

Evaluation of ASTM D6584 Method for Biodiesel Ethyl Esters from Sunflower Oil and Soybean/Tallow Mixture and for Biodiesel Methyl Esters from Tung Oil and Soybean/Tung Mixture

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Esse estudo descreve pela primeira vez o uso do método ASTM D6584 para a determinação de glicerol e glicérides em amostras de ésteres etílicos de biodiesel obtidas a partir de óleo de girassol e da mistura de óleo de soja/sebo; e amostras de ésteres metílicos de biodiesel produzidas a partir da mistura de óleo de soja/tungue e de óleo de tungue. Os resultados mostraram que o método ASTM D6584 pode ser empregado para outras fontes de biodiesel, as quais representam uma alternativa para a produção de biodiesel, principalmente no Brasil.

This study describes for the first time the determination of glycerol and glycerides in samples of biodiesel ethyl esters from sunflower oil and soybean/tallow mixture and biodiesel methyl esters from soybean/tung mixture and tung oil by employing ASTM D6584 method. The results showed that ASTM D6584 can be used for other sources of biodiesel which represent an alternative to biodiesel production, mainly in Brazil.

Keywords: biodiesel ethyl and methyl esters, by-product contaminants, gas chromatography

Introduction

Biodiesel is obtained mainly by basic catalytic transesterification of fatty substances, oils and fats, with short chain alcohols, such as methanol and ethanol.^{1,2} It can be used as a substitute for petrodiesel and as an additive to fossil fuels.³ Therefore, the use of the biodiesel as fuel is promising due to advantages such as biodegradability, reduction of most regulated exhaust emissions, higher lubricity and flash point.

In Brazil, soybean oil is the main raw material used in the biodiesel production.⁴ Besides, other biodiesel sources such as castor oil, palm tree, sunflower, babassu, peanut, animal fat, waste cooking oil and microalgae have also been used.⁵⁻⁸

Non-edible vegetable oils, which are mostly produced by seed-bearing trees and shrubs, could be an alternative to common raw materials. Non-edible oil crops, which can be grown in large scale on non-cropped marginal lands and wastelands, are considered to be the best feedstock for biodiesel because they do not affect the edible oil market. For

example, tung oil is a promising raw material for biodiesel production.⁹ Another example that does not compete with edible oil market and it can also be used as feedstock for biodiesel production is the tallow. It is a residual material from slaughterhouses with a large annual production.¹⁰

Biodiesels from oils with high degree of unsaturation, such as tung oil, have low oxidative stability and care should be taken in the handling and storage process.¹¹ Studies indicated that biodiesel from tung oil showed excellent cold temperature behavior, but its viscosity, fatty acid methyl esters (FAME) content, and oxidation stability did not satisfy EN 14214.⁹ However, these properties of biodiesel from tung oil can be improved by mixing it with other biodiesels such as biodiesel from soybean oil.¹² On the contrary, animal fat with high degree of saturated fatty acid, such as beef tallow, has good oxidation stability and poor low temperature properties.⁹ In this case, the mixture with biodiesel from soybean oil also can be a solution.

In this study, biodiesel ethyl esters from soybean/tallow mixture, and sunflower oil; and biodiesel methyl esters from soybean/tung oil were produced. Due to its importance,

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the presence of by-product contaminants, such as glycerol, mono-, di- and triglycerides in the biodiesel produced was verified. The reference methods for the determination these by-product contaminants in biodiesel methyl esters are ASTM D6584 and EN 14105 methods. This article is a continuation of a previously published study by our group.¹³ The aim of our research is the magnification of samples of biodiesel that could be analyzed by ASTM D6584 and EN 14105 methods, since these methods are indicated for the biodiesel methyl esters from rapeseed oil and biodiesel, which have similar chemical composition as the studied ones.

To the best of our knowledge, studies of the applicability of the ASTM D6584 method for the determination of by-product contaminants in biodiesel ethyl esters from soybean/tallow 80:20 (m/m) mixture and sunflower oil; and in biodiesel methyl esters from soybean/tung oil mixture and tung oil have not been published yet. Therefore, the aim of this study was to verify the applicability of the ASTM D6584 for these biodiesels, since the ASTM D6584 method is indicated only for biodiesel methyl esters from rapeseed, sunflower, soybean and used frying oils.

Experimental

Analytical standards and reagents

Glycerol (99.5%), 1-mono[*cis*-9-octadecenoyl]-*rac*-glycerol (monoolein) (99%), 1,3-di(*cis*-9-octadecenoyl) glycerol (diolein) (99%), 1,2,3-tri(*cis*-9-octadecenoyl) glycerol (triolein) (99%), 1,2,3-tridecanoylglycerol (tricaprin) (99%), 1-monohexadecanoyl-*rac*-glycerol (monopalmitin) (99%), 1-monooctadecanoyl-*rac*-glycerol (monostearin) (99%), 1-([*cis,cis*]-9,12-octadecadienoyl)-*rac*-glycerol (monolinolein) (99%) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) standards were purchased from Sigma-Aldrich (USA). (*S*)-(-)-1,2,4-butanetriol was bought from Fluka (99.5%) (USA). The solvents pyridine and heptane, chromatographic grade, were supplied by J.T. Baker (USA).

