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Simultaneous gas chromatographic analysis of total esters, mono-, di- and triacylglycerides and free and total glycerol in methyl or ethyl biodiesel

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ABSTRACT

The purpose of this paper was to develop and validate a simple adapted method for the quality control of the determination of total esters, mono-, di-, and triacylglycerides and free and total glycerol content in methyl or ethyl biodiesel. Advantages of the method over the standard EN 14103 and EN 14105 methods include reduced sample preparation and analysis time and wide applicability to biodiesel containing naturally occurring heptadecanoic acids. The method correlates well with the standard EN 14103 and the EN 14105 methods.

1. Introduction

In Brazil, biodiesel quality control is carried out in accordance with ANP (National Agency of Petroleum, Natural Gas and Biofuels) Resolution 7 (2008), based on Brazilian, European and American standards [1,2]. Controlling biodiesel chemical composition and purity parameters presents a series of problems. In Brazil, a large number of feedstocks differ substantially from those used in Europe and the United States; however, the parameters are not applicable to biodiesels produced by the ethyl route [3–8].

EN 14103 (2003) [9], for total ester determination, uses methyl heptadecanoate (C17:0) as an internal standard despite the natural occurrence of this fatty acid in bovine tallow, chicken oil, and other fats and oils. The injector temperature of 250 °C is below the volatilization temperature of some long chain fatty acid esters. In addition, the low oven temperature (200 °C) hampers the elution of some long-chain esters [10]. Over a long analysis time at 200 °C, mono-, di-, and triacylglycerides do not elute under the chromatographic conditions of injector and oven temperature. GC oven temperature has been modified to determine short-chain fatty acid ester content [11]. This method does not permit the elution of acylglycerides, however.

The cold-on-column technique used in both EN 14105 [12] and ASTM D6584 [13] requires a long injector cooling time and the oven temperature of 370 °C is not suitable for biodiesels derived from oilseeds such as peanut and crambe which contain long-chain triacylglycerols.

The EN 14103 method requires total ester content to be obtained by the integration of C14 to C24:1 fatty acid methyl ester peaks. However, many oilseed-derived biodiesels, like biodiesels from tallow, babassu, and palm kernel, contain shorter chain fatty acids. In addition, the standard EN 14105 and ASTM D6584 methods to determine mono-, di-, triacylglycerides and total and free glycerol quantify mono-, di-, and triacylglyceride content by peak bands without adequately differentiating mono- and diacylglycerides from fatty acid methyl esters and other minor compounds present in biodiesel.

Thus, the purpose of this study was to develop a simplified method for controlling the quality of ethyl or methyl biodiesel from a variety of feedstocks which would permit the simultaneous determination of total esters, mono-, di-, and triacylglycerides, and free and total glycerol content.

2. Experimental

2.1. Production of biodiesel

Peanut, canola, sunflower, corn, jatropha, pequi, soybean, palm kernel, crambe, moringa, coffee, tallow and babassu oils underwent a methylic transesterification reaction.

The reaction was carried out with 25 g of each oil in a 9:1 molar ratio of alcohol/oil and 2% w/w catalyst (KOH) and a mechanical stirring speed of 200 rpm at 60 °C for 2 h. After glycerol was separated out, the product was washed three times in HCl 0.1 mol L^{-1}

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and distilled water. The biodiesel was dried in a 100 °C oven for 80 min and was filtered.

Ethyl babassu biodiesel was produced in a way similar to the methyl route. However, a molar ratio of alcohol to oil of 12:1 was used. As there was no phase separation of esters and glycerin, 5 mL of glycerol was added to cause the phase separation of fatty acid ethyl esters and glycerin.

2.2. Identification of fats and oils constituents

The fat and oil samples were subjected to an esterification reaction using a modification of the Hartman and Lago method [14] to evaluate fatty acid composition. The resulting fatty acid methyl esters were analyzed using a GC 7890 Agilent gas chromatograph equipped with a flame ionization detector (FID) and split/splitless injector. A split injection with a split ratio of 1:30 was used at 360 °C with a pressure of 37.9 psi and hydrogen 5.0 as the carrier gas. Oven temperature was set at 180 °C, increasing 5 °C min^{-1} to 240 °C for 5 min, then 2 °C min^{-1} to 250 °C for 8 min. The HP-88 capillary column (100 m × 250 μm × 0.2 μm i.d.) was used. The detector was operated at 385 °C.

