Fraunhofer
IGB

Final report

Screening of suitable monomer-crosslinker systems and experiments on molecular recognition of acylated Steryl glycosides (ASG)

Project 540/102

for
Union zur Förderung von Öl- und Proteinpflanzen e.V. (UFOP)
Claire-Waldoff-Str. 7
10117 Berlin

created by

Dr. Carmen Gruber-Traub, Annelie Hauser
Fraunhofer-Institut für Grenzflächen-
und Bioverfahrenstechnik IGB
Nobelstraße 12
70569 Stuttgart

Stuttgart,
01. September 2011

Contents

1	**Task**	**2**
2	**Materials and methods**	**5**
2.1	Molecularly imprinted polymer	5
2.2	Stigmasterol solubility test	7
2.3	Monomer-Screening- ASG	10
2.3.1	Production of Particles through miniemulsion polymerisation	11
2.4	Analysis	18
2.4.1	Purification of the particle suspensions produce	21
2.4.2	Adsorption tests	22
3	**Conclusions**	**25**
4	**Outlook**	**26**
5	**Chemicals used**	**28**
6	**Glossary**	**29**
7	**Bibliography**	**30**
8	**Appendix**	**31**

1 Task

The subject of this project, which was initiated by the AGQM, was a feasibility study for the analysis of the field of application of nanostructured molecularly imprinted polymer adsorbent particles (MIPs) for the isolation of minor bio-oil components (useful and/or contaminant materials). As an example of minor components – in the present example Stigmasteryl-6-O-palmitoyl-alpha-D-glucopyranosid - ASG was selected as a contaminant in biooils

Acylated Sterylglycosides (ASG) are minor components in vegetable oils. After the transesterification of the bio oil during the bio diesel production the acyl radical is separated, creating Sterylglycoside (SG). SGs are briefly soluble in the biodiesel but thereafter slowly crystallise slowly from the FAME; this process can be accelerated by cooling. The residual SGs can lead to a blockage of filter materials.[1] The separation of ASG from bio oils is therefore of significant importance for the quality improvement of the biodiesel.

In the context of this project the first preliminary work was implemented in order to demonstrate the feasibility of the application possibilities of polymer adsorber particles in bio oils. The further optimisation of the material production and introduction of the Molecularly imprinted materials into technical processes should, upon the conclusion of this feasibility study, occur in a publicly promoted project in collaboration with other Fraunhofer institutes – such as, for example Fraunhofer UMSICHT-.

Initially a monomer cross linker screening was carried out in combination with the production of the adsorbent particles. Here it should primarily be examined whether interaction exists between the goal molecule and the monomers (polymerisable chemical building blocks) and cross linkers (polymerisable network-developing chemical blocks) exist and whether the production of Molecularly imprinted nanoparticles is possible.

A miniemulsion polymerisation [2] was used for the production of the Molecularly imprinted nanoparticle. With this method a stable polymer network is generated around the template through the polymerisation of a functional monomer with a crosslinker which interacts with the template through different forces (e.g. hydrogen bonds, Van der Waals and electrostatic forces).
After the template molecule has been extracted from the nanoparticle by solvent exchange, binding sites remain in the polymer network (artificial receptors) which specifically absorb the SG or ASG goal molecules (molecular recognition) (see Figure 1).

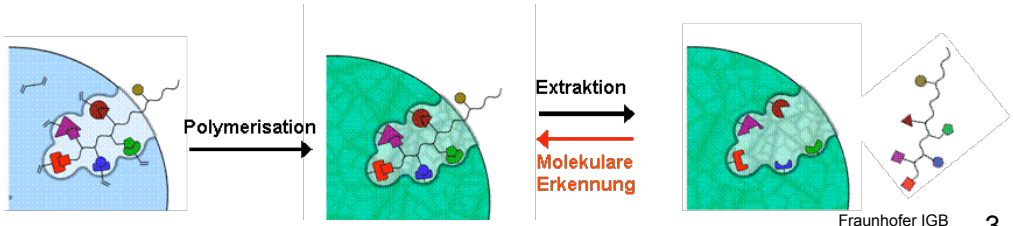


Figure 1: Diagram of the procedure principle for the chemical representation of selective substance-binding nano-particles by the molecule imprinting of nanoparticles through a template polymerisation of nano-droplets to spherical particles. After the polymerisation the templates, which cause the nano-structuring of the particle surface, are extracted and nanopores remain in the polymer network which have selective preferentiality for the template molecule and absorbs these (Tovar et al.).[3]

The molecular recognition mechanism was already described in 1894 by Emil Fischer through the hypothetic example of the specific connection of enzyme and substrate. [4] The so-called “key-lock-principle“ describes the interaction of two or more complementary structures which must fit spatially to each other in order to be able to fulfil a specific biological function

With the molecular imprinting procedure a suitable lock is developed around an existing key, the template molecule, (Figure 2).

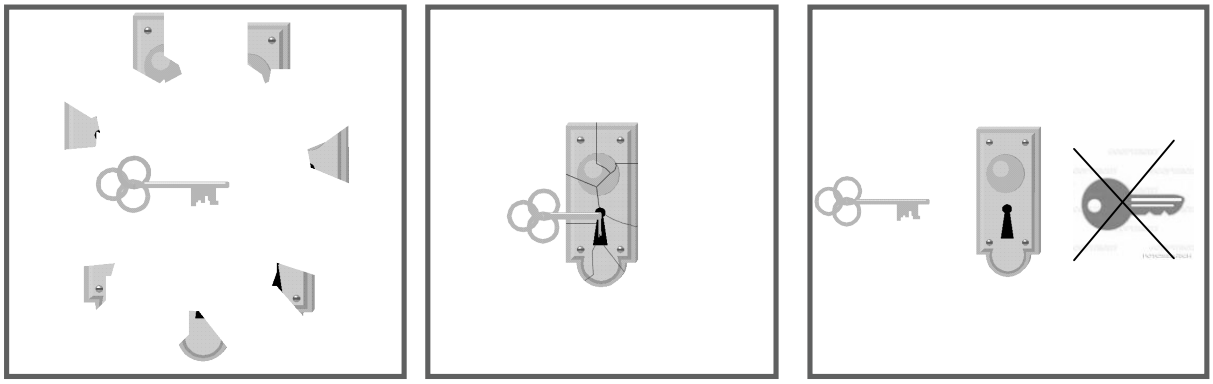


Figure 2: Diagram of the synthetic reproduction of Fischer´schen Key-Lock-principle (Graphics from Ramstrom, O., Molecular Imprinting Technology - A Way to Make Artificial Locks for Molecular Keys, http://www.molecular-imprinting.org/story/MIT.htm (1996)).

Molecularly imprinted polymers are synthetic adsorbent materials which make a highly selective binding of certain goal molecules possible. One can also use them, for example, in order to separate an unwanted by-product from a reaction mixture. Or also perhaps to mask contaminants such as unpleasant smelling substances: These materials then remain in a mixture such as, for example, paint colours, but are prevented from entering the gas phase and reaching the nose of the organism in the vicinity through their binding to the polymer surfaces.

2 Material and Methods

2.1 Molecularly imprinted polymers

Molecularly imprinted polymers are usually produced as polymer monoliths through bulk polymerisation which must then be crushed by grinding to expose the artificial receptor imprints.

