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Optimization of a method for the simultaneous determination of glycerides, free and total glycerol in biodiesel ethyl esters from castor oil using gas chromatography

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ABSTRACT

This paper describes the optimization of a method of simultaneous determination of glycerides, free and total glycerol in biodiesel ethyl esters from castor oil by using gas chromatography. Changes were proposed for the methods ASTM D 6584 and EN 14105 in order to determine these by-product contaminants in biodiesel from castor oil. The silylation reaction for this biodiesel was optimized, and 250 μL MSTFA was used. Its accuracy values were between 70% and 120% with RSD <11%. The identification of monoricinolein and diricinolein was made by gas chromatography with mass spectrometry detection (GC–MS). The matrix effect (ME) was investigated and considered low for glycerol, mono- and diolein; it was medium for triolein. The method was robust even when there were variations in the matrix. It was also successfully used for the determination of glycerides, free and total glycerol in samples of biodiesel from castor oil.

1. Introduction

The increasingly high demand for energy in the industrialized world, in households, transport and industry, besides the problems that result from the widespread use of fossil fuels, requires the development of renewable energy sources with limitless duration and lower environmental impact than traditional ones [1].

Biodiesel, a common term for long chain alkyl esters, is a renewable, biodegradable and non-toxic biofuel, which has become an important alternative source of energy. Biodiesel is derived from the transesterification of mono-, di- and triglycerides and the esterification of free fatty acids that naturally occur in biological lipids, such as animal fats and plant oils [2].

The presence of by-products contaminants, such as glycerol, mono-, di- and triglycerides after transesterification, is the main factor that determines fuel quality [3,4]. The determination of free glycerol, mono-, di- and triglycerides not only indicates the quality of the final product, but also shows the efficiency of the production process [5]. Free glycerol is a parameter that is used to assess the purification step of the biodiesel, whereas mono-, di- and triglycerides are used to check oils and animal fats in biodiesel.

The reference methods for the determination of free and total glycerol, mono-, di- and triglycerides in biodiesel methyl esters are ASTM D 6584 and EN 14105. Gas chromatography with flame ionization detection (GC–FID) and derivatization with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) are suggested as procedures. These methods can be applied, without any modification, to biodiesel methyl esters with a similar chemical composition to the one of the biodiesel methyl esters from rapeseed oil, sunflower oil, soybean oil and used cooking oil. Studies of raw materials, such as castor oil and the ethyl route – which is not included in ASTM D 6584 and EN 14105 – are necessary. Castor oil is a non-edible vegetable oil; its use for the production of biodiesel may be an alternative for edible oils as biofuel [6]. The advantage of castor oil for biodiesel production is that the oil is soluble in alcohol and its transformation requires neither heat nor energy expenditure (other vegetable oils do in order to transform them into biofuel) [7].

Brazil is a large tropical country; thus, it has various options to produce vegetable oils. A social project called *Brazilian Program for the Production and Use of Biodiesel* was developed in the northeastern region and has focused on the production of castor. The plant has adapted to the Brazilian semi-arid region and has become an alternative culture for the so-called family agriculture; that is why castor was chosen to be the flagship of the initial phase of this social program [8].

The castor seed, *Ricinus communis*, comes from a plant of the Euphorbiaceae species. It is the only member of the genus *Ricinus* and of the sub-species Ricininae. The seeds contain up to 60% oil, which is rich in triglycerides, mainly ricinolein. The production of

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castor seeds worldwide is around 1 million tons per year; India, China and Brazil are the main producers. It is easy in tropical and subtropical climates since new uses can be found for it [9].

