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The Identification and Quantification of Steryl Glucosides in Precipitates from Commercial Biodiesel

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Abstract There have been several discoveries of unexpected precipitates in manufacturing facilities, transport vessels, and storage tanks containing commercial biodiesel. In some cases these have been formed during storage at temperatures above the cloud point of the fuel. High performance liquid chromatography (HPLC) and mass spectrometry (MS) methods were applied to 24 field receipt samples of solids from such biodiesels. The analyses revealed the presence of steryl glucosides (SG), common phytosterols (plant sterol) found in crude soybean oil and many other plant materials, in these biodiesel precipitates. Quantitative analysis of the solids revealed SG concentrations as high as 68 wt% of the provided material (which had not been previously washed with solvent to remove entrained biodiesel). In some samples no SG were present. In others they constituted all of the non-biodiesel material present. Monacylglycerols and diacylglycerols, the products of incomplete transesterification of triacylglycerols, were also present in some samples. The normal phase and reverse phase methods described in this report could be used to analyze SG quantitatively from biodiesel precipitates with an HPLC instrument equipped with either an evaporative light-scattering detector (ELSD) or a more common UV detector operating at 205 nm.

Keywords Phytosterols · Biodiesel · HPLC · Mass spectrometry · Sterol glucoside · Steryl glucosides

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Introduction

Stimulated by the boycott of petroleum exports by the Organization of Petroleum Exporting Countries in the 1970s, research into alternatives to petroleum-based diesel fuels led to the identification of the monoalkyl esters of fatty acids, now known as 'biodiesel', as a suitable substitute. Within roughly the past decade a biodiesel industry has formed and grown rapidly in the United States, and elsewhere around the world, to the point where this fuel is now a component of the mainstream diesel fuel infrastructure in some locations. United States production is calculated to have been 225 million gallons in 2006, and is rapidly growing, while European production far surpasses that of the U.S.

As biodiesel has gone from a laboratory reagent to a generally used fuel, there have been instances of the occurrence of precipitates at various points in the chain of biodiesel production, transport and use. These have included manufacturing facilities, transportation vessels, storage tanks, and vehicle fuel filters. The latter has caused engine failure due to fuel filter plugging by solid materials that formed in the fuel after it left the site of manufacture. In some cases these have been shown to consist of acylglycerols or fatty acid alkyl esters containing high melting point (i.e., saturated) fatty acids [1]. In other instances,

Table 1 The levels of steryl glycosides and other constituents in biodiesels and biodiesel-derived solids from various points in the biodiesel production and fuel infrastructure

Sample	Supplier identifier	Identity, origin	Solid/liquid/turbid liquid (S/L/TL)	SG[a]	FAME[a]	TAG[a]	DAG[a]/sterol (co-elute)	MAG[a]	FFA[a]
1	S. Howell	Unknown	TL	68	32	0	0	0.	0
2	AURI[c] 2/06	B100	L	<1.7	n.d.[b]	n.d.	n.d.	n.d.	n.d.
3	Z[d]	B100	L	<1.7	98	0	0	0	0
4	A[d]	Solid from final biodiesel	TL	0.8	15	0	0.4	0.2	<1
5	A	Residue, final biodiesel filter	TL	3.7	98	0.3	2.1	0.94	<1
6	A	1.7% Filter residue, in biodiesel	TL	2.8	15	0.25	2.8	0.72	<1
7	W[d]	Final biodiesel polish; sock filter retentate	S	1.0	92	<0.25	0.4	0.8	<10
8	W	Residue, in-plant biodiesel transit pipe	S	21	74	<0.25	1.9	1.4	1.6
9	A	Solids from chilled B100	TL	2.0	99	0.25	1.5	0.99	<1
10	AURI 2/06	B100 storage tank bottoms	S	1.3	n.d.	n.d.	n.d.	n.d.	n.d.
11	AURI 10/06	B100 tank bottoms	S	62	30	0	0	0	0
12	F[d]	Railcar residue	TL	<0.8	65	0.25	1.7	3.3	<1
13	AURI 10/06	B20 blending tank	S	48	33	0	0	6.8	0
14	B[d]	B20 blend deposit	TL	<0.8	20	0.25	0.6	4.3	<1
15	AURI 10/06	B20 storage tank filter	S	0	5.7	0	0	7.4	0
16	AURI 2/06	Black gel, mixed tank of B100 and petrodiesel	S	52	n.d.	n.d.	n.d.	n.d.	n.d.
17	AURI 2/06	Light brown gel	S	0	n.d.	n.d.	n.d.	n.d.	n.d.
18	AURI 2/06	Brown gel	S	0.25	n.d.	n.d.	n.d.	n.d.	n.d.
19	AURI 2/06	Dark brown gel	S	2.5	n.d.	n.d.	n.d.	n.d.	n.d.
20	AURI 2/06	Black gel	S	0	n.d.	n.d.	n.d.	n.d.	n.d.
21	AURI 11/07	B100-10,000 gal storage, top	TL	0.89	n.d.	n.d.	n.d.	n.d.	n.d.
22	AURI 11/07	B100-10,000 gal storage, middle	TL	1.6	n.d.	n.d.	n.d.	n.d.	n.d.
23	AURI 11/07	B100-10,000 gal storage, bottom	TL	2.8	n.d.	n.d.	n.d.	n.d.	n.d.
24	AURI 11/07	B100-10,000 gal storage, drawdown	TL	2.8	94	n.d.	n.d.	n.d.	n.d.