In the monomer crosslinker template a specific spatial arrangement of the monomer around the template molecule takes place prior to the polymerisation through different interactions (hydrogen bonds, ionic interactions, hydrophobic interactions, Dipol-Dipol interactions, π-π interactions).

The networking of the monomers to a firm polymer material then occurs in one structures affected by the presence of the template molecule. A large number of monomers and crosslinkers are commercially available for this kind of molecular imprinting. One of the most frequently used monomers is Methacrylic acid. Alternatively, basic monomers (for example 4-Vinylpyridin) and neutral monomers (for example acrylamide) are also used.

The optimal monomer and/or the optimal monomer mixture must be selected for each template so that a maximum molecule specific interaction is achieved. [5] A polymer block develops with the materials formed by bulk polymerisation which must initially be ground into granulates. In the course of the production process the binding sites are distributed inhomogeneously in the granulate and are partly destroyed in the grinding. Afterwards the size distribution of the granulates is set at certain limits by a filter procedure. The individual grains are, however, irregular in size and their form cannot be controlled.

In Fraunhofer IGB **miniemulsion polymerisations** are used for the direct synthesis of molecularly imprinted adsorber particles. One advantage of this imprinting method – patented by IGB – in comparison to traditional approaches is the single step synthesis process and precisely defined nanoparticle morphology. The imprinted non-particles can be used immediately in suspension after the synthesis, grinding and sieving process omitted. For individual uses the nanoparticles can also be used as a selective layer in composite membranes.

With complex molecules individual molecule building blocks can be used as template molecules as well. With this so-called **Epitope-approach** (see Figure 3) the entire molecule is absorbed after the extraction of the template molecule.

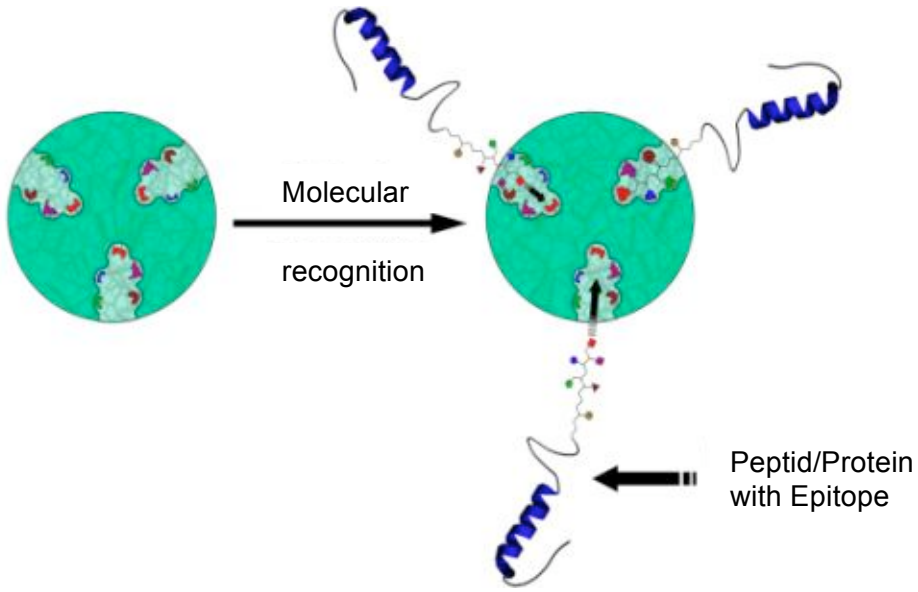


Figure 3: Diagram of the Epitope approach and its use in the formation of molecularly imprinted nanoparticles.

In the context of this project Stigmasterol (Figure 4) was identified as a suitable Epitope for the production of molecularly imprinted adsorber particles.

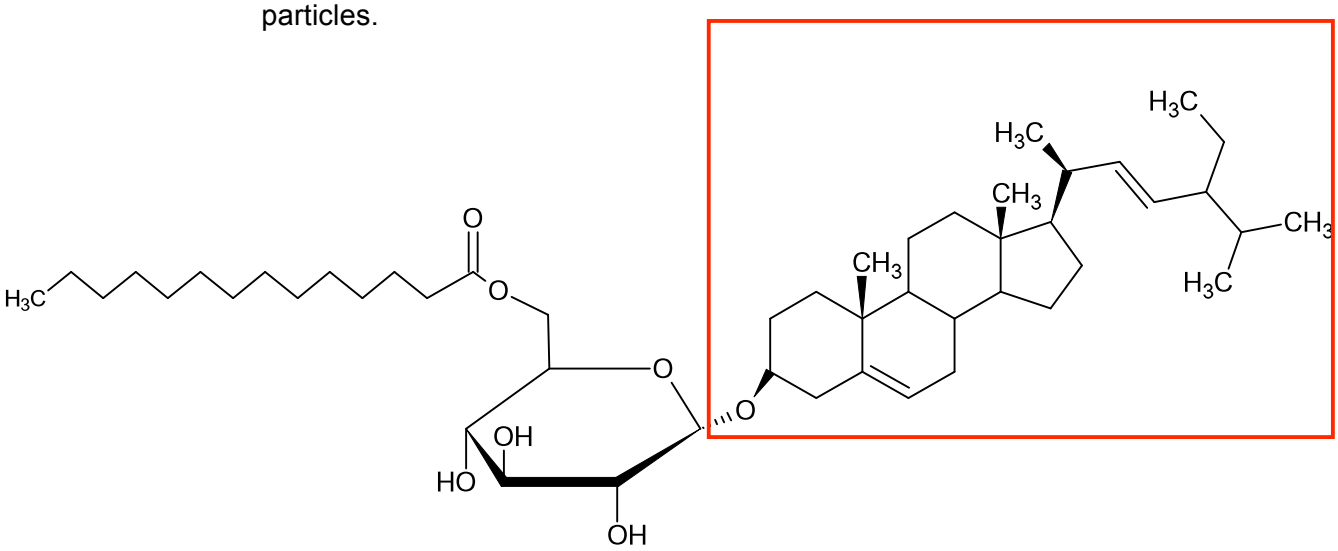


Figure 4: Structural formula of ASG; the Stigmasterol-Epitope is marked red.

2.2 Stigmasterol solubility test

Stigmasterol solubility tests were carried out prior to the forming of molecularly imprinted particles (MIPs). It was analysed in which solutions the Stigmasterol template module was soluble and in which quantity Stigmasterol could be dissolved in the Polymer systems. The amount on the dissolved template is very important for the optimal setting of the template / monomer behaviour. The maximum solubility of Stigmasterol is shown in the following tables. Table 1 shows the solubility in the individual Polymer systems and Table 2 shows the solubility in different solvents such as in a solvent/water mixture.

Table 1: Maximum solubility of Stigmasterol in the polymer mixtures used.