2.3. Identification of ethyl and methyl biodiesel chemical composition

The samples of methyl and ethyl biodiesel produced from different feedstocks were subjected to a silylation reaction with N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA, Sigma-Aldrich >97.0%) and diluted in n-heptane for HT-CGC (High Temperature Capillary Gas Chromatography) analysis [15]. The derivatized biodiesels were then analyzed with a GC 2010 Shimadzu gas chromatograph equipped with a flame ionization detector and a split/splitless injector. A split injection with a split ratio of 1:20 was used at a temperature of 360 °C, with a pressure programming of 5 psi for 5 min increasing 1 psi min^{-1} to 6 psi and 1.5 psi min^{-1} to 16 psi and this pressure was kept constant for 2.83 min. Hydrogen 5.0 was used as the carrier gas at 1.4 mL min^{-1}. The oven temperature was set at 60 °C for 1.5 min and increased at a rate of 25 °C min^{-1} to 110 °C. It was then kept at this temperature for 1 min, then increased to 215 °C at a rate of 25 °C min^{-1}, kept at this temperature for 5 min, increased to 350 °C at a rate of 25 °C min^{-1}, and was finally increased at a rate of 10 °C min^{-1} to 385 °C and kept at this temperature for 2.4 min. The capillary column was a DB-5-HT (25 m × 250 μm × 0.1 μm i.d.). The detector was operated at 385 °C and nitrogen was used as make up gas at 60 mL min^{-1}. These same chromatographic conditions were applied to the simultaneous determination of free and total glycerol, acylglycerides and total ester contents.

To identify lipidic constituents, fatty acid methyl and ethyl esters, and acylglyceride standards were purchased from Nuchek-Prep Inc.

2.4. Development of the chromatographic analysis

The procedure was developed by adding tricosanoic acid methyl or ethyl ester (C23:0) as internal standards for methyl or ethyl biodiesel respectively, in addition to the conventional internal standards (1,2,4-butanetriol and tricaprine) used in the standard EN and ASTM methods to quantify glycerol and free and total glycerol contents.

2.4.1. Determination of mono-, di-, triacylglycerides, and free and total glycerol

Stock solutions of internal standards and reference substances were prepared directly in 10 mL volumetric flasks as shown in Table 1. The volume was made up to the meniscus with pyridine. 1,2,4-butanetriol (Fluka® > 99.0) and tricaprin (Fluka® > 99.0) were the internal standards for glycerol and acylglycerides, respectively. A glycerol standard (Tédia® 99.7%) was used as a reference substance for glycerol; monolein and diolein (Nu-Chek Prep® > 99.0%), and triolein (Fluka® > 99.0%) was the reference for mono-, di-, and triacylglycerides. The internal standard analytical curve was used to quantify free and total glycerol and acylglycerides, as displayed in Table 1. The standard solutions were analyzed in triplicate under the same chromatographic conditions used in determining the chemical composition of biodiesel.

For each of the reference substances, the standardized response signal (the relation between the areas of the reference substance and the internal standard) and the standardized quantity (the relation between the masses of the reference substance and internal standard) were determined. In this way, the analytical curve of the standardized signal response based on standardized quantity was obtained.

2.4.2. Determination of total esters

Tricosanoic acid methyl ester C23:0 (Nu-Chek Prep® > 99.0%) was used as the internal standard for methyl biodiesel and tricosanoic acid ethyl ester (Nu-Chek Prep® > 99.0%) was the internal standard for ethyl biodiesel. The concentration of both internal standard solutions was 50 mg mL^{-1}. In addition, heptadecanoic methyl and ethyl esters C17:0 (Nu-Chek Prep® > 99.0%), were used to compare the internal standards of the proposed and standard methods.

To calculate the percentage of total ester in the samples, the response factor of C23:0 in relation to C17:0 was obtained by injecting 1 μL of a sample resulting from the mixture of 0.5 mL of two heptanic solutions containing 10 mg mL^{-1} of each fatty acid methyl or ethyl ester internal standard. The response factor for methyl and ethyl C23:0 was obtained in relation to methyl and ethyl C17:0, respectively.

2.4.3. Biodiesel sample preparation

One hundred mg of biodiesel was weighed directly into the 1.5 mL automatic injector vial. Then, 100 μL of each internal standard solution – 1,2,4-butanetriol, tricaprin, tricosanoic acid ethyl or methyl ester – was added. The samples were then analyzed under the same chromatographic conditions described in Section 2.3.