Samples of precipitates were dissolved (1 mg/ml) in chloroform/methanol (85/15, *v/v*) and a 10 μl sample was injected into the HPLC. Results expressed as wt%

[a] *SG* steryl glucosides, *TAG* triacylglycerols, *DAG* diacylglycerols, *MAG* monoacylaglycerols, *FFA* free fatty acids

[b] *n.d.* not determined

[c] *AURI* Agricultural Utilization Research Institute, Marshall, MN, USA

[d] Samples from suppliers who have chosen to remain confidential

these materials were absent, yet solids still formed. Irrespective of the causative agent, there is no tolerance in the transportation industry for fuels that render an engine inoperable. It is therefore vital to the survival of the biodiesel industry that the nature of such precipitation events be identified, and methods developed to prevent them.

It has been reported that these precipitates may be steryl glucosides (SGs, also called sterol glucosides, steryl glycosides or sterol glycosides), one of several common phytosterol lipid classes found in crude soybean oil and many other plant materials [2, 3]. In addition, fatty acylated steryl glucosides are also found in vegetable oils, and would undergo transesterification during conventional biodiesel production, releasing additional SG. At least one formal description of the effects of SG solids on the filterability of biodiesel, along with a protocol for SG removal, has appeared [2]. That report [2] notes that the melting point of SGs is 240 °C, that they are insoluble in biodiesel, and that SG thus would exist as 'dispersed fine solid particles' in biodiesel. Over time, especially under static conditions, these would be expected to settle out of solution, forming the observed precipitates. They may also aggregate with other components of biodiesel, exacerbating the tendency to form precipitates. Archer Daniels Midland Co. recently filed a patent application for a method to remove these precipitates, by a cooling process and filtration using diatomaceous earth [4]. A second patent application also describes a process to remove SGs and other precipitates by cold treatment and filtrations [5].

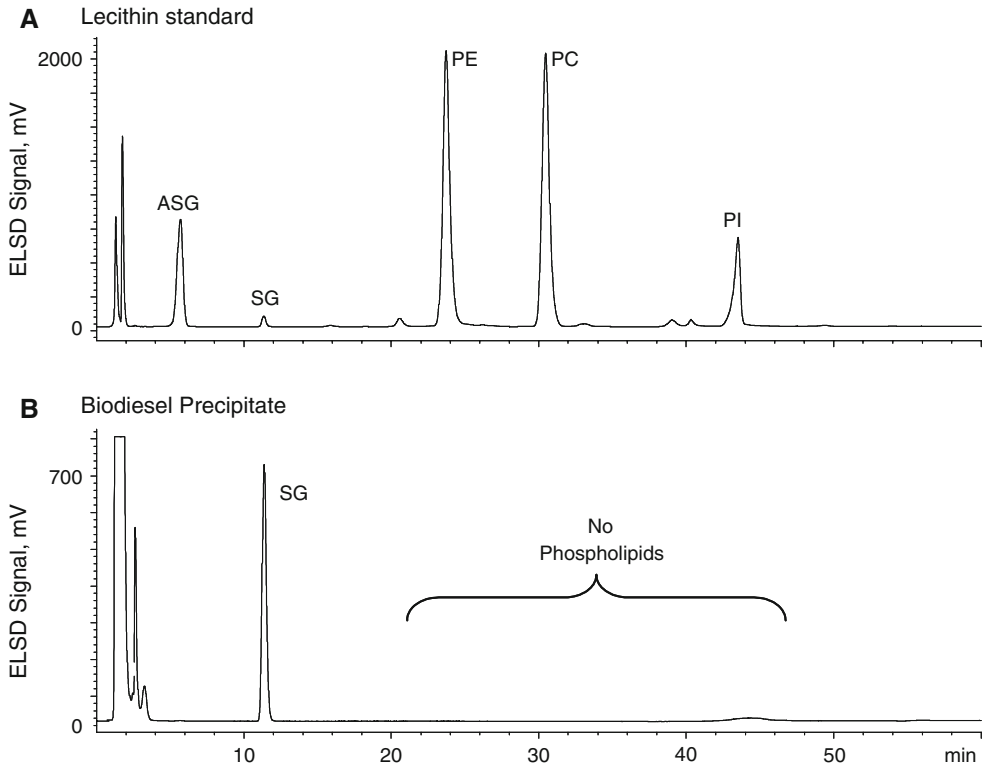

Fig. 1 Normal phase gradient HPLC (Method A) of: **a** lecithin standard and **b** biodiesel precipitate (Sample 1 from Table 1) with detection via an evaporative light scattering detector

In previous reports, thin layer chromatography (TLC) has been used for the qualitative analysis of SGs, and high temperature gas chromatography (GC) has been used for their quantitative analysis [6]. The objectives of the current study were to evaluate several high performance liquid chromatography (HPLC) and mass spectrometry (MS) methods previously developed in our laboratory to confirm the identity of SGs in these precipitates. When it was confirmed that SG was a major component in some of these precipitates, the methods were then used to quantitatively analyze the levels of SG in several precipitate samples.

Materials and Methods

Samples of biodiesel containing precipitates, or of solids isolated from biodiesel, were obtained from several providers, and originated at various locations in the biodiesel supply chain. Data on the source lipids used in the production of these biodiesels were not available. Given the preponderance of soy oil in the biodiesel feedstock stream it is most likely that these fuels were soy oil-derived. Three types of samples were received: clear liquid biodiesel, turbid liquid biodiesel, and homogeneous solid or semisolid materials. Clear liquids and solid/semisolid samples were prepared for HPLC analysis by dissolution in mobile phase solvent. Turbid liquids were centrifuged (Sorvall Model RC-3B, 5,900×*g*, 4 °C, 15 min), and the resulting pellet was dissolved in either chloroform/methanol, 85/15, *v/v*, for normal-phase HPLC or methanol/water, 96/4, *v/v*, for reverse phase HPLC. No attempt was made in any case to further purify the solids, e.g., by solvent washing to remove biodiesel and other entrained materials in advance of analysis, due to concerns that this could remove other materials of potential interest from the solids, such as triacylglycerols (TAG), diacylglycerols (DAG) and monoacylglycerols (MAG). Compositional data are expressed as wt% of the liquid phase for clear liquid samples, and as wt% of the solid phase for solid samples and for solid portions recovered from turbid liquid samples. Standards of SGs and acylated (esterified) SGs were obtained from Matreya (Pleasant Gap, PA, USA). Food grade lecithin, for use as a chromatographic standard, was obtained from the Vitamin Shoppe (North Bergen, NJ, USA). Acylglycerol standards (MAG, DAG, TAG) were obtained from Sigma Aldrich (St Louis, MO, USA). Commercial biodiesel for use as a standard was Soygold Brand (Ag Environmental Products, L.L.C., Lenexa, KS, USA). All solvents were J.T. Baker HPLC-grade (Mallinchrodt Baker Inc., Phillipsburg, NJ, USA).

