Volumetric Method for Free and Total Glycerin Determination in Biodiesel

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In the past few years, biodiesel has gained considerable attention due to the increasing concern for the environmental problems associated with the use of fossil fuels. The quality control of this renewable fuel involves numerous analyses, several of them requiring the use of analytical instruments. According to ASTM and EN standards, the analyses of free and total glycerin which are of particular relevance to biodiesel quality must be carried out by GC analysis. This work presents an alternative volumetric method, which does not need expensive equipment, and is therefore particularly useful for quality control in small facilities. Another advantage of the proposed method is that it also overcomes the shortcomings of the GC procedure, such as detection limits and type of raw material used to produce biodiesel. While the GC analysis is restricted to biodiesel obtained from soybean, rapeseed or sunflower oil, the volumetric method has no limitations in this sense.

1. Introduction

Biodiesel production has significantly increased in the past few years. Several plants with large production capacity have been built and several others are under construction in many countries. These plants mainly use rapeseed oil (Europe), soybean oil (USA, Brazil, Argentina), or palm oil (Asia) as raw materials. On the other hand, the use of alternative raw materials has also received considerable attention due to the increasing concern about the use of edible oil for fuel production. Among the oils used for biodiesel production other than soybean, rapeseed, or sunflower oil, we can mention oils of jatropha, algae, castor, linseed, or safflower oil. These raw materials represent an option for small farmers and communities located far from urban centers to produce the fuel they need for farming, heating, and transportation. The use of very small production facilities to produce biodiesel for self-consumption has also spread throughout many countries. For these latter cases, the quality control of biodiesel is a major concern.

The quality of biodiesel is specified in standards such as ASTM D-6751,[1] or EN 14214.[2] Among the numerous properties that must be controlled in order to meet these specifications, total and free glycerin are two of the most important ones, since they are related to the extent of unconverted triglycerides and the effectiveness of the purification procedure, respectively. According to ASTM or EN standards, these determinations must be carried out by chromatographic analysis. Samples have to be silylated, and two different internal standards must be used in order to quantify free glycerin and mono-, di-, and triglycerides. The procedure that must be followed for these analyses is described in ASTM D-6584[3] or in the EN 14105[4] and EN 14106.[5]

There are other methods to determine the mono-, di-, and triglycerides content in biodiesel, such as size exclusion chromatography,[6] viscosity,[7] TGA,[8] reversed-phase high-performance liquid chromatography (RP-HPLC),[9] free glycerol in biodiesel by capillary electrophoresis,[10] nonaqueous reverse-phase HPLC with a UV detector,[11] infrared spectroscopy,[12−14] Raman spectroscopy,[15] or NMR to identify intermediate and final products during the transesterification.[16] Different GC methods have been analyzed by Mittelbach et al.[17]

Each technique has advantages and drawbacks, and the selection of the analytical procedure will be finally made depending on whether it is for quality certification, pass or failed control, or process monitoring. In addition, quality, simplicity, cost, and duration of the analysis including possible sample pretreatments are very important aspects.

The gas chromatographic analysis as described in each of the above-mentioned standards (ASTM D6584[3] and EN 14105[4]) has limitations since it has to be used only for biodiesel obtained from soybean, rapeseed, or sunflower oils; besides, it cannot be used with other oils, such as coconut, or animal fats, since peak overlapping might occur. This is a severe limitation of the method since one of the reasons why biodiesel is gaining acceptance is because it allows the use of alternative raw materials such as chicken, pork, or cow fat, in addition to many other vegetable oils such as coconut, cotton, algae, or palm oil.

A volumetric method, described in the AOCS Ca 14-56,[18] involves saponification with an alcoholic solution of KOH, followed by the addition of chloroform and acetic acid, separation, washing, addition of periodic acid first and then KI, and finally iodine titration with sodium thiosulfate. However, even though this method does not require any complicated equipment, it implies the handling of several harmful substances and is not quite suitable for the determination of total and free glycerin at low levels with high precision. Besides, it includes many chemical steps.

In this work, we present a different volumetric procedure, which has been tested for several years already. This method has no limitations regarding the raw material used to produce biodiesel or the level of combined or free glycerin; it has a very high precision and repeatability, and does not require the use of expensive and/or sophisticated equipment. In fact, several biodiesel industries in Argentina, Paraguay, and Chile have been using this procedure for some time, as well as small farmers. This method does not allow the individual determination of mono-, di-, and triglycerides.