Polymer system	*Mixture /g*	*Dissolved Stigmasterol-quantity /mg*
MAA/EGDMA	MAA (0,2) EGDMA (1,8) Hexadekan (0,083) AMBN (0,04)	6,5
TFMAA/EGDMA	TFMAA (0,3) EGDMA (1,7) Hexadekan (0,083) AMBN (0,04)	17,02
MAA/DVB	MAA (0,127) DVB (0,77) Hexadekan (0,0375) AIBN (0,0225)	51,11

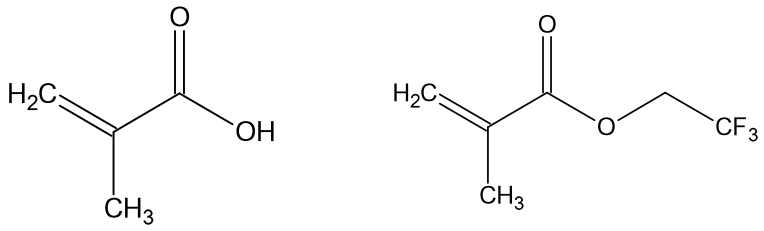
Table 2: Solubility analysis of the Stigmasterol in different solvents and in solvent/water mixtures.

Solvent	*Solubility of Stigmasterol*	*Comment*
Acetonitril	No	Floculates; cloudy
Methanol	No	Partially dissolves; recogniseable residue
Hexadekan	No	
Water	No	Not mixable
Isopropanol	Yes	
THF	Yes	
Ethanol	Yes	
THF/Water (50:50)	Yes	
IsoP/Water (50:50)	No	
IsoP/Water (80:50)	No	
Ethanol/Water (50:50)	No	

2.3 Monomer-Screening- ASG

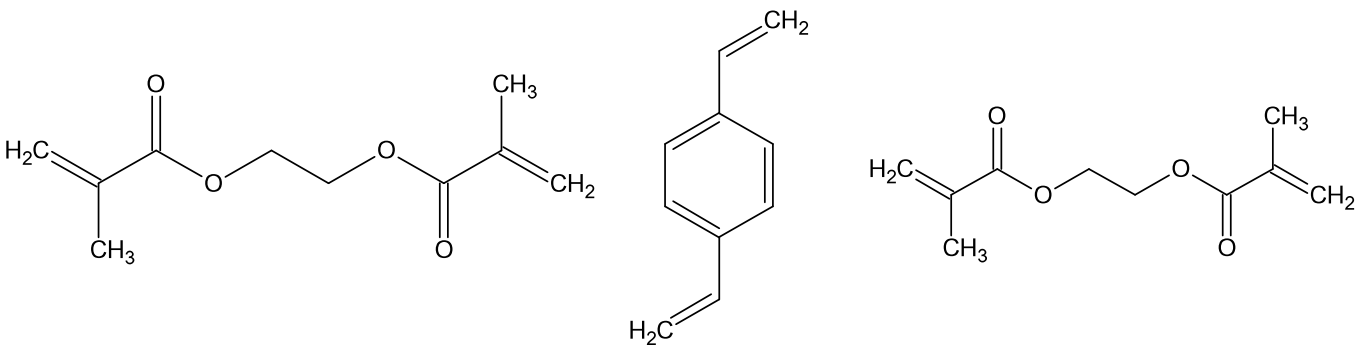
Initially different monomer- crosslinker-systems were selected. Methacrylic acid (MAA) and 2-(Trifluoroethylmethyl)methacrylic acid (TFMAA) (Figure 5) were selected as monomer and Ethylenglykoldimethacrylate (EGDMA), Bis[2-(methacryloyloxy)ethyl]phosphate (MEP) and Divinylbenzen (DVB)

(Figure 6) were selected as crosslinkers. Stigmasterol was used as template molecule as this is a significant component of the ASGs and SGs and was available in the context of this Fraunhofer IGB project. The formation of the particle is described below.



TFMAA MAA

Figure 5: Structural formula of the monomers used.



EGDMA DVB MEP

Figure 6: Structural formula of the cross linker used.

2.3.1 Production of particles through miniemulsion polymerisation

Miniemulsions are emulsions of monomer droplets which are 50 nm – 500 nm in water. These monomer droplet sizes can be achieved through

ultrasonic or high pressure treatment. An equilibrium is produced between coalescence and splitting of the monomer droplets through this.

Emulsifiers as well as hydrophobic auxiliary materials are used as stabilisers. If these hydrophobic auxiliary materials possess two functions they are described as co-stabilisers. On the one hand they can work together with the emulsifier to protect droplets against coalescence, as both accumulate on the oil- water interface and, on the other, they can suppress the Ostwald-ripening, as an osmotic pressure builds in the droplets and thereby prevents the diffusion of monomers from the smallest to the largest drops, as shown in Figure 7. If a hydrophobic auxiliary aid only possesses the latter function, then this substance is described as a hydrophobic agent. The most important requisite of the co-stabiliser and/or the hydrophobic agent is a low water solubility.[6]

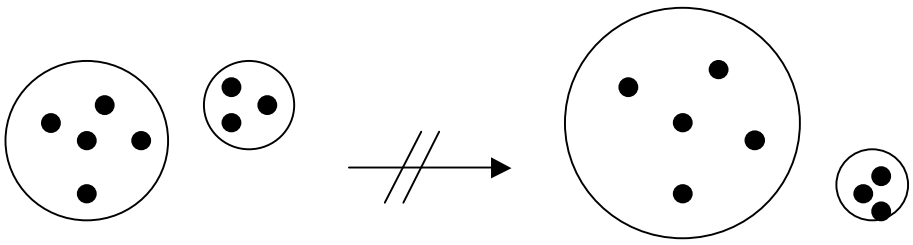


Figure 7: Suppression of the Ostwald-ripening during the miniemulsions - polymerisation through the use of a hydrophobe agent.

With the mini emulsion polymerisation the monomer droplet size before the polymerisation almost corresponds with the particle size after the polymerisation, which corresponds to a 1:1 copying process

This copying process can be proven through a combination of surface tension measurements and conductivity. The miniemulsion polymerisation process is shown in Figure 8 below.

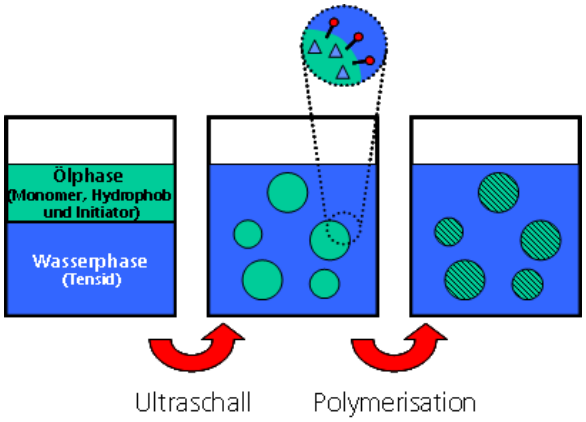


Figure 8: Diagram of the miniemulsion polymerisation.

The permitted amount of surfactant is so small with the mini emulsion polymerisation that CMC is not exceeded and therefore the micelle aggregate is prevented.

In the following the syntheses are described. The homogenisation for the production of the miniemulsion took place by means of ultrasound. Different monomers and cross-linkers were analysed, as well as the varied monomer template - behaviour. This depended on the solubility of the templates in the respective system.