Normal phase gradient HPLC analysis for SG determination (Method A) was conducted as previously described [7] using an Agilent 1100 HPLC, with autosampler, and detection by both an Agilent Model 1100 diode-array UV–

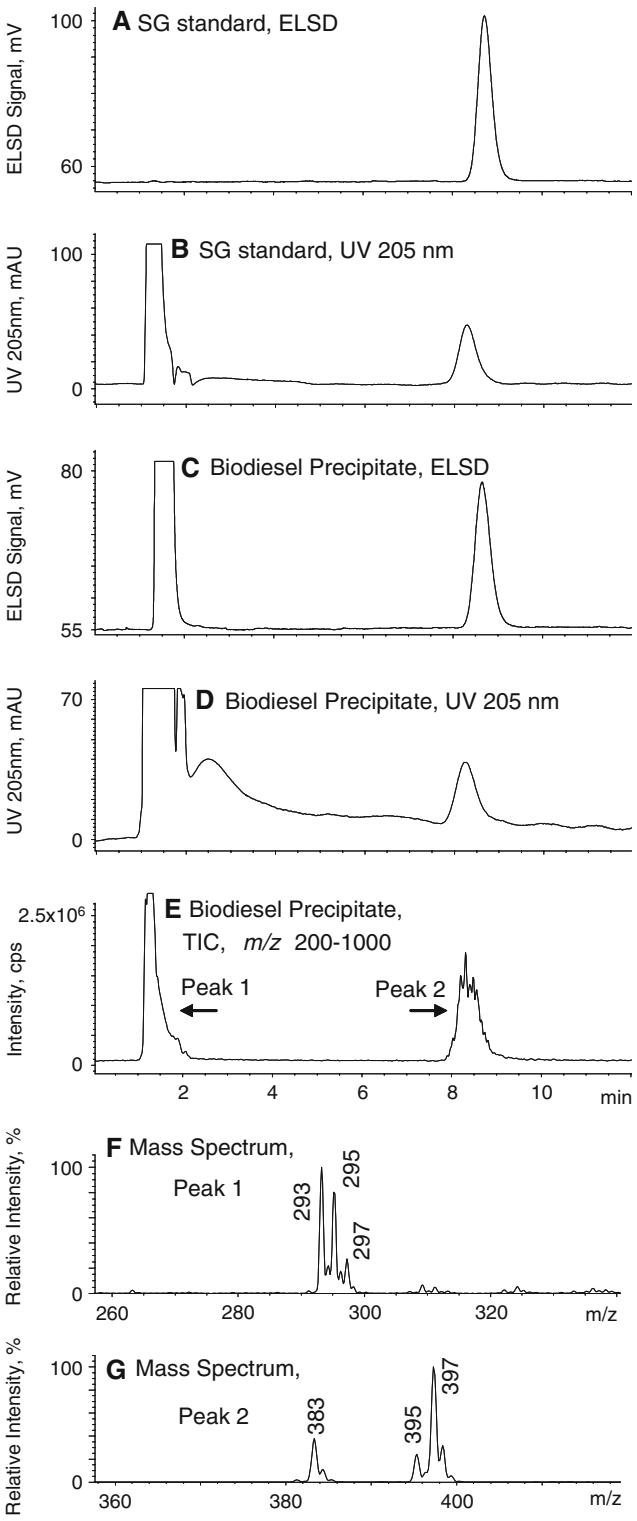

Fig. 2 Normal phase isocratic HPLC (Method B) of SG standard and biodiesel precipitates with detection via ELSD, UV 205 nm, and positive APCI mass spectrometry. **a** ELSD chromatogram of SG standard, **b** UV 205 nm chromatogram of SG standard, **c** ELSD chromatogram of a biodiesel precipitate, **d** UV 205 nm chromatogram of a biodiesel precipitate (Sample 1 from Table 1), **e** total ion chromatogram (*TIC*) *m/z* 200–1,000 of a biodiesel precipitate (Sample 1 from Table 1), **f** mass spectrum of peak 1 in **e**, **f** mass spectrum of peak 2 in **e**

visible detector operated at 205 nm (Agilent Technologies, Avondale, PA, USA) and a Sedex Model 55 evaporative light scattering detector (ELSD) (Richard Scientific, Novato, CA, USA), operated at 40 °C and a nitrogen gas pressure of 2 bars. A LiChrosorb five diol column, 3 × 100 mm, equipped with a guard column (Chrompack Polar Bonded, 2.1 × 10 mm, both from Varian, Lake Forest, CA, USA) were used at a flow rate of 0.5 ml/min. The ternary gradient consisted of: Solvent A = hexane/acetic acid, 1,000/1 (*v/v*), Solvent B = isopropanol, and Solvent C = water. Gradient timetable: at 0 min, 90/10/0 (vol %A/vol %B/vol %C); at 30 min, 58/40/2; at 40 min, 45/50/5; at 50 min 45/50/5; at 51 min, 50/50/0; at 52 min, 90/10/0; and at 60 min 90/10/0. This method separates SG and acylated steryl glucoside (ASG) from one another and from less polar materials such as glycerides and petroleum alkanes. The minimum limits of quantitative detection were about 1 μg per injection. Mass versus peak area calibration curves were constructed for the range of 1–20 μg of SG standard per injection.

