

# Free and Total Glycerin Analyses in Biodiesel–Diesel Blends

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**ABSTRACT:** An analytical method for the analysis of free glycerin and total glycerin in biodiesel/diesel blends is presented. The total glycerin is related to the amount of nonconverted glycerides present in the biodiesel, while the free glycerin is related to its purification steps. The method is based on the conversion of mono-, di-, and triacylglycerides in methyl esters and glycerin, and then the glycerin is extracted and quantified with a standard chemical procedure. It is possible to measure the total and free glycerin with very high repeatability and reproducibility. The limits of detection and quantification for the total glycerin analysis are  $3.1 \times 10^{-5}$  and  $9.1 \times 10^{-5}$  wt %, respectively, making it possible to determine this parameter in biodiesel/diesel blends containing a low amount of biodiesel of good quality.

## 1. INTRODUCTION

The increasing importance of sustainability in energy production has motivated a global compromise toward the use of renewable resources for fuels production. The biodiesel is the second more important liquid biofuel used in the world, behind the ethanol. The global supply of liquid biofuels in 2015 was approximately 35 billion gallons, being ethanol and biodiesel produced in a 3 to 1 ratio;<sup>1</sup> i.e., the biodiesel production in 2015 was approximately 7.8 billions gallons. Similar situations occurred during 2016, with a global production of 103 and 31 billion liters of ethanol and biodiesel, respectively, and in 2017, with 106 and 31 billion liters of each of these biofuels.<sup>2</sup> The global production of diesel fuel in 2017 was approximately 20 millions of barrels of oil equivalent.<sup>3</sup> Taking into account that a ton of biodiesel is equivalent to 5.89 barrels of oil equivalent, the biodiesel produced during 2017 represents approximately 2.2% of the diesel produced in the world.

Biodiesel is produced by transesterification of vegetable oils or animal fats with methanol (or ethanol), forming alkyl esters of fatty acids.

Many modern diesel engines can operate with pure biodiesel (B100), although it is commonly used in mixtures below 10% v/v (B10). In the European Union and in the USA, according to the EN-590<sup>4</sup> and ASTM D-975,<sup>5</sup> respectively, the fuel sold as diesel may contain up to 5%v/v without notification to the consumers. The mandatory use of biodiesel is a fact in many countries. For example, in Argentina, the diesel must contain 10%v/v of biodiesel.<sup>6</sup>

Two of the more important quality parameters of biodiesel are the free glycerin (FG) and total glycerin (TG) contents. The former is the glycerin present in the mixture, while the TG is the sum of the FG and the bound glycerin (BG). This latter parameter is related to the amounts of unreacted mono- (MAG), di- (DAG), and triacylglycerides (TAG) present in the fuel expressed as glycerin. Biodiesel containing values of TG and FG higher than those specified in the standards (ASTM D-6751<sup>7</sup> and EN 14214<sup>8</sup>) may cause engine malfunction. Unreacted glycerides are responsible for carbon

deposition in injectors, pistons, and valves, and high FG values lead to phase separation during storing.

ASTM D6584<sup>9</sup> and EN 14105<sup>10</sup> are the standard procedures to determine the FG and the unreacted mono-, di-, and triacylglycerides (MAG, DAG, TAG) expressed as bound glycerin (BG) in biodiesel by gas chromatography. These procedures cannot be applied to the biodiesel–diesel (B-D) blends.

Our research group developed an alternative volumetric method to determine FG and TG content in biodiesel.<sup>11</sup> This method proved to be useful for small and medium sized industries, since it does not need delicate or expensive analytical equipment such as a gas chromatograph. In addition, this volumetric method is not limited for analysis of biodiesel obtained from certain raw materials, as is the case of the analytical procedure described in the EN 14105 standard, which is applicable only to biodiesel obtained from soybean, rapeseed, or sunflower oil.<sup>10</sup>

The volumetric method developed to determine FG involves an extraction step with water of the glycerin present in the sample, and its quantification. To measure the TG, in a first step, the glycerides left in the sample due to an uncompleted conversion during the biodiesel production process are transformed in glycerin. In a second step, the glycerin is extracted and quantified using a chemical procedure. This procedure has shown to have a very good precision and repeatability.<sup>12</sup>

The analysis of FG and TG in the B-D blends is more complicated due to the dilution and the presence of a large number of compounds in the diesel that overlap with the biodiesel components in the GC analysis. There is only one procedure reported to analyze the FG and the TG in the B-D blends. This method uses anion exchange chromatography to detect the glycerin, after the transformation of the glycerides in glycerin and methyl esters.<sup>12</sup> Besides the equipment cost, this methodology presents as another disadvantage the high

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possibility of interferences during the analysis. There are several compounds that could have similar retention time than the glycerin. Also, the excess of sodium hydroxide used during the sample preparation to determine TG could lead to an unbalanced pH in the anion exchange column.

