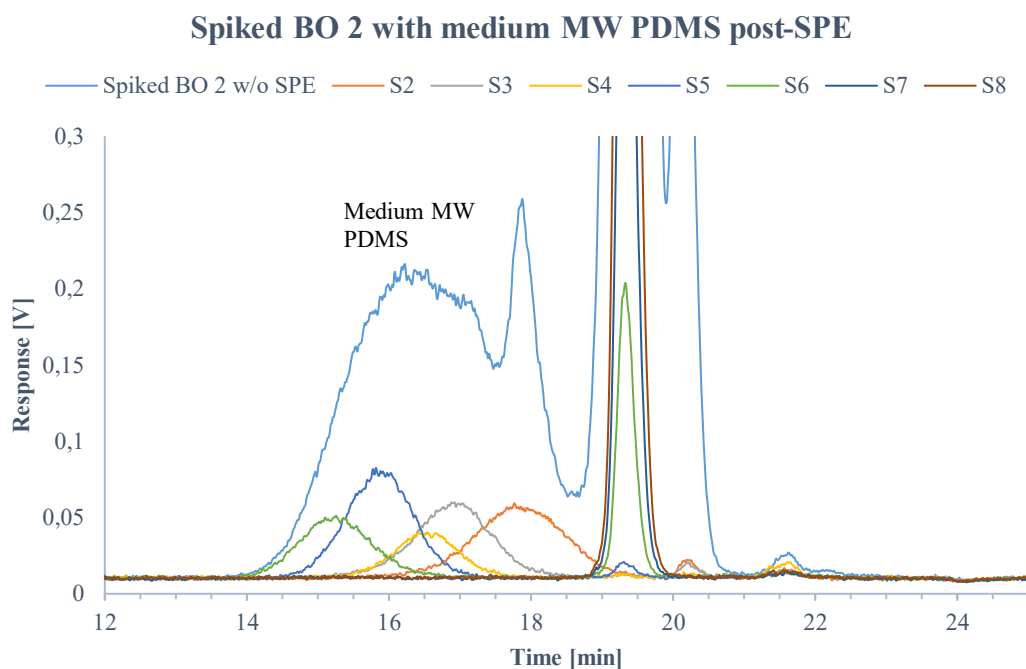*Figure 40. HP-SEC chromatogram of the fractionation of spiked BO 2 with medium MW PDMS, where spiked BO 2 works as a reference for the material without SPE. Gain 6 and simplified solvent combinations were used.*
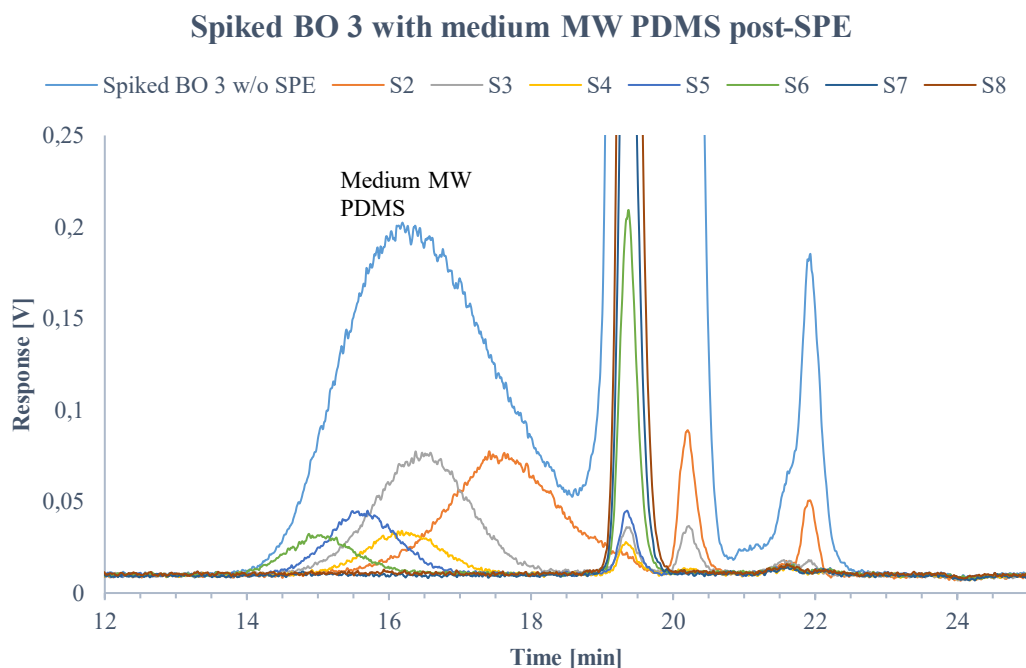
Figure 41. HP-SEC chromatogram of the fractionation of spiked BO 3 with medium MW PDMS, where spiked BO 3 works as a reference for the material without SPE. Gain 6 and simplified solvent combinations were used.

The molecular weight distribution of the eluted medium MW PDMS in the spiked BO 2 and the spiked BO 3 samples was also studied. The molecular weight distributions are illustrated in Figures 42 and 43 for BO 2 and BO 3, respectively. The integral molecular weight curves for the same samples are found in appendix E (Figures E4 and E5). There was no clear difference in the average molecular weights when comparing it to the pure medium MW PDMS or the medium MW PDMS in BO 1 matrix. A complete comparison of all the tested samples and the elution for each fraction is summarized in appendix E Table E1.
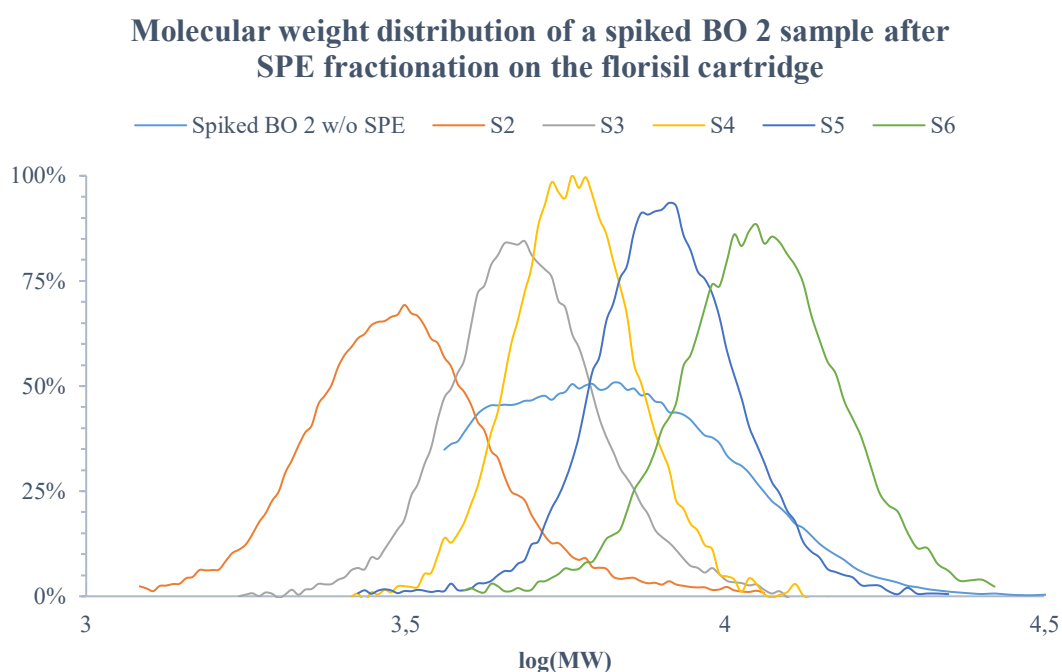
Figure 42. The molecular weight distribution of the medium MW PDMS in a spiked BO 2 sample post-SPE.

Molecular weight distribution of a spiked BO 3 sample after SPE fractionation on the florisil cartridge
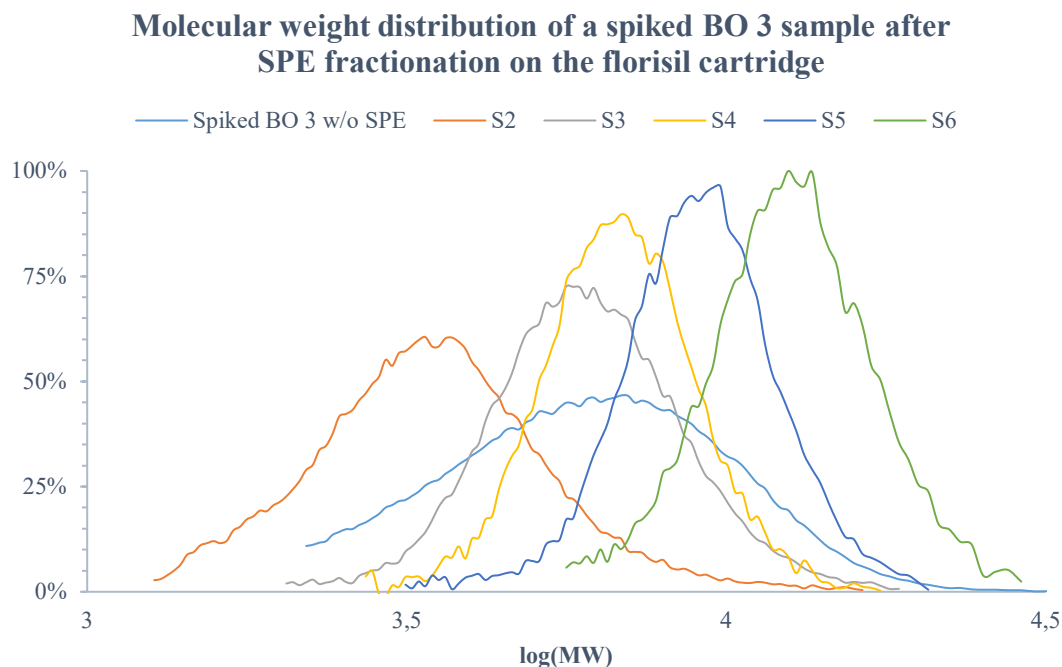
Figure 43. The molecular weight distribution of the medium MW PDMS in a spiked BO 3 sample post-SPE.

5.4.10 Pure low MW PDMS

Like the medium MW PDMS, the low MW PDMS also eluted in the earliest fraction. For low MW PDMS, the elution was more concentrated to only fraction S1 and S2. When comparing the different cartridges with each other, there were some differences. The florisil cartridge was separating the elution of the PDMS between both fraction S1 and S2, while the SiOH cartridge was eluting mostly in fraction S2, although there was also some minimal amount of eluted PDMS in fraction S1 and S3. Figure 44 illustrates the PDMS eluting fractions for the florisil cartridge, and Figure 45 illustrates the fraction with the most eluted PDMS for the SiOH cartridge.

For both chromatograms (Figures 44 and 45), the PDMS peaks are numbered as 1, 2, 3, 4, etc. Only the five first PDMS peaks are numbered, though the numbering continues for every continuously repeated peak. The peak areas are summarized in appendix B (Table B3), where both the separate peak areas and the total peak area of the low MW PDMS are available. Differences in the total peak area for the low Mw

PDMS without SPE and post-SPE were due to the sample work-up. The low MW PDMS was slightly volatile and some of it might have evaporated from the sample during evaporation or drying in the oven.
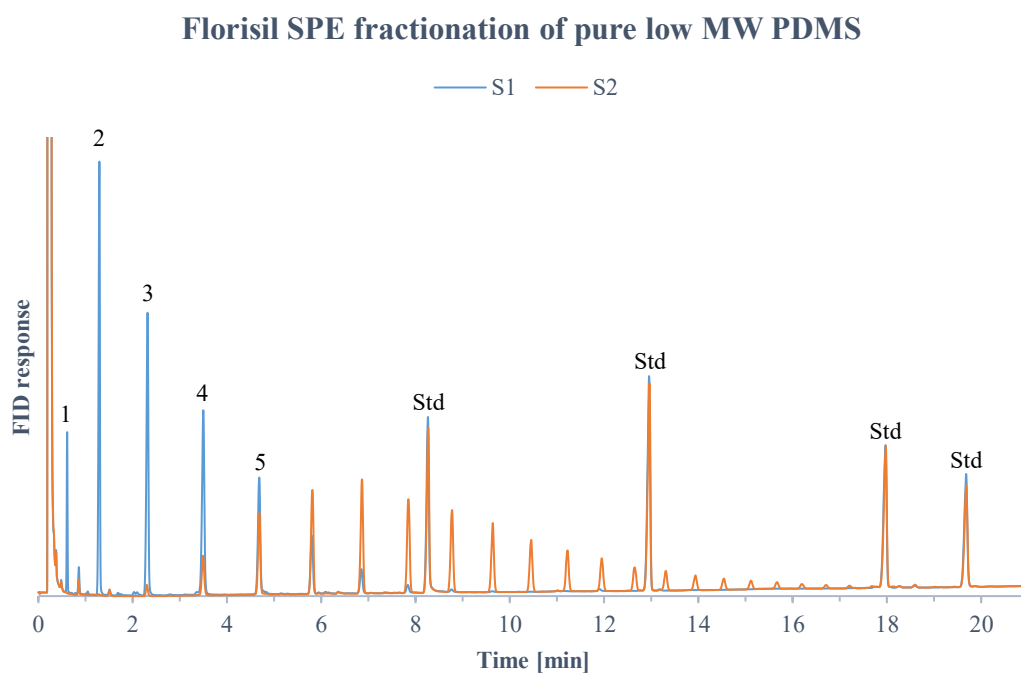
Figure 44. GC-FID chromatogram of the fractionation of pure low MW PDMS on the florisil cartridge. Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column and simplified solvent combinations were used.