Normal phase isocratic HPLC with ELSD analyses for SG determination (Method B) were conducted using the HPLC, ELSD and column described above. The isocratic mobile phase was hexane/isopropanol/acetic acid, 85/14.9/0.1 (*v/v/v*), with a flow rate of 0.5 ml/min. MS was conducted with an Agilent Model 1100 MSD, operating with an atmospheric pressure chemical ionization (APCI) chamber in the positive ion mode with the following parameters: 200–1,000 *m/z*, fragmentor 5 V, drying gas 5 l/min, nebulizer pressure 50 psi, drying gas temperature 350 °C, corona current of 4.0 μA, and capillary voltage of 4,000 V. This method separates and detects compounds with the polarities of common plant glycolipids such as SGs, glucocerebrosides and mono- and digalactosyldiacylglycerol and can be used with various detectors (ELSD, UV, or mass spectrometric detection).

The reverse phase isocratic HPLC analyses for SG determination (Method C) were with the same HPLC and MS systems as above. The column was a Prevail C18 3 μm column (2.1 × 150 mm, Alltech Associates, Deerfield, IL, USA) operated at a flow rate of 0.2 ml/min. The mobile phase was methanol/water, 96/4, (*v/v*), pre-mixed, with 20 mM ammonium formate added to the pre-mixed mobile phase. An electrospray ionization chamber was used, with the following parameters: 200–1,000 *m/z*, fragmentor 5 V, drying gas 5, nebulizer pressure 50, drying gas temperature 300 °C, and capillary voltage 5,500. This method allows resolution and detection of compounds with polarities between and including those of fatty acid methyl esters (FAME), glycolipids, and phospholipids.

Glyceride and FAME components of the samples were detected and quantified by normal phase HPLC on a silica column (LiChrosorb SI 60-5, 100 × 3 mm, Varian, Walnut

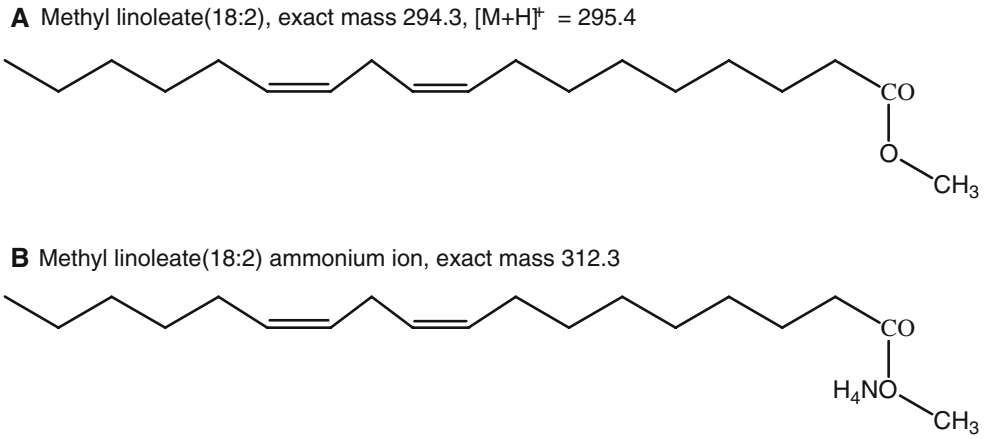

Fig. 3 Structures and ions from fatty acid methyl esters (*FAMEs*). **a** Structure of methyl linoleate, the most abundant FAME in soy biodiesel, **b** structure of the ammoniated ion of methyl linoleate

Creek, CA, USA) using a Hewlett Packard (now Agilent) Series 1050 system (Method D). The column was developed by gradients of isopropanol and water in hexane-0.6 vol% acetic acid [8]. The gradient was isocratic at 98.4/0.6/1.0/0, hexane/acetic acid/isopropanol/water, *v/v/v/v*, for the first 6 min, and then increased 39.4/0.6/51.0/9.0 at 20 min, and remained isocratic at this composition until 30 min. Analyte peaks were detected with an ELSD (Varex MKIII, Alltech Associates) operated with a nebulizer temperature of 60 °C and a nitrogen flow rate of 3.5 l/min. Peaks were quantitated by reference to standard curves constructed with known pure compounds.

Results and Discussion

Method A: Normal Phase Gradient HPLC with ELSD

A sample of biodiesel precipitate obtained from S. Howell (MARC IV Consulting, Kearney, MO, USA) (Table 1, sample 1) was analyzed with a normal phase HPLC system (Method A) optimized to quantitatively analyze plant glycolipids and phospholipids [7] using ELSD (Fig. 1). The standard, a sample of a food grade lecithin supplement, was found to have two glycolipid peaks (ASG and SG) and three major peaks of phospholipids (phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol), all identified by having identical retention times with those of commercial standards. When the biodiesel precipitate sample was analyzed in this HPLC system, only two peaks were observed, a broad peak eluting at about 2 min that was shown by comparison with commercial biodiesel to consist of FAME, and a sharp peak eluting at about 11 min, a retention time equal to that of the SG peak in lecithin and to authentic SG standard (chromatogram not shown). No peaks were observed in the region of 20–45 min, indicating that there were no phospholipids in the biodiesel precipitate sample.