Standard solutions and sample preparation

Stock solutions with 0.5 mg mL⁻¹ glycerol, 5 mg mL⁻¹ glycerides, 1 mg mL⁻¹ (*S*)-(-)-1,2,4-butanetriol and 8 mg mL⁻¹ tricaprins were prepared in pyridine. Different volumes of these solutions were used for the preparation of standard mixtures. For the samples, 100 µL of the (*S*)-(-)-1,2,4-butanetriol stock solution and 100 µL of the tricaprins stock solution were added to 100 mg of sample. The standard mixtures and samples were silylated with 100 µL MSTFA and, after 20 min, 8 mL *n*-heptane were added.

Quantification of free and total glycerol, mono-, di- and triglycerides in biodiesel

The content of these contaminants in the samples was determined according to ASTM D6584 and for samples from soybean/tung oil mixture and tung oil, glyceride peaks related to α -eleostearic acid (C18:3c) were also taken into account.

Analytical curves were constructed for glycerol, monoolein, diolein and triolein using the peak areas. The analytical curve obtained from monoolein allowed the quantification of all monoglycerides; and the analytical curves from diolein and triolein allowed the determination of the diglycerides and triglycerides, respectively.

The quantification of total glycerol was made by the Equation 1:

$$\text{Total glycerol \% (m/m)} = \text{free glycerol} + 0.2591 \text{ monoglycerides} + 0.1488 \text{ diglycerides} + 0.1044 \text{ triglycerides} \quad (1)$$

Chromatographic analyses

The chromatographic analyses were carried out using a Shimadzu GC-2010 gas chromatograph, equipped with on-column injector, technique of simple on-column injection and flame ionization detector. An SGE HT-5 capillary column (25 m × 0.32 mm i.d., 0.1 µm film thickness) was used for the GC separation (Ringwood, VIC, Australia). Injection volume of 1 µL, hydrogen as carrier gas with linear velocity of 50 cm sec⁻¹ and flame ionization detector at 380 °C were used. The column oven temperature program was 50 °C (1 min hold), 15 °C min⁻¹ up to 180 °C, 7 °C min⁻¹ up to 230 °C; 30 °C min⁻¹ up to 380 °C (15 min hold); on-column injector with direct injection mode and temperature at 70 °C (1 min hold), 20 °C min⁻¹ up to 380 °C (10.31 min hold).

Analytical curves and applicability of the method

The analytical curves were prepared according to ASTM D6584. The linear ranges for the analytes were: glycerol 0.005 to 0.05%; monoolein 0.1 to 1%; diolein and triolein 0.05 to 0.5% (m/m).

ASTM D6584 was applied to samples of biodiesel produced in the Organic Chemistry laboratories at the Federal University of the Rio Grande. The samples were: biodiesel ethyl esters from soybean/tallow 80:20 (m/m) mixture and sunflower oil; and biodiesel methyl esters from soybean/tung mixture and tung oil. The experimental protocol used in the production of biodiesel was based on

the literature. In the process of alkaline alcoholysis studies reported that mostly 6:1 or 9:1 alcohol:oil molar ratio and 0.5 to 1.5% catalyst were used.^{14,15} In the esterification reaction it is common to employ 10% catalyst.¹⁶

The production of ethyl esters from soybean/tallow (m/m) mixture was performed by esterification and transesterification reactions. The esterification process was performed for 3 h at 60 °C. In this stage, 10% catalyst (H₂SO₄) and the molar ratio ethanol:oil of 80:1 were used. Afterwards, sodium carbonate was added to neutralize the catalyst and the mixture was subjected to a rotary evaporator to remove the excess of alcohol. The resulting mixture was washed with hexane and warm water and the hexane was evaporated. Then, the mixture was transesterified during 1 h at 60 °C with an ethanol:oil molar ratio of 6:1 and 1% catalyst (NaOH). After that, the solvent was evaporated, the mixture was centrifuged and decanted and the biodiesel was separated from the glycerol.

A mixture of sunflower oil and ethanol, 1:6 molar ratio, and sodium hydroxide 1% m/m (based on oil) was heated at 60 °C for 1 h. After the reaction was complete, concentrated sulfuric acid was added on pot and the reaction temperature was kept for 0.5 h.¹⁷ After the precipitation of the salt (Na₂SO₄), the solvent was evaporated, the mixture was decanted and the biodiesel was subjected to esterification. The ethyl ester phase containing free fatty acids was esterified using 60:1 and 80:1 (alcohol:free fatty acids) molar ratios and concentrated sulfuric acid 5 and 10% m/m (based on fatty acids) at 60 °C for 2 h. When the reaction was complete, fatty acid ethyl esters were purified by washing with potassium carbonate and distilled water to remove residual inorganic salts, glycerol and alcohol, dried and taken to the storage tank.