SiOH SPE fractionation of pure low MW PDMS

Figure 45. GC-FID chromatogram of the fractionation of pure low MW PDMS on the SiOH cartridge. Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column and simplified solvent combinations were used.

5.4.11 Low MW PDMS in BO 1 matrix

The elution of low MW PDMS in a spiked BO 1 sample was quite similar to the elution of pure low MW PDMS (i.e., most PDMS eluted in fractions S1 and S2). However, the separation from the BO 1 components, on the florisil cartridge, was not quite optimal in the case for the spiked sample. The short column GC-FID method for the detection of PDMS was although a well-working analytical approach. The chromatogram of the PDMS eluting fractions and the unfractionated spiked sample is illustrated in Figure 46.

Spiked BO 1 with low MW PDMS post-SPE on the florisil cartridge

Figure 46. GC-FID chromatogram of the fractionation of low MW PDMS in a spiked BO 1 sample on florisil cartridge. Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column and simplified solvent combinations were used.

5.4.12 Low MW PDMS in BO 2 and BO 3 matrices

The elution of low MW PDMS and the separation from the components of the matrix were also tested for spiked BO 2 and BO 3 samples. The elution of low MW PDMS was still the same as in BO 1 matrix (i.e., most was elution in S1 and S2). The separation of the PDMS from the BO 2 matrix on the florisil cartridge worked very well, however, for the BO 3 matrix there were some components of BO 3 present in the PDMS eluting fractions. The chromatograms of the spiked BO 2 and BO 3 fractions are illustrated in Figures 47 and 48, respectively. From the chromatograms, it is visible that the components of the matrix were not present for BO 2 at RT 20–22 min, which is the opposite of BO 3. The irregular peaks in both chromatograms (Figures 47 and 48) at RT 4–7 min were identified by GC-MS to be methyl esters and ethyl esters, which most likely were derived from the components of the matrix. The GC-MS chromatograms, which were identifying the methyl and ethyl esters in the

spiked BO 2 and BO 3 samples, are available in appendix F (Figures F1 and F2, respectively).

Spiked BO 2 with low MW PDMS post-SPE
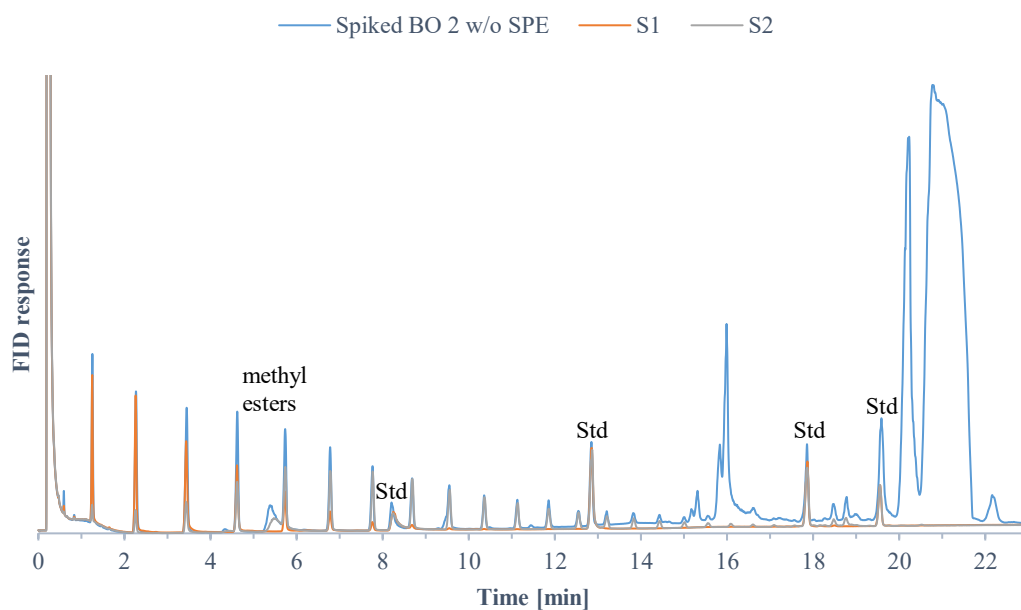
Figure 47. GC-FID chromatogram of the fractionation of low MW PDMS in a spiked BO 2 sample on the florisil cartridge. Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column and simplified solvent combinations were used.
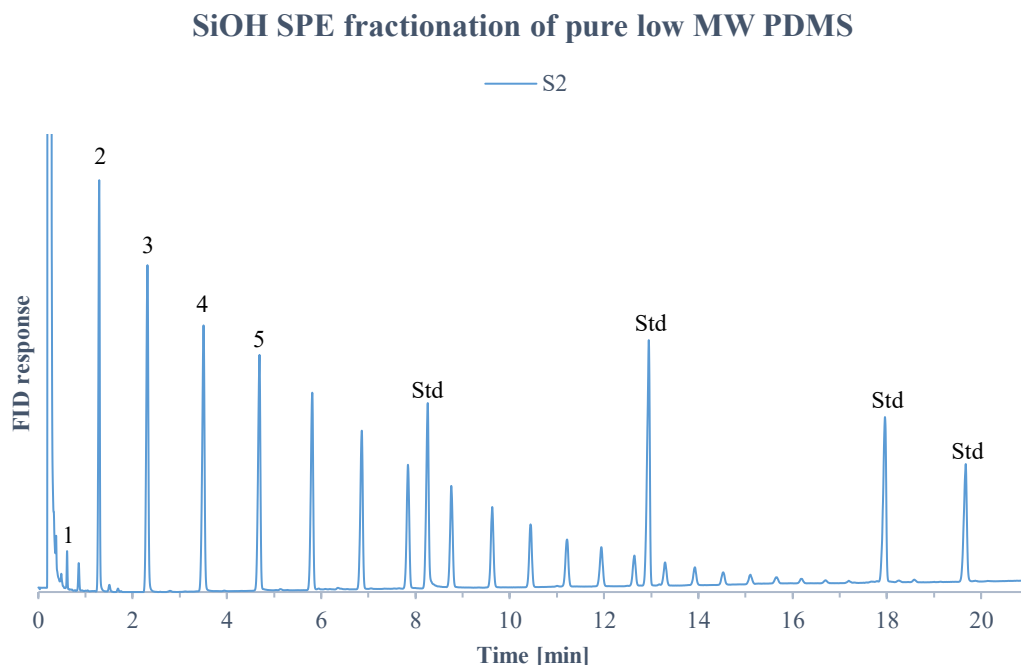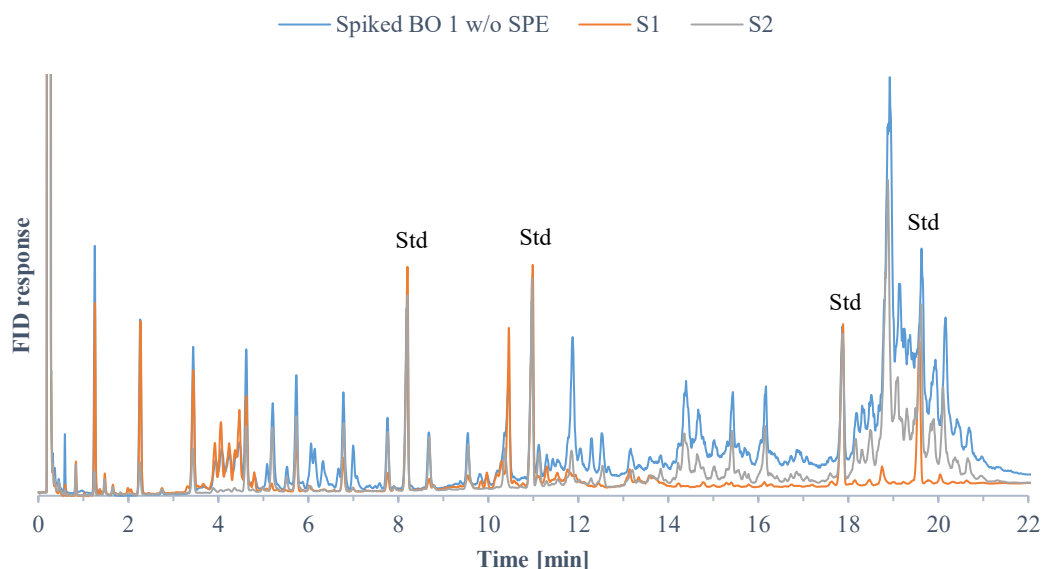
Spiked BO 3 with low MW PDMS post-SPE
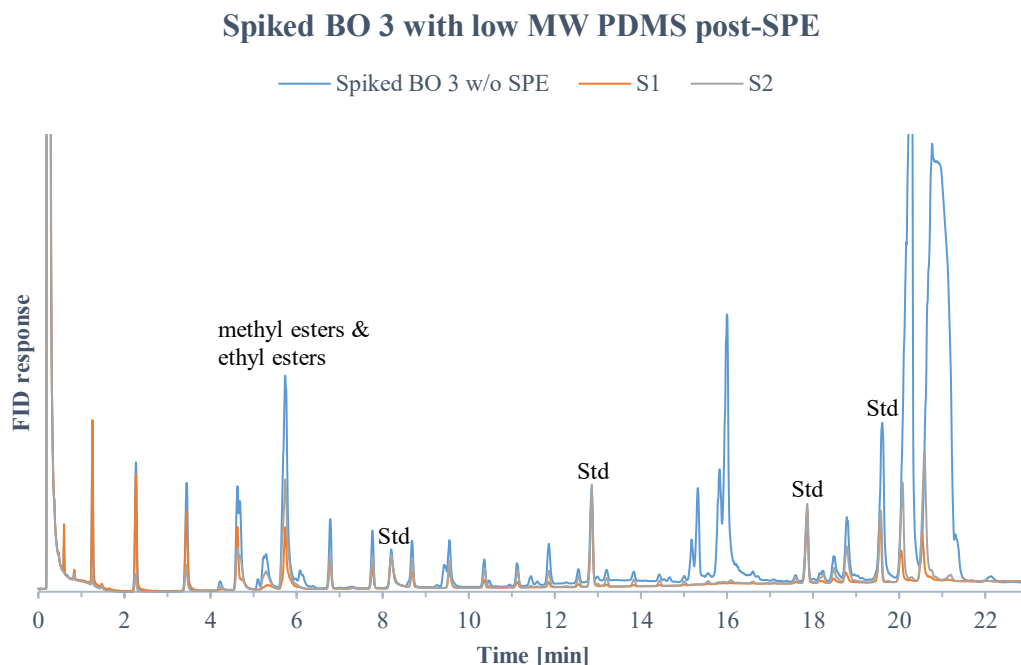
Figure 48. GC-FID chromatogram of the fractionation of low MW PDMS in a spiked BO 3 sample on the florisil cartridge. Internal standards from left to right: acid 21:0, betulinol, CH17, and TG-standard. Short column and simplified solvent combinations were used.

5.4.13 Pure D_3 and D_4 PDMS oligomers

For D_3 fractionated on the florisil cartridge (Figure 49), the elution was divided between fraction S1 and S2. For D_3 fractionated on the SiOH cartridge (Figure 50), the elution was concentrated only to S2. This was the same behavior as the low MW PDMS model compound. The elution of D_4 on the florisil and the SiOH cartridge was like the elution of D_3. Figures 51 and 52 illustrate the elution of D_4 on the florisil and the SiOH cartridge, respectively. The peak areas of D_3 and D_4 on both the florisil and the SiOH cartridge are summarized in appendix B (Table B4).

SPE fractionation of D₃ on the florisil cartridge
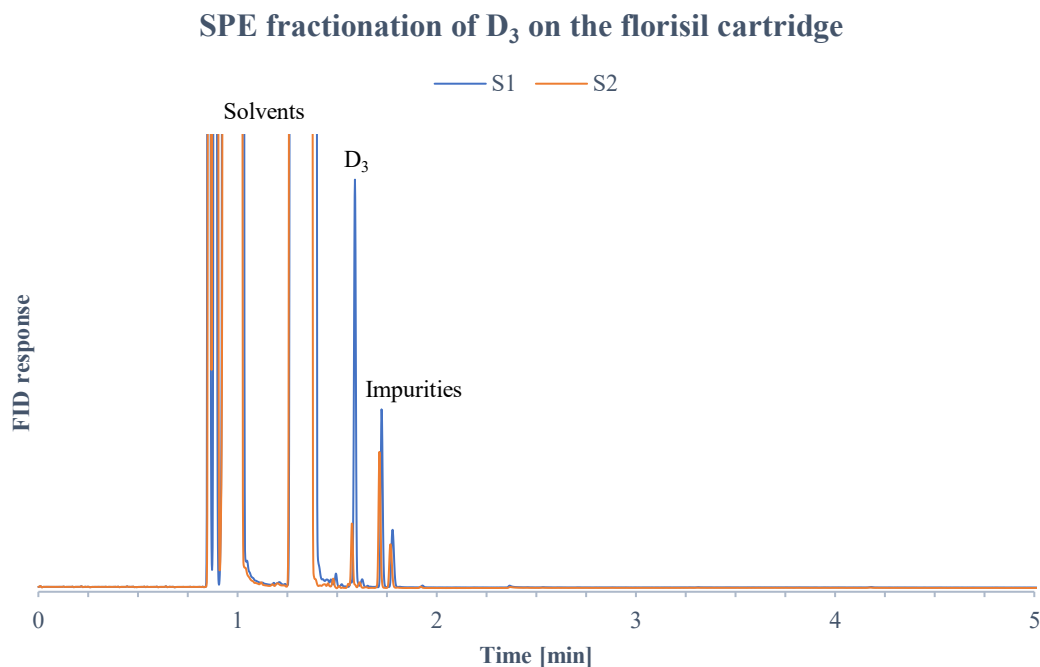
Figure 49. GC-FID chromatogram of the fractionation of the D₃ PDMS degradation product on the florisil cartridge. Long column and simplified solvent combinations were used.