On the other hand, several methodologies have been proposed to determine the ester content in B-D blends, such as infrared (IR),<sup>13</sup> ultraviolet (UV),<sup>14</sup> ultraviolet–visible (UV–vis) spectroscopy,<sup>15</sup> <sup>1</sup>H NMR,<sup>16</sup> and chromatography.<sup>17</sup> The standard procedure used in the quantification of the biodiesel content in B-D blends is based on IR spectroscopy, as indicated in the ASTM D-7371<sup>18</sup> and EN 14078<sup>19</sup> standards. This technique is based on the high absorption of the carbonyl groups in the ester molecule, in a region of wave numbers in which the diesel does not absorb. The IR technique may present problems of interferences in the case that the blend contains other compounds that have carbonyl groups, for example, vegetable oil. Then, if the biodiesel used in the blend does not meet the limits established by the EN 14214 regarding the composition of the unreacted glycerides (MAG, DAG, TAG), it is not possible to be detected and quantified with the analysis based on IR spectroscopy. On the other hand, most of the techniques developed to quantify the biodiesel content of B-D blends do not take into account the presence of glycerides (MAG, DAG, TAG).<sup>14–17,20,21</sup>

Due to the importance of the presence of these contaminants in the fuel, there have been several reports of studies related to techniques to quantify specifically the adulterations with vegetable oils in the B-D blends, such as NIR,<sup>22–25</sup> MIR,<sup>26,27</sup> FTIR,<sup>28</sup> FT-Raman,<sup>29</sup> UV–vis,<sup>30,31</sup> spectrofluorimetric,<sup>32,33</sup> Electro Spray Ionization Mass Spectrometry (EIS-MS),<sup>34</sup> and Easy Ambient Sonic-Spray Ionization Mass Spectrometry (EASI-MS).<sup>35</sup> All of these works used different techniques to analyze vegetable oil–biodiesel–diesel blends (O-B-D). In most cases, the vegetable oil concentrations are relatively high. In other words, the vegetable oil content used in the B-D blends corresponded to partial or total substitution of the biodiesel; therefore, the vegetable oil concentration related to the biodiesel concentration was in the range 10–100%, and even more.<sup>25–35</sup> The objective of these techniques is to detect adulteration of B-D blends with vegetable oil, but they cannot detect the level of triacylglycerides that could be present in the blend if a good quality biodiesel is used in its preparation. The biodiesel used in the blends can contain up to 0.2 wt % of triacylglycerides.<sup>8</sup> There are only few studies of O-B-D blends that used lower concentrations of vegetable oil in biodiesel, in the range of 0.2–6 wt %.<sup>13,22–24</sup> However, none of these methodologies took into account the presence of mono- and diacylglycerides, as well as the glycerin. Therefore, these alternatives for identifying the presence of vegetable oil in B-D blends have a major drawback due to the lack of identification of mono- and diacylglycerides that could be present due to the incomplete reaction during biodiesel production. Since the chemical nature of these compounds is very similar to that of vegetable oil, they could have interference in the analytical procedure. The unreacted glycerides are always present in the biodiesel used to prepare the blends.