Production method 1

The water phase, consisting of SDS (24 mg) dissolved in water (16.7 mL) was prepared. The oil phase was prepared separately. For this the AMBN initiator (0.208 mmol), the crosslinker, the monomer and the template were dissolved in Hexadekan (0.083 g) and agitated until everything was solved. The oil phase was added to the water

phase and agitated for one hour. After this the mixture was homogenised for two minutes with an amplitude of 60% by means of an ultrasonic finger. Thereafter the emulsion was heated up with 80°C at 500 RPM for 4 hrs and polymerised at this temperature. Subsequently, the particles were washed twice with distilled water.

Table 3 contains the amounts of Monomer, Crosslinker and, if necessary, the template molecules used. NIP describes the non-imprinted polymer particle which is produced without template molecule. MIP describes the Molecularly imprinted polymer particles, which were produced in the presence of the Stigmasterol template molecule. The use of the EGDMA crosslinkers enables the production of individual systems both with MAA as well as TFMAA. The MAA/EGDMA and TFMAA monomer-crosslinker-Systems could thereby be successfully polymerised. Particles could not be produced by using the MEP as crosslinker. The polymerisation was unsuccessful. MEP is not suitable as a crosslinker in the formation production of molecularly imprinted materials.

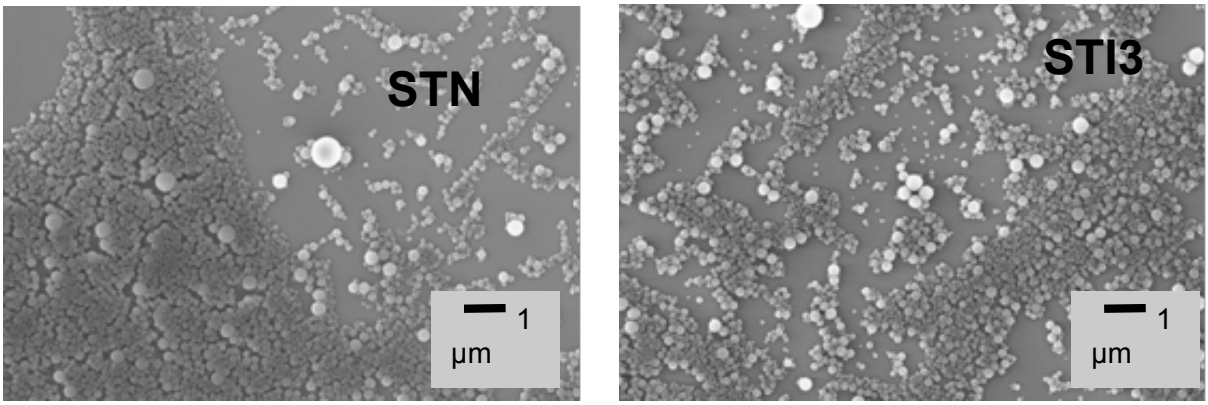
Table 3: Quantities used on monomer, cross linker and acrylic acid with the particle production in production method 1. STN stands for the polymerisation of NIPs (without template) and STI stands for the production of MIPs (with template).

Starting solution	Template /g	Monomer /g	Cross linker /g	Comment

Starting solution	Template /g	Monomer /g	Cross linker /g	Comment
STN1 (NIP)	_	MAA /0,2	EGDMA /1,8	_
STI3 (MIP)	Stigmasterol /0,0196	MAA /0,2	EGDMA /1,8	_
STN4 (NIP)	_	TFMAA /0,3	EGDMA /1,7	_
STN5 (NIP)	_	TFMAA /0,196	MEP /1,8	No polymerisation
STI6 (MIP)	Stigmasterol /0,017	TFMAA /0,3	EGDMA /1,7	_

synthesised the that individual

Scanning electron microscopic pictures were taken of the successfully particle systems. In Figure 9 the photographs of the non-imprinted (left) and imprinted particles (right) are compared. It can clearly be seen systems can be successfully produced in the desired size.



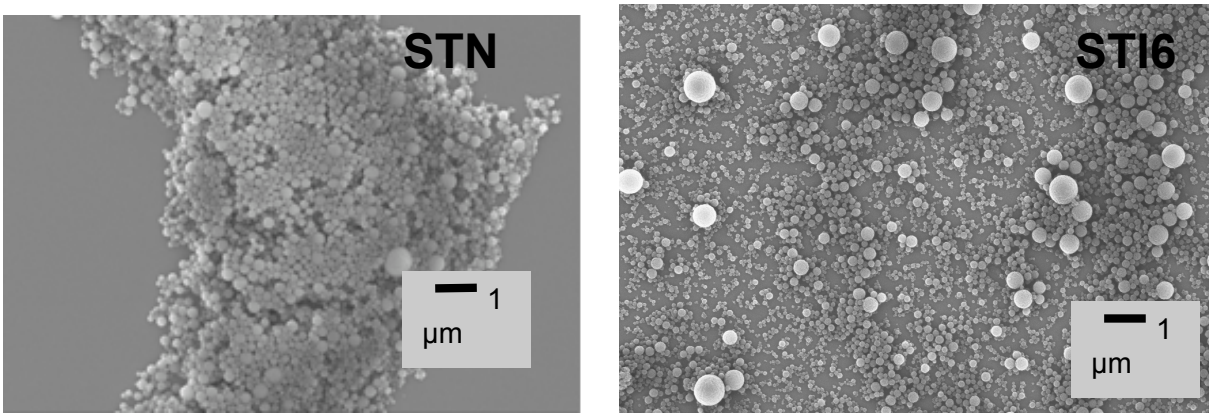


Figure 9: scanning electron microscopic pictures of the miniemulsions polymerisate (STN1, STI3 and STN4).

The production method was modified for the production of the particle system with Divinylbenzol as cross linker. The AIBN initiator was used. The production of the particle is described below.

Production method 1:

The water phase, consisting of SDS (21.6 mg) dissolved in water (15 mL) was prepared. The oil phase was prepared separately. For this the AMBN initiator (0.276 mmol), the crosslinker, the monomer and the template were dissolved in Hexadekan (0.075 g) and agitated until everything was solved. The oil phase was added to the water phase and agitated for one hour. After this the mixture was homogenised for two minutes with an amplitude of 60% by means of an

ultrasonic finger. Thereafter the emulsion was heated up with 80°C at 500 RPM for 4 hrs and polymerised at this temperature. Subsequently, the particles were washed twice with distilled water.

The monomer and crosslinker quantities used in production method 2 are shown in table 4. Individual systems were developed in the synthesis using DVB as crosslinker (see Figure 10). The particles are, however, - except for few exceptions - very small. A separation of the particles, even by repeated centrifugation, was not possible. The particles were eventually able to be separated with an appropriate filter but these could not, however, be used in the context of this project due to supply difficulties.

Table 4: Quantities of monomer and crosslinkers used in particle production according to production method 2. STN stands for the polymerisation of a NIPs (without template) and STI stands for the production of MIPs (with template).