The *Agência Nacional do Petróleo, Gás Natural e Biocombustível* (ANP) in Brazil has established reference methods for the analyses of biodiesel from castor oil, according to Resolution No. 4, issued in February 2010. ABNT NBR 15341 is the method that determines free glycerol; ABNT NBR 15342 regulates the analysis of mono- and diglycerides; and ABNT NBR 15344 establishes total glycerol. When methods ABNT NBR 15341 and ABNT NBR 15342 are applied, it is possible to determine the content of triglycerides. Therefore, in order to assess the contents of free and total glycerol, mono-, di- and triglycerides in biodiesel from castor oil, it is necessary to use three distinct methods: ABNT NBR 15341, ABNT NBR 15342 and ABNT NBR 15344; the third one is a classical method.

No research has been published so far on any validated method of simultaneous determination of glycerides, free and total glycerol in biodiesel ethyl esters from castor oil using gas chromatography. This study proposes a new method for the simultaneous determination of glycerides, free and total glycerol in biodiesel ethyl esters from castor oil using gas chromatography with flame ionization detection and gas chromatography with mass spectrometry detection (GC–MS). The silylation reaction was optimized, and linearity, sensitivity, accuracy, precision, robustness and the matrix effect (ME) were evaluated.

2. Experimental

2.1. Analytical standards and reagents

The standards 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-glycerol1 (monolinolein) (99%) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased at Sigma-Aldrich (USA). (S)-(−)-1,2,4-butanetriol was bought at Fluka (99.5%) (USA). The solvents pyridine and heptane, chromatographic grade were supplied by J.T. Baker (USA).

2.2. Standard solution preparation

The preparation of standard solutions was carried out according to ASTM D 6584. Stock solutions with 0.5 mg mL^{-1} for glycerol, 5 mg mL^{-1} for glycerides, 1 mg mL^{-1} for (S)-(−)-1,2,4-butanetriol and 8 mg mL^{-1} for tricaprin were prepared in pyridine. Different volumes of these solutions were transferred for the standard mixtures preparation. With 100 μL of MSTFA, the standard mixtures were silylated, and after 20 min, 8 mL n-heptane was added.

2.3. Optimization of the silylation reaction for the biodiesel ethyl esters from castor oil

The biodiesel ethyl esters from castor oil were produced by base-catalyzed transesterification and followed by on pot addition of sulfuric acid [10].

A 100 μL of the (S)-(−)-1,2,4-butanetriol stock solution and 100 μL of the tricaprin stock solution were added to 100 mg of the sample. After that, the sample was silylated with 100 μL of MSTFA, according to ASTM D 6584 (recommended for other types of biodiesel). Due to the particularity of biodiesel from castor oil initially, 250, 500 and 750 μL of MSTFA were tested. In sequence, 180, 250 and 300 μL were evaluated. After the addition of the silylating reagent, the sample was shaken. The silylation reaction took 20 min and, then, 8 mL n-heptane was added.

In the optimization of the volume of MSTFA, the variations in the concentrations of the analytes were evaluated and it compared by Normalization.

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

The content of free and total glycerol, mono-, di- and triglycerides in the sample was determined according to ASTM D 6584, but glyceride peaks related to C18:1-OH were also taken into account.

2.5. Chromatographic analysis

The chromatographic system was based on ASTM D 6584.

For the identification of glycerides, an analysis by GC–MS was necessary for the sample of biodiesel ethyl ester from castor oil. The conditions were the following: HT5 capillary column (25 m long × 0.32 mm i.d., 0.1 μm film thickness) by SGE (Ringwood, VIC, Australia), injection volume of 1 μL; oven at 50 °C (1 min hold), 15 °C min^{-1} at 180 °C, 7 °C min^{-1} at 230 °C and 30 °C min^{-1} at 350 °C (30 min hold); split/splitless injector with split injection mode, split ratio of 50:1 and temperature at 250 °C; helium as carrier gas with linear velocity of 64.7 cm s^{-1} and mass spectrometry with electron impact ionization at 70 eV, mass range of 70–1090 *m/z*, ion source temperature at 250 °C and interface temperature at 320 °C.

2.6. Validation parameters

2.6.1. Analytical curve and linearity

The linear ranges were established by ASTM D 6584. Each level of concentration was injected three times into the chromatographic system. The linearity of the method for each compound was evaluated by the Pearson coefficient (r), after the construction of the analytical curves.