Reactions for the production of methyl esters from soybean/tung oil mixture and tung oil were performed by using 100 g raw material, with 0.5, 1 and 1.5% catalyst (NaOH) for blended samples and 0.5% for pure oil samples. The molar ratio methanol:oil was 6:1 and 9:1 for blended samples and 6:1 for pure oil samples at 60 °C for 90 min, under constant magnetic stirring and reflux. After decanting for 24 h, the biodiesel was separated from the glycerol. Then, the biodiesel was subjected to washing and drying with magnesium sulfate.

Results and Discussion

Identification and quantification of the glycerides and free glycerol

The elution order of the glycerides in the conditions proposed by the ASTM D6584 method is related with the

carbon number and those that have double bonds and the same carbon number coelute.

The saturated and unsaturated ones that possess the same carbon number are separated; the unsaturated ones elute first. The glyceride peaks related to α -eleostearic acid (C18:3c) from tung oil, due to the presence of three conjugated double bonds (at carbons 9 *cis*, 11 *trans*, 13 *trans*), elute after the other glycerides.

In this study, monoeleostearin was identified as a peak of higher intensity than the one of the other monoglycerides; elution should be subsequent to the other monoglycerides; and peak should be absent in the chromatogram of the sample of biodiesel methyl esters from soybean oil.¹³ This is because the analytical standard of glycerides from eleostearin is not available.

Figure 1 shows the identification of the monoglycerides. The retention times of diglyceride and triglyceride bands were established from 22 to 22.4 min and from 28 to 30 min, respectively. Free glycerol was identified with $t_R = 5.4$ min.

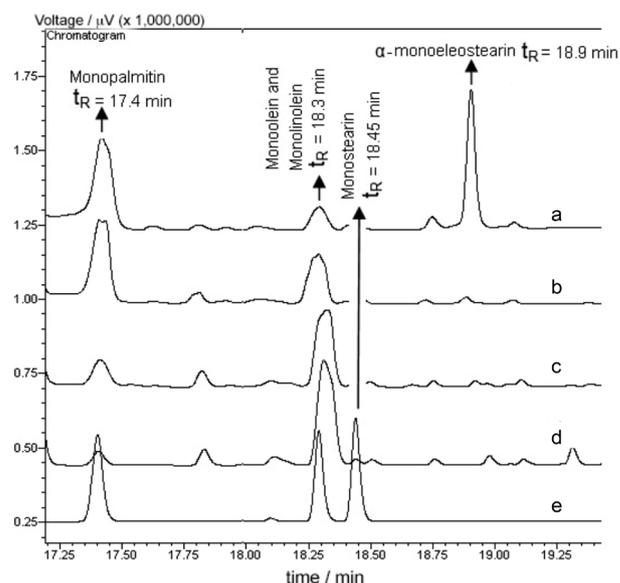


Figure 1. Chromatographic profile of monoglyceride band for biodiesel methyl esters from tung oil (a); sample of biodiesel methyl esters from soybean/tung oil 75:25 (m/m) mixture (b); sample of biodiesel methyl esters from soybean/tung oil 90:10 (m/m) mixture (c); sample of biodiesel methyl esters from soybean oil (d); and standard monopalmitin, monoolein, monolinolein mixture at third level of concentration (e), under the analysis conditions of the ASTM D6584 method.

Analytical curves and applicability

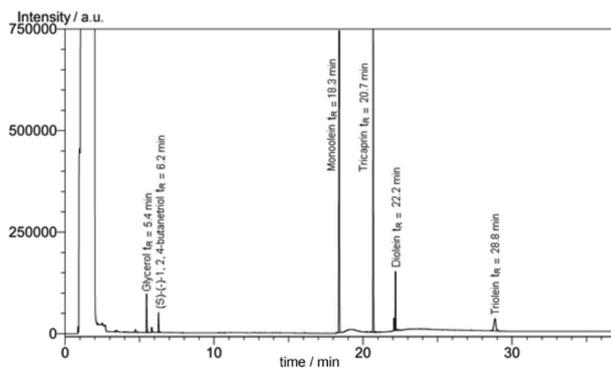
The method presented r values > 0.999 for all compounds, resulting in excellent linearity. Figure 2 shows the chromatographic profile of the mixture of the standards under the analysis conditions of the ASTM D6584 method.