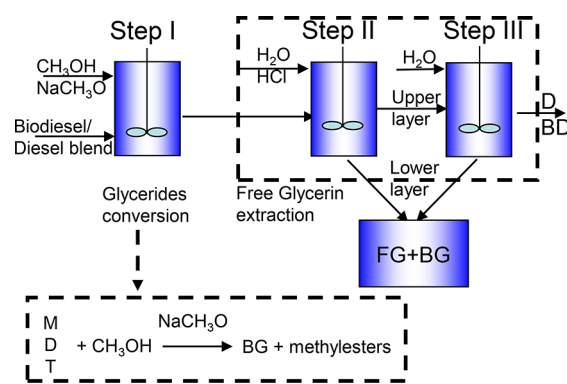
In this work, a volumetric procedure to determine the FG and TG content in B-D blends is presented. This procedure only requires simple laboratory equipment, not being necessary calibration curves. It is shown that, with proper modifications, the procedure previously reported to determine these

parameters in biodiesel<sup>11</sup> is adequate to measure the free and total glycerin contents in B-D blends with high accuracy. This procedure has been used in our laboratory to perform the analysis for petroleum companies that buy biodiesel to prepare the B10 mixture for more than 4 years.

## 2. FUNDAMENTALS

The BG parameter represents the amount of glycerin that is present in the mixture forming MAG, DAG, and TAG. Therefore, by converting these glycerides into methyl esters and glycerin by a transesterification reaction, the parameter “bound glycerin” (BG) can be determined. To do this, after the glycerides conversion, the glycerin has to be extracted and then titrated according to standard procedures. Scheme 1 shows the

**Scheme 1. Procedure Used To Generate the Aqueous Samples for Free and Total Glycerin Analyses**



simple sequence of steps needed to measure the TG and the FG parameters. The TG, which is the sum of the BG and the FG, is measured after the glycerides are converted in glycerin and methylesters in step I, and then extracted in consecutive steps using acidified water in the first one, and water in the following steps (steps II and III). In order to ensure that all the glycerides are converted to methylesters and glycerin, an excess of methanol and catalyst is used.

The FG content is determined by titration after extraction with water (steps II and III, Scheme 1). Then, the bound glycerin is obtained as the difference between the TG and the FG.

These methods are very sensitive, and it is possible to perform the TG and FG determinations on B-D blends using 200 and 350 g of sample, respectively.

The glycerin present in the aqueous phase after extraction from the reaction media, is determined using a similar procedure to that used to measure the glycerin content in soaps (IRAM 5571<sup>36</sup>), industrial glycerin (IRAM 41089,<sup>37</sup> BS-5711,<sup>38</sup> AOCs Ea 6-94<sup>39</sup>), alkylid resins (ASTM D-1615<sup>40</sup>), and analogous to FG and TG determinations in biodiesel.<sup>11</sup> This method is based on the oxidation of the glycerin using sodium metaperiodate, a reaction that produces formic acid. This acid is then titrated using sodium hydroxide. The production of formic acid upon oxidation occurs if the molecule has more than 2 hydroxyl groups, as is the case of the glycerin.<sup>11</sup>

In order to avoid interferences, the sample under analysis should not contain compounds having three or more adjacent hydroxyl groups, such as sugars. To consume the excess of

sodium periodate, ethylene glycol is added after the glycerin oxidation, forming formaldehyde and sodium iodate.

### 3. EXPERIMENTAL SECTION

**3.1. Materials.** The fossil diesel without biodiesel was provided by a refining company in Argentina (Refinor S.A.). Diesel grade 2 (less than 500 ppm of S) and grade 3 (less than 10 ppm of S) have been included in this study. Biodiesel samples were provided by different companies in Argentina, all of them having proven technologies that meet all the international quality standards.

Free and total glycerin contents in the biodiesel samples that were used to prepare the B-D blends were determined using the EN 14105 standard.<sup>10</sup> Blends containing different proportions of a previously characterized biodiesel were prepared, and analyzed with the procedure presented in this paper.

Blank analyses on the diesel fuel were carried out 30 times, for both free and total glycerin contents. Limits of detection (LOD) and limits of quantification (LOQ) were calculated according to IUPAC,<sup>41</sup> using the following equations:

$$\text{LOD} = X_B + 3\sigma_B \quad (1)$$

$$\text{LOQ} = X_B + 10\sigma_B \quad (2)$$

$X_B$  represents the average of the blank experiments measurements, and  $\sigma_B$  is its standard deviation.

**3.2. Procedures.** The detailed procedures that must be followed in order to obtain good results are similar to those described for the analysis of free and total glycerin in biodiesel samples previously published,<sup>11</sup> and are presented in sections 3.2.1 and 3.2.2, that correspond to Total and Free Glycerin determinations, respectively. The differences between the methods adjusted to determine these parameters in the diesel/biodiesel blends compared to that used in biodiesel samples are in the quantities of sample used to perform the analyses and in the aliquot used to titrate the glycerin.