2.6.2. Sensitivity

The sensitivity of ASTM D 6584 and EN 14105 was compared by the slope of the equation ($y = ax + b$) of each compound, because the higher the slope, the more sensitive the method is [11].

2.6.3. Accuracy and precision

The accuracy and the precision of the method were evaluated due to the modification of the silylation reaction carried out for the biodiesel ethyl esters from castor. Their evaluation was performed in the first, third and fifth concentration levels through the spiking of the sample with the compounds. Three tests were performed for each level with subsequent injection in triplicate in the GC–FID. This procedure was carried out on different days for the evaluation of the intermediate precision.

Precision was expressed by the relative standard deviation (RSD%). Accuracy was checked by the standard addition method and by recovery essays [12]. The standard addition method was used for compounds, which were present in the matrix, such as glycerol, monoolein and diolein. The recovery essays were employed when the matrix did not have the compound under study, such as triolein.

2.6.4. Robustness

The robustness of a method measures the sensitivity that it presents at small variations. In this work, two variations regarding source (castor oil) and production process (route ethylic) were studied. The EN 14105 and ASTM D 6584 reference methods are recommended for biodiesel methyl esters from rapeseed, sunflower and soybean oil, besides esters with similar chemical

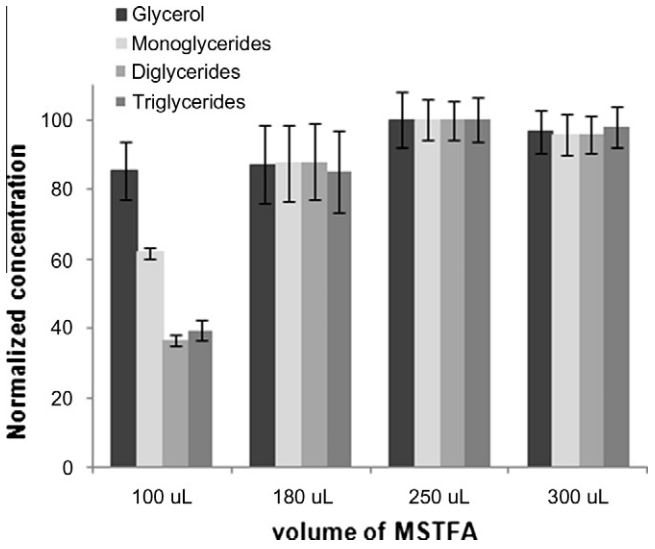

Fig. 1. Comparison between different volumes of MSTFA in the silylation reaction ($n = 9$).

composition. This study employed another matrix, the biodiesel ethyl from castor oil, and this resulted in a different volume of MSTFA in the preparation sample.

2.7. Evaluation of the ME

The evaluation of the occurrence of the ME was performed by the analytical curves of the solvent and of the matrix [13].

The matrix curves were prepared by adding volumes of the stock solutions of the standards and internal standards to 100 mg of sample, in order to obtain five levels of concentration, according to ASTM D 6584. Each level was derivatized with 250 μL MSTFA for 20 min, and after that, 8 mL heptane was added:

$$\mathrm{ME}\% = \frac{\mathrm{slope}(X_1) - \mathrm{slope}(X_2)}{\mathrm{slope}(X_2)} \times 100 \quad (1)$$

, where X_1 is the slope of the curve obtained by the injection of the analytical solutions of each compound, prepared in the biodiesel ethyl esters from castor oil (matrix), X_2 is the slope of the curve obtained by the injection of the analytical solutions of each compound, prepared in n-heptane (solvent).

The ME was considered low for a range of signal suppression/enhancement $-20\% < C\% < +20\%$, medium, for the ranges $-50\% < C\% < -20\%$ or $+20\% > C\% > +50\%$ and high, for the ranges $C\% < -50\%$ or $C\% > +50\%$ [14].