SPE fractionation of D₃ on the SiOH cartridge
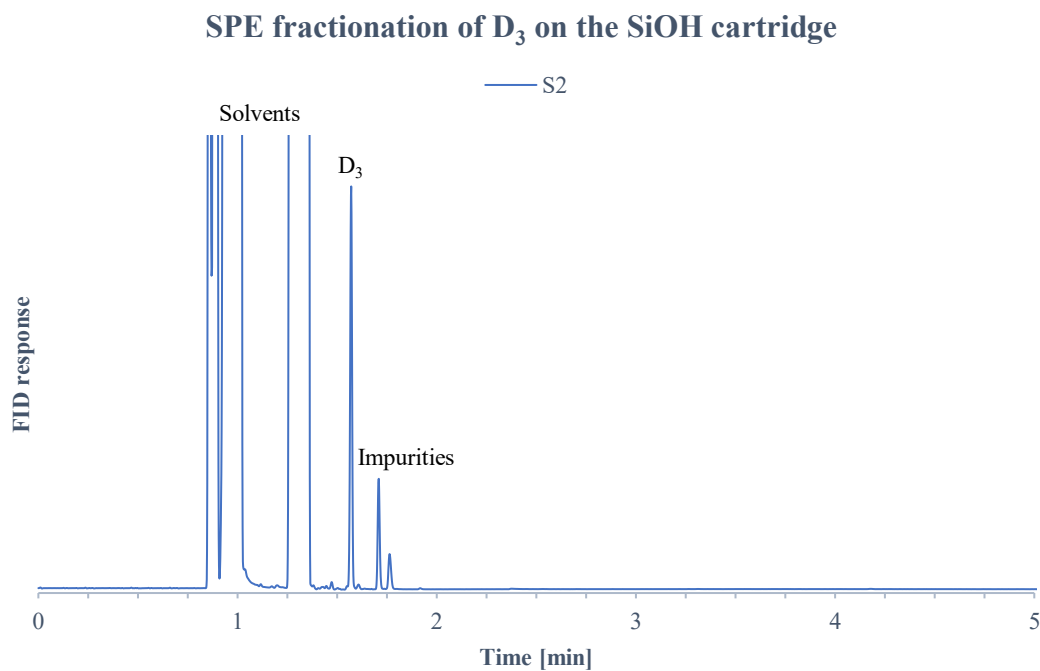
Figure 50. GC-FID chromatogram of the fractionation of the D₃ PDMS degradation product on the SiOH cartridge. Long column and simplified solvent combinations were used.

SPE fractionation of D$_4$ on the florisil cartridge
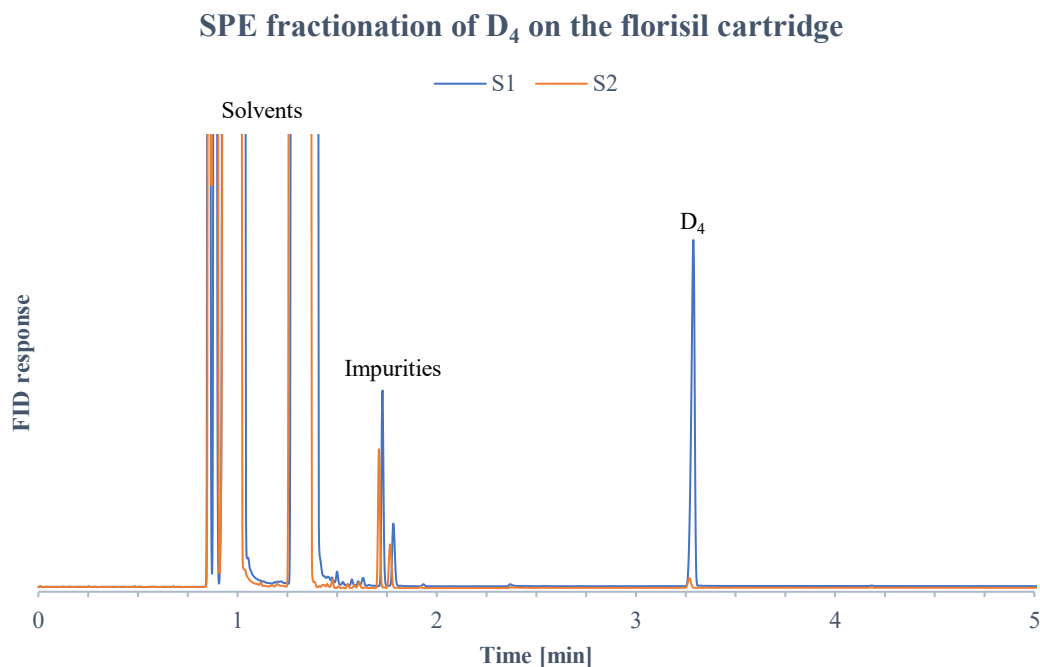
Figure 51. GC-FID chromatogram of the fractionation of the D$_4$ PDMS degradation product on the florisil cartridge. Long column and simplified solvent combinations were used.

SPE fractionation of D$_4$ on the SiOH cartridge
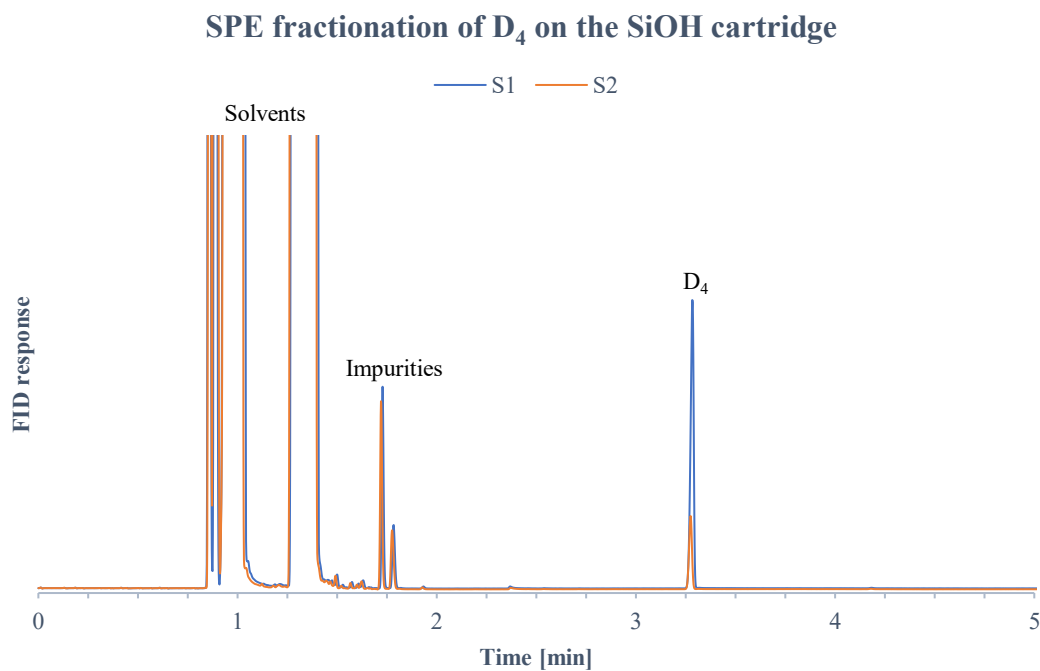
Figure 52. GC-FID chromatogram of the fractionation of the D$_4$ PDMS degradation product on the SiOH cartridge. Long column and simplified solvent combinations were used.

5.4.14 D₃ and D₄ PDMS oligomers in BO 1 matrix

The elution of the degradation products D_3 and D_4, respectively, in a spiked BO 1 sample was also studied. The theory before the test was that it might be possible to separate the D_3 and D_4 products completely from the BO 1 matrix. A successful separation of both D_3 and D_4 from the BO 1 matrix was obtained. The chromatograms of the D_3 and D_4 are illustrated in Figures 53 and 54, respectively. The D_3 and D_4 products were eluting in fractions S1 and S2. These fractions were, however, not completely free of BO 1 material. At RT 16–20 min, fractions S1 and S2 of both spiked samples contained very small amounts of BO 1 components. Nevertheless, these BO 1 components were such a small amount that they were neglected. The peak areas of D_3 and D_4 in the spiked sample are summarized in appendix B (Table B5).
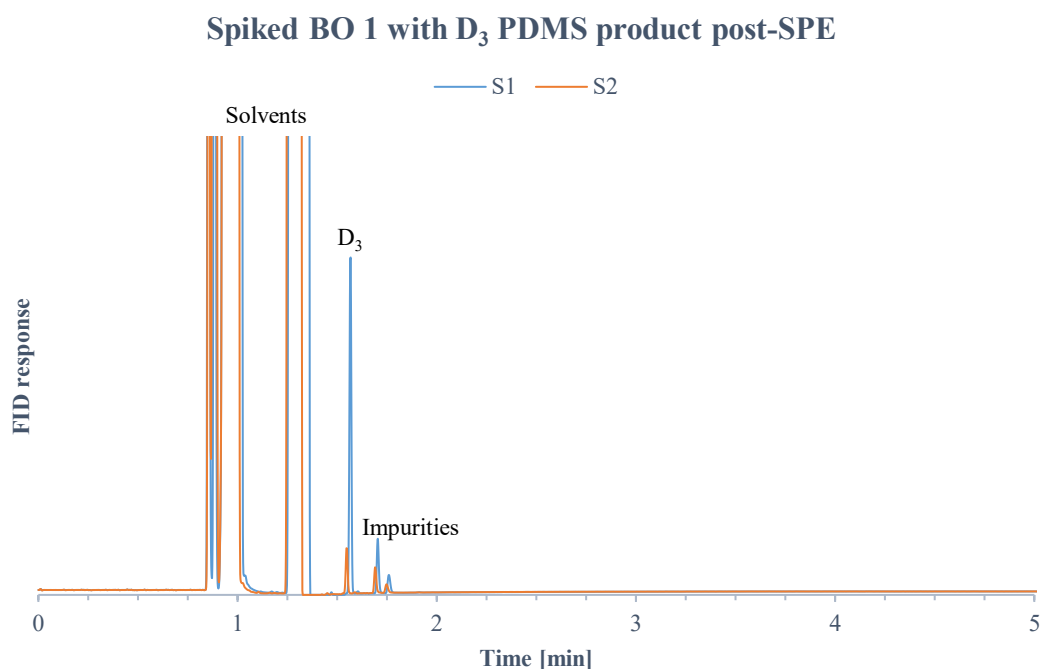
Figure 53. GC-FID chromatogram of the elution of D₃ product in a spiked BO 1 sample on the florisil cartridge. Long column and simplified solvent combinations were used.

Spiked BO 1 with D$_4$ PDMS product post-SPE
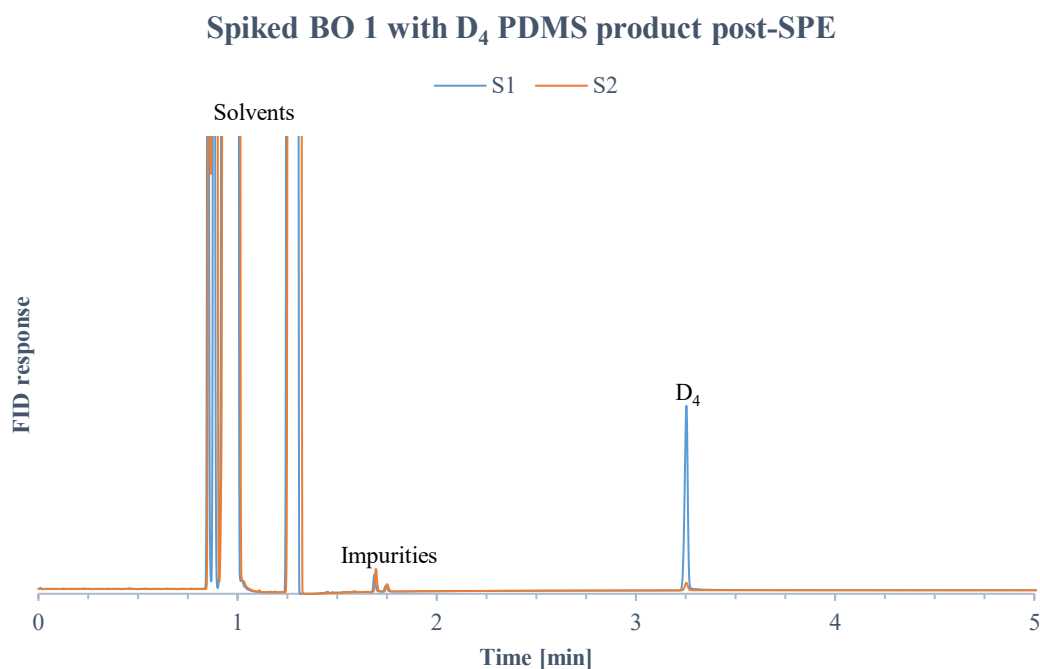
Figure 54. GC-FID chromatogram of the elution of D$_4$ product in a spiked BO 1 sample on the florisil cartridge. Long column and simplified solvent combinations were used.