Method B: Normal Phase Isocratic HPLC with ELSD or APCI MS

When unknown samples are analyzed via HPLC with either ELSD or UV detections and they have peaks eluted at the same retention times as standards, it is generally assumed that they are the same analytes. However, it is preferable to employ other identification methods, such as MS, to confirm the chemical structures of the components in peaks of new samples, since different compounds can have the same retention times. Therefore, in the next experiment normal phase HPLC-MS was used to try to confirm the identity of the peak in this biodiesel precipitate sample (Table 1, sample 1) as SG (Fig. 2). Because no phospholipids were observed in the previous experiment, it was decided that isocratic conditions could be used to shorten the time needed for the separation of SG. With the isocratic method employed here (Method B), the SG standard eluted at about 9 min. The biodiesel precipitate also contained a prominent peak of putative SG that eluted at the same time. In addition to being detected via ELSD (Fig. 2a, c), the 9-min peaks in both the SG standard and the biodiesel precipitate were also detected via UV detection at 205 nm (Fig. 2b, d), a wavelength used to detect carbon–carbon double bonds, such as are found in phytosterols.

When the peaks generated by this HPLC analysis of the biodiesel precipitate were analyzed via APCI MS (Fig. 2e), scanning in the range of 200–1,000 *m/z*, two peaks were observed in the total ion chromatogram (TIC), one at about 2 min and one at about 9 min. The mass spectrum of the peak at 3 min revealed that it contained three major ions (293, 295, and 297 *m/z*), corresponding to FAMEs of linolenic, linoleic, and oleic acids, respectively (Figs. 2f, 3). It is probable that these are common methyl esters, e.g., biodiesel, consistent with the fact that the precipitate material was obtained by centrifugation of a biodiesel and analyzed without further washing of the derived pellet. The

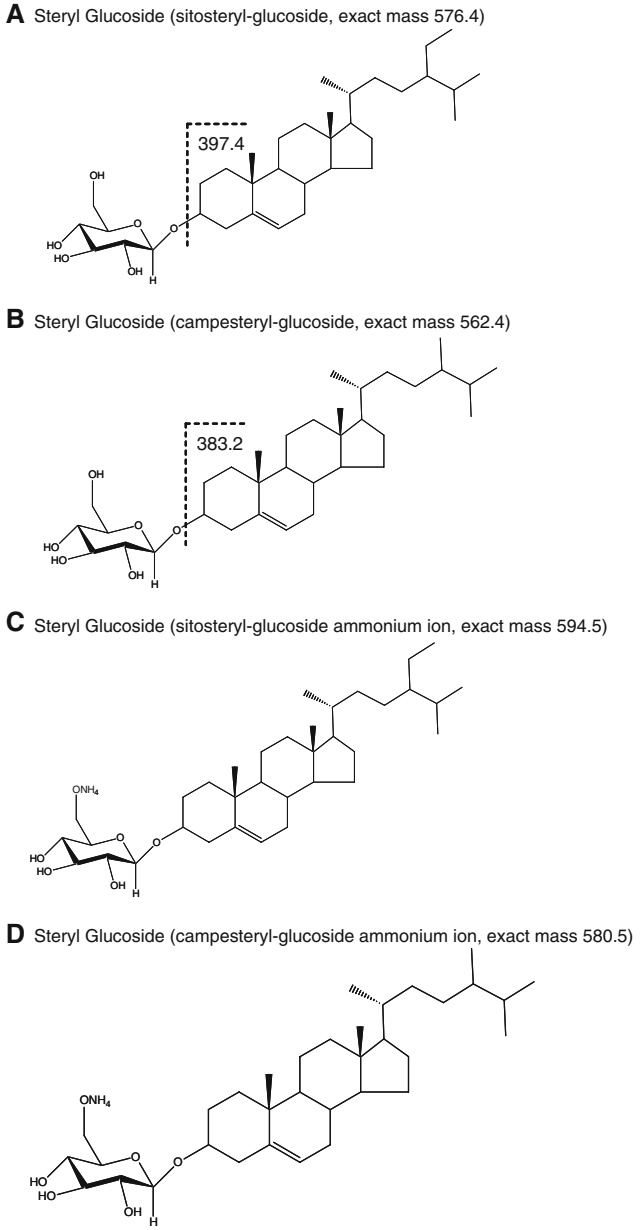

Fig. 4 Structures and ions from steryl glucosides. **a** Structure and probable fragmentation of sitosteryl-glucoside, **b** structure and probable fragmentation of campesteryl glucoside, **c** structure of ammoniated ion of sitosteryl-glucoside, **d** structure of the ammoniated ion of campesteryl glucoside

mass spectrum of the peak at 9 min revealed that it contained three distinct ions (383, 395, and 397), corresponding to the sterol fragment from campesterol, stigmasterol, and sitosterol, respectively (Figs. 2g, 4). We [7] and others [9] have previously reported that when phytosteryl conjugates are analyzed via APCI MS, no molecular ion is observed, and instead one observes major ions for the sterol moieties with the glucose fragmented at the ether bond. As reported above, this fragmentation was observed with these samples.