2.5. Comparison with official methods

To validate the method proposed for simultaneous determination of free and total glycerol, acylglycerides and ester contents, all the biodiesel samples were analyzed using the proposed method and the EN 14105:2003 and EN 14103:2003 official standards.

3. Results and discussion

The chromatographic analyses described in Section 2.2 were used to determine the fatty acid composition of the feedstocks used to produce the biodiesel samples, as shown in Table 2. The natural presence of the C17:0 ester in sunflower, corn, crambe, peanut, and tallow biodiesel compromises the total ester results determined according to the EN 14103:2003 standard [11]. The norm EN 14103:2011 [16] uses methyl nonadecanoate (C19:0) as the internal standard for ester analysis. EN 14105:2011 [17] uses M19 (monoacylglyceride with 19 carbon atoms) as the internal standard to quantify monoacylglycerides. However, C19:0 and its respective monoacylglyceride are present in oils from various species of microalgae, which makes the use of these compounds as an internal standard impossible [18].

Samples such as crambe and peanut also contain many long chain fatty acids, and this indicates that the triacylglycerols found

Table 1
Concentration of stock solutions and respective dilutions for analytic curves to quantify free and total glycerol, mono-, di- and triacylglycerides.

Standard stock solutions	Concentration (mg mL^{-1})	Stock solution standard volumes (μL) Levels				
		1	2	3	4	5
Glycerol	0.5	10	20	40	80	120
Monoolein	10	10	40	80	120	160
Diolein	5	10	10	40	80	120
Triolein	5	10	10	40	80	120
1,2,4-butanotriol	1	100	100	100	100	100
Tricaprin	8	100	100	100	100	100

in these samples need a high oven temperature to elute. Thus, while the maximum oven temperature used in EN 14103 is 250 °C, the proposed method uses 385 °C, which is sufficient to elute triacylglycerols containing more than 60 carbon atoms of the sum of carbon atoms of all fatty acids chains [19,20].

It is important to point out that there is no C23:0 ester in any of the biodiesel samples. This fatty acid was chosen as an internal standard in the proposed method because it is a highly stable solid that can be acquired commercially in extremely pure form.

The analysis of several biodiesel samples permitted the identification of the constituents of ethyl and methyl biodiesel from various oilseeds (Tables 3 and 4). In the elution region of tricosanoic acid ethyl and methyl esters (C23:0), there was no elution of any other compounds, including mono-, and diacylglycerols. This confirms that it is possible to use C23:0 as an internal standard for any commercial biodiesel produced from conventional vegetable or animal fats or oils.

HT-CGC was used for efficient separation with a short analysis time. The use of high linear carrier gas velocity (46.6 cm s^{-1}) and the high oven temperature (385 °C) made it possible to obtain appropriately shaped peaks and assured the elution of all sample constituents with well defined peaks and baseline stability. Thus, it was possible to obtain analytical curves to quantify mono-, di-, and triaclylglycerides with good repeatability at all calibration levels.

The coefficients of variation for all the points on the analytical curves were acceptable (CV < 15%), and actually quite low with the exception of point 1 on the triacylglyceride quantification curve (7.32%). High linearity was obtained for all analytical curves, with r values higher than 0.998 and RSD values lower than 0.03%.

Fig. 1 is an example of how the proposed method efficiently separates all biodiesel constituents and shows that there was no co-elution of fatty esters, mono-(M), di-(D), or triacylglycerides (T) with the C23:0 internal standard. This chromatographic method does not permit the proper separation of C19:0 either, since in the region between 10 and 12.5 min, a series of compounds elute which would coelute with C19:0. The identification of the fatty acids, methyl and ethyl esters and acyglycerides in biodiesel samples is described in Tables 3 and 4. The area of the C23:0 internal standard is similar to the major component in the biodiesel samples. In addition, the concentration of C23:0 was similar to that of C17:0 in EN 14103.