5.4.15 Real unspiked BO 1 samples

The HP-SEC results of the real samples (i.e., unspiked BO 1 samples) are illustrated in Figure 55. The approximate concentrations of PDMS in the real samples were as follows: high concentration for BO 1 (3), medium concentration for BO 1 (4), and low concentration for BO 1 (5). A comparison of only the peaks of the PDMS in the chromatogram revealed that the BO 1 (5) sample had a considerably lower concentration of PDMS present, which would correspond to the approximate concentration values. The BO 1 (3) and BO 1 (4) samples, however, seemed to have quite similar concentrations of PDMS.

Real (unspiked) samples post-SPE
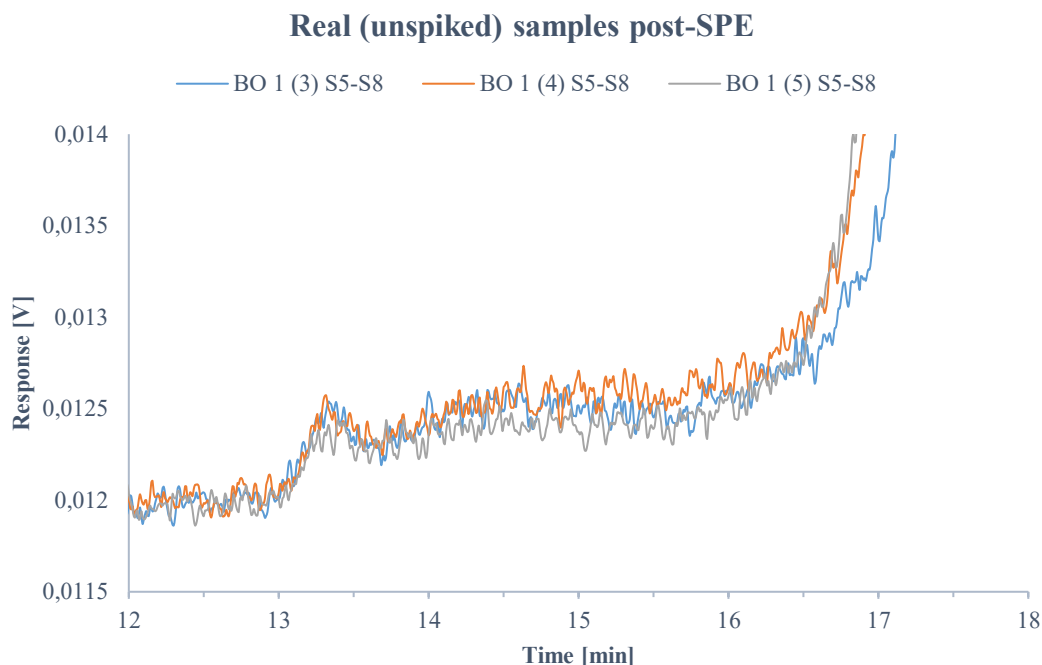
Figure 55. HP-SEC chromatogram of the elution of the real (unspiked) BO 1 samples after SPE fractionation on the florisil (2 000 mg) cartridges and the simplified solvent combinations, with focus on the elution of the PDMS. Sample concentration in THF varies between 3.0–3.89 mg/ml. Gain 6 simplified solvent combinations were used.

A comparison between the alternative SPE method and the previously developed method showed some differences. For the alternative SPE method, the BO 1 components had a longer retention time. This was a sign that the florisil cartridge could retain the BO 1 material better than the SiOH cartridge. Another clear difference between the two methods was that the peak for the PDMS was slightly more defined with the alternative SPE method. Figure 56 illustrates the results from the previously developed SPE method (i.e., SiOH cartridge with the primary solvent combinations from Table 9).

One important thing to remember when comparing the results is that the final concentration of the samples in THF was different. The concentration of the samples in THF for the previously developed method was the same for all the samples: 5 mg/ml. For the alternative method, the concentration of the samples in THF varied between 3.0 mg/ml and 3.89 mg/ml. One other slight difference was the sample load.

The total sample load for the florisil SPE method was 1.2 ml, while the sample load for the SiOH SPE method was 1.5 ml.

Previously developed method for SPE separation of the real samples
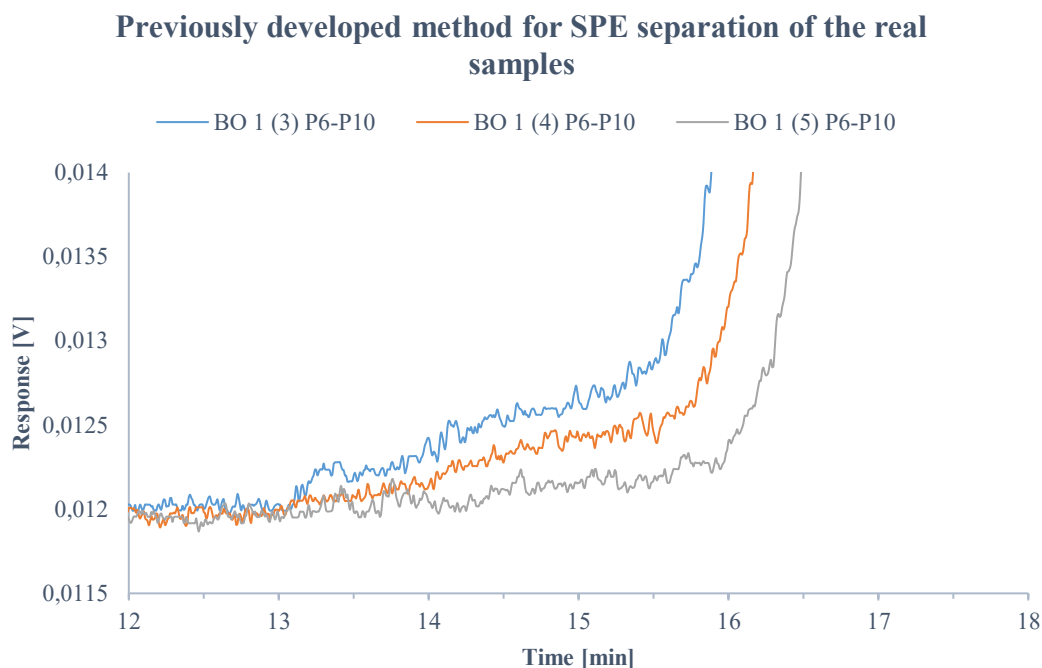
Figure 56. HP-SEC chromatogram of the elution of the real (unspiked) BO 1 samples after SPE fractionation on the SiOH cartridge and the primary solvent combinations, with focus on the elution of the PDMS. Sample concentration in THF is 5 mg/ml. Gain 6 and primary solvent combinations were used.

5.4.16 Realistically spiked BO 1 samples

An attempt to determine the LOD and LOQ for the florisil SPE method was performed by fractionating a set of spiked BO 1 samples. The samples were spiked with realistic amounts of high MW PDMS. The sample with 75 ppm of spiked PDMS became contaminated during work-up and was therefore not included here. Figure 57 illustrates the chromatogram of the spiked samples. Comparing appearance of all the samples in the same HP-SEC chromatogram, showed a good differentiation between the four highest spiked samples (i.e., 700–150 ppm). The difference among the samples with 150–25 ppm of spiked PDMS was not very clear. It seemed the samples

were all on the same level, which was quite unexpected since they were spiked with different amounts of PDMS.
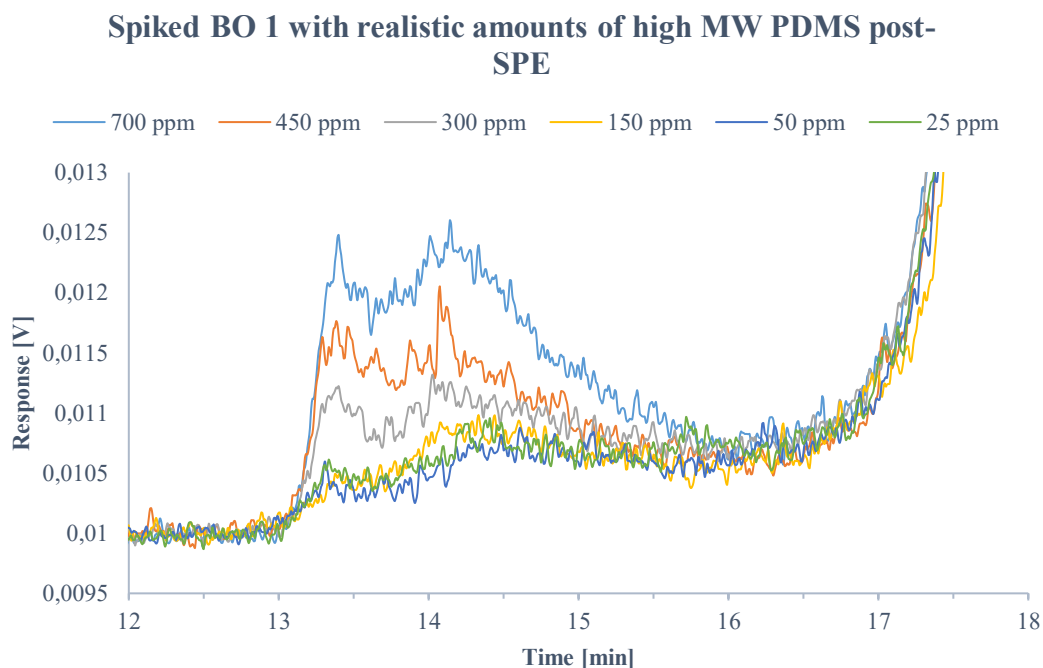
Figure 57. HP-SEC chromatogram of the elution of high MW PDMS from the spiked BO 1 samples after three parallel SPE fractionations of florisil (2 000 mg) cartridges. Gain 6 and simplified solvent combinations were used. Combined as fractions S5–S8.

For the calculation of the LOD and LOQ, the peak areas from the HP-SEC chromatogram were plotted into a graph. Figure 58 illustrates the calibration curve for the obtained set of data. The peak areas used for the plotting are summarized in appendix G Table G1.
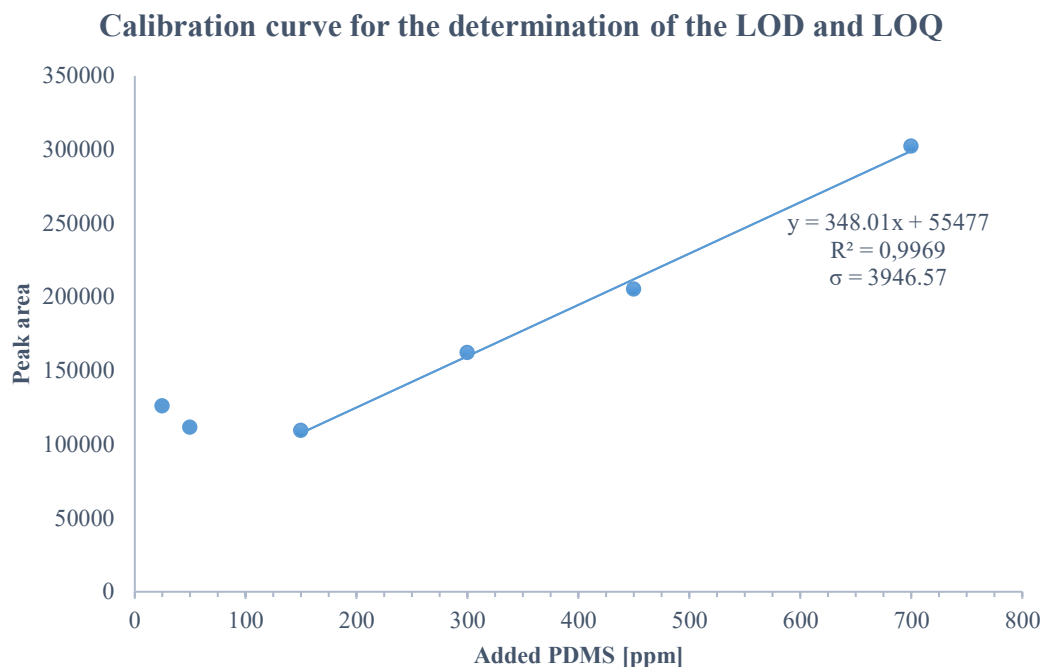
Figure 58. Calibration curve for the determination of the LOD and LOQ.

