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Novel sensitive determination of steryl glycosides in biodiesel by gas chromatography–mass spectroscopy

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ABSTRACT

A new method was developed for the quantitative analysis of steryl glycosides in biodiesel (fatty acid methyl esters). This method is much more sensitive than existing methods and has minimum limits of quantification of 50 μg/kg, compared to previously published minimum limits of quantification of about 15 mg/kg. The analysis is based on gas chromatography–mass spectroscopy determination of simple pre-treated and silylated samples via single ion monitoring at 204, 217, 247 *m/z*, which are specific ions for the silylated sugar moiety. Quantification was carried out using cholesteryl β-D-glucopyranoside as internal standard. The modified synthesis and purification of the internal standard is also presented as well as the characterization by NMR and mass spectroscopy. The advantage of the method compared with other approaches is the simplified sample preparation avoiding extra pre-treatment steps coupled with complete derivatization of the sugar hydroxyl groups by using *N,O*-bis(trimethylsilyl)acetamide with 5% trimethylchlorosilane as derivatization reagent. On the given conditions high recovery rates ≥89% can be obtained. Evaluation of lab specific variance and intermediate precision underline the robustness of the method which will be further assessed by Round robin tests.

1. Introduction

The content of minor compounds in fats and oils, which is used for biodiesel production has been mainly neglected during the last decades. However, steryl glycosides (SG's) respectively were assumed to be (co-) responsible for poor filterability leading to fuel filter plugging and in worst case engine failures and therefore became of increasing importance even from the scientific point of view [1–11]. The crucial point is that esterified steryl glycosides (ESG's) which can be identified in varying concentrations in vegetable oils and fats are converted (side reaction) into SG's during the transesterification process of oil into fatty acid methyl esters (FAME) by alkaline catalysis as reflected in Fig. 1.

Now, the problem occurs that the primarily oil soluble ESG species are transformed into an insoluble compound class. Usually the concentration of SG's is very low (<20 mg/kg) and evenly dispersed within the biodiesel matrix. However, if the fuel is stored at lower temperatures over a longer period of time the primarily spread SG's agglomerate and form insoluble precipitates which fail to completely re-dissolve if the fuel is re-heated. Especially biodiesel prepared from palm- or soybean oil with its known content of up to 2300 mg/kg of ESG's showed increased formation and contents of SG insolubles [2,4]. The composition of the most common SG's found in plant material, respectively oil is characteristic with β-sitosteryl, stigmasteryl, and campesteryl glycosides as predominant species [5]. Besides the problems that might occur with SG's it is essential to have analytical tools and appropriate methods to determine such minor compounds within or outside of a complex matrix. The main pre-requisite is the selection of appropriate internal standards, but also sampling and sample pre-treatment can be even a bottleneck in the context of several proposed methods. Especially commercially available SG standards are extremely costly and represent mostly mixtures of different compounds so that additional purification steps are necessary. Assessing the different analytical approaches several new adopted techniques dealing with both SG's as well as ESG's have been recently reported. First trials have been performed in the 1960s by characterization of such compounds in plant extracts via TLC [12–14]. These techniques are still the method of choice for quick qualitative assessment of fats and oils and corresponding fatty acid methyl esters (FAME, biodiesel) especially if samples have to be pre-treated in order to remove or reduce interfering compounds of the matrix [5]. If higher sensitivity is required especially HPLC and GC methods coupled with different pre-treatment steps and detection systems have been proven. Both techniques show good reliance but in order to establish them as standard methods some difficulties still have to be overcome. HPLC methods are characterized by rapid measurements and simple sample preparation. However, an overlap with

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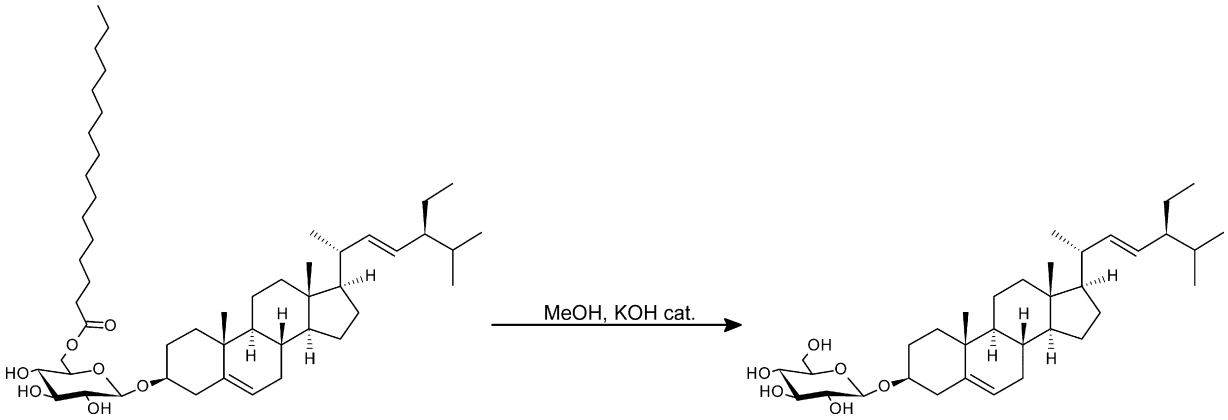

Fig. 1. Degradation of stigmasteryl (6′-*O*-palmitoyl)-β-D-glucopyranoside into stigmasteryl β-D-glucopyranoside during transesterification.

other compounds cannot be avoided and the limit of detection is over 10 mg/kg [6–8].