3. Results and discussion

3.1. Optimization of the silylation reaction for the biodiesel ethyl esters from castor oil

Ninety percent of castor oil is comprised of a triglyceride derivative of the ricinoleic acid; this composition distinguishes the biodiesel obtained from this oil from other types of biodiesel. Thus, the main constituent of the biodiesel from castor oil and the contaminants mono-, di- and triglycerides are mostly hidroxylated.

ASTM D 6584 and EN 14105 employ a silylation reaction in the sample preparation. The reaction occurs by replacing the acidic

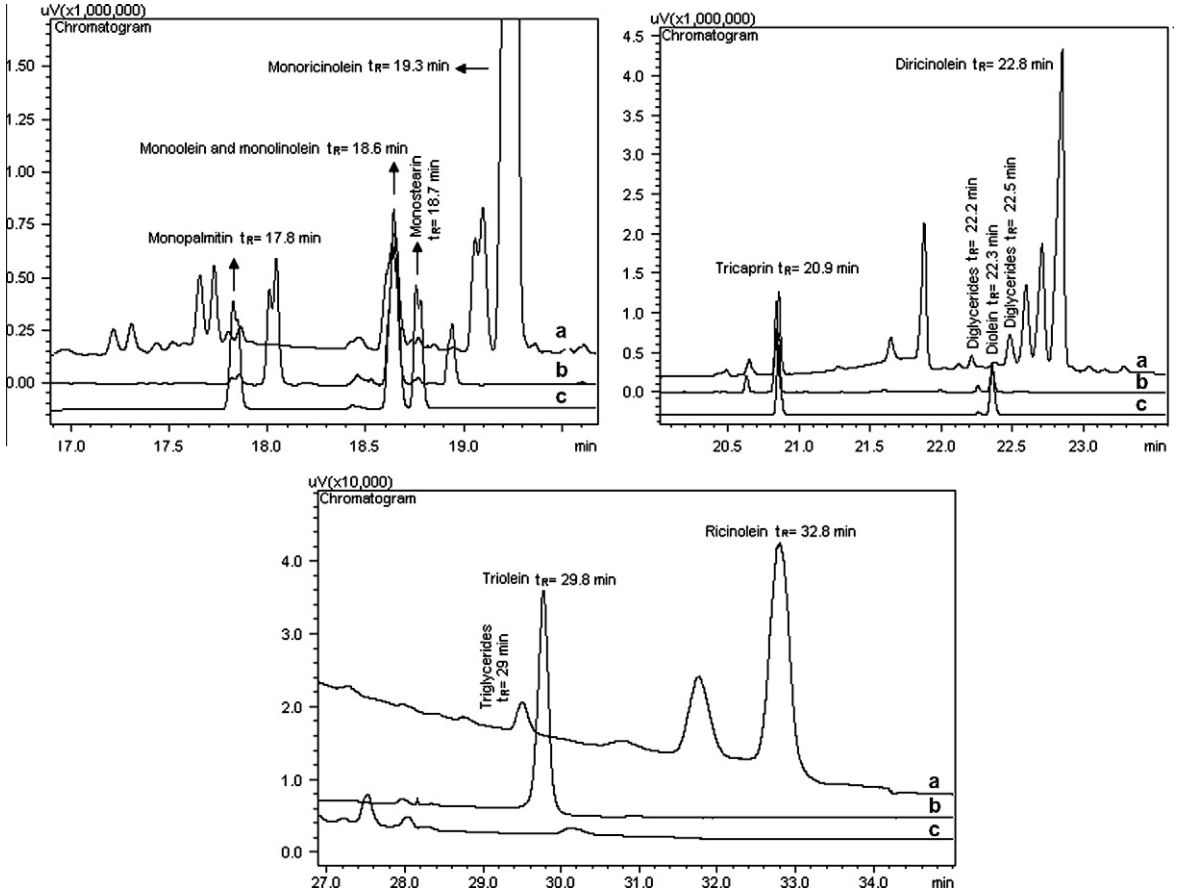

Fig. 2. Chromatographic profile of biodiesel ethyl esters from castor oil (a), sample of biodiesel ethyl esters from sunflower oil (b), and standard mono-, di- and triglycerides mixture at third level of concentration (c), under the analysis conditions of ASTM D 6584 method.