The LOD and LOQ were calculated according to the guideline from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).[55] In guideline Q2 (R1), it is stated that the LOD and LOQ can be calculated by equation 1 and 2, respectively.

$$LOD = 3.3 \times \frac{\sigma}{S} \tag{1}$$

$$LOQ = 10 \times \frac{\sigma}{S} \tag{2}$$

In equations 1 and 2, σ is the standard deviation of the residuals and S is the slope of the calibration curve. The LOD for the florisil SPE method was determined to be 37 ppm and the LOQ was determined to be 113 ppm. The LOD and LOQ were calculated for the monomer of the PDMS. The values of LOD and LOQ would have been more accurate if there were more data points available. In that sense, the LOD and LOQ were not completely precise and the calculated values here were therefore more indicative. The lowest spiked samples (i.e., 25 ppm and 50 ppm) showed to be inconsistent compared to the rest of the spiked samples (Figure 58) and were therefore not included in the calculations for the LOD and LOQ.

A final comparison of the elution of each type of PDMS in each fraction is summarized in appendix H, Table H1. The information in the table consists of the different fractions (i.e., percentage of DCM in hexane), the matrix used during the fractionation, and the material of the used cartridge.

5.5   Florisil SPE method versus SiOH SPE method

Herein follows a rough comparison between the florisil SPE method (FL-SPE) developed during this work and the SiOH SPE method (SI-SPE) developed during the work of Kenneth Arandia.[54] The first thing to notice is that the solvent combinations of hexane and DCM worked well for both the florisil and the SiOH cartridges. The solvent combinations allowed for a relatively good separation of the PDMS from the BO 1 matrix material, however, complete absent of BO 1 matrix material was not achieved.

The main difference between FL-SPE and SI-SPE was the sample volume that could be loaded onto the cartridges. With the same sized cartridges (e.g., 1 000 mg), the SiOH had the capacity to fractionate 1.5 ml sample, while the florisil only could fractionate 0.2 ml sample before overload was observed. The lower sample volume made combined parallel fractionations much more important, in order to reach up to the same total sample volume loaded onto both cartridges.

It seemed though like the combined parallel fractionations resulted in a more defined isolation of the PDMS, as for the case with the real unspiked BO 1 samples. With the combined parallel fractionations, it also seemed like more BO 1 matrix material was absorbed onto the florisil cartridge compared to a single sample load onto the SiOH cartridge. Instead of using parallel cartridges, another alternative could be to use one florisil cartridge of a much bigger size compared to the SiOH cartridge.

The determined values for the LOD and LOQ for both FL-SPE and SI-SPE were quite similar. The LOD and LOQ for SI-SPE were 30 ppm and 92 ppm, respectively. The same values for FL-SPE were 37 ppm and 113 ppm, respectively. The similar values would indicate that the methods are quite comparable. However, the values for FL-SPE

are not to be completely trusted, as they were determined only based on four data points. In order to receive more accurate values of the LOD and LOQ for FL-SPE, it is advised to obtain more data points.

6   SUMMARY AND CONCLUSIONS

The previously developed analytical procedures for concentration of high MW PDMS from the BO 1 matrix by SPE were further studied in order to find an improved procedure. The focus was the isolation of high MW PDMS from the BO 1 matrix and an alternative analytical procedure for this purpose was developed based on using florisil SPE cartridges. However, the separation of medium and low MW PDMS from the BO 1 matrix was also tested with the newly developed FL-SPE. The BO 1 samples were also spiked with D_3 and D_4 PDMS degradation products and an attempt to isolate D_3 and D_4 PDMS products was performed. The matrices BO 2 and BO 3 were also studied with FL-SPE and compared to the performance of the BO 1 matrix. The detection of high MW and medium MW PDMS was achieved by HP-SEC-ELSD. The detection of low MW PDMS was possible through GC-FID.

Prior to any SPE fractionation, the matrices were fractionated by solvent extraction between methanol and hexane. The fraction of interest was the hexane-soluble fraction. The gravimetric content of the hexane-soluble fraction for the BO 1 samples varied between 54% and 79%. The gravimetric content of the hexane-soluble fraction of BO 2 and BO 3 was more than 85%. This difference is due to the different compositions of the three bio-oils. BO 1 consisted of steryl esters, fatty acids, and resin acids, while BO 2 and BO 3 consisted mostly of triglycerides and small amounts of diglycerides and fatty acids.

The SPE tests were many and varied somewhat between each other. DCM was proved to be the best alternative for solvent, when comparing different solvents (i.e., DCM, chloroform, EtAc, acetone, ethanol, THF, MTBE, and toluene). Comparing different material of the SPE cartridge (i.e., CN, florisil, OH as diol, NH_2, and SiOH), proved CN to be the best alternative. However, that applied only for pure PDMS and the conclusion was made that the CN cartridge would not separate PDMS from BO 1 at all. The SPE tests continued then with the florisil SPE cartridge.

For many of the experiments, the florisil cartridge was compared with the SiOH cartridge. One example was when trying an alternative solvent combination for the SPE fractionation. The elution of the high MW PDMS seemed to be quite similar with

both the florisil and the SiOH cartridge. There was, however, a difference in the elution of the BO 1 matrix material. For the florisil cartridge, the PDMS could be more concentrated since there was not as much of the BO 1 matrix that was eluting compared to the SiOH cartridge. This could be a sign that the florisil cartridge could retain the BO 1 matrix better than the SiOH cartridge. Another example was the effect of sample overload. The SiOH cartridge (1 000 mg) was able to handle 1.5 ml sample load without signs of overload. The florisil cartridge (1 000 mg), however, showed signs of overload already with a 0.4 ml sample volume. The highest sample load without overload for the florisil cartridge was then determined to be 0.2 ml. One conclusion of this is that single cartridges of the same size do not fractionate the same volume of sample. To resolve this problem, the fractionation through the florisil cartridge could instead be performed by using parallel cartridges or by using one single cartridge of a much bigger size.

The established florisil SPE method was also applied to spiked BO 1 samples with medium MW and low MW PDMS. The medium MW PDMS was nicely separated based on the molecular sizes, with all collected fractions containing differently sized PDMS molecules. Comparing the florisil and the SiOH cartridge in the ability to separate the pure medium MW PDMS molecules, revealed that the SiOH cartridge separated the differently sized molecules slightly better than the florisil cartridge. Spiked BO 2 and BO 3 with medium MW PDMS showed the same tendency of the differently sized PDMS molecules nicely separated. The low MW PDMS was also spiked in all three matrices. The isolation of the PDMS from BO 2 and BO 3 was working well. In the BO 1 matrix, the PDMS eluting fractions also contain some BO 1 material. It was also concluded that the composition of BO 2 was different from the BO 3. This was proved from the GC-FID chromatogram of the spiked samples with low MW PDMS, where none of the BO 2 material and much less of the methyl ester derivatives were present compared to the BO 3 matrix.

The established FL-SPE was also applied to the fractionation of spiked BO 1 with D_3 and D_4 cyclic oligomeric PDMS products, and the method was working successfully. Both D_3 and D_4, respectively, were isolated from the rest of the matrix material. As the cyclic oligomers are highly volatile, the samples were not allowed to evaporate

prior to analysis. Analysis of the eluted fractions without any work-up showed to be working well, as the oligomers were detected.

The main comparison between the FL-SPE and the SI-SPE was the concentration of PDMS from the unspiked real BO 1 samples. Since the FL-SPE required several parallel fractionations, the peak of the PDMS in the HP-SEC chromatogram was much more defined and more visible. The BO 1 material in the analyzed fractions was much less for the FL-SPE than the SI-SPE, which indicated that the florisil cartridge could retain the matrix material better. The advantages of FL-SPE were therefore a better separation and isolation of the PDMS from the matrix. The disadvantage was the much lower sample volume and the requirement of several parallel fractionations. The LOD and LOQ for both methods were quite similar, which indicated similar abilities to separate components although the SI-SPE was slightly better. However, it is advised that the LOD and LOQ for the FL-SPE instead would be determined by using much more data points.

Overall, the newly established SPE method (i.e., FL-SPE) showed good applicability to all the different MW PDMS and the different matrices. The analytical techniques for the detection of the PDMS was as follows: the PDMS in the first fractions was detected by GC-FID and the PDMS in the rest of the fraction was detected by HP-SEC. The eluted PDMS in the first fractions was low MW. The high MW and medium MW PDMS were sometimes eluting at the same percentage of DCM in the eluent. In the case of a sample with a mixture of all three MW PDMS, it might be impossible to detect the medium MW PDMS, because of overlapping peaks with the matrix material in the HP-SEC chromatogram. The analytical procedure allowed for detection of the volatile PDMS products, low MW PDMS, medium MW PDMS, and high MW PDMS, if they are all in separate bio-oil samples. However, the problem with the procedure was the quantification of PDMS post-SPE.

For future considerations, this attempt to improve the detection of PDMS showed that an absolute bottom has already been reached with the SI-SPE. It was not possible to improve the SI-SPE as much as hoped, however, the FL-SPE showed to separate the PDMS better from the matrix. If any further improvements should be tested, it should be to improve the detection and quantification of PDMS after florisil SPE. Another

improvement that could be tested is to somehow treat the eluted SPE fractions in order to separate the low and medium MW PDMS from each other, if they are present in the same fraction.

7   SUMMARY IN SWEDISH – SVENSK SAMMANFATTNING

Metodutveckling för analys av polydimetylsiloxan i biooljor.

Avhandlingen är uppdelad i två delområden: en teoretisk del och en experimentell del. I den teoretiska delen behandlas trä- och papperskemi, samt de olika talloljeprodukterna. Trä- och papperskemin ger en djupare inblick och förståelse av de olika talloljeprodukterna samt hur de bildas. Den teoretiska delen behandlar även PDMS och fokuserar på vissa analytiska metoder som kan detektera PDMS. Den experimentella delen baserades på ett försök att optimera separationen mellan biooljekomponenter och silikonolja genom SPE. En viktig parameter för SPE var att uppnå ett högt utbyte för elueringen av silikonolja. Andra parametrar som undersöktes för optimeringen var till exempel kolonnens stationära fas och lösningsmedelskombinationer för elueringen.

7.1   Bakgrund

Alla träd delas in i antingen barrträd eller lövträd. Trots indelningen består ändå alla träd av celler, som består av både strukturella och icke-strukturella komponenter.[5] De strukturella komponenterna i träd är cellulosa, hemicellulosor och lignin, vilka också är trädens huvudkomponenter. De icke-strukturella komponenterna i träd är extraktivämnen och andra vattenlösliga komponenter. I jämförelse med huvudkomponenterna förekommer extraktivämnena endast i små mängder, men trots det är de intressanta med tanke på att de påverkar trädets egenskaper i hög grad, speciellt under tillverkningsprocessen för pappersmassa.[7] Ett exempel på hur extraktivämnena påverkar massakokningen är att utbytet av massan minskar i och med att mängden extraktivämnen i utgångsmaterialet ökar.

Massakokning är en metod som tillverkar pappersmassa från trästockar eller -flis.[8] Det är vanligast att massakokningen är antingen mekanisk eller kemisk. Den största biprodukten från massakokningen är svartlut. Bioraffinering utnyttjar svartluten för att tillverka värdefulla produkter så som energi, biomaterial eller kemikalier.[19] Genom

olika kemiska metoder kan man utgående från svartluten framställa olika talloljeprodukter, till exempel CTO eller TOP[1] eller till och med bränsle.[20]

Olika talloljeprodukter innehåller olika mängder av tallfettsyror, tallharts och oförtvålbara föreningar.[21] Figur 3 illustrerar den kemiska strukturen för de vanligaste tallfettsyrorna. Figur 4 illustrerar strukturen för de vanligaste föreningarna av tallharts.[1] Figur 5 illustrerar strukturen för de vanligaste oförtvålbara föreningarna.[21] Användningsområden för tallolja delas in i vilken typ av talloljeprodukt det är frågan om.[1] Tallfettsyror kan användas som skyddande beläggningar, tvålar, rengöringsmedel, smörjmedel, textiler eller gummiprodukter. Tallharts kan användas för produktion av kemikalier eller bläck, bindemedel, beläggningar samt gummi.

Kännetecknande för silikonpolymerer är den upprepande kisel–syre-enheten, vilken även förekommer i PDMS.[28] Förutom kisel–syre-skeletten har silikonpolymerer oftast förgreningar, vilka kan vara organiska föreningar såsom metyl, fenyl eller vinyl. Specifika egenskaper för silikonpolymerer är väldigt låg ytspänning och en hög gaspermeabilitet.[29] För övrigt är egenskaperna för silikonpolymerer modifierbara och polymererna har därmed ett brett användningsområde. Exempelvis kan silikonpolymerer användas inom livsmedelsindustrin eller som tillsatsämnen i skönhetsprodukter. Silikonpolymerer har även en stor medicinsk användning.

En vanlig produkt som består av silikonpolymerer är antiskummedel. Antiskummedel är viktigt för processer i bland annat livsmedelsindustrin eller inom trä- och pappersindustrin.[29] Antiskummedel inom trä- och pappersindustrin är oftast baserade på en dispersion av kiselpartiklar[25] genererade från PDMS.[4] PDMS används som källa för kiselpartiklar på grund av den låga ytspänningen.[26] Det finns även antiskummedel som är baserade på kolväten. Utan antiskummedel skulle både produktionen och säkerheten för miljön reduceras, på grund av skumbildningen.[23] Kisel från antiskummedel anses dock även vara ett problem speciellt vid bioraffinering av svartluten från massatillverkningen.[4]

Analys av silikonpolymerer kan göras med ett flertal allmänt använda analysmetoder.[29] Exempel på användbara metoder är FTIR, GC, NMR, GPC eller SEC.