By using GC/FID as analysis tool SG's first have to be separated from the biodiesel (or oil) matrix mostly carried out by column chromatography (flash or solid phase extraction) prior to determination [9,10]. A pre-treatment procedure via distillation of the biodiesel and analysis of SG's out of the distillation residue has also been reported [4]. Other possibilities are adsorbent treatment, cold soak filtration or centrifugation prior to analysis [8]. The GC determination itself is more or less comparable within the different methods mentioned above. Samples containing SG's are derivatized prior to analysis which, depending on the reagent used, can be problematic in terms of incomplete silylation of the glucose hydroxyl groups leading to broader and overlapping peaks. Columns used for GC have to be high temperature applicable, typically 15–30 m × 0.25 mm × 0.1 μm. Due to the high boiling point of the SG's temperature ramps up to finally 370 °C are necessary to get reasonable chromatograms. Usually cool on column (COC) injection techniques are used.

The here described method is based on high temperature GC with COC injection but with GC/MS detection of the target compounds via SIM mode to decrease the limit of quantification and simultaneously simplify sample preparation. For quantification of SG's in biodiesel a new internal standard (IS) was synthesized because classically used 5α-cholestan-3β-ol does not really fit (overlapping with matrix signals and correction by response factor is required) and commercially standards from Matreya (Pleasant Gap, US) are not of highest purity and mostly mixtures of different compounds [4,9,10].

2. Experimental

2.1. Chemicals and reagents

Silver carbonate p.a., β-D-glucosepentaacetate (98%), diethyl ether (technical grade), chloroform (HPLC grade), acetone (HPLC grade), sodium sulphate anhydrous, sodium bicarbonate, ammonium acetate, silica gel 60 (≥400 mesh) and *N*,*O*-bis(trimethylsilyl)acetamide with 5% trimethylchlorosilane (BSA + TMCS) were purchased from Sigma (Sigma–Aldrich, Steinheim, Germany), cholesterol and hydrogen bromide (33% in acetic acid) were obtained from Merck chemicals (Darmstadt, Germany). Pyridine (99%+), dichloromethane (HPLC grade) and methanol (HPLC grade) came from Acros organics (Geel, Belgium).

Crude steryl glycoside reference material was gained from a biodiesel plant and was purified by dissolving 5 g in 100 ml $CHCl_3$/MeOH 2:1 and stirring the solution with charcoal. After 30 min the solution was filtrated and the solvent was removed

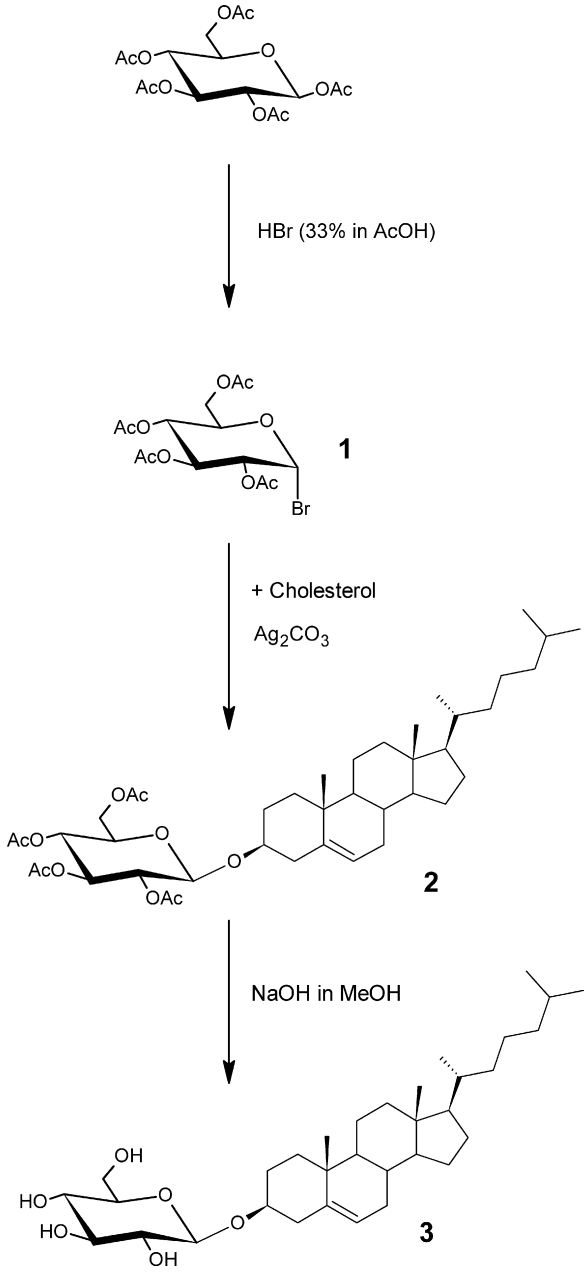

Fig. 2. Synthesis of cholesteryl β-D-glucopyranoside.