2. Fundamentals

Total glycerin content is obtained after all the glycerides, that is, mono-, di-, and triglycerides are quantitatively transformed

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into methyl esters and glycerin, by transesterification. Afterward, the glycerin is extracted first with acidified water, and then with water. Finally, the glycerin is titrated according to standard procedures.

This method is very sensitive, and it is possible to perform the total glycerin determination with 50 g of sample or less.

Free glycerin is determined just by extraction with water, followed by titration, as indicated in the ASTM D-1615,[19] IRAM 41089,[20] BS-5711,[21] or AOCS Ea 6-94.[22] ASTM D-1615[19] and IRAM 5571[23] refers to the determination of glycerin in alkyd resins and was discontinued in 2004. BS-5711 is specific for glycerin analysis and has also been discontinued. Nevertheless, since it has not been replaced by any other standard, international commerce is based on BS-5711.

In all these standards glycerin titration is based on its oxidation by sodium periodate. This reaction leads to the formation of formic acid when more than 2 hydroxyl groups are present in the molecule. Primary hydroxyl groups react forming formaldehyde upon oxidation and secondary hydroxyl groups lead to formic acid production, according to the following reaction:

$$C_3H_8O_3 + 2NaIO_4 \rightarrow 2HCHO + HCOOH + 2NaIO_3 + H_2O \quad (I)$$

Formic acid is then titrated with sodium hydroxide. This method can be applied to samples free from sugars or other organic compounds with more than two adjacent hydroxyl groups.

To consume the excess of sodium periodate, ethylene glycol is added when reaction I is completed. The reaction that takes place is

$$C_2H_4(OH)_2 + NaIO_4 \rightarrow 2HCHO + NaIO_3 + H_2O \quad (II)$$

3. Results and Discussion

3.1. 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. This procedure cannot be carry out during normal biodiesel production, because of the severe purification problems that arise when large amounts of catalyst are used. Besides, the energy requirement involved in methanol recovery would also be a major problem. In our case, since the objective is not the production of biodiesel according to standards, using a large excess of catalyst is not a problem. After the reaction, the system is neutralized with an aqueous solution of HCl 5 wt %, followed by two additional washing steps, in order to recover all the glycerin present in the biodiesel phase. In this work, we have checked that under these conditions, no mono-, di-, or triglycerides are found on the final product, and therefore the glycerin formed under these conditions exactly reflects the amount of unreacted glycerides originally present in the sample.

Figure 1a shows an example of a biodiesel that contains 0.24 wt % of total glycerin, as determined by EN14105,[4] before and after the first step of this procedure, that is, the reaction with methanol and NaOH as catalyst. It can be clearly seen that no di- and triglycerides are left after the reaction. However a very small peak that elutes at the same time as the monoolein is still present after this reaction step. According to our results, it is very likely that this peak is not a monoolein, as discussed below. Therefore, as above-mentioned, this means that the glycerin formed during this step of the procedure is an exact representation of the amount of unconverted glycerides. Figure 1b shows another example of a GC analysis of a biodiesel sample, and the same sample after the first step of this procedure. In this case, the biodiesel contains 1.08 wt % of total glycerin (EN14105[4]). Again, all the di- and triglycerides are converted, and a very small peak comes out at the same retention time as the monoolein. In both cases (Figure 1 panels a and b), this peak represents an equivalent of total glycerin content lower than 0.01 wt %, if assumed to be monoolein. In any case, this is a very small value, and as discussed below, it is well below the repeatability and reproducibility of the total glycerin content determination by the GC-technique.

Even though this procedure does not allow the determination of the individual amounts of mono-, di-, or triglycerides, it provides a reliable and relatively fast determination of the total glycerin content. It should be remarked that the ASTM standard does not specify the individual content of each type of glycerides, while the EN standard does.

The detailed procedure that must be followed in order to obtain good results is presented in Appendix A.