7.2   Experimentell del

Detta arbete är baserat på, och fungerade som en fortsättning på, ett tidigare arbete skrivet av Kenneth Arandia.[54] Målet med den experimentella delen av arbetet var att öka separationen mellan PDMS och materialet av bioolja då SPE utförs. Andra mål med arbetet var också att öka utbytet för PDMS som eluerar genom SPE-kolonner samt att öka detektionen för PDMS med HP-SEC.

De experimentella procedurerna i detta arbete var lösningsmedelsextraktion och SPE. De analytiska metoderna var HP-SEC och GC. För lösningsmedelsextraktion användes metanol och hexan, eftersom de är två icke-blandbara lösningsmedel. De olika biooljorna fraktionerades mellan metanol och hexan, varefter hexanfasen användes för vidare experiment. SPE utfördes många gånger, med lite varierande parametrar varje gång. De viktigaste testen i början var kontroll av vilket lösningsmedel samt vilket material på SPE-kolonnen som fungerade bäst. Därefter testades elueringen av PDMS, både som rent och blandat med olika biooljor. En jämförelse mellan molekylvikten för PDMS utfördes också i och med att alla tre olika molekylvikter (hög, medel, låg) testades genom fraktionering med SPE.

7.3   Resultat, diskussion och slutsatser

Lösningsmedelsextraktionen fördelade komponenterna av biooljorna mellan metanol och hexan. Gravimetriskt förekom 54–79 % av komponenterna i hexanfasen för en typ av bioolja. För två andra typer av bioolja innehöll hexanfasen gravimetriskt mera än 85 %. Denna skillnad berodde på de olika sammansättningarna för de tre olika biooljorna.

Slutsatsen från testerna med SPE är att diklormetan var det bästa lösningsmedlet. De olika lösningsmedlen som testades var diklormetan, kloroform, etylacetat, aceton, etanol, tetrahydrofuran, metyl-tert-betyleter och toluen. De olika kolonnmaterialen som jämfördes var CN, florisil, OH i form av diol, NH_2 och SiOH. Kolonner med CN-material visade sig eluera mest av ren PDMS, men dessa kolonner förkastades i och med att de inte kunde separera en blandning av PDMS och bioolja. Kolonner med

florisilmaterial var det näst bästa alternativet och dessa användes därmed för alla efterföljande experiment.

I och med de olika testerna för SPE jämfördes florisilkolonnen ofta med SiOH-kolonnen. En av skillnaderna som observerades var att PDMS ur ett blandat prov (PDMS och bioolja) kunde möjligtvis bli mera koncentrerat efter SPE genom en florisilkolonn jämfört med efter SiOH-kolonnen. Detta observerades i och med att mindre material av biooljan eluerade i samma fraktioner som PDMS. En annan skillnad som upptäcktes var att vid jämförelse med kolonner av samma storlek kunde SiOH-kolonnen fraktionera en betydligt större mängd prov. Florisilkolonnen klarade inte av att fraktionera lika stor volym, eftersom tecken på överbelastning då framträdde. En SiOH-kolonn med 1000 mg material kunde fraktionera 1,5 ml prov, medan en florisilkolonn av samma storlek kunde fraktionera endast 0,2 ml prov. En lösning till skillnaden i provvolymen var att utföra flera parallella fraktioneringar med florisilkolonnen eller att använda endast en florisilkolonn av en mycket större storlek.

Den utvecklade FL-SPE-metoden kunde även anpassas till både medel- och lågmolekylärt PDMS samt till cykliska oligomerer av D_3 och D_4 PDMS-degraderingsprodukter i bioolja som bakgrundsmedium. Fraktioneringen av medelmolekylärt PDMS visade sig vara tydlig, i och med att varje fraktion bestod av PDMS med olika molekylära storlekar. För varje fraktion, då polariteten ökade, eluerades högre molekylärt PDMS. Lågmolekylärt PDMS eluerades i de mest opolära fraktionerna, vilket även gällde för D_3 och D_4 PDMS-degraderingsprodukterna.

FL-SPE jämfördes även med den tidigare utvecklade SI-SPE-metoden. Som tidigare nämnt, krävdes flera parallella fraktioneringar med florisilkolonnen jämfört med SiOH-kolonnen. De parallella fraktioneringarna gjorde därmed att den fraktionerade PDMS uppvisades mycket tydligare i HP-SEC kromatogrammet än för SI-SPE. LOD och LOQ räknades också ut för FL-SPE och jämfördes med SI-SPE. Värdena för LOD och LOQ är 37 ppm och 113 ppm för FL-SPE, jämfört med 30 ppm och 92 ppm för SI-SPE, vilket indikerar att båda metoderna ändå är ganska jämförbara.

Den utarbetade analytiska proceduren möjliggjorde detektion av de flyktiga D_3 och D_4 PDMS-degraderingsprodukterna samt låg-, medel- och högmolekylärt PDMS,

förutsatt att de förekom i skilda prover av bioolja. De flyktiga D_3 och D_4 PDMS-degraderingsprodukterna och lågmolekylärt PDMS kunde detekteras med GC-FID. Medel- och högmolekylärt PDMS detekterades bäst med HP-SEC. Generellt sett kunde den utvecklade FL-SPE-metoden anpassas för separation av alla de olika PDMS-produkterna som testades samt var separationen av PDMS-produkterna även möjlig i olika bakgrundsmedier.

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9   APPENDICES

Appendix A. HP-SEC results
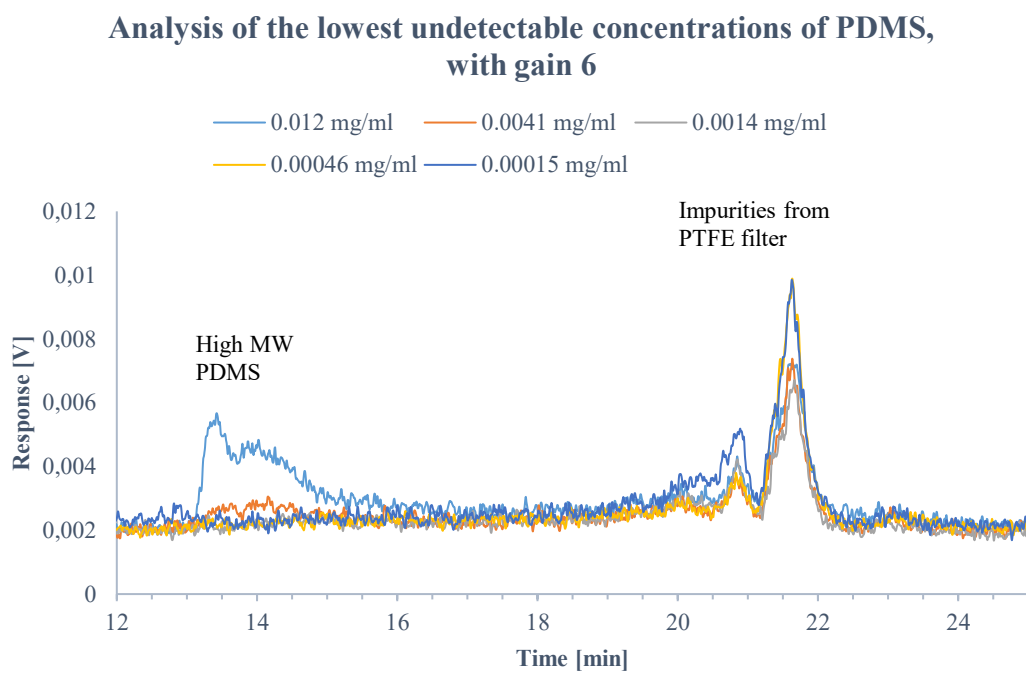
Figure A1. HP-SEC chromatograms of the lowest undetectable concentrations of PDMS, with gain 6.

Appendix B. Peak areas for GC-FID

Table B1 Peak areas of pure low MW PDMS.

PDMS peak starting from left	Area
PDMS 1	208 379
PDMS 2	928 846
PDMS 3	861 143
PDMS 4	807 594
PDMS 5	696 261
PDMS 6	582 628
PDMS 7	487 493
PDMS 8	405 023
PDMS 9	323 693
PDMS 10	272 015
PDMS 11	217 502
PDMS 12	178 203
PDMS 13	150 368
PDMS 14	115 751
PDMS 15	99 726
PDMS 16	72 244
PDMS 17	58 191
PDMS 18	45 754
PDMS 19	36 197
PDMS 20	26 885
PDMS 21	23 657
PDMS 22	18 060
PDMS 23	14 905
PDMS 24	13 941
PDMS 25	20 895
PDMS 26	7 814
PDMS 27	6 817
Total	6 679 985

Appendix B. Peak areas for GC-FID

Table B2 Peak areas of pure D₃ and D₄ PDMS degradation product. Internal standards could not be used since D₃ and D₄ PDMS products are volatile. Peak areas therefore not completely accurate.

PDMS product	Area	Concentration	Amount in GC sample
D_3	361 408	0.33 mg/ml	0.0495 mg/150 µl
D_4	362 842	0.33 mg/ml	0.0495 mg/150 µl

Table B3 Peak areas of pure low MW PDMS post-SPE.

PDMS peak	Area of FL-S1	Area of FL-S2	Area of SI-S1	Area of SI-S2	Area of SI-S3
PDMS 1	251 110	-	22 311	49 098	828
PDMS 2	1 154 159	6 463	90 090	950 676	2 117
PDMS 3	1 074 914	42 430	1 113	1 121 801	-
PDMS 4	864 420	193 958	-	1 042 896	1 205
PDMS 5	515 583	368 686	-	920 765	-
PDMS 6	276 285	507 942	5 154	774 620	2 471
PDMS 7	122 857	543 818	7 572	648 021	7 868
PDMS 8	40 508	492 247	-	527 398	-
PDMS 9	14 938	432 027	2 814	438 272	2 920
PDMS 10	7 019	350 602	310	355 868	563
PDMS 11	1 444	282 474	-	284 688	1 282
PDMS 12	-	230 123	-	232 081	4 085
PDMS 13	13 308	192 059	11 917	196 023	14 823
PDMS 14	-	146 654	-	149 530	3 680
PDMS 15	-	113 114	-	115 138	4 856
PDMS 16	469	93 144	-	93 898	6 621
PDMS 17	-	69 222	240	69 012	7 378
PDMS 18	-	53 259	-	50 589	10 467
PDMS 19	-	41 551	482	36 558	12 614
PDMS 20	-	31 487	-	25 321	13 229
PDMS 21	173	23 189	-	16 712	12 804
PDMS 22	470	16 202	2 368	13 496	15 483
PDMS 23	-	15 773	-	8 243	15 854

PDMS 24	-	10 830	-	-	14 718
PDMS 25	12 115	18 931	11 706	12 850	25 878
PDMS 26	-	5 835	-	1 294	9 123
PDMS 27	-	4 935	-	-	7 805
PDMS 28	-	5 363	-	-	8 794
Total	**8 642 090 (FL)**		**8 498 391 (SI)**		

Table B4 Peak areas of D_3 and D_4 PDMS degradation products post-SPE.

Cartridge/fraction	Area of D_3	Area of D_4
FL S1	275 220	419 677
FL S2	46 268	10 377
Total	**321 488**	**430 054**
SI S1	277 629	323 663
SI S2	-	76 625
Total	**277 629**	**400 288**

Table B5 Peak areas of D_3 and D_4 PDMS degradation products from a spiked BO 1 sample.

Fraction	Area of D_3	Area of D_4
S1	238 465	207 231
S2	33 871	8 177
Total	**272 336**	**215 408**

Appendix C. Peak areas for HP-SEC

Table C1 Peak areas for SPE fractionation using the primary solvent concentration combination scheme.

PDMS eluting fractions	Peak area of PDMS	Peak area of BO 1
E1	87 488	3 663 864
E5	62 563	3 916 168
E7	83 537	2 998 508
E8	1 345 705	5 795 402
E9	74 044	6 316 790
Total	**1 653 337**	**22 690 732**

Table C2 Peak areas for SPE separation using the alternative solvent concentration combination scheme.

PDMS eluting fractions	Peak area of PDMS	Peak area of BO 1
E1	148 570	4 980 783
E5	111 420	5 235 150
E7	890 531	2 747 151
E8	192 758	1 590 062
E9	139 868	1 138 462
E10	36 316	3 134 499
Total	**1 519 463**	**18 826 107**

Table C3 Peak areas for the SPE separation using HyperSep florisil cartridge and the alternative solvent concentration combination scheme.

PDMS eluting fractions	Peak area of PDMS	Peak area of BO 1
E1	65 414	12 609 434
E5	276 554	8 518 224
E6	135 989	3 734 269
E7	2 008 934	3 376 505
E8	228 986	2 134 930

E9	90 950	1 643 169
E10	49 444	7 447 593
Total	2 856 271	39 464 124

Table C4 Peak areas for the SPE separation using HyperSep SiOH cartridge and the alternative solvent concentration combination scheme.