Each constituent's content percentage (w/w) was calculated using Eq. (1), which is the same equation used in both the EN 14105 and ASTM D6584 methods for quantifying glycerol, mono-, di- and triacylglycerol content:

$$C = \frac{[(A/A_{pi}) - b] \cdot M_{pi} \cdot 100}{a \cdot m} \quad (1)$$

where C is the content in percent (w/w) of glycerol, mono-, di-, or triacylglycerides present in the biodiesel sample; A the glycerol peak area or the sum of the areas of mono-, di- or triacylglyceride peaks; A_{pi} the peak area of the 1,2,4-butanetriol or tricaprin internal standard; M_{pi} the mass of the 1,2,4-butanetriol or tricaprin internal standard; m the mass of the biodiesel sample (mg); a the inclination (angular coefficient) of the calibration function for glycerol, mono-, di- or triacylglycerides; and b is the intercept (linear coefficient) of the calibration function for glycerol, mono-, di- or triacylglicerides.

Total glycerol content is determined by Eq. (2), specified in the EN 14105 standard:

$$GT = GL + (0.255 \cdot M) + (0.146 \cdot D) + (0.103 \cdot T) \quad (2)$$

Where GT is the percentage (w/w) of total glycerol present in the biodiesel sample; GL the content in percent (w/w) of free glycerol determined by Eq. (1); and M, D and T is the content in percent (w/w) of mono-, di-, and triacylglycerides, respectively, determined by Eq. (1).

After all the fatty acid monoalkyl ester peaks were identified and their respective areas were obtained, the total ester content in the biodiesel sample was determined using Eq. (3). In comparison to the calculations used in the EN 14103 method, this equation includes a response factor ($A_{C23:0} \cdot m_{C17:0}/A_{C17:0} \cdot m_{C23:0}$) reflecting differences in C17:0 and C23:0 response on the flame ionization detector. Experimentally, response factors of 0.993606 and 0.995411 were found for ethyl esters and methyl esters, respectively.

$$Et = \frac{[(\sum Ae - A_{pi}) \cdot C_{pi} \cdot V_{pi} \cdot 100]}{A_{pi} \cdot m} \times \left[\frac{A_{C23:0} \cdot m_{C17:0}}{A_{C17:0} \cdot m_{C23:0}}\right] \quad (3)$$

where Et is the percentage (w/w) of total esters in the biodiesel sample; $\sum Ae$ the sum of the areas of all the ester peaks; C_{pi} the concentration in mg/mL of tricosanoic acid or methyl or ethyl ester; V_{pi} the volume in mL of tricosanoic acid methyl or ethyl ester solution; A_{pi} the peak area of the C23:0 internal standard used in the biodiesel analysis; m the mass of the biodiesel sample (mg); $A_{C17:0}$ the peak area for C17:0 (methyl/ethyl ester); $m_{C17:0}$ the weighted mass of C17:0 standard to prepare the 10 mg/mL solution of C17:0 (methyl/ethyl ester); $A_{C23:0}$ the peak area for C23:0 (methyl/ethyl ester); and $m_{C23:0}$ is the weighted mass of C23:0 standard to prepare the 10 mg/mL solution of C23:0 (methyl/ethyl ester).

It is worth mentioning that the use of response factors to quantify total ester content also makes it possible to use a tricosanoic acid methyl ester as an internal standard for an ethyl biodiesel if no long fatty chain is eluting with the internal standard. It also make it possible to use a tricosanoic acid ethyl ester as an internal standard for a methyl biodiesel.

The results of the simultaneous quantification of free glycerol, total glycerol, mono- (M), di- (D), triacylglycerides (T), and total esters using the proposed and standard methods are shown in Table 5. It is worth noting that the use of C23:0 as the internal standard to quantify total ester causes modifications such as higher total ester content in tallow samples. However, the results of the proposed method demonstrate more consistency between total glycerol and total ester content than those of the official EN 14103 and EN 14105 methods. This is because, differently from

Table 2
Fatty acids constituents of oil and fat samples.