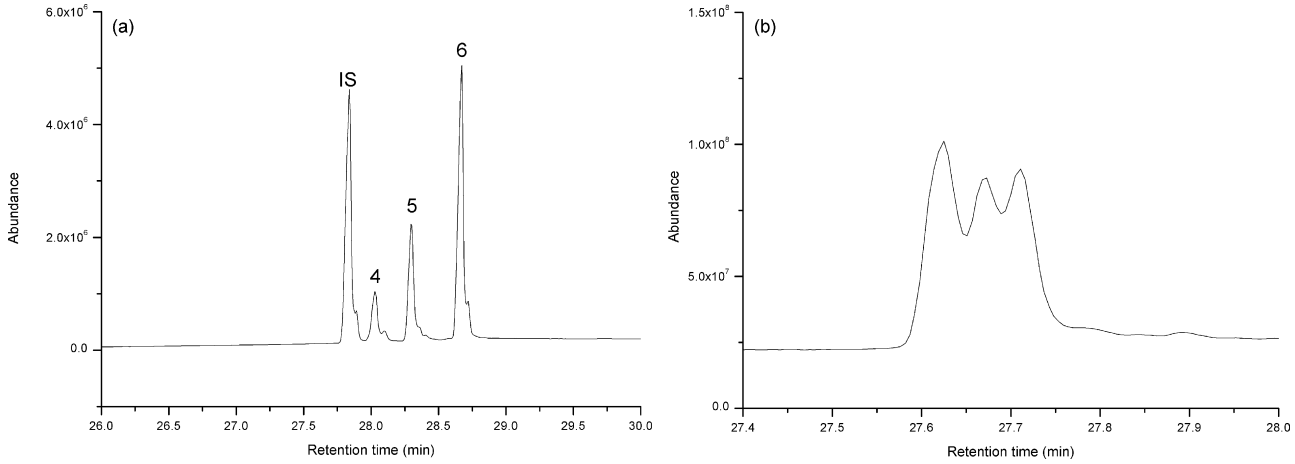

Fig. 3. (a) Total ion chromatogram of synthesized cholesteryl β-D-glucopyranoside (IS) and isolated steryl glycosides which are campesteryl-**4**, stigmasteryl-**5** and β-sitosteryl-glycoside **6** (compounds were derivatized with BSA + TMCS). (b) Total ion chromatogram of compound **4** derivatized with MSTFA.

under reduced pressure. The obtained waxy solid was washed with *n*-hexane to yield a steryl glycoside mixture without other compounds.

Biodiesel samples free of precipitates were obtained from local distributors.

2.2. Instrumentation

GC/MS analysis was performed on an Agilent 7890A GC system equipped with an 5975C inert XL MSD with Triple-Axis, an 7693 auto sampler, a G4513A injector, and a DB5-ht column (30 m × 250 μm × 0.1 μm). Data analysis was carried out using MSD-Chemstation E.02.00.493.

Mass spectra for determination of silylation level of the internal standard were recorded on a Synapt HDMS Q-TOF MS by direct infusion with a flow rate of 20 μl/min. An ESI ion source was used with the following settings: capillary voltage: 3 kV; sampling cone: 20 V; extraction cone: 4 V; scan time: 1 s.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 300 instrument (300 MHz ^{1}H frequency, 75 MHz ^{13}C frequency) and additional ^{1}H and 2D NOESY spectra on a Bruker Avance DRX 500 instrument (500 MHz ^{1}H frequency). Chemical shifts are reported in ppm from TMS, residual solvent signals were used as internal standard and are given in δ-units. Evaluation of NMR spectra was performed using Topspin 1.1.

2.3. Synthesis of the internal standard cholesteryl β-D-glucopyranoside (Fig. 2)

2.3.1. 1-Bromo 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside **1**

A solution of 5.0 g (13 mmol) β-D-glucosepentaacetate in 100 ml CH_2Cl_2 was treated slowly with 9.4 g (38 mmol) HBr (33% in AcOH) and stirred for 3 h at room temperature. The mixture was diluted with 100 ml CH_2Cl_2, washed two times with 100 ml ice/water and 100 ml saturated $NaHCO_3$ solution. The organic phase was dried with Na_2SO_4. After removal of the solvent the crude 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside bromide was recrystallized with Et_2O to yield 4.8 g (12 mmol, 91%).