**3.2.1. Total Glycerin Analysis: Analytical Procedure.** The analysis should be carried out in a 500 cc flask, loading a sample of approximately 200 g, weighed with a precision of 0.01 g. In order to fully convert the acylglycerides, add 20 mL of a solution of sodium methoxide in methanol (40 g/L). As an alternative, a solution of sodium hydroxide in methanol can also be used (35 g/L). Put the flask in a water bath at 60–65 °C, and connect a condenser. The mixture has to be vigorously stirred, using a magnetic stirrer, maintaining these conditions during 2.5 h approximately.

Once the acylglycerides are converted, the glycerin has to be extracted from the reacting media, as shown in Steps II and III in Scheme 1. In order to do this, remove the condenser and add 20 mL of a 5 wt % HCl solution while stirring, maintaining the temperature at 60–65 °C during 15 min. Then transfer the content of the flask to a separatory funnel, and after the phases are separated, transfer the water phase to a 250 cc Erlenmeyer, and the biodiesel–diesel blend to the 500 cc flask used during the reaction and first extraction steps. Add 10 mL of 5 wt % HCl solution and 10 mL of distilled water in order to continue with the glycerin extraction, and maintain the flask in the water bath during 15 min with gentle stirring. Then separate phases in the separatory funnel, adding the water phase to the Erlenmeyer and returning the biodiesel–diesel phase to the 500 cc flask for a new extraction step, in this case, using only 20 mL of distilled water, and maintain again during 15 min at 60–65 °C. After this extraction, phases are separated collecting the water phase in the Erlenmeyer, and the biodiesel–diesel phase can be discarded. The flask and the separatory funnel may be washed with 20 mL of water, adding this water to the Erlenmeyer.

Finally, the water phase has to be analyzed in order to quantify the glycerin extracted from the biodiesel–diesel blend. Add a few drops of phenol red indicator to the Erlenmeyer, and then adjust the pH, adding sodium hydroxide (2 N) until the color changes to fuchsia. Then the pH is adjusted to an acid value, adding 5 wt % HCl solution until the solution changes its color to yellow, and then adding an extra amount of 0.5 mL of the HCl solution. This pH is needed in order to

remove the carbon dioxide absorbed in the aqueous solution. Boil this solution during 3 min and then cool it, connecting the Erlenmeyer to a carbon dioxide trap, prepared using a short glass tube filled with sodium hydroxide. This trap avoids the reabsorption of carbon dioxide. Once the solution is at room temperature, adjust the pH adding the same sodium hydroxide solution that will be used in the final titration, (e.g., 0.03 or 0.1 N). The addition is stopped exactly when the color changes from yellow to fuchsia. The oxidation of glycerin to formic acid is achieved by adding 15 mL of sodium periodate with a concentration of 6 g/100 mL, swirl the Erlenmeyer, put a stopper, and leave it in the darkness during 30 min. Then, in order to eliminate the excess of sodium periodate, add 2.5 mL of ethylene glycol, and wash the walls of the Erlenmeyer with distilled water, swirl the Erlenmeyer, and leave it in the darkness with a stopper during 20 min. The final step is the titration of the aqueous phase using sodium hydroxide with a concentration of 0.03 N, even though a concentration of 0.1 N is also adequate.

The amount of Total Glycerin (TG%) is calculated as follows:

$$\text{TG} (\%) = 0.0921 \times V_{\text{NaOH}} \times N_{\text{NaOH}} \times 100/w_{\text{sample}} \quad (3)$$

TG% is expressed as g of glycerin/100 g of sample,  $V_{\text{NaOH}}$  is the volume of sodium hydroxide used in the titration (mL),  $N_{\text{NaOH}}$  is the concentration of the sodium hydroxide solution (normality), and  $w_{\text{sample}}$  is the amount of sample loaded in the analysis (g).