Samples	Fatty Acid Composition (%)																		
	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0	C24:1
Peanut	–	–	–	–	–	11.1	0.1	3.1	2.9	36.8	0.6	37.7	1.3	3.6	–	1.7	0.2	0.2	–
Canola	–	–	–	–	0.1	5.3	0.4	–	2.4	61.0	3.3	19.6	7.2	0.3	0.1	0.2	–	0.1	–
Sunflower	–	–	–	–	0.1	6.1	0.1	0.6	3.1	30.0	0.8	57.9	0.5	0.6	0.2	–	–	–	–
Corn	–	–	–	–	0.1	11.8	0.3	1.0	2.1	35.5	0.8	46.6	1.4	0.2	–	–	0.2	–	–
Jatropha	–	–	–	–	0.1	13.8	0.9	–	6.2	42.4	1.5	34.6	0.3	0.1	0.1	0.1	–	–	–
Pequi	–	–	–	–	0.1	31.4	0.7	–	2.2	47.3	1.7	15.6	0.7	0.1	0.1	–	–	–	–
Soybean	–	–	–	–	0.1	11.2	0.2	–	3.3	23.3	1.6	52.6	6.7	0.4	0.1	–	–	–	–
Palm Kernel	0.6	1.1	2.2	45.4	16.6	10.0	–	–	2.7	18.0	0.2	2.9	0.1	–	–	–	–	–	–
Crambe	–	–	–	–	–	1.8	–	2.1	0.8	15.4	0.6	9.6	8.9	–	2.1	–	56.9	0.7	1.0
Moringa	–	–	–	–	–	5.1	1.3		4.4	69.7	7.3	2.8	2.1	–	6.2	–	–	1.1	–
Coffee	–	–	–	–	–	32.1	–		0.7	10.6	0.5	46.1	2.2	0.8	–	–	–	–	–
Tallow	–	–	0.4	1.6	7.5	24.9	4.9	7.2	17.9	31.1	0.5	2.3	1.7	–	0.2	–	–	–	–
Babassu	0.4	5.3	5.9	44.2	15.8	8.6	–	–	2.9	14.6	0.5	1.7	0.1	–	–	–	–	–	–

Table 3
Absolute and relative to the T30:0 retention times for acylglycerides according to the developed method.

Acylglycerols	Retention time (min)	
	Absolute	Relative to T30
Monoacylglycerides		
M8:0	7.02	0.39
M10:0	8.68	0.49
M12:0	9.58	0.54
M14:0	10.92	0.62
M16:1	13.13	0.74
M16:0	13.42	0.76
M18:2; M18:1	15.41	0.87
M18:0	16.32	0.92
M20:0	16.93	0.96
M22:0	17.23	0.97
M24:1	17.37	0.98
Diacylglycerides		
D16:0	10.84	0.61
D20:0	15.23	0.86
D24:0	16.83	0.95
D28:0	17.75	1.01
D32:2	18.42	1.04
D32:0	18.49	1.05
D36:2	19.09	1.07
D36:0	19.14	1.08
D40:0	19.82	1.12
D44:0	20.59	1.16
Triacylglycerides		
T30	17.30	1.00
T36	18.74	1.06
T42	19.71	1.11
T48	20.86	1.18
T50	21.23	1.23
T52	21.62	1.25
T54	22.11	1.28
T56	22.58	1.31
T60	23.84	1.38
T62	24.60	1.42

the official methods. The proposed method's total ester content is always inversely proportional to the total glycerol content.

For feedstocks like soybean, canola and corn, in contrast to the other materials monoacylglyceride values are similar. The standard (EN 14105) showed higher values for diacylglyceride content, with the exception of canola and tallow biodiesel. Free glycerol content was higher with the official method than with the proposed method. Those differences occur because the EN and ASTM standards determine components in bands, differently from the proposed method, which identifies the compound peaks individually. Therefore, the proposed method can be applied to a much larger number of biodiesel feedstocks. In addition, the chromatographic conditions of EN 14105 do not allow separation of some long-chain fatty acid esters or monoacylglyceride peaks in the case of peanut and crambe methyl biodiesel.

Triacylglyceride results of the proposed method proved quite similar to those of the standard method for most biodiesel feedstocks. However, the EN 14105 quantification underestimated triacylglyceride content in peanut, crambe, pequi, tallow and babassu. This was due to EN 14105's lower final oven temperature compared to the proposed method. The official methods' lower final oven temperature results in peak tailing and consequently hinders peak integration. This shows how important the complete and efficient elution of all sample components is for obtaining an accurate quantitative analysis.

Another advantage of the proposed method derives from the quantification of all the acylglycerides found in biodiesel samples. Use of oils with high short chain fatty acid content, such as pequi and palm kernel can result in biodiesel with greater quantities of short chain acylglycerides, which the EN 14105 method ignores. That method considers only the acylglycerides, usually only medium- and long-chain, eluted in restricted regions of the chromatogram. In addition, the proposed method quantifies even low-concentration acylglycerides using analytical curves with a wide concentration range.

The proposed method also makes it possible to identify and include peaks of short chain fatty esters and differentiates monoacylglycerides and diacylglycerides from long chain esters, thus avoiding overestimations or underestimations in quantitative analysis.