2.3.2. Cholesteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside **2**

The synthesis was accomplished by a Koenigs-Knorr reaction according to Kunz and Harreus [15]. A stirred mixture of 1.64 g (4 mmol) **1**, 1.93 g (5 mmol) cholesterol, 1.24 g (7 mmol) Ag_2CO_3 and 7.00 g molecular sieves (3 Å) in 30 ml dry Et_2O was kept under inert conditions for 9 h. The suspension was filtrated and the residue washed with dry Et_2O. Afterwards the solution was washed with 50 ml saturated $NaHCO_3$ solution and 50 ml water. The organic phase was dried with Na_2SO_4 and the solvent was evaporated. Column chromatography using 25 g silica gel and 3 l petroleum ether/acetone 12:1 and recrystallization from acetone/MeOH yielded 1.01 g (1 mmol, 35%) cholesteryl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside. M.p. 155–157 °C (acetone/MeOH). Data in accordance with [15]: yield 41%; M.p. 156–159 °C (ethanol).

2.3.3. Cholesteryl β-D-glucopyranoside **3**

18 ml of a 0.1 M solution of NaOH in MeOH was added to a vigorously stirred solution of 1.01 g (1 mmol) of compound **2** in 36 ml CH_2Cl_2/MeOH 1:1. The solution was allowed to react for 3 h at room temperature. After neutralization with about 1.8 ml 1 M HCl the mixture was treated with 50 ml H_2O in order to precipitate the reaction product, which was filtrated and washed with water to obtain 0.71 g (92%) cholesteryl β-D-glucopyranoside. Product purity was determined by GC/MS as well as NMR and can be given at a proven level of ≥98.5%.

^{1}H NMR (500 MHz, DMSO) δ 5.11 (brs, 1H, C=C*H*CH$_2$), 4.62 (m, 3H, OH-2-4), 4.19 (t, J = 5.75 Hz 1H, OH-6), 4.00 (d, J = 7.7 Hz, 1H, H-1), 3.42 (m, 1H, H-6a), 3.24 (m, 1H, H-6 cholesterol), 3.19 (m, 1H, H-6b), 2.93-2.75 (m, 3H, H3-H5), 2.67 (m, 1H, H2), 2,15 (dd, J = 13.2 Hz, 3.7 Hz, 1H), 1,90 (t, J = 11.9 Hz, 1H), 1.74 (d, J = 11.4 Hz, 1H), 1.71 (d, J = 16 Hz, 1H), 1.59 (brs, 2H), 1.57 (m, 1H), 1.56 (brs, 1H), 1.37–1.15 (m, 7H), 1.15–1.06 (m, 4H), 1.04 (s, 1H), 1.02 (brs, 1H), 1.01 (s, 1H), 0.89 (m, 6H, 3CH$_2$), 0.85 (m, 2H), 0.74 (bs, 6H, 2CH$_3$), 0.67 (d, J = 6.5 Hz, 3H, CH$_3$), 0.65 (m, 1H), 0.62 (m, 3H, CH$_3$), 0.43 (s, 3H, CH$_3$). ^{13}C NMR (75 MHz, DMSO) δ 121.7, 101.2, 77.3, 77.2, 73.9, 70.5, 61.6, 56.6, 56.0, 50.0, 42.3, 38.7, 37.3, 36.7, 36.1, 35.7, 31.9, 29.7, 28.2, 27.9, 24.3, 23.7, 23.2, 22.9, 21.1, 19.6, 19.0, 18.9, 12.1 MS (EI) compound derivatized with BSA + TMCS: m/z 369 ($C_{27}H_{45}$; 29%), 353 ($C_{26}H_{41}$; 1%), 319 ($C_{13}H_{31}Si_3O_3$; 1%), 305 ($C_{12}H_{29}Si_3O_3$; 2%), 243 ($C_{11}H_{23}Si_2O_2$; 3%), 217 ($C_9H_{21}Si_2O_2$; 24%), 204 ($C_8H_{20}Si_2O_2$; 100%), 191 ($C_7H_{17}Si_2O_2$; 7%), 189 ($C_7H_{19}Si_2O_2$; 4%), 147 ($C_5H_{15}Si_2O$; 24%), 117 ($C_4H_9Si_2O_2$; 5%) 103 ($C_4H_{11}SiO$; 11%), 73 (C_3H_9Si; 36%) Mass spectra data of the glucose moiety in accordance to DeJongh et al. [16].