Free glycerol, monoglycerides, diglycerides and total glycerol were determined in biodiesel ethyl esters from

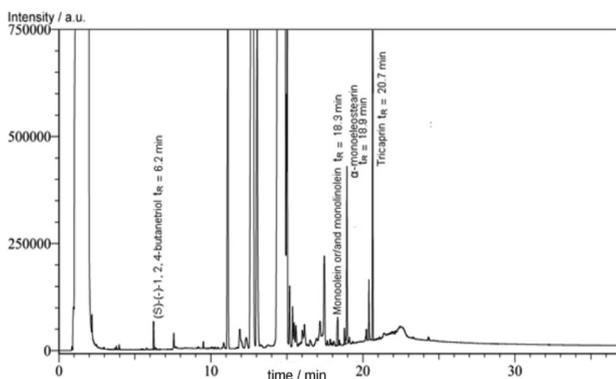
Table 1. Concentrations for free and total glycerol, mono-, di- and triglycerides in several biodiesel samples

Biodiesel	Reaction conditions	Concentration / %, m/m				
		Free glycerol	Monoglycerides	Diglycerides	Triglycerides	Total glycerol
Sunflower ^a	1% catalyst ^c and molar ratio ^d of 6:1	< 0.005	0.130	0.082	0.013	0.047
Soybean/tallow 80:20 ^b	Esterification process: 10% catalyst ^c and molar ratio ^d of 80:1; Transesterification process: 1% catalyst ^c and molar ratio ^d of 6:1	0.07	1.641	1.189	0.213	0.694
Tung ^a	0.5% catalyst ^c and molar ratio ^f of 6:1	< 0.005	0.694	< 0.005	< 0.005	0.180
Soybean/tung 90:10 ^a	1% catalyst ^c and molar ratio ^f of 6:1	< 0.005	0.358	0.2	< 0.005	0.123
Soybean/tung 90:10 ^a	1% catalyst ^c and molar ratio ^f of 9:1	< 0.005	0.486	0.058	< 0.005	0.135
Soybean/tung 90:10 ^a	0.5% catalyst ^c and molar ratio ^f of 6:1	< 0.005	0.419	0.128	< 0.005	0.128
Soybean/tung 90:10 ^a	1.5% catalyst ^c and molar ratio ^f of 9:1	< 0.005	0.665	0.176	< 0.005	0.198
Soybean/tung 90:10 ^a	1.5% catalyst ^c and molar ratio ^f of 6:1	< 0.005	0.632	0.175	< 0.005	0.190
Soybean/tung 75:25 ^a	1% catalyst ^c and molar ratio ^f of 6:1	< 0.005	0.483	0.134	< 0.005	0.145

^aBiodiesel produced by transesterification reaction; ^bbiodiesel produced by transesterification and esterification reactions; ^cNaOH; ^dethanol:oil; ^eH₂SO₄; ^fmethanol:oil.

**Figure 2.** Chromatographic profile of the standard mixture under the analysis conditions of the ASTM D6584 method with glycerol at 0.05%, monoolein at 1%, diolein and triolein at 0.5% (m/m) of concentration.

soybean/tallow 80:20 (m/m) mixture and sunflower oil, biodiesel methyl esters from soybean/tung oil 75:25 (m/m) and 90:10 (m/m) mixtures and tung oil. Table 1 shows the results. Figure 3 shows a chromatogram of a sample under analysis.

**Figure 3.** Chromatographic profile of a sample of biodiesel ethyl esters from tung oil.

The concentrations of by-products detected in soybean/tallow mixture were only an estimate because they exceeded the last level of concentration of the linear range. Therefore, the concentrations found are not in accordance with the legislation. The results for other samples comply with the standards required by ANP 14/2012,¹⁸ ASTM D 6751 and EN 14214 (Table 2).

Table 2. Maximum residue limits for free and total glycerol, mono-, di- and triglycerides in the biodiesel according to the ANP 14/2012, ASTM D 6751 and EN 14214 standards

Parameter	ANP 14/2012 Limit / %, m/m	ASTM D 6751 Limit / %, m/m	EN 14214 Limit / %, m/m
Free glycerol	0.02	0.02	0.02
Monoglycerides	0.8	–	0.8
Diglycerides	0.2	–	0.2
Triglycerides	0.2	–	0.2
Total glycerol	0.25	0.24	0.25

Conclusions

Free glycerol, monoglycerides, diglycerides and total glycerol were successfully determined using the ASTM D6584 method for biodiesel from different raw materials which are presented as alternative to common biodiesel.

The results show that the reference method ASTM D6584 can include the biodiesel ethyl esters from sunflower oil, soybean/tallow mixture and biodiesel methyl esters from soybean/tung mixture and tung oil as matrices to be analyzed. It is important to highlight that, for samples of biodiesel from soybean/tung mixture and tung oil, the glyceride peaks related to α -eleostearic acid (C18:3c) also have to be taken into account.

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