PDMS eluting fractions	Peak area of PDMS	Peak area of BO 1
E2	82 047	4 223 069
E4	61 055	48 870 522
E5	1 117 296	37 019 267
E6	470 273	4 584 545
E7	1 724 769	6 188 410
E8	687 116	7 887 118
E9	84 471	2 436 516
Total	4 227 027	111 209 447

Appendix D. The ratio between PDMS and BO 1 for each increasing sample volume

Table D1 A summary of the ratio of PDMS to BO 1 for the prepared samples, when increasing the loaded sample volume but keeping the amount of PDMS loaded onto the cartridge constant.

Sample volume	Volume PDMS : volume BO 1	Conc. of PDMS in sample	PDMS loaded onto cartridge
0.2 ml	3 ml PDMS : 3 ml BO 1	1 mg/ml	0.2 mg
0.4 ml	1 ml PDMS : 2 ml BO 1	0.5 mg/ml	0.2 mg
0.8 ml	0.50 ml PDMS : 2 ml BO 1	0.25 mg/ml	0.2 mg
1.0 ml	0.40 ml PDMS : 2 ml BO 1	0.2 mg/ml	0.2 mg
1.2 ml	0.48 ml PDMS : 3 ml BO 1	0.16 mg/ml	0.192 mg
1.5 ml	0.39 ml PDMS : 3 ml BO 1	0.13 mg/ml	0.195 mg

Appendix E. Molecular weight distribution

Molecular weight distribution after SPE fractionation on the florisil cartridge

Figure E1. Integral molecular weight curve of the eluted fractions, where 1 mg/ml represents the material without SPE.

Molecular weight distribution after SPE fractionation on the SiOH cartridge
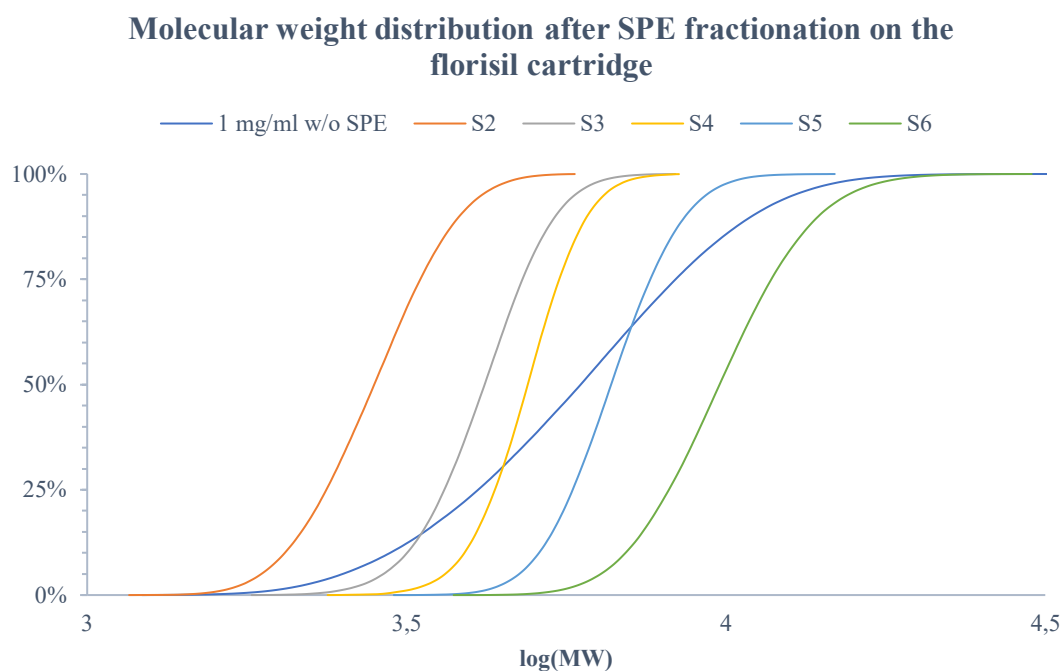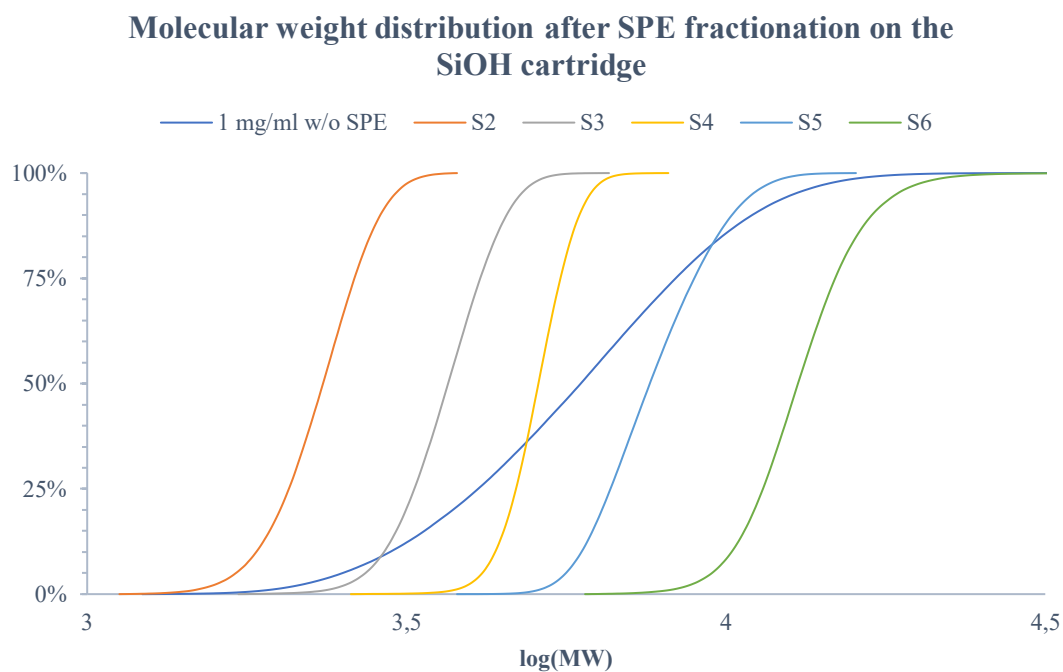

Figure E2. Integral molecular weight curve of the eluted fractions, where 1 mg/ml represents the material without SPE.
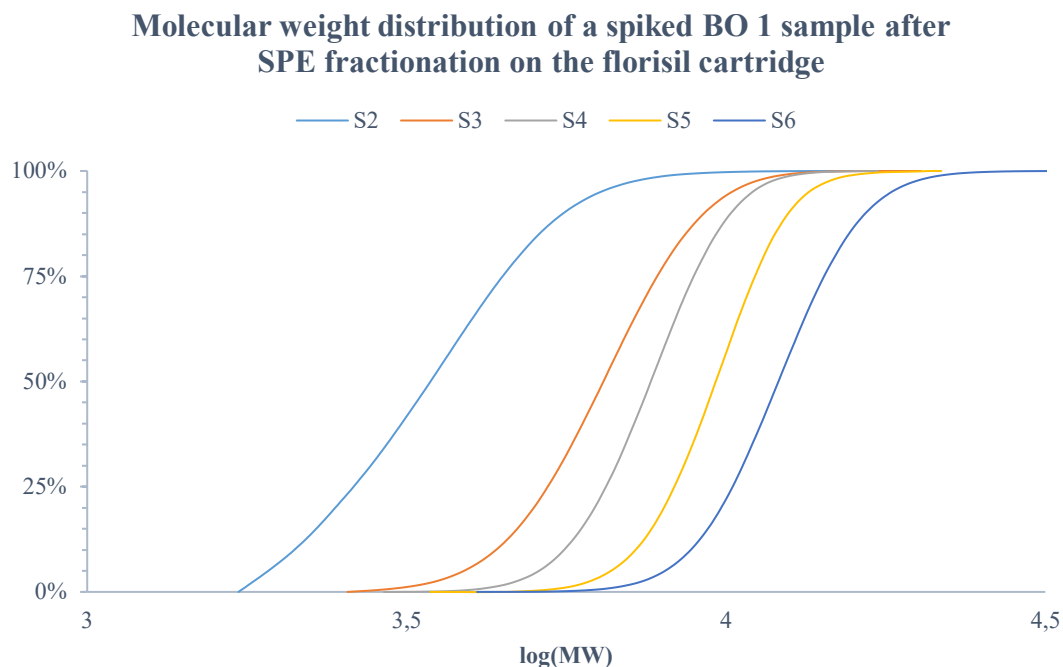
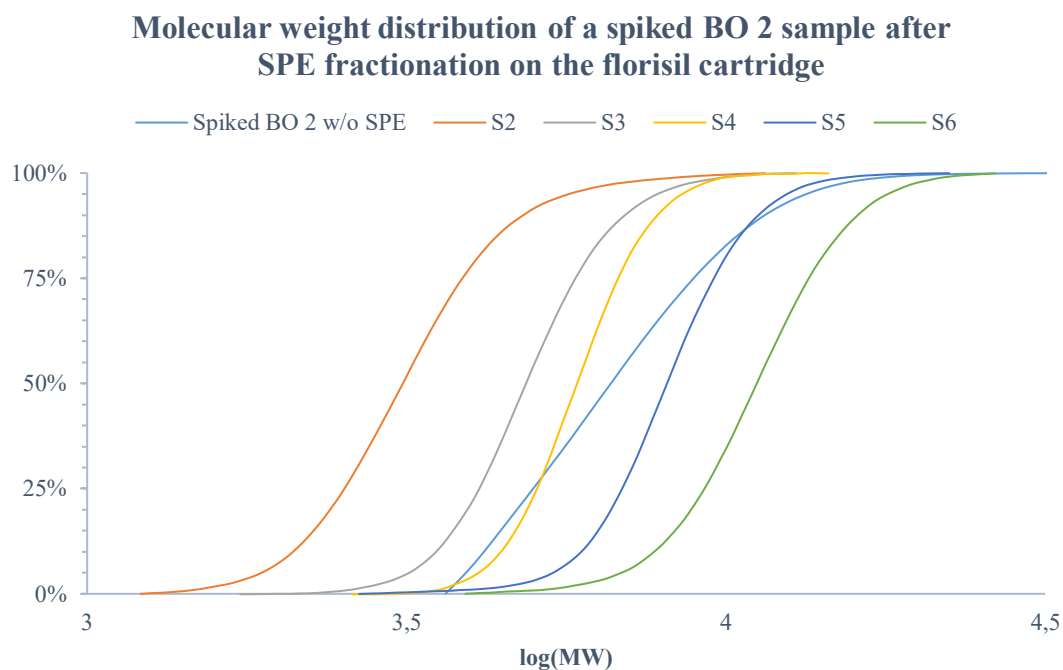*Figure E3. Integral molecular weight curve of the eluted fractions from the spiked BO 1 sample.*

Figure E4. Integral molecular weight curve of the eluted fractions from the spiked BO 2 sample.
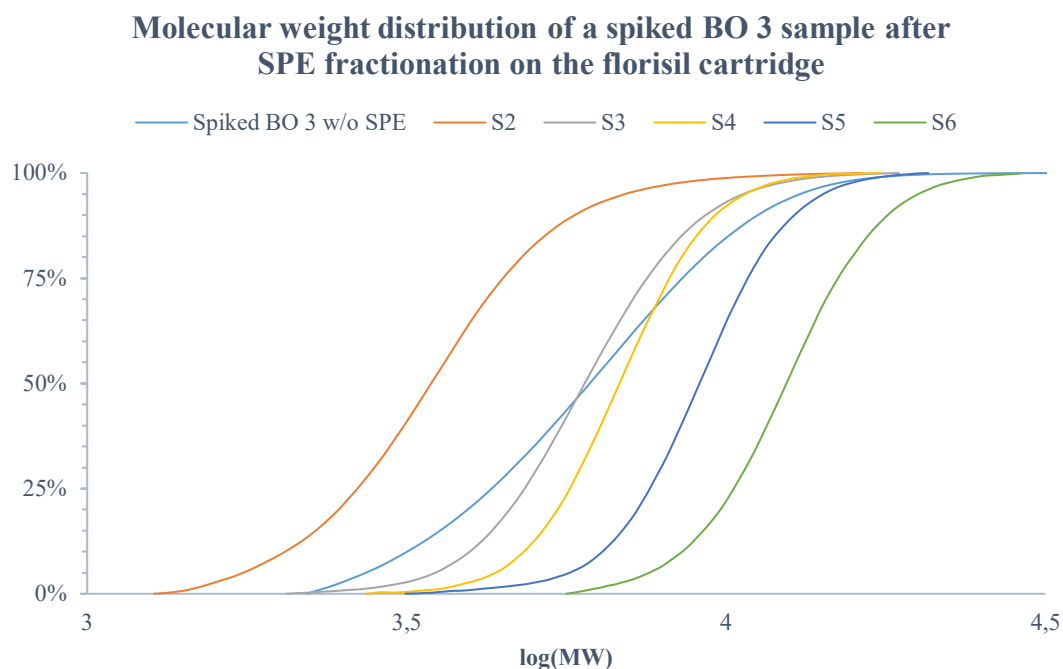
Molecular weight distribution of a spiked BO 3 sample after SPE fractionation on the florisil cartridge

Figure E5. Integral molecular weight curve of the eluted fractions from the spiked BO 3 sample.

Table E1 A summary of all the different samples (with medium MW PDMS) and the elution for each fraction, for the simplified solvent combinations.