2.4. Analysis of steryl glycosides

2.4.1. GC/MS conditions

Helium was used as carrier gas at a constant flow rate of 1 ml/min. 1 μl of sample was injected using a cold on column injection part. The GC temperature program was set as follows: 100 °C

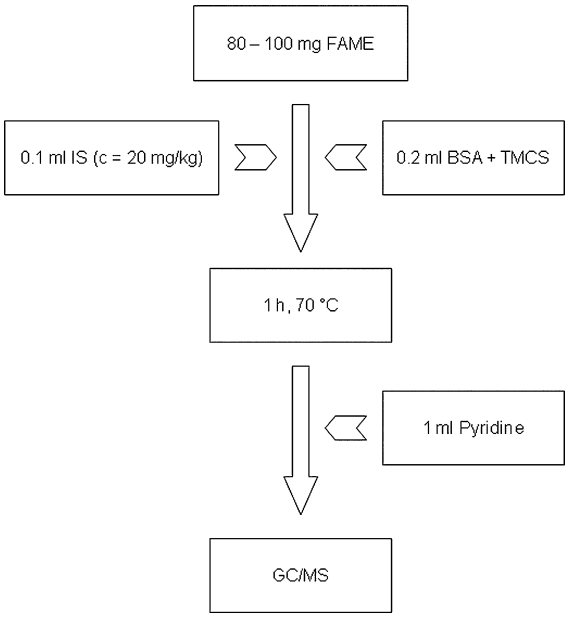

Fig. 4. Simple analytical procedure for the analysis of steryl glycosides in FAME.

hold for 2 min, ramp to 370 °C at 10 °C/min and hold for 15 min. The temperature of the MS source was set at 350 °C and the quadrupole at 180 °C. Using scan mode a mass range from 50 to 1000 *m*/*z* at 1400 scan/s was defined. In SIM mode 247, 204 and 217 *m*/*z* were used.

2.4.2. Identification of steryl glycosides in FAME

The identification of the compounds of interest was accomplished using a pure steryl glycoside mixture isolated from biodiesel as well as the synthesized internal standard **3**. Both were dissolved in 100 μl pyridine and derivatized with 200 μl BSA + TMCS as silylation reagent. The solution was allowed to react for 1 h at 70 °C and was further diluted with 1 ml pyridine, injected into the GC/MS system and detected via scan mode to determine retention times (Fig. 3a) and mass spectra for all substances. The silylation was also accomplished with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) whereby no full silylation could be achieved (Fig. 3b). Further testing with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and BSA also resulted in incomplete derivatizations. To verify a full silylation, mass spectra were recorded on a HDMS Q-TOF MS. For that, 4.7 mg (8.6 μmol) cholesteryl β-D-glucopyranoside were dissolved in 60 μl pyridine and 7 μl BSA + TMCS (27 μmol BSA) were added. The mixture was allowed to react for 1 h at 70 °C. After cooling the solution to room temperature it was diluted with 5 μl dry $CHCl_3$/MeOH 2:1. Afterwards, 1 ml of the solution was diluted with 10 ml of a 10 mM solution of NH_4Ac in dry $CHCl_3$/MeOH 2:1 and injected into the MS system.

2.4.3. Quantification of steryl glycosides in FAME

2.4.3.1. Sample preparation. The internal standard was dissolved and diluted with pyridine to obtain a 20 mg/kg solution since the amount of SG in biodiesel can be typically quantified in this concentration range, as mentioned by Lacoste et al. [10]. 80–100 mg of each sample was weight directly into a GC vial. If a liquid sample was inhomogeneous or showed some precipitates it was previously diluted with 2 ml pyridine to ensure a representative sampling. Further 100 μl of the internal standard solution and 200 μl BSA + TMCS were added and the mixture was allowed to react for 1 h at 70 °C. After cooling to room temperature the solution was diluted with 1 ml pyridine and analyzed by GC/MS SIM mode (Fig. 4).