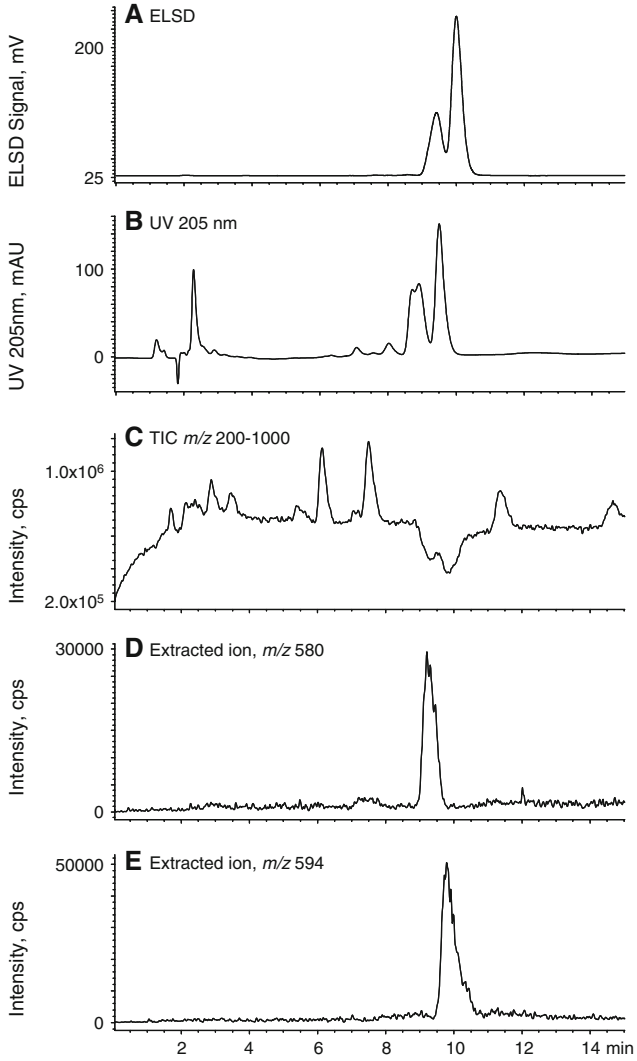

Fig. 5 Reverse phase isocratic HPLC (Method C) of SG standard with detection via ELSD, UV 205 nm and positive electrospray ionization mass spectrometry. **a** ELSD chromatogram of SG standard, **b** UV 205 nm chromatogram of SG standard, **c** total ion chromatogram (*TIC*) *m/z* 200–1,000, of SG standard, **d** extracted ion chromatogram (*EIC*) of *m/z* 580, representing the ammoniated ion of campesteryl-glucoside (see Fig. 4d), and **e** extracted ion chromatogram (*EIC*) of *m/z* 594, representing the ammoniated ion of sitosteryl-glucoside (see Fig. 4c)

Method C: Reverse Phase Isocratic HPLC with ELSD, UV 205 nm or MS (Electrospray Ionization)

Analysis of the SG standard via reverse phase HPLC revealed that it contained two major peaks using ELSD (Fig. 5a) and UV 205 nm (Fig. 5b) detections. The bigger peak eluted at about 10 min and the smaller at about 9 min. Based on the ELSD peak areas, the proportion of the two SG molecular species were approximately 20% campestery-glucoside (Fig. 4d) and 80% sitosteryl-glucoside (Fig. 4c), similar to the 1:4 ratio reported previously in bell

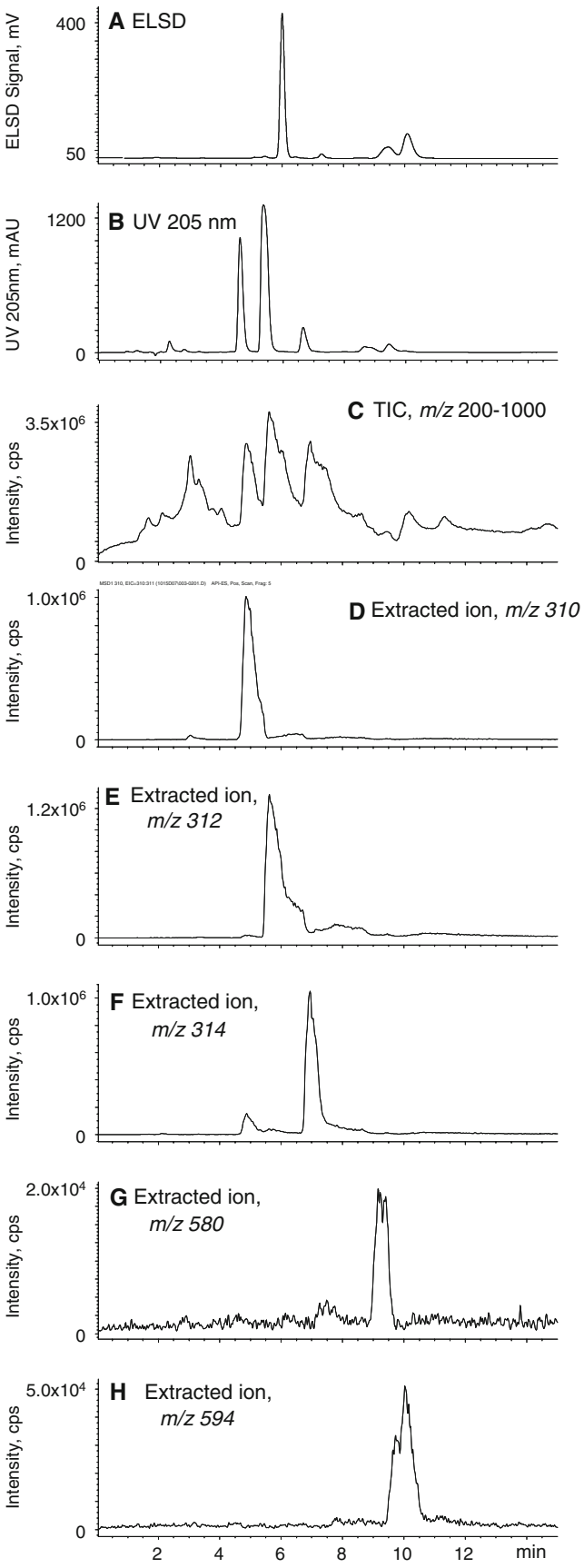

Fig. 6 Reverse phase isocratic HPLC (Method C) of biodiesel precipitate with detection via ELSD, UV 205 nm, and positive electrospray ionization mass spectrometry. **a** ELSD chromatogram of a biodiesel sample, **b** UV 205 nm chromatogram of a biodiesel sample, **c** total ion chromatogram (*TIC*) *m/z* 200–1,000 of a biodiesel sample, **d** extracted ion chromatogram of *m/z* 310, representing the ammoniated ion of methyl linoleate, **e** extracted ion chromatogram of *m/z* 312, representing the ammoniated ion of methyl linoleate, **f** extracted ion chromatogram of *m/z* 314, representing the ammoniated ion of methyl oleate, **g** extracted ion chromatogram of *m/z* 580, representing the ammoniated ion of campesteryl-glucoside, and **h** extracted ion chromatogram of *m/z* 594, representing the ammoniated ion of sitosteryl-glucoside