It has to be checked that the amount of sodium periodate added to the solution containing the glycerin was enough to fully transform it to formic acid. Each mole of glycerin is oxidized consuming two sodium periodate moles and leads to the formation of 1 mol of formic acid. Therefore, if the number of moles of formic acid detected in the titration is very similar to the half of the number of moles of periodate added to the aqueous solution to be titrated, the sodium periodate may be the limiting reactant, and the quantification of glycerin may be wrong. As a reference, if the volume of 0.1 N sodium hydroxide used to titrate the solution is close to 21 mL, it means that the analysis must be repeated with a larger amount of  $\text{NaIO}_4$  or with a smaller amount of sample.

**3.2.2. Free Glycerin Analysis: Analytical Procedure.** As indicated in Scheme 1, the procedure for the free glycerin analysis does not include the acylglycerides conversion. Since the amount of free glycerin content in the blend is approximately 1 order of magnitude smaller than the total glycerin, a larger mass of sample is used in the former. Typically, using 300 g of sample is enough to determine the free glycerin content of the biodiesel–diesel blend with the precision described in this study. Once the sample is loaded in a 500 cc flask, put it in a water bath at 60–65 °C and follow the extraction procedure and glycerin analysis described in section 3.2.1.

The free glycerin content (g of glycerin/100 g of sample) of the biodiesel–diesel blend can be calculated as follows:

$$\text{FG} (\%) = 0.0921 \times V_{\text{NaOH}} \times N_{\text{NaOH}} \times 100/w_{\text{sample}} \quad (4)$$

The variables have been defined above.

Since the free glycerin content is usually very low, the volume of sodium hydroxide used in the titration is normally a small value. Therefore, to increase this volume and the precision of the method, the free glycerin analysis can be carried out using 0.03 N sodium hydroxide.

## 4. RESULTS AND DISCUSSION

**4.1. Total Glycerin Determination.** To obtain reliable quantitative results, the total conversion of the glycerides present in the sample must be assured. This is achieved using a large excess of methanol and catalyst in Step I of the analytical procedure (see Scheme 1), transforming all the glycerides in glycerin and methyl esters. After the reaction step, the system is neutralized with an aqueous solution of 5 wt % HCl (Step II in Scheme 1), followed by two additional washing steps (Step III), in order to recover all the glycerin present in the diesel

Table 1. Total Glycerin Analyses, after Addition of a Biodiesel with TG = 0.2080 wt % to a Diesel Sample

amount of biodiesel in the blend, wt %	total glycerin, wt %		absolute difference ( $x_c - x_m$ ), wt %	difference % $[(x_c - x_m)/x_c] \times 100$
	calculated $x_c$	measured $x_m$		
1.1	0.0022	0.0021	0.0001	4.5
2.9	0.0061	0.0067	0.0006	9.5
4.7	0.0099	0.0092	0.0007	7.1
6.6	0.0136	0.0127	0.0009	6.9
6.6	0.0136	0.0138	0.0002	1.4
6.6	0.0136	0.0140	0.0004	2.7
6.8	0.0142	0.0144	0.0002	1.0
8.8	0.0184	0.0194	0.0010	5.3
9.1	0.0189	0.0192	0.0003	1.6
13.3	0.0276	0.0277	0.0001	0.4

phase. The water phases obtained in Steps II and III are collected.

In a previous work,<sup>11</sup> we have checked that, under these conditions, no mono-, di-, or triacylglycerides were found on the biodiesel samples after this procedure, and therefore, the glycerin formed under these conditions exactly reflects the amount of unreacted glycerides originally present in the sample.

A set of experiments were carried out by adding a known amount of a previously analyzed biodiesel to fossil diesel. Table 1 shows examples of the real amount of total glycerin (TG) added to the diesel ( $x_c$ ) and the experimental results ( $x_m$ ) obtained following the procedure described in section 3.2.1. The biodiesel used in these analyses had a TG content of 0.2080 wt %. It can be observed that the values of TG obtained with this procedure in the B-D blend ( $x_m$ ) is very close to the theoretical value calculated using the TG content of the biodiesel and the amount of biodiesel added to the diesel ( $x_c$ ). In all cases, the difference between these two values is lower than 10% (Table 1), which is a better reproducibility than the chromatographic analysis used to measure the total glycerin content variable in biodiesel, as described in the EN 14105 standard. In the case of the replicated experiments shown in Table 1, with 6.6 wt % of added biodiesel to the blend, the average measured value of total glycerin resulted to be 0.0135 wt %, while the calculated value was 0.0136 wt %. The differences between the average (0.0135 wt %) and each measured value shown in Table 1 for 6.6 wt % of added biodiesel (0.0127, 0.0138, 0.0140 wt %) are 5.9, 2.2, and 3.7%, respectively. These values are significantly better than those presented in the EN 14105 standard. For example, according to this standard, the repeatability (absolute difference between two measurements) for total glycerin analysis in a biodiesel containing 0.013 wt % is 0.00489 wt %, representing a percentage difference of 37%. It has to be mentioned that it is unusual to find such low values of total glycerin in pure biodiesel.