As shown in Table 2, C17:0 occurs naturally in crambe and tallow oil, so the EN 14103 method may underestimate total ester content in these samples. For example, the proposed method showed higher ester content in tallow biodiesel than EN 14103 did (Table 5) due to the natural occurrence of C17:0 in these samples. This means that C23:0 is a very useful internal standard for this type of sample.

With EN 14103, total ester content must be obtained by the integration of fatty acid methyl ester peaks from C14 to C24:1. Biodiesels from oilseeds such as babassu, coffee, moringa, pequi, palm kernel and many others contain short chain fatty acids that may compromise analysis. In addition, the EN 14105 and ASTM D6584 standards integrate the peaks of mono-, di-, and triacylglycerides by bands without correctly differentiating mono- and diacylglycerides from fatty acid methyl esters.

To verify the dispersion of the results of the EN 14103, EN 14105 and proposed methods, the same commercial methyl biodiesel sample was analyzed. The results (Table 6) showed standard deviation similar and close to zero. This demonstrates that the

Table 4
Absolute retention times and relative to the C23:0 for ethyl and methyl esters according to the developed method.

Ester	Retention time of methyl esters		Retention time of ethyl esters	
	Absolute (min)	Relative to methyl C23:0	Absolute (min)	Relative to ethyl C23:0
C6:0	1.7	0.12	2.2	0.15
C8:0	3.3	0.24	3.2	0.22
C10:0	4.9	0.35	5.4	0.37
C12:0	6.3	0.45	6.9	0.47
C14:0	7.3	0.53	7.7	0.53
C15:0	7.8	0.56	8.1	0.55
C16:0	8.2	0.59	8.5	0.58
C17:0	8.7	0.63	8.9	0.61
C18 (C18:0, C18:1, C18:2, C18:3)	8.9	0.64	9.3	0.64
C20:0	10.3	0.74	10.2	0.70
C22:1	11.9	0.86	12.5	0.86
C22:0	12.4	0.89	13.2	0.90
C23:0	13.9	1.00	14.6	1.00
C24:0	14.9	1.07	15.4	1.05

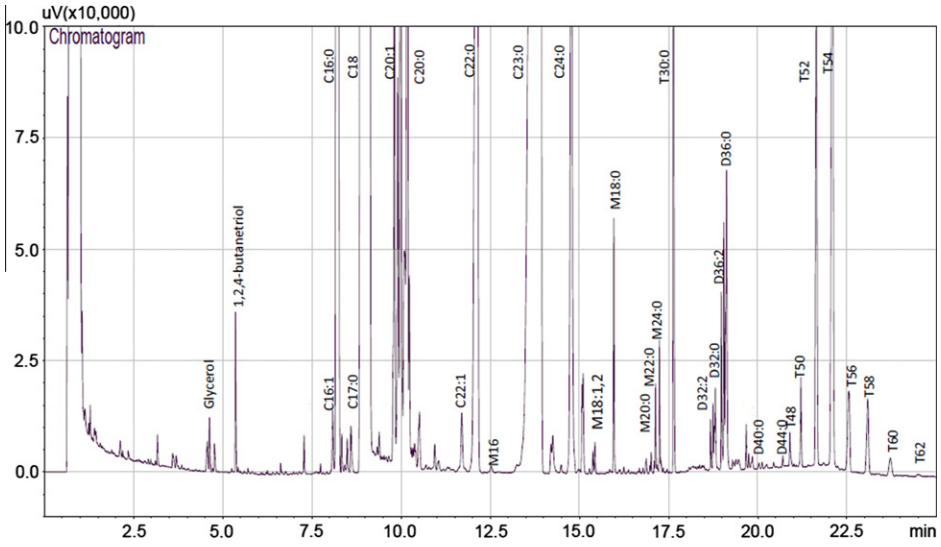

Fig. 1. Chromatogram of peanut methyl biodiesel with addition of internal standards.

Table 5
Results of the quantification of mono- (M), di- (D), triacylglycerides (T), total and free glycerol according to the standards methods (SM) and according to the developed method (DM).