pepper [10]. When the molecular species of SG in the standard were analyzed using electrospray ionization LC-MS (Fig. 5c), the TIC did not show distinct peaks in the 9–10 min ranges (negative peaks), but the extracted ion of 590 *m/z* confirmed that the smaller peak at 9 min (Fig. 5d) had the same mass as the ammonium adduct of campesteryl-glucoside. Similarly, the extracted ion of 594 *m/z* confirmed the major peak at 10 min (Fig. 5e) was the ammonium ion of sitosteryl-glucoside.

When the biodiesel precipitate (Table 1, sample 1) was analyzed with reverse phase HPLC coupled with electrospray ionization MS, putative SG peaks were also observed at 9 and 10 min (Fig. 6). Interestingly, the ELSD chromatogram revealed an additional major peak at 6 min (Fig. 6a). The UV 205 nm chromatogram (Fig. 6b) and MS TIC (Fig. 6c) revealed three major peaks at about 4.5, 5.5 and 6.5 min. By examining their extracted ions (Fig. 6d–f) these three peaks were identified as ammonium ions of the three common 18-carbon fatty acid FAMEs linolenate, linoleate (Fig. 3b), and oleate, respectively. This indicates the presence of included biodiesel in this sample obtained by centrifugation from a turbid biodiesel liquid sample. It is probable that some or all of these three FAME were nebulized and not detected with the ELSD (Fig. 3a) but were detected in the UV 205 nm (Fig. 3b) and TIC-MS chromatograms (Fig. 3c). We previously demonstrated that FAME were almost completely nebulized with a Sedex ELSD operated at a nebulizer temperature of 40 °C, the same temperature used in the current study [11]. Similarly, like the SG standard (Fig. 5) the peaks at 9 and 10 min were identified as campesteryl-glucoside and sitosteryl-glucoside, respectively (Fig. 6g, h), because their extracted ions corresponded to the mass of the ammonium ion of these molecular species (Fig. 4c, d).

The reverse phase methods have shown that steryl-glucosides are present in the biodiesel precipitate examined, and that they can be separated, detected and identified. We decided to evaluate our normal-phase isocratic HPLC-ELSD method (Method B) as in Fig. 2 as a convenient

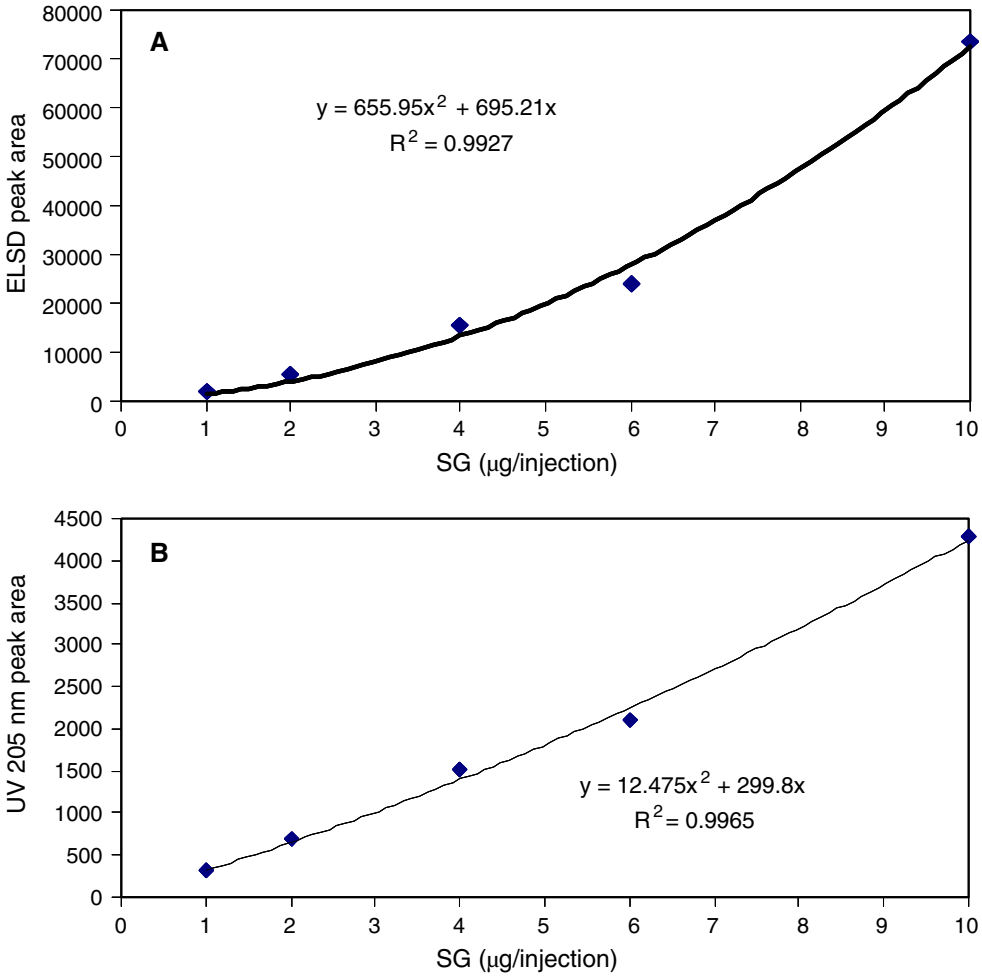

Fig. 7 Calibration of ELSD and UV 205 nm peak areas (using Method B) versus mass of SG