3.1.1. Glycerin Titration. In many standards related to glycerin analysis (ASTM D-1615, IRAM 5571, IRAM 41089, AOCS Ea 6-94), a blank must be performed. However, we have checked that when following the procedure described in IRAM 41089 or IRAM 5571, in all cases only one drop of 0.1 N NaOH solution was used. Nevertheless, each lab must check whether or not with the procedure being followed, the blank has an influence on the final calculation. Other standards, such as BS 5711 carry out the final titration at an acidic pH and, therefore, in these cases it is necessary to perform the blank determination. In our procedure we used the glycerin analysis as described in IRAM 5571, in which the blank experiment was always negligible. This procedure is similar to that described in ASTM D-1615. However, the latter is designed to determine glycerin, ethylene glycol, and pentaerythritol in alkyd resins. In this latter standard, the solution is not boiled prior to the titration. Therefore the blank could be more relevant since the CO_2 absorbed from the air during the sample handling is not stripped by boiling the solution, and consequently certain amount of the titrating reactant (NaOH) will be used to neutralize the carbonic acid.

3.1.2. Analytical Results. A set of experiments was carried out, by adding a known amount of triglycerides (refined soybean oil) to a biodiesel which was previously analyzed by GC in order to determine the total glycerin content.

Table 1 shows the results. In this table, examples of the determinations that were carried out are presented. A plot of the theoretical total glycerin amount as a function of the measured amount (not shown) displays a general trend exactly on the line at 45°, which indicates that the procedure has no bias either at low or high total glycerin content. The absolute difference between the theoretical value and the measure value of approximately 0.1 wt % is the larger deviation found at high total glycerin content, and a difference of approximately 0.05 wt % is the larger error at total glycerin contents below 0.25 wt %. It has to be emphasized that this set of results corresponds to experiments in which additions of given quantities of vegetable oils were carried out, and the exact amount of total glycerin that corresponds to this addition is not known due to small uncertainties, such as for example, the oil molecular weight.

The volumetric procedure was also used with a sample employed in an interlaboratory test, organized by CEMITEC (Spain), in which 9 European laboratories took part. Table 2 shows these results, as well as a comparison of several other samples analyzed by the method presented in this work, and