Type	Sample	Free glycerol (%w/w)		M (%w/w)		D (%w/w)		T (%w/w)		Total glycerol (%w/w)		Total Esters (%w/w)	
		SM	DM	SM	DM	SM	DM	SM	DM	SM	DM	SM	DM
Methyl	Peanut	0.02	0.04	2.90	1.01	1.92	1.46	1.46	1.27	1.20	0.58	96.9	96.8
	Crambe	0.01	0.08	1.82	0.59	0.13	0.05	0.05	0.04	0.50	0.27	99.0	97.8
	Moringa	0.01	0.03	1.13	0.43	0.07	0.07	0.07	0.07	0.32	0.21	97.7	95.1
	Coffee	0.01	0.03	0.46	2.39	0.07	0.05	0.05	0.33	0.14	0.69	95.2	95.1
	Sunflower	0.01	0.09	0.91	1.19	0.34	0.13	0.13	0.12	0.31	0.47	96.7	96.2
	Soybean	0.01	0.03	0.27	0.29	0.10	0.05	0.05	0.04	0.10	0.15	93.6	98.8
	Corn	0.01	0.04	0.41	0.41	0.40	0.20	0.20	0.21	0.20	0.25	97.6	98.3
	Pequi	0.02	0.05	0.49	0.63	0.37	0.82	0.82	0.48	0.28	0.29	94.7	95.2
	Canola	0.02	0.04	0.50	0.42	0.38	0.12	0.12	0.16	0.21	0.21	96.7	98.2
	Palm Kernel	0.00	0.05	0.47	0.62	0.96	0.01	0.01	0.02	0.26	0.23	96.3	97.4
	Jatropha	0.01	0.03	0.38	0.46	0.14	0.00	0.00	0.03	0.13	0.18	97.0	97.9
	Tallow	0.02	0.02	0.32	2.30	0.28	0.49	0.49	0.33	0.20	0.65	77.0	98.1
	Babassu	0.08	0.05	0.32	0.59	1.35	0.07	0.07	0.66	0.32	0.61	94.8	94.5
Ethyl	Babassu	0.00	0.04	0.30	0.53	1.26	0.13	0.13	0.01	0.26	0.20	94.7[a]	95.8

[a] Obtained by adaptation of the EN 14103 standard using ethyl C17:0 as internal standard.

proposed method provides results with low dispersion as the official methods.

Table 7 presents ANOVA results to determine whether the two methods are comparable. The results shown are means (μ) of the values expressed in Table 5. Calculated F exceeded tabulated F for di- and triacylglicerides only. This occurred because the diacylglycerides are quantized individually and the triacylglycerides are eluated totally in the proposed method. And the results

Table 6
Results for the repeatability of quantification of mono- (M), di- (D), triacylglycerides (T), total and free glycerol according to the EN 14105 method, total esters according to the EN 14103 method and according to the developed method.

Method	Analysis	Free glycerol (%)	M (%)	D (%)	T (%)	Total glycerol (%)	Total ester (%)
EN*	Average	0.01	0.50	0.08	0.03	0.15	89.3
	Standard deviation	0.00	0.00	0.00	0.00	0.00	0.1
Developed** method	Average	0.01	0.25	0.29	0.03	0.12	98.5
	Standard deviation	0.00	0.01	0.02	0.00	0.00	0.2

Number of replicates (n).
* $n = 4$.
** $n = 7$.

Table 7
Results of ANOVA to comparing the standard methods (SMS) to the developed method (DM).

Analysis	μ Standard method	μ Developed method	F_{calc}	F_{tab}
Free glycerol	0.02	0.04	1.047	2.577
M	0.76	0.85	0.982	
D	0.55	0.26	3.066	
T	0.26	0.27	11.168	
Total glycerol	0.32	0.36	1.805	
Total ester	94.8	96.8	0.837	

are comparable for free and total glycerol, total ester and monoacylglicerides [21]. However, for samples whose fatty acid compositions are very different from canola, soybean and corn, the results such as total ester content in tallow biodiesel, differ greatly.

4. Conclusion

The proposed method offers several advantages over the official EN e ASTM methods, in particular the greater consistency between total glycerol and total ester content. Moreover, the internal standard used in the determination of ester content is not a constituent of the biodiesel samples. The method proposed here also offers a satisfactory routine analysis time (25 min), and permits the elution of long-chain triacylglycerides.

The method also provides adequate separation of monoacylglycerides and both methyl and ethyl ester peaks. The procedure is applicable to biodiesels from different feedstocks produced by both methyl and ethyl routes.

Furthermore, it was possible to unify the determination of free and total glycerol, acylglycerides and ester content in a single chromatographic procedure, which is not possible using the official methods.

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