method to quantitatively analyze the levels of steryl-glucosides in the precipitates isolated from a number of field-receipt biodiesel samples. Calibration curves (quadratic, $R^2 > 0.99$) with a range of 1–10 μg SG were constructed using detection by both ELSD (Fig. 7a) and UV at 205 nm (Fig. 7b). Although the ELSD detection was more sensitive (resulted in larger peak areas), UV detection was also very good in this range of masses (and provided higher R^2 than ELSD). The remainder of the reported analyses were conducted using the mass versus ELSD area calibration curve (Fig. 7a). For those laboratories that need to analyze SGs and only have a UV detector, Fig. 7b suggests that UV detection can be used for the accurate quantitative analysis.

Using the normal-phase isocratic HPLC-ELSD method (Method B) and the calibration curve in Fig. 7a, we analyzed the levels of SG in samples of biodiesel, or solids from biodiesel, obtained from several providers, and from a variety of locations in the fuel supply chain (Table 1). Unfortunately, these were not generated in controlled experiments, and little is known of the handling conditions that led to formation of these solids. They do, however, represent a cross-section of the kinds of interfering materials found in the modern biodiesel production, storage and distribution system. As, mentioned above, the detection limits of SG in our HPLC method are about 1 μg per injection, which translates to about 100 ppm. Because there is evidence that 50 ppm or less of SG in biodiesel is enough to cause filtration problems under some conditions (1), our method is useful for quantifying SG in precipitates but it is not sufficiently sensitive to analyze SG at the lowest levels of interest in biodiesel samples.

The chromatographic methods employed here are also able to detect and quantify fatty acylated steryl glucosides. Using either Method A or Method B the retention time of ASG is about 4–5 min. One would expect any ASG present in feedstock oil to be converted to SG during the transesterification reaction of biodiesel production.

The SG content of biodiesel-derived solids from various locations in the production/distribution chain ranged from <0.8 to 68 wt% (Table 1). Filtration is sometimes employed in an attempt to reduce the SG levels of biodiesel, and solids retained on such filters contained SG.

Levels were on the order of 1–4 wt% of the recovered material (Table 1, no. 5–7). FAME, presumably entrained in the solids, predominated in these solids. However, untransesterified partial glycerides (mainly MAG or DAG) in amounts comparable to the levels of SG were also detected in these precipitates.

In a number of samples the SG content of the solid was low, and some lacked SG altogether (no. 15, 17, 20). Thus, SG is not the only species comprising solids isolated from biodiesels, and in some cases SG may not be a component of these materials. MAG, especially those containing saturated fatty acids, are known also to cause precipitation problems in biodiesel. Significant amounts of MAG were found in two samples (no. 13, 15), both from B20 blending and storage operations. The addition of (non-polar) diesel fuel during production of B20 may hasten precipitation of these relatively polar lipids from the mixture. In one of these samples (no. 13), the solid also contained a substantial portion of SG, while SG was absent from the other sample (no. 15). In other samples the non-SG and non-fuel components of the solid could not be identified with the methods employed here.

Sample no. 8 contained 21 wt% SG, with the balance consisting largely of FAME (Table 1). This material was recovered from a pipe used to move finished biodiesel. After an extended period of continuous use the inside of the pipe became substantially occluded by accumulated solids. Regular maintenance may be required to alleviate problems due to system blockage. It is also possible that slow flow through an ambient temperature system may represent a strategy for the removal of precipitated SG from a biodiesel.

Chilling of biodiesel (final temperature and duration unspecified) produced a solid material containing 2 wt% SG (Table 1, sample 9). Although the balance of the material was largely FAME, minor amounts of TAG, DAG and MAG were also present. These compounds may interact to potentiate the formation of biodiesel solids.

There was notable heterogeneity in the compositions of the materials, even those isolated from similar locations. Thus, in some cases (no. 11) the sludge at the bottoms of biodiesel storage tanks contained substantial amounts of SG (62%), while in others (no. 10) little SG was found in this location. One cannot make a general conclusion that all solids from biodiesel systems contain high levels of SG (no. 16–20). An interesting insight into the stratification of solids that can occur in a bulk storage tank containing biodiesel is presented by samples 21–24, which show increasing SG content in samples taken from increasing depths in a tank of B100.

In summary, we have demonstrated the utility of several state-of-the art forms of HPLC and MS in detecting SGs in precipitates from biodiesel. Using HPLC with APCI MS, we were able to confirm that the precipitates contained sterols. Then using reverse phase HPLC with electrospray MS we were able to identify molecular ions of two major "molecular species" SGs, sitosteryl glucoside and campesteryl glucoside. We have also found that a rapid (10 min), simple normal phase isocratic HPLC method (Method B), with detection by either ELSD or UV, can be used to quantitatively analyze the levels of SG in biodiesels and solids formed in biodiesels.

There is no tolerance for solids-related fuel system failures among users of diesel engines. This work has established the presence of SG in some, but not all, solids found at a number of points in the biodiesel handling chain. The data suggest that SG-containing solids can form at virtually any point after a biodiesel cools down following transesterification. The amounts of SG in these precipitates can be quite variable, with some lacking detectable SG while in others SG are the only detectable non-fuel components of the material.

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