Figure 1 shows the relationship between the measured and the theoretical values of TG in the B-D blends listed in Table 1. It can be observed that the points follow the line at 45°, which indicates that the precision of this procedure is very good. The highest absolute difference between the theoretical value and the measured value was approximately 0.0010 wt %. Figure 1 also shows the 95% confidence and prediction intervals. The former refers to the interval in which the mean of a measured value should be in 95 out of 100 measurements, and the latter referred to the interval in which a single measurement should be detected with the same confidence. It

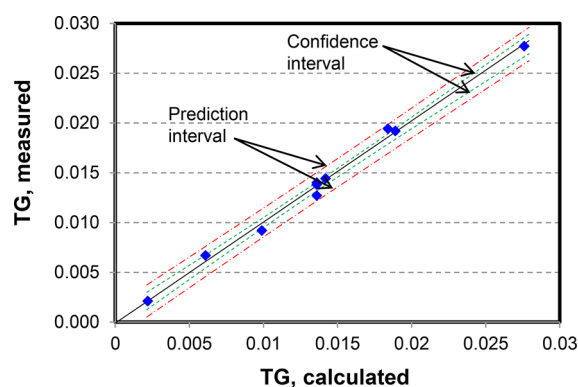


Figure 1. Relationship between the measured and theoretical values of total glycerin in different diesel/biodiesel blends.

can be observed in this figure that the repeatability of this procedure is very good and makes it possible to determine the total glycerin content in the B-D blend with high accuracy and precision. Figure 2 compares the maximum error that,

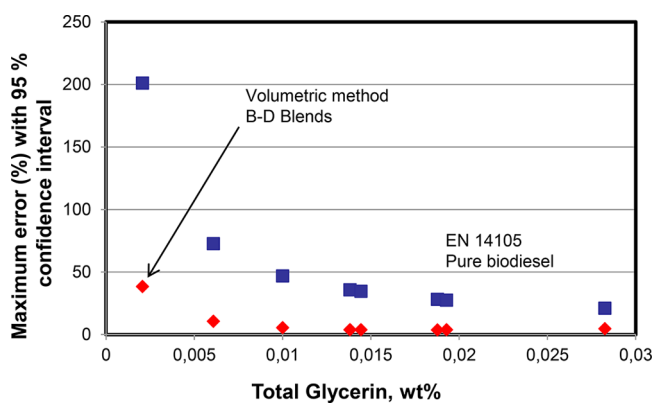


Figure 2. Errors (differences between the mean value and the expected value) with 95% confidence, for the volumetric method and method proposed in EN 14105.

according to the confidence interval shown in Figure 1, can have the mean value of the total glycerin content determination using the procedure described in this work. This figure also shows the same magnitude that the chromatographic procedure has for the total glycerin analysis in pure biodiesel, according to the statistical information provided in the EN 14105 standard.<sup>10</sup> It can be observed that the repeatability of the method presented in this work has, at the same level of total glycerin, a significantly better

Table 2. Free Glycerin Analyses, after Addition of Biodiesel Containing 0.0169 wt % of FG to a Diesel Sample

amount of biodiesel in the blend, wt %	glycerin, wt %		absolute difference ( $x_c - x_m$ ), wt %	difference % $[(x_c - x_m)/x_c] \times 100$
	calculated $x_c$	measured $x_m$		
3.0	0.00051	0.00052	0.00001	2.6
5.5	0.00093	0.00090	0.00003	2.6
7.0	0.00118	0.00111	0.00007	6.3
10.1	0.00170	0.00178	0.00008	4.5

Table 3. Free Glycerin Analyses, after Addition of Biodiesel Containing 0.00825 wt % of FG to a Diesel Sample

amount of biodiesel in the blend, wt %	glycerin, wt %		absolute difference ( $x_c - x_m$ ), wt %	difference % $[(x_c - x_m)/x_c] \times 100$
	calculated $x_c$	measured $x_m$		
1.0	0.00008	0.00007	0.00001	8.5
3.0	0.00025	0.00028	0.00003	11.8
5.1	0.00042	0.00041	0.00001	1.9
6.3	0.00052	0.00052	0.00000	0.0
6.5	0.00053	0.00050	0.00004	6.6

repeatability than the method used worldwide for total glycerin analysis in pure biodiesel.