Starting solution	Template/g	Monomer /g	Crosslinker /g	Comment
STN7 (NIP)	_	MAA /0,255	DVB /1,54	Particles cannot be centrifuged
STI8 (MIP)	Stigmasterol /0,102	MAA /0,255	DVB /1,54	Particles cannot be decentrifuged

The scanning electron microscopic photograph of the non-imprinted particles which were produced with DVB as crosslinker is shown in Figure 10. The very small particle sizes are clearly recognised

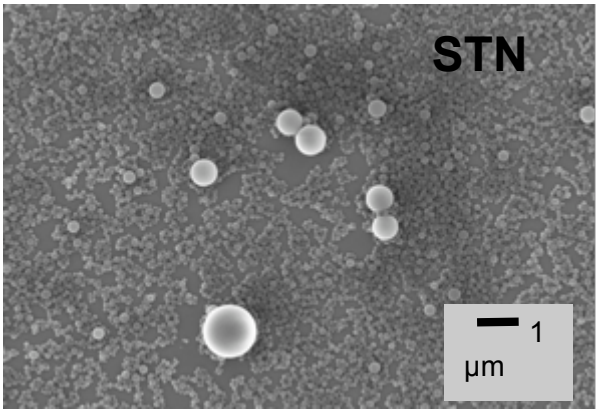


Figure 10: Scanning electron microscopic photograph of the miniemulsion - polymerisates (STN7).

Table 5 contains the individual experiments which were implemented in the scope of this project.

Table 5: Overview table of the individual experiments with the monomer: Cross linker, Template: Monomer- Behaviours and the particle size.

Starting solution	STN1	STI3	STN4	STI6	STN7	STI8
Monomer	MAA	MAA	TFMAA	TFMAA	MAA	MAA
Crosslinker	EGDMA	EGDMA	EGDMA	EGDMA	DVB	DVB
Template	-	Stigmasterol	-	Stigmasterol	-	Stigmasterol
Monomer: Crosslinker Behaviour	1:4	1:4	1:4	1:4	1:4	1:4
Template: Monomer Behaviour	-	1:147		1:54	-	1:12
Particle size / nm	163,7 ±1,6	163,9 ±1,4	183,3 ±1,3	151,1	133,9	-
PdI	0,079	0,086	0,084	-	0,054	-
Volume / %	-	-	76,2	89,2	77,9	77,8

2.4 Analysis

A suitable method for Stigmasterol. analysis was developed for the examination of the specific and/or nonspecific adsorption of Stigmasterol on the polymer adsorber particles (NIPs and/or MIPs) developed in the context of this project. Both the HPLC chromatography and the LC-MS-method were used. For the detection of the Stigmasterol by means of the HPLC chromatography, solubility tests were first implemented with different solvents (see chapter 2.2).

These solubility tests demonstrated that Stigmasterol is soluble in the Isopropanol and THF solvent. The HPLC measurements in isopropanol and THF as eluent were, therefore, implemented. The tests concerning HPLC analytics are specified in the appendix. With the preliminary tests specified therein a signal was assigned to the Stigmasterol, which only arose in this THF-charge. During the repetition of the tests for the examination of the reproducibility another THF-charge was used.

During the measurement of the HPLC standards, which were produced with this THF charge, no Stigmasterol signal could be proven. As a control attempt pure THF, which was used as eluent, THF from which new standards were made, and a newly produced standard (250 µg/mL) were all measured and compared with the results from the first HPLC. The result is shown in Figure 11.

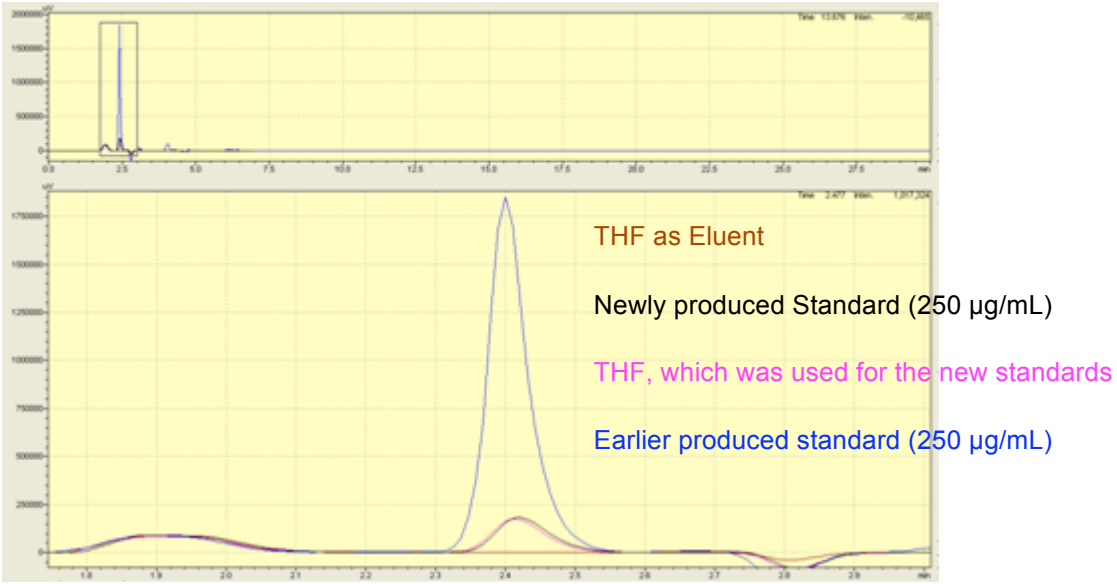


Figure 11: HPLC-Chromatogram of Stigmasterol (standards produced with different THFs) and THF (different charges) determined through HPLC-Method 3.

From the Figure it follows that the THF which was used as eluent, exhibits no peak with the retention time of Stigmasterol (approx. 2.3 min - 2.56 min). While with the THF which was used for the new standard, a small peak appears. The newly created standard exhibits exactly the same peak

With the standard which was produced by an earlier THF-charge and with which the method was initially developed, a clear peak appears at the alleged retention time of Stigmasterol, which was regarded as the Stigmasterol peak, since it arose only with samples with Stigmasterol.

Due to the results shown in Figure 11 it is assumed that the peak must be pollution or a component of the THF which does not concern the Stigmasterol peak. The template cannot, therefore, be proven with this method.

As the Stigmasterol could not be detected with the HPLC-method, a new method for the detection of Stigmasterol was developed. The LC/MS-

method was selected for this. The adsorption tests samples in chapter 2.4.2 were determined with this method.

LC/MS-Method 1

LC/MS-System:	
Mass spectrometer	LCQ Deca; Thermo Scientific
HPLC	Surveyor; Thermo Scientific
Column:	Phenomenex Luna C18(2), 150 mm x 2,0 mm, 3 µm
Eluent:	100 % Methanol
Flow:	200 µL/min
Injection volumes:	20 µL

2.4.1 Purification of the particle suspensions produced

The particle suspensions produced were purified in different wash steps, if necessary, and the bound template was extracted. The purification of the particles without template (NIPs) took place by twice washing with water via centrifugation (15,000 RPM; 25 min) and a dialysis thereafter.

With the particles which were manufactured with the template molecule Stigmasterol (MIPs), the bound Stigmasterol was extracted for the following adsorption tests in order to make the binding places freely accessible again for the adsorption of Stigmasterol. The particles were washed at least six times with THF, by centrifugation (15,000 RPM; 20 min) and thereafter transferred in water. After the removal of the Stigmasterols the binding places are freely accessible again and it is possible to examine whether Stigmasterol can be bound specifically by the MIPs produced.