Fraction	Sample	Molecular weight [kDa]	Retention time	Amount of matrix based on peak areas
S2	Pure PDMS (SI)	2.3	13.5–19.9	-
	Pure PDMS (FL)	2.8	13.5–19.8	-
12.5%	20:80 BO 1 (FL)	3.6	12.9–19.0	79.6% steryl esters
DCM	20:80 BO 2 (FL)	3.3	13.4–19.7	5.2% triglycerides
	20:80 BO 3 (FL)	3.7	13.3–19.6	22.5% triglycerides
S3	Pure PDMS (SI)	3.6	13.5–19.9	-
	Pure PDMS (FL)	4.2	13.5–19.8	-
15%	20:80 BO 1	6.6	12.9–19.0	62.8% steryl esters
DCM	20:80 BO 2	5.0	13.4–19.7	5.5% triglycerides
	20:80 BO 3	6.3	13.3–19.6	14.5% triglycerides

	Pure PDMS (SI)	5.0	13.5–19.9	-
S4	Pure PDMS (FL)	4.9	13.5–19.8	-
15%	20:80 BO 1	7.7	12.9–19.0	52.4% steryl esters
DCM	20:80 BO 2	5.9	13.4–19.7	-
	20:80 BO 3	6.9	13.3–19.6	15.1% triglycerides
	Pure PDMS (SI)	7.8	13.5–19.9	-
S5	Pure PDMS (FL)	6.7	13.5–19.8	-
20%	20:80 BO 1	9.7	12.9–19.0	37.9% steryl esters
DCM	20:80 BO 2	8.2	13.4–19.7	5.0% triglycerides
	20:80 BO 3	9.2	13.3–19.6	20.9% triglycerides
	Pure PDMS (SI)	13.2	13.5–19.9	-
S6	Pure PDMS (FL)	10.1	13.5–19.8	-
30%	20:80 BO 1	12.3	12.9–19.0	26.5% steryl esters
DCM	20:80 BO 2	11.4	13.4–19.7	52.4% triglycerides
	20:80 BO 3	12.7	13.3–19.6	67.9% triglycerides

Appendix F. GC-MS chromatograms

Figure F1. GC-MS chromatogram of the spiked BO 2 sample with low MW PDMS, confirming identification of methyl esters.
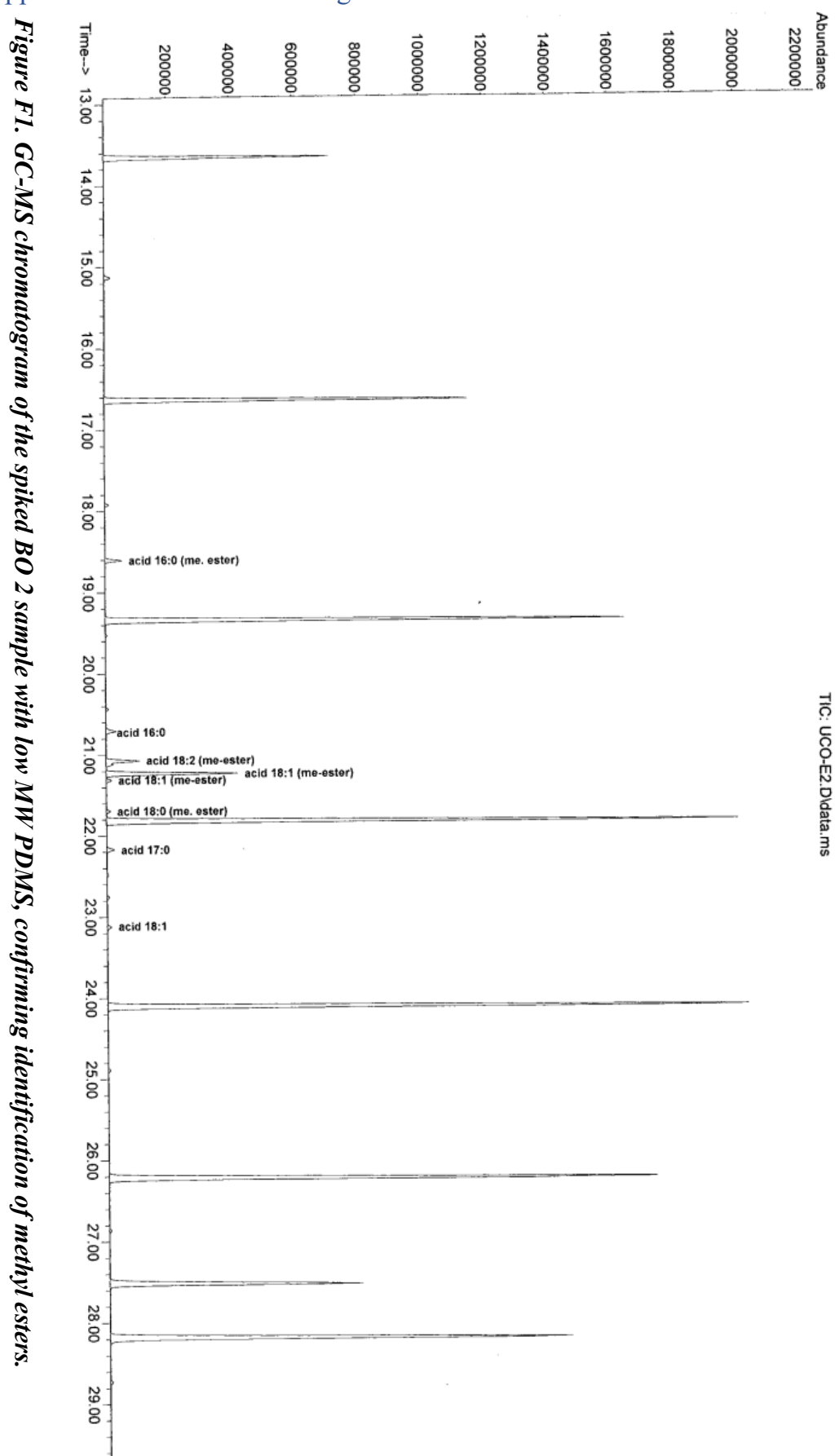
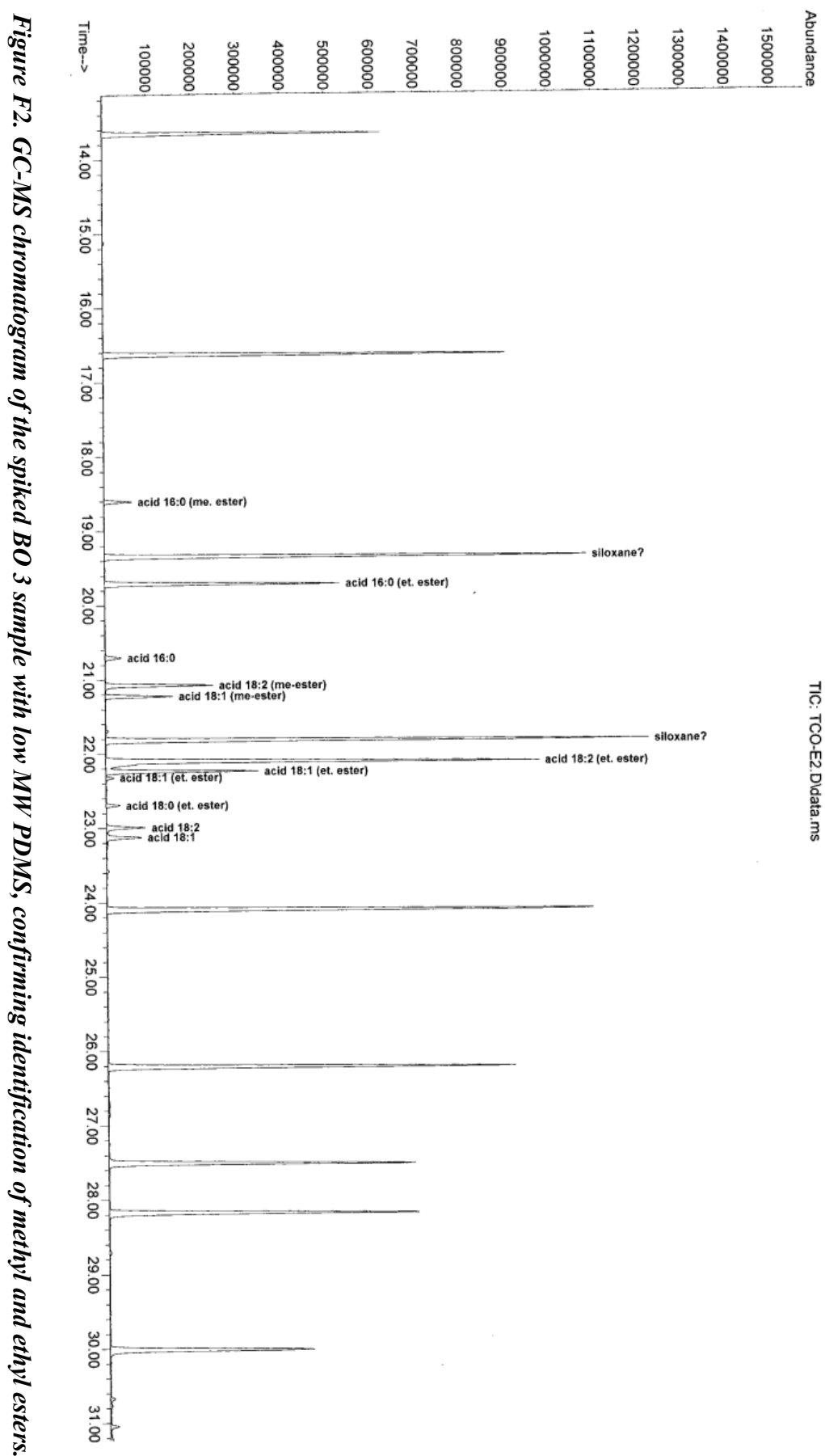*Figure F2. GC-MS chromatogram of the spiked BO 3 sample with low MW PDMS, confirming identification of methyl and ethyl esters.*

Appendix G. Determination of LOD and LOQ

Table G1 Peak areas for each of the added PDMS.

Added PDMS [ppm]	Peak area from HP-SEC chromatogram
700	302 188
450	205 317
300	162 031
150	109 189
50	111 478
25	126 146

Appendix H. Type of eluted PDMS model compound in each SPE fraction

Table H1 Summary of which fraction elutes the different types of PDMS model compounds for each of the used type of cartridges and matrices.

Fraction	Matrix (cartridge)	Eluted PDMS
P1, A1, S1 Pure hexane	Pure PDMS (CN)	High MW
	Pure PDMS (SI)	-
	Pure PDMS (FL)	High MW, low MW
	BO 1 (SI)	-
	BO 1 (FL)	-
	BO 2 (FL)	Low MW
	BO 3 (FL)	Low MW
P2–P5, A2, S2 12.5% DCM	Pure PDMS (CN)	High MW
	Pure PDMS (SI)	High MW, medium MW, low MW
	Pure PDMS (FL)	High MW, medium MW, low MW
	BO 1 (SI)	-
	BO 1 (FL)	High MW, medium MW
	BO 2 (FL)	High MW, medium MW, low MW
	BO 3 (FL)	High MW, medium MW, low MW
P6, A3, S3 15% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW, medium MW, low MW
	Pure PDMS (FL)	High MW, medium MW
	BO 1 (SI)	-
	BO 1 (FL)	Medium MW
	BO 2 (FL)	High MW, medium MW
	BO 3 (FL)	High MW, medium MW
A4, S4 15% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW, medium MW
	Pure PDMS (FL)	Medium MW
	BO 1 (SI)	-
	BO 1 (FL)	Medium MW
	BO 2 (FL)	High MW, medium MW
	BO 3 (FL)	High MW, medium MW

P7, A5, S5 20% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW, medium MW
	Pure PDMS (FL)	High MW, medium MW
	BO 1 (SI)	High MW
	BO 1 (FL)	High MW, medium MW
	BO 2 (FL)	High MW, medium MW
	BO 3 (FL)	High MW, medium MW
A6 20% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW
	Pure PDMS (FL)	-
	BO 1 (SI)	-
	BO 1 (FL)	High MW
	BO 2 (FL)	High MW
	BO 3 (FL)	High MW
A7, S6 30% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW, medium MW
	Pure PDMS (FL)	High MW, medium MW
	BO 1 (SI)	High MW
	BO 1 (FL)	High MW, medium MW
	BO 2 (FL)	High MW, medium MW
	BO 3 (FL)	High MW, medium MW
A8, S7 35% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW, medium MW
	Pure PDMS (FL)	High MW
	BO 1 (SI)	High MW
	BO 1 (FL)	High MW, medium MW
	BO 2 (FL)	High MW
	BO 3 (FL)	High MW
P8, A9, S8 40% DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW
	Pure PDMS (FL)	High MW
	BO 1 (SI)	High MW
	BO 1 (FL)	High MW

	BO 2 (FL)	High MW
	BO 3 (FL)	High MW
P9–P10, A10 Pure DCM	Pure PDMS (CN)	-
	Pure PDMS (SI)	High MW
	Pure PDMS (FL)	High MW
	BO 1 (SI)	High MW
	BO 1 (FL)	High MW
	BO 2 (FL)	-
	BO 3 (FL)	-