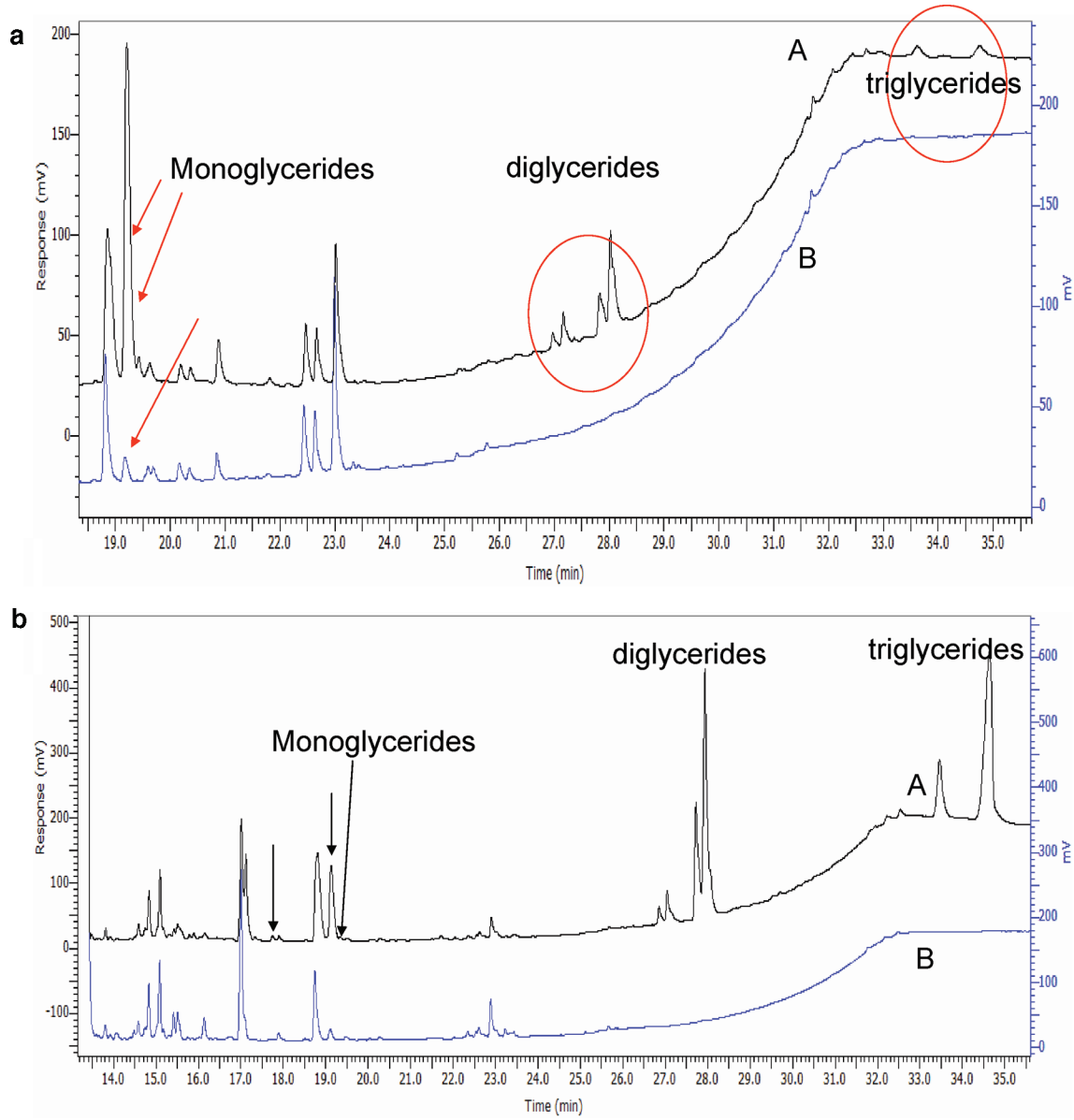

Figure 1. GC analyses of biodiesel samples, before (A) and after (B) the first step of the volumetric procedure: (a) sample with 0.24 wt % total glycerin, (b) sample with 1.08 wt % total glycerin.

by the GC methology. It can be seen that our results are in good agreement with the average of other laboratories that used the EN 14105 procedure.

Regarding the repeatability, ASTM D 6584 establishes that for total glycerin determination, the repeatability is $r = 0.009$. The EN 1405 gives the following equation for repeatability: $r = 0.0687x + 0.004$ (where x is the mean value), and for reproducibility it provides the following information: $R = 0.4472x - 0.01$. Therefore, in the latter case, it can be expected that for a total glycerin content of 0.15 the reproducibility is 0.057 wt %, which represents a difference of 38% between two measurements carried out in different laboratories. Our determination in this case differs from the mean value in 0.03 wt %, which represents a deviation of 20%. Therefore, the volumetric procedure results to be well within the quality standards regarding the statistics criteria. The z-criterion establishes that z should be less than 2, to have a reliable result, z being defined as

$$z = x - \bar{x}/\sigma$$

The volumetric measurement is well within the average of the other nine laboratories, with $z = 0.55$, being $\sigma = 0.055$. A value of z less than 2 is acceptable and means that the measured value differs from the real value less than two times the standard deviation, and this would occur in 95% of the cases for a normal distribution.

3.1.3. GC Analysis: Influence of Free Fatty Acids. As above-discussed, the GC analysis of a biodiesel sample reacted with excess of methanol and catalyst, shows a small peak that comes out at the same retention time as the monoolein (see Figure 1a,b).

We explored the possibility that it could correspond to a fatty acid. The fatty acid elutes at longer retention times than the corresponding methylester. As indicated in the example given in the ASTM D-6584 standard, the C24 methylester comes out in the chromatogram right before the main peak that corresponds to the monoglycerides. Therefore, the small peak shown in Figure 1a,b could be related to the C24 fatty acid. Figure 2 shows GC analyses of a biodiesel sample before and after addition of a mixture of free fatty acids. It can be clearly seen

Table 1. Total Glycerin Analyses, After Addition of Vegetable Oil to a Biodiesel Sample

	glycerin, wt %			
triglyceride added wt %	calculated x_c	measured x_m	Absolute difference wt %	error % $((x_c - x_m)/x_c) \times 100$
1.028	0.112	0.154	0.042	37.5
1.713	0.184	0.203	0.01	5.4
4.310	0.462	0.462	0.000	0.0
0.430	0.049	0.060	0.011	22.4
0.965	0.105	0.113	0.007	6.7
1.463	0.162	0.148	0.015	9.5
0.970	0.732	0.750	0.017	2.3
		0.730	0.002	0.3
2.010	0.842	0.900	0.058	6.9
		0.840	0.001	0.1
3.000	0.946	0.870	0.076	8.0
		0.830	0.116	12.2
0.940	0.949	0.880	0.069	7.3
1.930	1.053	1.000	0.053	0.05
0.450	0.897	0.950	0.052	5.8
0.630	0.916	0.860	0.056	6.1
1.530	1.011	0.940	0.071	7.0
2.800	1.145	1.040	0.105	9.2
0.600	0.863	0.860	0.003	0.3
1.100	0.916	0.930	0.014	1.5
1.550	0.963	1.000	0.036	3.7