2.4.2 Adsorption tests

After the complete purification of the particle suspension the adsorption tests were implemented. The adsorption tests were implemented in different incubation mediums such as isopropanol and THF:Water (50:50). The polymer quantity was not varied. NIPs were examined in order to determine the non-specific adsorption of Stigmasterol as well as MIPs, in order to examine the specific adsorption

Detemination method 1

Repeat determinations were in each case implemented for the adsorption tests. The particle suspensions (10 mg NIPs and MIPs) were centrifuged and afterwards the excess was completely removed. Subsequently, the pellet was re-suspended in the desired adsorption medium (THF/Water 50:50 and isopropanol). The template (100 µg/mL) was added to the particle suspension and vibrated at 10 °C, at 1.200 RPMfor two hours. After the incubation the particle with the template was centrifuged again. The excess was examined by means of the LC/MS method.

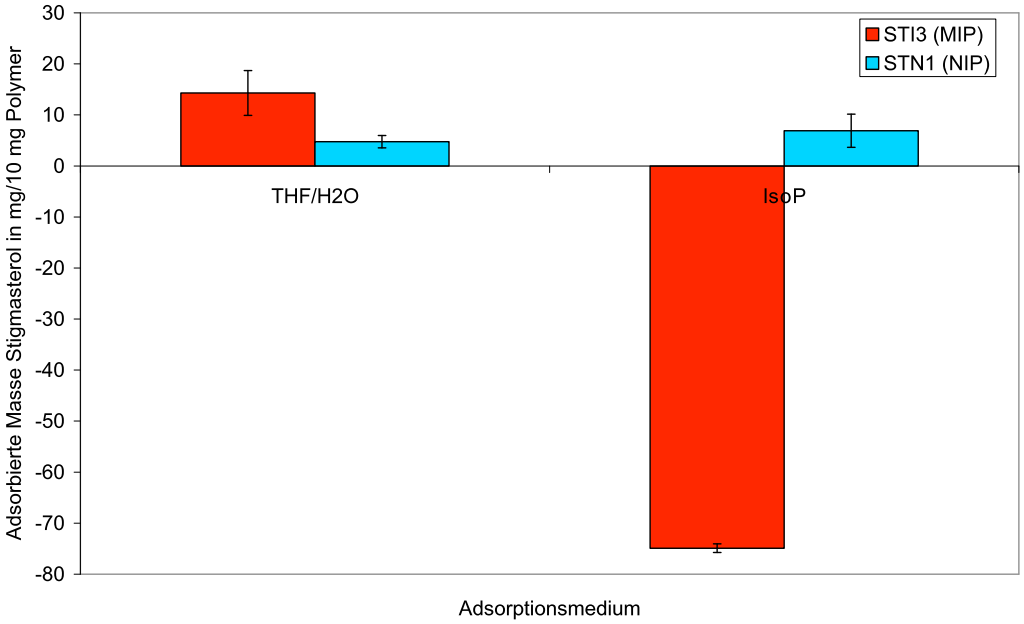


Figure 12: Results of the adsorption experiments of STI3 (MIP) and STN1 (NIP) and Stigmasterol, in the THF/Water (50:50) incubation medium and Isopropanol.

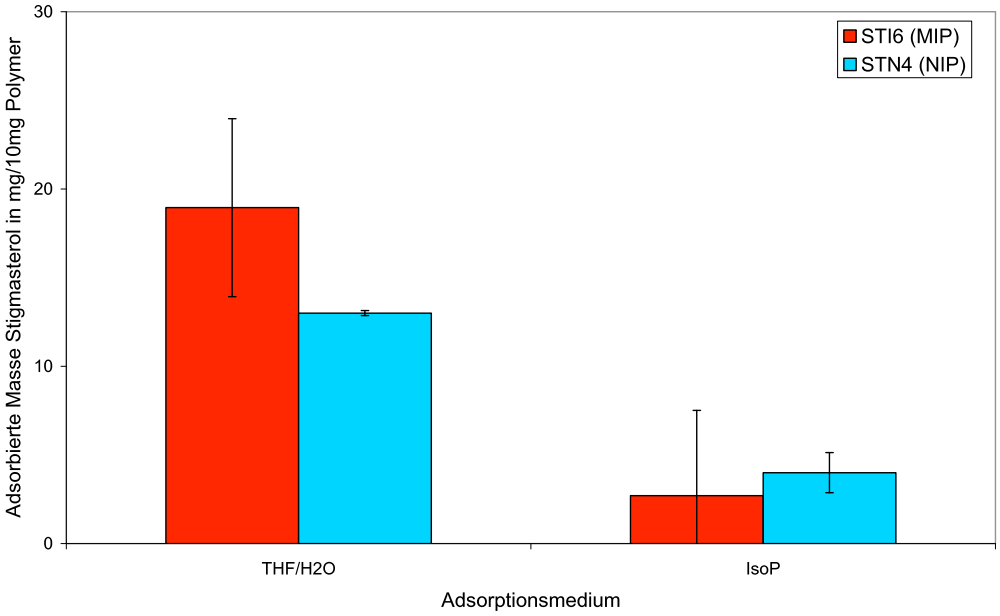


Figure 13: Results of the adsorption experiments of STI6 (MIP) and STN4 (NIP), in the incubation process media THF/Water (50:50) and Isopropanol.

The results of the adsorption tests are represented in Figure 12 and Figure 13. As shown in Figure 12, a specific connection of Stigmasterol to the MIP (STI3) could be detected, however only in the THF/Water (50:50) incubation medium. No specific adsorption could be detected in isopropanol. In both incubation mediums, however, a non-specific connection (STN1) of Stigmasterol to the polymer particles could be detected.

With the results represented in Figure 13, a likewise specific connection of Stigmasterol to the MIP (STI6) in the incubation medium THF/Water (50:50) could be detected. With isopropanol no specific adsorption could be detected. In both incubation mediums a nonspecific connection (STN4) could again be detected.

A possible explanation for this behaviour could be that the polymer systems are slightly hydrophobe due to their MAA, TFMAA and EGDMA educts. Therefore the affinity of the templates for the polymer is clearly greater than to the incubation medium (THF/water), whereby more template is bound to the particles. With pure solvent interaction can occur between the template and solvent, whereby the template also possesses an affinity for the solvent and so less template is bound to the particles

If the two polymer systems are compared with one another, it becomes clear that with the TFMAA/EGDMA polymer system, shown in Figure 13, somewhat more template, nonspecific and specific, was bound in comparison with the MAA/EGDMA polymer system. This could be due to the Template: Monomer Behaviour, which stands at 1:54 with the TFMAA/EGDMA system and 1:147 with the MAA/EGDMA system. Due to these behaviours more binding are available with the TFMAA/EGDMA system.

3 Conclusions

The subject matter of the project was a sounding out feasibility study for the analysis of the main applicability possibilities of nano-structured molecularly imprinted polymer particles (MIPs) for the separation of ASG – here, for example, Stigmasteryl-6-O-palmi¬toyl-alpha-D-glucopyranosid - from biooils.