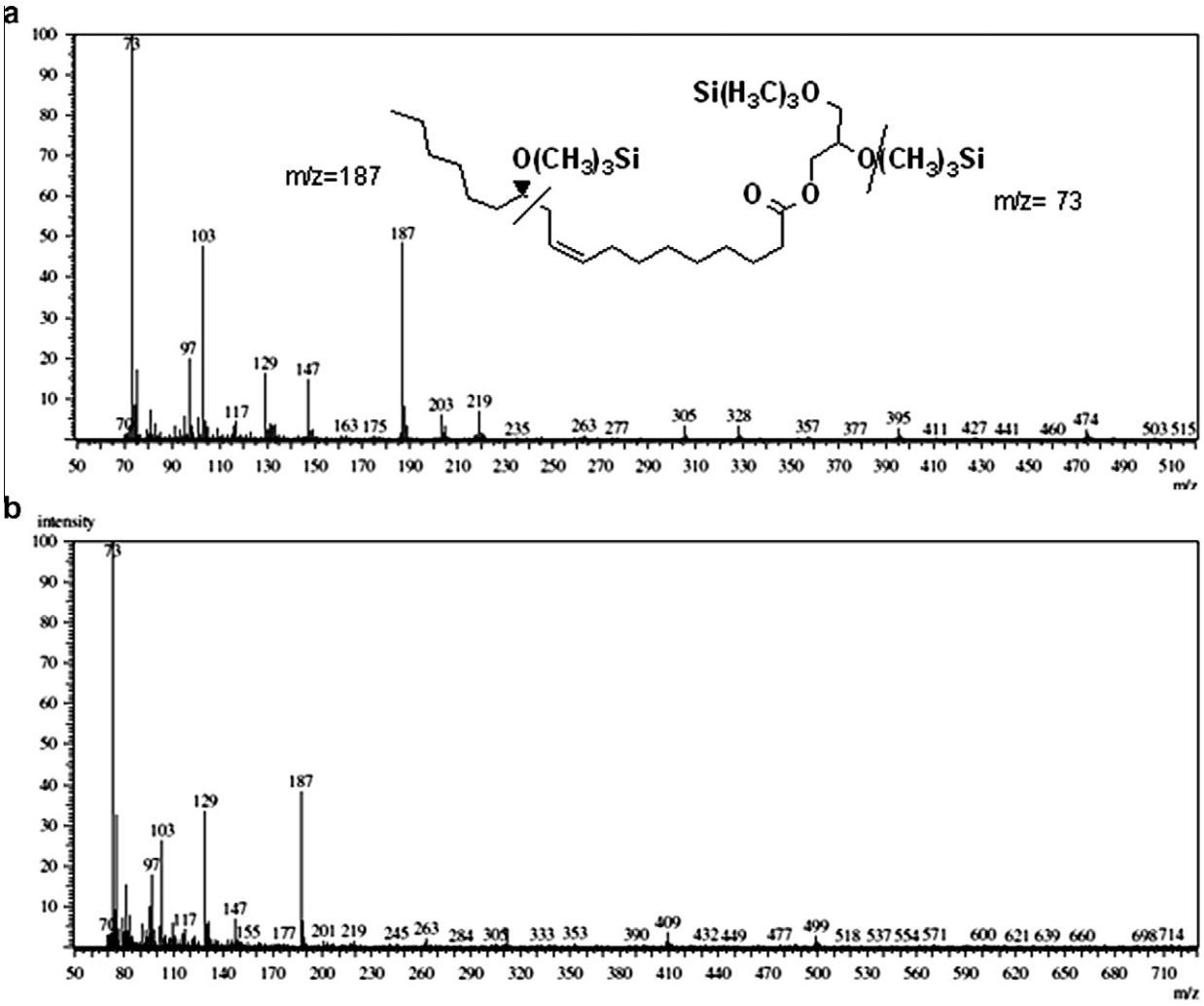

Fig. 3. Mass spectra for monoricinolein derivatized with MSTFA (a) and mass spectra for 1,3-diricinolein derivatized with MSTFA (b).