**4.2. Free Glycerin Determination.** The FG determination was carried out by extracting it in three consecutive washings steps from the sample (B-D blend) with distilled water. This procedure is similar to that presented in Scheme 1, but without Step I. Only Steps II and III are carried out. The international standards establish a maximum value for this parameter in biodiesel of 0.02 wt %.<sup>7,8</sup> Typical values of FG in biodiesel of very good quality are around 0.005 wt %. Therefore, if a biodiesel with this level of free glycerin is blended at a 10 wt % with diesel, the blend will have an FG content of 0.0005 wt %, which is a very low concentration. However, since the method presented in this work is based on the glycerin extraction using a rather small volume of water, and it is possible to use a large amount of sample, this concentration is within the detection limit of the procedure described in section 3.2.2. In Argentina, we have found that normal values of FG in biodiesel obtained from plants of proven technologies are in the range of 0.005–0.015 wt %.

A set of experiments were carried out, by adding a known amount of a previously analyzed biodiesel to fossil diesel. The calculated ( $x_c$ ) and experimental ( $x_m$ ) results for the FG determinations carried out on several B-D blends are presented in Tables 2 and 3. The former displays results obtained in blends that were prepared using a biodiesel containing 0.0169 wt % of FG, which is a rather high value, while, in Table 3, the results correspond to blends prepared using a biodiesel with 0.00825 wt % of FG. It can be observed in both tables that the measured values of FG are very close to the expected values, which were obtained by calculation using the content of FG determined in the biodiesel used to prepare the blends, and the percentage of biodiesel added to the diesel. In all cases, the difference between the expected and the measured values was lower than 12%.

**4.3. Detection and Quantification Limits.** **4.3.1. Total Glycerin Determination.** Pure diesel was used in the procedure presented in Scheme 1, carrying out the total glycerin analysis 30 times, as requested in the IUPAC procedure in order to calculate the limit of detection and limit of quantification. All the analyses fell in the range  $0-2 \times 10^{-5}$  wt %. These experiments show that there are no interferences in the diesel that can affect the FG and TG determinations. The analyses of the biodiesel used to prepare

the blends with the diesel fuel showed that its total glycerin content was 0.2080 wt %. These analyses were carried out using the GC procedure described in the EN 14105 standard,<sup>10</sup> and also verified using the volumetric method already reported.<sup>11</sup> The maximum deviation in this set of analyses was 2.6% referred to the mean value.

The average value of the blank determination in the TG blank analyses was  $5.7 \times 10^{-6}$  wt %, and the standard deviation of these measurements was  $8.6 \times 10^{-6}$  wt %. Using the equations mentioned in section 3.1, the LOD and LOQ for the Total Glycerin analysis resulted to be as follows:

$$(\text{LOD})_{\text{TG}} = 3.1 \times 10^{-5} \text{ wt \%}$$

$$(\text{LOQ})_{\text{TG}} = 9.1 \times 10^{-5} \text{ wt \%}$$

There are very different regulations across the world regarding the content of biodiesel in the diesel fuel. One of the highest mandatory values is used in Argentina, being 10%. Therefore, in this study, it has been adopted an upper limit of 10 wt % for the addition of biodiesel to the diesel, and a lower limit of 1 wt %. If it is assumed that the biodiesel has the maximum content of TG and FG allowed in the international standards (EN 14214), i.e., 0.25 and 0.02 wt %, respectively, the B-D blend with 10 wt % of biodiesel (B10) will have a TG content of 0.025 wt %, and an FG content of 0.002 wt %. The quantification capacity of this analytical procedure can also be appreciated in the following example. Using a biodiesel containing 0.15 wt % of total glycerin to prepare a B01 mixture (1% biodiesel in diesel), the total glycerin of this blend will be 0.0015 wt %, and this quantity is well above the quantification limit ( $9.1 \times 10^{-5}$ ).