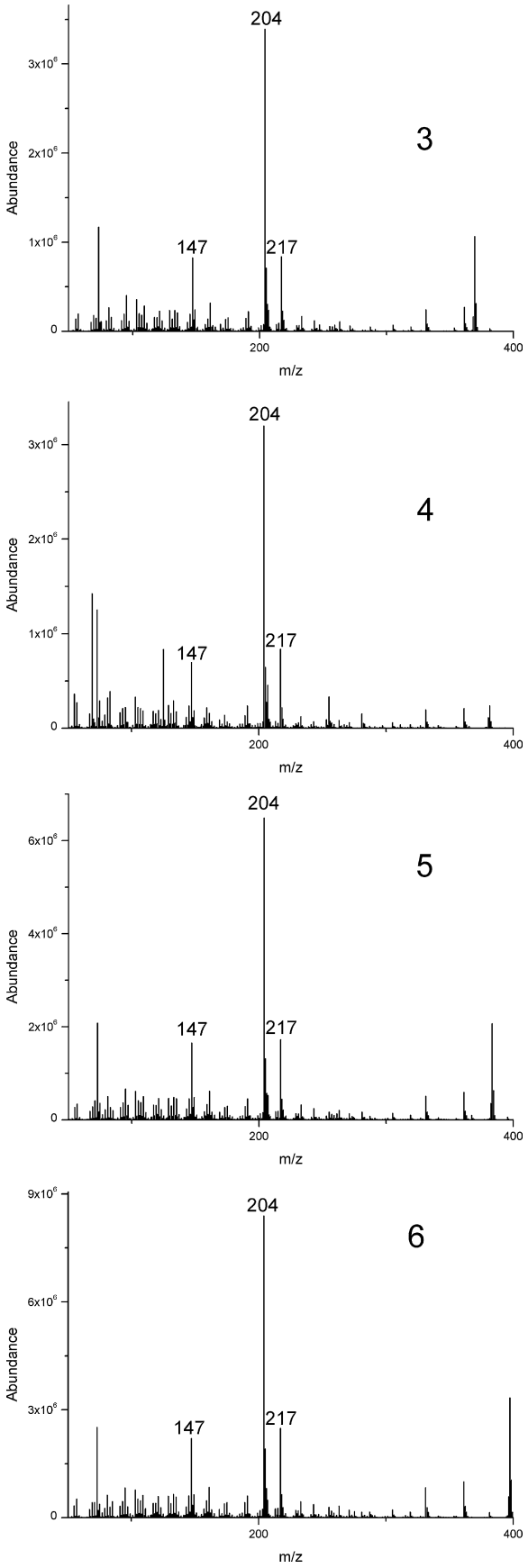

Fig. 5. Mass spectra (EI) of steryl glycosides with cholesterol **3**, campesterol **4**, stigmasterol **5** and β-sitosterol **6** as aglycone parts.

2.4.3.2. Validation. The determination of the detection limit was carried out with a steryl glycoside sample isolated from biodiesel which had been dissolved, derivatized with BSA + TMCS and measured at concentrations from 1 mg/kg downwards.

Recovery rates were defined with a distilled Soya FAME by spiking with steryl glycosides and measuring each sample three times.

Furthermore, intermediate precision was accomplished by varying days, operators and sample weights. Also, lab specific variance was evaluated. Therefore, one sample was prepared and measured five times in a row.

3. Results and discussion

Quantification is carried out using cholesteryl β-D-glucopyranoside as internal standard. The advantages of this approach are the much higher sensitivities down to 50 μg/kg coupled with simplified sample preparation and the absence of negative matrix interferences. The derivatization step has been optimized and reference SG's have been synthesized and isolated from biodiesel to guarantee unambiguous allocation. Statistical evaluation concerning intermediate precision, reproducibility, recovery, limit of determination (LOD) and quantification (LOQ) have been accomplished on reference as well as authentic natural samples.

3.1. Identification

Fig. 5 shows the mass spectra of the silylated steryl glycosides isolated from biodiesel samples and the silylated cholesteryl β-D-glucopyranoside **3**. Aglycone parts as campesterol **4**, stigmasterol **5** and β-sitosterol **6** are in accordance to results published by Bondioli et al. [9]. The pattern of **3**, **4**, **5** and **6** shows several analogies as reflected in Fig. 5.

The main correlation in all spectra is represented by the ions with a mass to charge ratio of 147, 204 and 217. These ions are characteristic fragments of the silylated glucose residue as already described by DeJongh et al. [16]. Interestingly, all glycopyranoside moieties derivatized with TMS show the presence of those specific ions. Hence it is also possible to identify SG's like sitosteryl 3-*O*-β-D-xylopyranoside, which can be found in e.g. *Bauhinia candicans* or 3-*O*-Î²-D-galactopyranosyl-stigmasta-5,25-diene, a component of marine green algae [17,18]. These additional data were combined in order to set up a quantification method in SIM mode based on those fragments.

3.2. Quantification

Due to the fact that all steryl glycoside species can be determined within a set of three ions in SIM mode (147, 204, 217 *m*/*z*) it was possible to keep the sample preparation much more simple compared to other reported methods [9,10]. Moreover it can be guaranteed that no other biodiesel components, like diglycerides (DG), triglycerides (TG) and even sterol esters are eluting in the retention range of interest as shown in Fig. 6. Quantification refers to the area sum of the individual SG species. Additionally, response factors have been determined of the different SG's and are equal to 1 (~0.9998) due to almost negligible difference of the corresponding compounds investigated. Therefore response factors are not given.

Another advantage of this method is that there is no negative impact whether the sample is homogenous or SG's are already precipitated, because in this case it is just necessary to dilute the biofuel sample with pyridine to re-dissolve all analytes. From another point of view, precipitated SG's are the main problem in sampling due to the fact that they hardly re-dissolve in the FAME matrix and therefore a representative sampling is quite difficult.