hydrogens from the compounds by the trimethylsilyl group ($(CH_3)_3Si$) from the derivatizing reagent.

The trimethylsilylation of the free hydroxyl groups of glycerol, mono-, diglycerides and of ricinolein ensures excellent peak shapes, good accuracy and low quantification limits, besides improving the robustness of the procedure.

Therefore, the study of the silylation reaction is necessary since the biodiesel from castor oil has more acidic hydrogens, which can react by silylation.

Studies which are considered the basis for reference methods report that the internal standard (*S*)-(−)-1,2,4-butanetriol serves as a very sensitive indicator of incomplete derivatization [15]. In case of insufficient silylation (not all three hydroxyl groups are silylated), the (S)-(−)-1,2,4-butanetriol peak is split and drastically reduced in height.

In first experiment, 100, 250, 500 and 750 μL of MSTFA in the silylation reaction of biodiesel from castor oil were employed. The results showed peaks with less intensity for a volume of 100 μL of derivatizing reagent and peaks with similar intensity for volumes of 250, 500 and 750 μL. The height of the (S)-(−)-1,2,4-butanetriol peak with 100 μL of MSTFA in the sample of biodiesel from castor oil was half compared at height (S)-(−)-1,2,4-butanetriol peak in the standard mixtures.

With other sample more pure of biodiesel from castor oil, 100, 180, 250 and 300 μL of MSTFA were evaluated. It did not was observed differences in the height of the (S)-(−)-1,2,4-butanetriol peak, that's why the concentrations of the analytes with these volumes were determined and it were compared by Normalization Method (Fig. 1).

Fig. 1. to prove that a volume of 100 μL of MSTFA, according to ASTM D 6584 (recommended for other types of biodiesel), is insufficient for a full silylation of the biodiesel from castor oil. The best results were for volumes of 250 and 300 μL. Therefore, a volume of 250 μL MSTFA was chosen for a sequence this work, because the consumption of silylating reagent is lower, thus, resulting in lower costs.

3.2. Identification and quantification of mono-, di- and triglycerides

The elution order of the mono-, di- and triglycerides in the conditions under study is related to the number of carbon. Those with same number of carbon and with double bonds coelute, but the saturated and unsaturated ones that have the same number are separated; the unsaturated ones elute first.

The biodiesel from castor oil presents glycerides (monoricinolein, diricinolein and ricinolein) that are not commonly found in other biodiesels.

Fig. 2. shows the retention time (t_R) of monopalmitin 17.8 min, monoolein and monolinolein t_R = 18.6 min, monostearin t_R = 18.7 min and monoricinolein t_R = 19.3 min.

The analytical standard of monoricinolein is not available. Therefore, the monoricinolein was identified according to three

requirements: the peak should be higher 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 ethyl esters from sunflower oil.

The retention band for the identification and quantification of diglycerides in the sample of biodiesel ethyl esters from sunflower was established from 22.2 to 22.5 min. For the biodiesel ethyl esters from castor oil, besides this band, the diricinolein with retention time of 22.8 min was identified (Fig. 2).

The requirements considered for the identification of diricinolein were the same ones that were used for monoricinolein, because there is not any analytical standard available for this compound, either.

For the triglycerides, a band of retention times between 29 and 31 min for the samples of the biodiesel ethyl esters from sunflower was established.