The upper limit of quantification using the quantities of sodium periodate mentioned in the section 3.2.1 is 0.065 wt %. If the amount of glycerin is higher than this value, periodate is not enough to fully oxidize it to formic acid. Therefore, the upper limit of quantification can be as high as desired, just increasing the amount of sodium periodate used in the analysis. No calibration is needed to modify the range of application of this procedure. According to the stoichiometry of the reaction between glycerin and sodium periodate, 4.65 g of  $\text{NaIO}_4$  are needed to oxidize each gram of glycerin to be titrated. To guarantee the complete oxidation of glycerin in the sample, 6 g of  $\text{NaIO}_4$  is used for each gram of glycerin. As indicated in sections 3.2.1 and 3.2.2, the volume of NaOH

used to titrate formic acid is also used as a guide to know if the  $\text{NaIO}_4$  added was enough, or if the analysis has to be repeated with a lower amount of sample, or a higher amount of  $\text{NaIO}_4$ . On the other hand, if the volume of  $\text{NaOH}$  used is too small, titration has to be repeated using a higher amount of sample in order to improve the accuracy.

**4.3.2. Free Glycerin Determination.** The free glycerin analysis was carried out in the pure diesel before the addition of biodiesel as the blank analyses. It was repeated 30 times as in the case of the total glycerin analysis. The results of these analyses were very similar to those found in the total glycerin analysis with the pure diesel sample. This confirms that, in this diesel sample, there are no components that interfere with the procedures described in sections 3.2.1 and 3.2.2. The mean value of these analyses was  $4.7 \times 10^{-6}$  wt %, and the standard deviation was  $7.3 \times 10^{-6}$  wt %. The limits resulted to be

$$(\text{LOD})_{\text{FG}} = 2.7 \times 10^{-5} \text{ wt \%}$$

$$(\text{LOQ})_{\text{FG}} = 7.8 \times 10^{-5} \text{ wt \%}$$

In the case in which the biodiesel used in the blend contains 0.01 wt % of FG, and is used in a level of 1 wt % in the blend, the concentration of free glycerin in the final mixture is  $1 \times 10^{-4}$  wt %, which is higher than the LOQ.

**4.4. Repeatability.** In Tables 1, 2, and 3, results obtained in replicated experiments are included, for both TG and FG analyses. In the case of the B-D mixtures, the central points have been replicated. In the TG analyses of a B-D mixture containing 6.6 wt % of biodiesel, the difference among the three analyses was lower than 7% (Table 1). In the case of the FG analyses, the differences were also lower than 7%.

It is important to emphasize that the analyses shown in these tables have been carried out by different operators and on different days. Therefore, these results do not correspond strictly to the definition of repeatability that requires the same operator in order to compare the results. It can be expected that the repeatability be even better than that shown in Tables 1, 2, and 3, as normally found when the same operator runs all the analyses.

The TG and FG analyses of the biodiesel samples used in this study were carried out both by the EN14105 standard and using the volumetric procedure previously reported.<sup>11</sup> The latter, when used directly on the B100 sample, has high repeatability, with average errors lower than 3% referred to the mean value, both for TG and FG determinations (results not shown). This percentage is valid only for B100 samples. In the case of B-D blends, the errors are higher, as above presented.

In the procedure reported in the ASTM D7591-12 to determine free and total glycerin in B-D blends by ion exchange chromatography,<sup>12</sup> both the repeatability and reproducibility are in some cases similar to those found in the present work, and in some other cases much higher, depending upon the level of biodiesel in the blend.

## 5. CONCLUSIONS

The volumetric method presented in this work allows determining the TG and FG parameters in B-D blends with very good repeatability, and have LOD and LOQ that makes it possible to determine low concentrations of glycerin, enough to conclude if the biodiesel used in the blend had the quality standards required in ASTM D-6751 or EN 14214. The range of concentrations of free and total glycerin for which this method can be applied has no limitations. On the other hand,

this analytical procedure has a low cost compared to other available methods.

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### Notes

The authors declare no competing financial interest.

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