A further important requirement to obtain comparable results in this context is to guarantee complete silylation of the analyte (4 hydroxyl groups of each SG). Therefore the choice of an appropriate derivatization reagent is essential. Earlier works on this research field utilized standard derivatization reagents as BSTFA or MSTFA [9,10]. However, using these silylation chemicals in the complex biodiesel matrix no full silylation of SG's could be achieved. Reasons for that could be on one hand sterically hindered hydroxyl groups on the glucose moiety and on the other hand the attendance of multiple hydroxyl groups, like free glycerol, monoglycerides (MG) or DG in the matrix. Our experiments have demonstrated that in presence of the silylation catalyst trimethylchlorosilane in combination with the silylation reagent in major excess a fairly quantitative silylation is possible within a reasonable time (1 h at 70 °C). We could prove a complete silylation by mass spectroscopy. Our measurement on a HDMS Q-TOF showed the ammonium adduct of the fully silylated cholesteryl β-D-glucopyranoside as reflected in Fig. 7. However, it is possible that not all analyte and IS molecules are completely derivatized but the HDMS Q-TOF experiments show that more than 80% of SG are totally silylated.

Due to the fact that the retention times of quantification (SIM mode) and identification experiments (scan mode) are equal we were also able to prove a full derivatization in the complex biodiesel matrix. However, a complete derivatization is required for reproducible results and under these specific conditions this is the most crucial issue.

Based on the sensitivity and recovery results mentioned (see below) several biofuel samples have been analyzed on their SG content (Table 1). The obtained data are comparable with previously published results [10].

All values showed expected distributions for SG's in the different biodiesel samples. The low SG content of the soybean FAME

Table 1
Quantification of SG's in different biodiesel samples.

Sample	SG content (mg/kg)
Palm/soybean mixture FAME	11
Palm/soybean mixture FAME	12
Rapeseed FAME	9
Degummed soybean FAME	11
Rapeseed FAME with lower content of soybean and palm	10
Soybean FAME	4

Table 2
Recovery of SG quantification in distilled Soya FAME (sample initially contains 0.55 mg/kg).

Sample		Soybean FAME + 5.70 mg/kg SG	Soybean FAME + 9.55 mg/kg SG	Soybean FAME + 15.18 mg/kg SG	Soybean FAME + 19.10 mg/kg SG
SG content (mg/kg)	Trial 1	5.87	9.24	14.84	19.26
	Trial 2	5.63	8.20	14.65	19.15
	Trial 3	4.88	8.16	14.52	19.35
	Mean value	5.46	8.53	14.67	19.25
Recovery (%)		96	89	97	101
RSD (%)		9	7	1	1

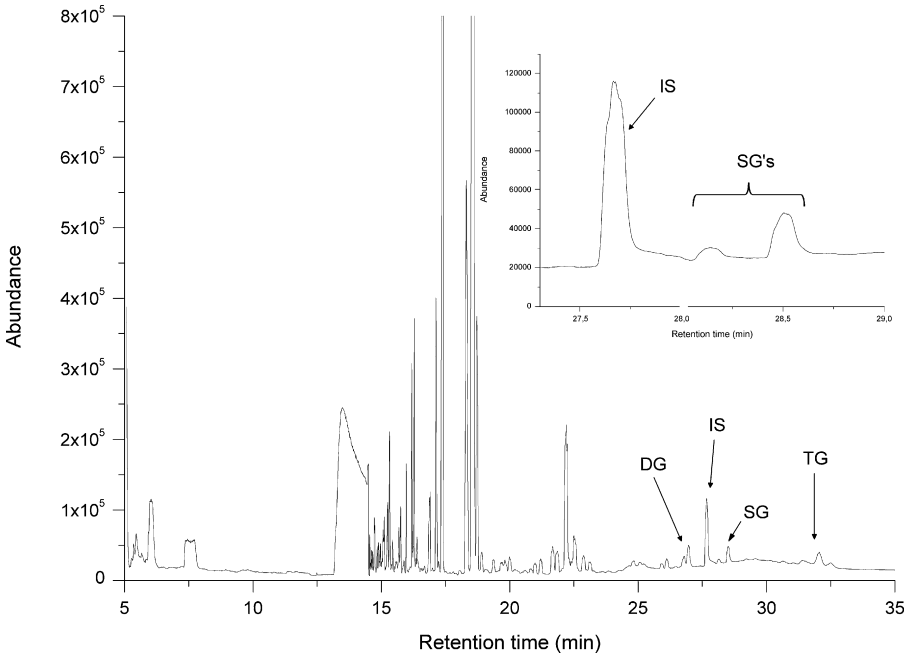

Fig. 6. Chromatogram (GC/MS-SIM) showing cholesteryl β-D-glucopyranoside (IS), steryl glycosides (SG), diglycerides (DG), triglycerides (TG) and biodiesel main components (5–25 min).

indicates that this biodiesel was the result of processing chemically refined soybean oil. This can be assumed because the content of ESG's in the oil which are then transferred into SG's during transesterification is additionally influenced by the type of refining. Especially physically refined oils are prone to have higher contents of ESG's. Rapeseed FAME shows a lower SG content as soybean and palm samples, which contain about equal amounts. Although the concentrations are low (4–12 mg/kg), it cannot be excluded that during cooling down of these samples some precipitation takes place. On the current experience contents between 15 and 20 mg/kg SG are representing the critical limits.