On the other hand, for the samples of biodiesel ethyl esters from castor oil, the band of retention times was larger, 29–33 min, due to the presence of ricinolein with t_R = 32.8 min (Fig. 2). There is no analytical standard available for ricinolein. Therefore, the same requirements used for the monoricinolein were applied. In this case, the total time of analysis was changed to 36.81 min, to enable the elution of ricinolein.

3.2.1. GC–MS for the confirmation of compounds

Since standards for monoricinolein and 1,3-diricinolein are not available, tests were carried out in the GC–MS to confirm these compounds. Ricinolein did not elute, because it is little volatile and it needs higher temperatures, which are not allowed in the ion source and in the interface of the GC–MS. The region of the mono- and diglycerides was similar to the profile obtained by GC–FID.

Through the mass spectra, it was possible to confirm the identities of the monoricinolein and of 1,3-diricinolein (Fig. 3.), once the ions *m/z* 73 ($(CH_3)_3Si^+$) are characteristic of the trimethylsilylated compounds and *m/z* 187 originated from breaking the α bound at the ether silyl group present in the mass spectra [16].

3.3. Validation parameters

3.3.1. Analytical curve, linearity and sensitivity

The methods presented *r* values >0.999 for all compounds, resulting in excellent linearity [12].

By comparing the slope of each compound and the analytical curve obtained by each method, it can be concluded that there is no difference in sensitivity between the methods, because the slopes were similar. Therefore, the oven temperature program of ASTM D 6584 was chosen because it results in shorter analysis time (31.81 min) when compared to EN 14105 (42.81 min). Fig. 4.shows a chromatogram of the mixture of the standards in the ASTM D 6584.

3.3.2. Accuracy and precision

Accuracy was satisfactory since values were between 70% and 119.8% (Table 1) [17]. Precision was acceptable with RSD values below 20% (Table 1) [12].

Among the compounds under study, triolein is the only one that does not have hydrogens, which can react by sylilation; it is not present in the matrix under study.

3.3.3. Robustness

The reference methods were robust against variations because the accuracy and precision were not compromised as showed in Table 1.

It must mention that the results obtained in the applicability do not show a representative profile of the samples produced in the laboratories at FURG.

3.4. ME

The presence of the ME of castor oil was evaluated by the analytical curves of the compounds under study. The ME was negative for all compounds, indicating a suppression of the signal. It represented a different behavior from the one described in the literature regarding the ME analyzed by GC: the enrichment of the signal is usually observed [5].

The ME was low for glycerol (−12.9%), monoolein (−18.7) and diolein (−15.5%) and medium for triolein (−48.7%) [15].

Because the addition standard method is one of the ways to correct or to reduce the ME, the results of the accuracy previously

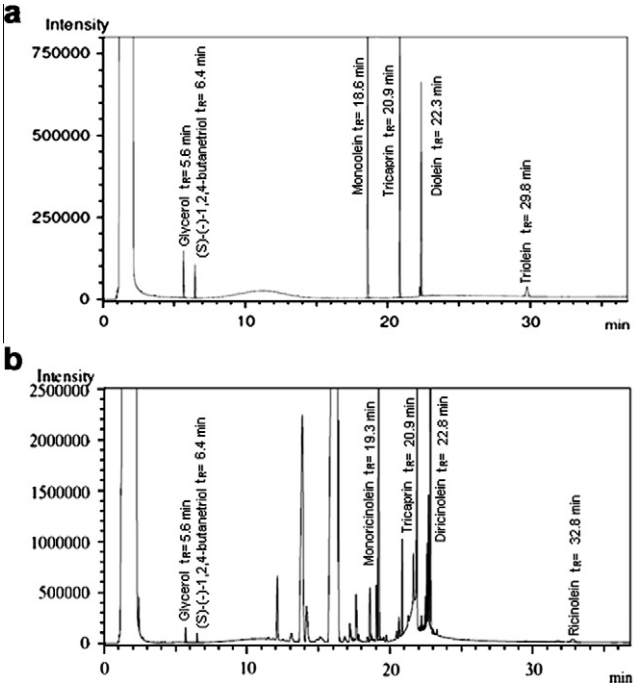

Fig. 4. Chromatographic profile of the standards mixture at fifth level of concentration, under the analysis conditions of ASTM D 6584 (a) and a chromatographic profile of a sample of biodiesel ethyl esters from castor oil (b).