In the context of this project different monomers and crosslinkers were initially used for the production of nanoparticles by means of miniemulsion polymerisation. In each case both non-imprinted particle systems (NIPs) as well as molecularly imprinted particle systems (MIPs) were synthesised. Stigmasterol was identified as a suitable template molecule. Stigmasterol occurs as Stigmasteryleinheit in the ASG. In the presence of Stigmasterol the molecularly imprinted adsorbent particles were formed. Ethylenglycoldimethacrylate proved to be a suitable crosslinker. With Divinylbenzol (DVB) as crosslinker nanoparticles could, likewise, be successfully produced. The further purification of these particle systems was not, however, possible due to the prohibitive costs of the too small particle sizes.

For the determination of the adsorption behaviour of the nano-particles a suitable analysis at the IGB could be developed and established. The adsorbent quantity of Stigmasterol was successfully determined according to optimisation of the method through LC-MS-analytics.

A specific adsorption of Stigmasterol could be detected in the THF/Water solvent mixture. Here the TFMAA/EGDMA particle adsorbed a maximum 18.95 mg Stigmasterol and the MAA/EGDMA particle 14.3 mg Stigmasterol per 10 mg polymer.

The results of this project show that the production of polymer adsorber particles for the specific adsorption of ASG is possible.

4 View

In the context of this project it has been shown that the production of adsorber particles for the specific adsorption of ASG is possible. The successful experiments concerning the adsorption and thus the separation of Stigmasterol using polymer adsorbent materials developed here provide solid reasons for the continuation of this project in the broader context with appropriate means.

A comparison of the adsorption behaviour of classic adsorbent materials with the polymer nanoparticles developed here concerning the adsorption of ASG would be significant at this point.

In this comparison both polymer adsorber particles without Stigmasterol, polymer adsorbent particle with greater affinity for Stigmasterol and its derivatives as well as the Molecularly imprinted nano-particles were used.

Furthermore, trials with real, technical samples, which come directly from production, are significant. For these trials to be implemented it is, however, important to work with companies and a research establishment which have experience in analysing these material samples with regards to their ASG content as well as provide the technical samples.

Furthermore, procedures for the technical use of these particle systems are to be developed and a cost-use-calculation provided. For the employment of

process engineering of the nanoparticles, the nanoparticles can be applied as filling materials (there are promising results for this from the Fraunhofer IGB) or built in as a selective layer in composite membranes. After the development of a test facility at laboratory scale this system is to be tested locally directly in the oil mills or with the biodiesel manufacturers.

5 Chemicals used

The chemicals used in the context of this project are summarised in the table below.

Table 6: Chemicals used

Chemicals	Function	Purity	Company	Order-N°.

Chemicals	Function	Purity	Company	Order-N°.
Methacrylic acid	Monomer	≥ 99%	Sigma-Aldrich	15,572-1
Ethylenglycoldimethacrylate	Crosslinker	≥ 97 %	Fluka	03808
Stigmasterol	Template	95 %	Sigma-Aldrich	S2424
2-Trifluormethacrylic acid	Monomer	98 %	Sigma-Aldrich	367346
Divinylbenzen	Crosslinker	80 %	Fluka	43908
2-(Methacryloyloxy)ethyl phosphat	Crosslinker	-	Sigma-Aldrich	496758
Hexadekan	Solvent	≥ 98 %	Fluka	52210
Sodiumdodecylsulfate	Emulsifier	-	Sigma-Aldrich	436143
Azobis-2-methylbutyronitryl	Initiator	≥ 98 %	Fluka	11596
2,2-Azoisobutyronitryl	Initiator	≥ 98 %	Fluka	11630
Tetrahydrofuran	Solvent	≥ 99,9 %	Sigma-Aldrich	34865
2-Propanol	Solvent	≥ 99,5 %	J.T. Baker	8067

Chemicals	Function	Purity	Company	Order-N°.
Hydrochinon		≥ 99	Sigma-Aldrich	

6 Glossary

Adsorber Name of mainly solid materials with large, active surfaces, which can selectively capture certain substances of gaseous or liquid mixtures on their interface

HPLC High performance liquid chromatography; Analytical method in chemistry with which substances can be identified and quantified.

Molecular imprinting Method for producing technically adapted, fully synthetic highly specific adsorbers.

Monomer Single components or basic units from which polymers are built through the assembly of larger connections

Polymer According to the IUPAC-Definition a substance which is built up of a number of molecules in which a type or many types of molecules or atom groupings.

Polymerisation Chemical reaction in which monomers react to polymers. Catalysers are often used for this.

Template Molecule which is inserted as a template for the production of molecular imprinting.

7 Bibliography

[1] J. Haupt, J. Fischer, K. Witt, T. Wilharm, in *Studie zum SG- und ASG-Gehalt von Pflanzenölen und FAME*, Arbeitsgemeinschaft Qualitätsmanagement Biodiesel e. V., **2011**.

[2] G. E. M. Tovar, I. Kräuter, C. Gruber, *Topics in current chemistry* **2003**, *227*, 125-144.

[3] M. Herold, G. E. M. Tovar, C. Gruber, M. Dettling, S. Sezgin, H. Brunner, *Polymer Prepr.* **2005**, *46*, 1125-1126.

[4] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 673 - 679.

[5] C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony, M. J. Whitcombe, *Journal of Molecular Recognition* **2006**, *19*, 106 - 180.

[6] V. Holzapfel, A. Musyanovych, K. Landfester, M. R. Lorenz, V. Mailänder, *Macromolecular Chemistry and Physics* **2005**, *206*, 2440–2449.

8 Annex

HPLC-Method 1: Isopropanol eluent

The SHIMADZU HPLC system used is composed of a number of different individual components.

- Degasser: DGU-20 A_5
- Liquid Chromatograph: LC-20 AT

- Auto Sampler: SIL-20 AC_{HT}
- Column Oven: CTO-20 AC
- Diode Array Detector: SPD-M2OA
- Communications Bus Module: CBM-20 A

Column:	C18 (inner diameter 4 mm; length 125 mm; column packing 4 µm)
Eluent composition:	85 % Isopropanol + 15 % Water
Flow:	1 mL/min
Injection volumes:	10 µL
Detection:	UV, 205 nm

Result

As shown in Figure 14, a Stigmasterol-Standard (100 µg) and pure Isopropanol were injected. It should be noted that the supposed Stigmasterol-Peak appeared at a retention time of approximately 5,8-6,6 min.