3.3. Validation

3.3.1. Sensitivity

The LOD for the presented method was determined in scan mode as 40 μg/kg, the LOQ of 50 μg/kg was obtained in SIM mode. Earlier gas chromatographic methods for the determination of SG's are dealing with detection limits between 10 and 15 mg/kg [9,10]. Therefore the improved sensitivity can be highlighted as big advantage of this method.

Especially for the development of process treatment steps for feedstock or biodiesel product in order to remove ESG or SG, it is

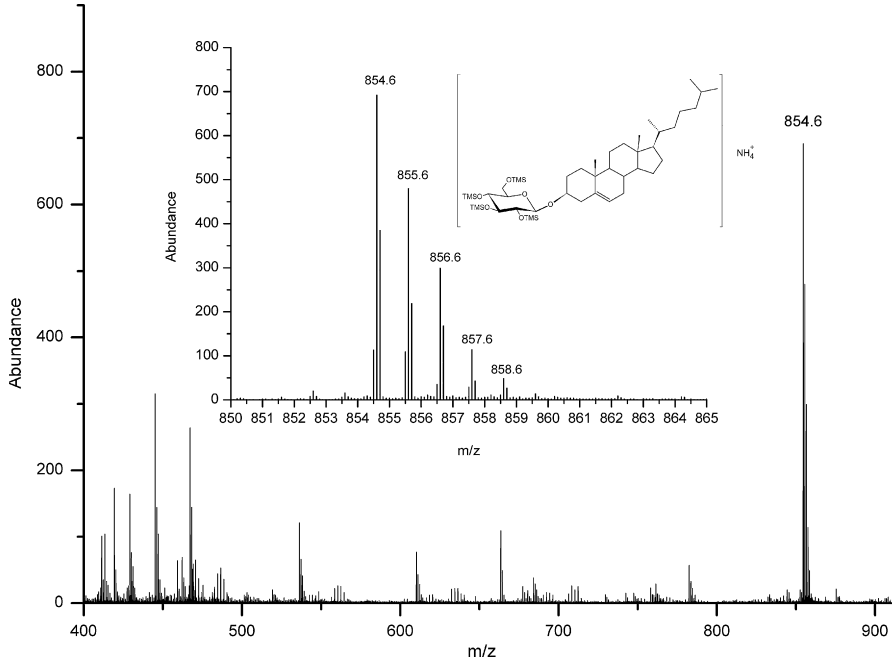

Fig. 7. ESI mass spectrum of silylated cholesteryl β-D-glucopyranoside ammonium adduct.

Table 3
Assessment of intermediate precision and variance.

	Palm/soybean mixture FAME	
Intermediate precision		
SG content (mg/kg)	Trial 1	12.22
	Trial 2	10.96
	Trial 3	11.80
	Trial 4	13.28
	Trial 5	13.02
	Mean value	12.26
	SD	0.94
	RSD (%)	7.65
Lab specific variance		
SG content (mg/kg)	Trial 1	10.96
	Trial 2	12.19
	Trial 3	12.12
	Trial 4	11.85
	Trial 5	11.15
	Mean value	11.65
	s_x^2	0.32
	SD	0.56
	RSD (%)	4.84

essential to have suitable analytical methods with very low quantification limits.

3.3.2. Recovery

The recovery rates have been identified between 89 and "101%" of SG in biodiesel (Table 2). The chosen concentrations represent the average appearance of the compounds of interest in FAME. Hence our recovery rates are improved compared with Bondioli et al. who have determined recovery rates between 71 and 88% as well as results of Lacoste et al., who showed recovery rates from 75 up to 90% [9,10].

3.3.3. Intermediate precision and lab specific variance

Our obtained data from 5 trials (Table 3) show that using our conditions reliable results could be achieved for both, precision and variance. Moreover, the values approve further evaluation of the method by round robin tests which are currently set up.

4. Conclusion

The occurrence of SG's which are formed during transesterification of oils into methyl esters can lead to poor filterability of the fuel. To analyze the content of such compounds a method has been developed based on high temperature GC/MS via SIM mode and quantification by using cholesteryl β-D-glucopyranoside as internal standard. The main advantage of this procedure is the simplified sample preparation without any additional matrix removal prior to analysis. Moreover, high recovery rates and remarkable low values for LOQ and LOD can be obtained. Furthermore, the derivatization of the SG's has been optimized using *N*,*O*-bis(trimethylsilyl)acetamide with 5% trimethylchlorosilane as silylation catalyst. Statistical evaluation of the method showed satisfiable results on variance and precision so that the method is appropriate to be further assessed by upcoming interlaboratory tests.

Acknowledgements

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