Table 1
Accuracy (%) and RSD (%) of the method for the compounds in the biodiesel ethyl esters from castor oil in different concentration levels.

Compounds	Fortification level (% w/w)	Repeatability		Intermediate precision	
		Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Glycerol	0.005	80.6	3.5	82.6	7.5
	0.025	96.0	2.6	88.8	3.4
	0.05	101.6	4.1	119.3	5.6
Monoolein	0.1	91.9	2.3	70.0	8.8
	0.5	96.4	4.2	114.2	6.6
	1	94.1	4.7	101.7	6.5
Diolein	0.05	104.9	2.6	98.5	4.3
	0.2	104.6	4.0	119.8	4.2
	0.5	107.6	4.4	106.8	5.0
Triolein	0.0522	100.9	10.7	115.5	6.8
	0.2088	82.5	4.7	85.6	5.7
	0.5220	76.5	5.1	80.2	1.8

Accuracy was evaluated by addition standard method for glycerol, monoolein and diolein and by recovery for triolein.

Table 2
Method recovery (%), expressed in terms of repeatability (R_r) and intermediate precision (R_{ip}), for triolein calculated by curves in the matrix and in solvent.

Fortification level (% w/w)	Solvent		Matrix	
	R_r (%)	R_{ip} (%)	R_r (%)	R_{ip} (%)
0.05	100.9	115.5	91.0	119.3
0.2	82.5	85.6	128.2	140.5
0.5	76.5	80.2	138.6	145.9

Table 3
MRLs for free and total, mono-, di- and triglycerides in the biodiesel of according to the ANP 04/2010, ASTM D 6751e EN 14214 norms.

Parameters	ANP 04/2010 Limit (% w/w)	ASTM D 6751 Limit (% w/w)	EN 14214 Limit (% w/w)
Free glycerol	0.02 max	0.02 max	0.02 max
Monoglycerides	To note	–	0.8 max
Diglycerides	To note	–	0.2 max
Triglycerides	To note	–	0.2 max
Total glycerol	0.25 max	0.24 max	0.25 max

shown for glycerol, monoolein and the diolein did not need to be corrected (Table 1). However, for triolein, these results were calculated again considering the curve in the matrix; it is another way to compensate the ME (Table 2).

Although the ME has been observed for all compounds, the quantification by analytical curves in the solvent is preferable. To use the curves in the matrix, it is necessary to build one curve for each type of biodiesel, because the ME can vary depending on the characteristic of the matrix. The addition standard method needs one curve for each sample.

3.5. Applicability of the method

The method was applied to samples of biodiesel ethyl from castor oil produced in the Organic Chemistry laboratories at FURG.

The values of glycerides, free and total glycerol in the sample were only an estimative, because they exceeded the last level of concentration of the liner range. The sample presented 0.053, 6.164, 4.507, 1.315 and 2.458 (% w/w) for free glycerol, monoglycerides, diglycerides, triglycerides and total glycerol, respectively. It did not comply with the standards required by ANP 04/2010, ASTM D 6751 and EN 14214 regarding the content of free and total glycerol, mono-, di- and triglycerides (Table 3).

Fig. 4. shows a chromatogram of a sample under analysis.

4. Conclusions

The main contribution of this study is the use of only one method to determine glycerides, free and total glycerol in biodiesel from castor oil, while the ANP recommends the use of three methods. From results obtained in this work, the reference methods ASTM D 6584 and EN 14105 can include the biodiesel ethyl esters from castor oil as matrix to be analyzed, with the condition that the volume of MSTFA should be 250 uL.

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