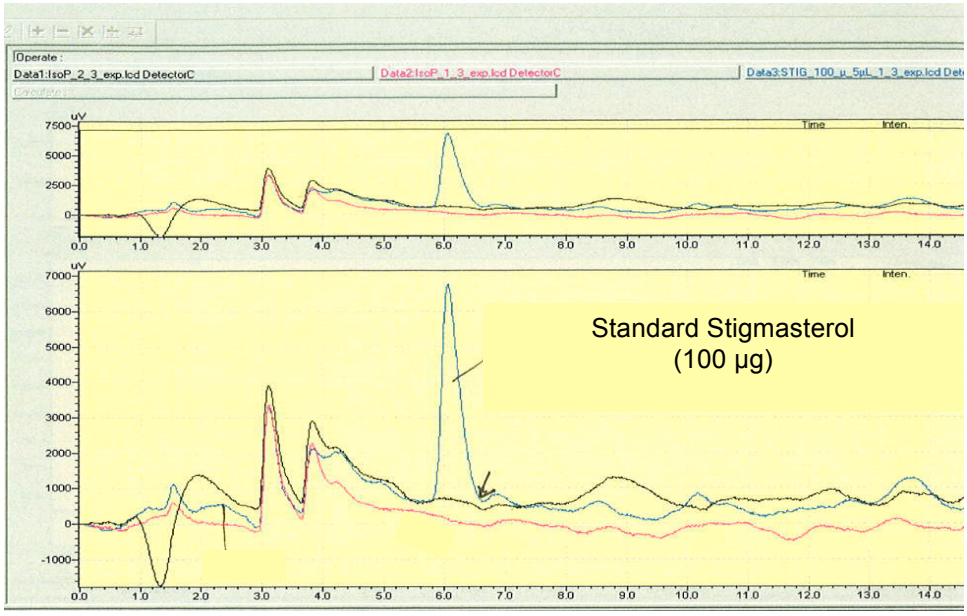


Figure 14: HPLC-Chromatogram of Stigmasterol and Isopropanol determined by HPLC-Method 1

The method was further optimised in that the eluent was changed to THF. The result was HPLC-method 2.

HPLC-method 2: THF eluent

A HPLC Prominence from Shimadzu, see HPLC method 1, with the following settings was introduced for the measurements:

Column:	C18
Eluent composition:	80 % THF + 20 % water
Flow:	1,3 mL/min
Injection volumes:	20 µL

Detection: UV, 205 nm

Result

As shown in figure15, a Stigmasterol-Standard (250 µg/mL) such as pure THF was injected. It should be noted that the supposed Stigmasterol-Peak appeared at a retention time of approximately 2,2-2,5 min.

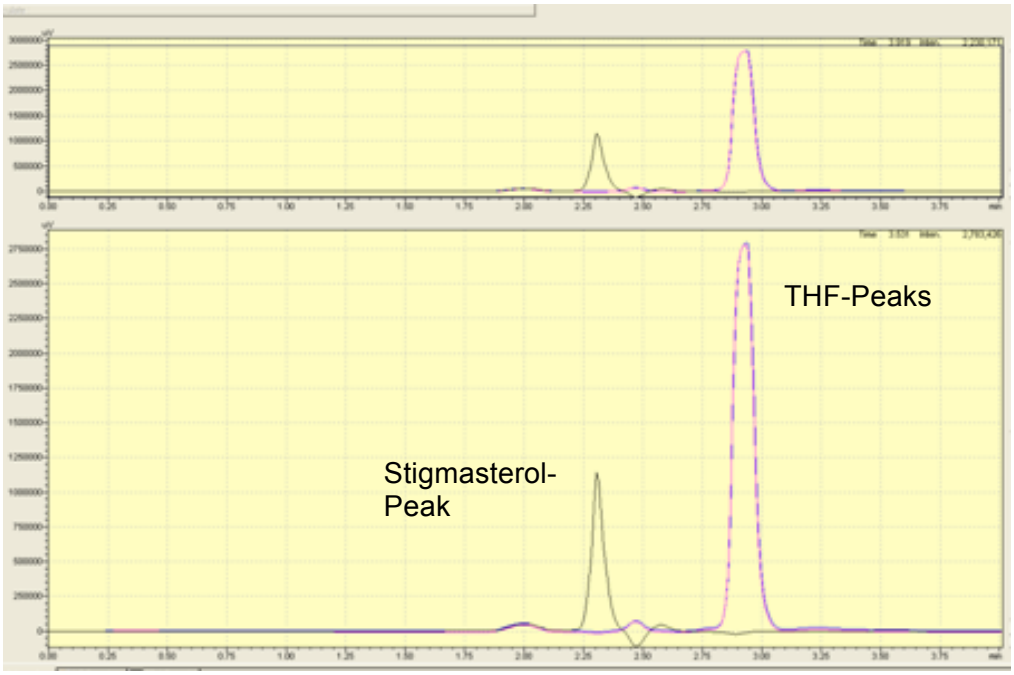


Figure 15: HPLC-Chromatogram of Stigmasterol and THF determined by HPLC-method 2

The method was further optimised in that the eluent composition and the method times were varied. The result was the HPLC-Method 3 .

HPLC-Method 3: Variation of the eluent composition

A HPLC Prominence from Shimadzu, see HPLC method 1, with the following settings was introduced for the measurements:

Column:	C18
Eluent composition:	60 % THF + 40 % water
Flow:	1,3 mL/min
Injection volume:	20 µL
Detection:	UV, 205 nm

Result

As in shown in figure 16, a Stigmasterol standard (250 µg/mL) such as pure THF was injected. It should be noted that the alleged Stigmasterol peak appears with a retention time of approx. 2.3-2.56 min. In addition the THF peak could be shifted before the sample peak.

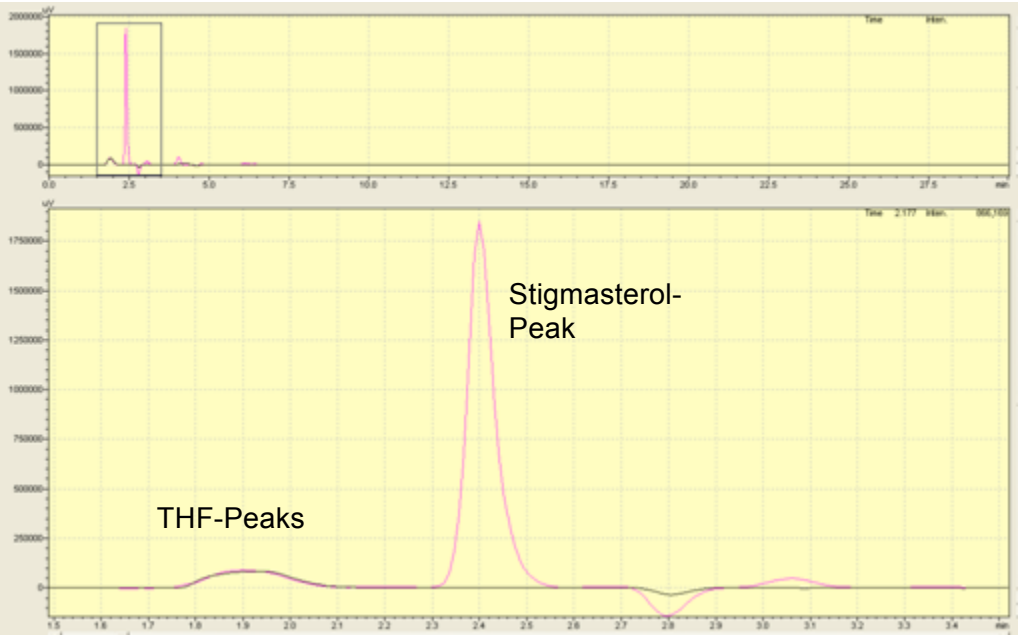


Figure 16 : HPLC-Chromatogramm of Stigmasterol and THF determined through HPLC-Method 3