Table 2. Comparison of Volumetric Method with the EN14105

analysis	volumetric	EN14105	comments
total glycerin	0.18	0.15	Interlab control, nine European laboratories
	0.88	0.82	different European control laboratories
	0.16	0.15	
	0.33	0.31	
	0.26	0.24	biodiesel production plant laboratory
	0.29	0.26	
free glycerin	0.01	0.01	Interlab control, nine European laboratories
	0.0067	0.01	different European control laboratories
	0.019	0.02	

that the peak assigned to the monoolein increases after the addition of free fatty acids, which indicates that in the GC analysis there might be an interference of the free fatty acid in the total monoglyceride analysis, most probably due to the C24 fatty acid. We carried out many reaction tests, using different methanol to oil ratios, catalyst amount, reaction times, and performing also up to three reaction cycles without being able to eliminate this peak, what strongly suggests that it is not a nonconverted monoolein. Either a free fatty acid or another type of compound could be responsible for this small peak. Nevertheless, we did not try to further identify it, since whatever it is, it is included in the analysis of monoglycerides as indicated by the EN14105 or ASTM D-6584. On the other hand, the peak represents a total glycerin content of 0.01 wt % or less. This is the reason why even after two or three reactions carried out with a large excess of methanol, starting with a raw material with low total glycerin content, a small peak can still be observed at approximately the same retention time as the monoolein peak. Therefore, either the acidity of the sample or other nonidentified oil component could lead to an overestimation of the total glycerin content when using the GC method. This problem is not found when using the volumetric procedure.

3.1.4. Repeatability. Figure 3 shows results obtained when replicated determinations were carried out in the same sample. It can be observed that the difference between two determinations is seldom larger than 10%. Only in one out of 14 samples was the deviation between two measurements larger than 10%, including experiments carried out by different operators and experiments carried out on different days. This is an excellent result compared with the expected repeatability of the instrumental procedure described in EN14105 or ASTM D6584. It is important to note also that many of the samples included in Figure 3 have total glycerin content in the range required by the standards.

The titration of the glycerin collected in water after the three washings, is very reliable. Results for replicated titrations of this glycerin are shown in Table 4. The difference between two analyses is very small.

3.1.5. Influence of Each Step Length. The chromatographic procedure as described in the above-mentioned standards requires a total analysis time of approximately 1.5 h, taking into account sample preparation with two internal standards, silanization, and GC analysis. The time involved in each step of the volumetric procedure as indicated in the Appendixes, are very conservative, and if needed, they can be substantially reduced, as follows.

Glycerin Titration Times. The time required to oxidize glycerin with $NaIO_4$ according to the ASTM standard is 30 min, followed by 20 min in darkness after ethylenglycol (EG) is added. We compared results obtained using these times (as prescribed in the standards) with results obtained using 5 min in each interval. It was found that the differences between these results are within the experimental error of this procedure (not shown).

Reaction. Experiments with times shorter than 2.5 h were carried out, varying also the methanol/sodium methoxide proportion loaded to the reactor. Table 5 shows the results. From these data, it can be concluded that using 40% methanol referred to the volume of oil loaded to the reactor, with 35 g NaOH/L of methanol and a reaction time of 30 min, it is enough to ensure total conversion of mono-, di-, and triglycerides.

Extraction Steps. To determine whether three washing steps are necessary to fully extract the glycerin from the reaction media, the aqueous phase coming from each of these washing steps was analyzed to determine the glycerin content. It was found that the first extraction recovered around 97% of the total glycerin formed during the reaction, the second extraction 2%, and the third 1% or less. These results indicate that in order to make sure that all the glycerin is extracted, three washings should be carried out. On the other hand, it can also be concluded that a very small error could be introduced if the third washing is not carried out.

3.2. Results for Free Glycerin Determination. The free glycerin determination is carried out by extracting it from the biodiesel sample with three consecutive washings. The first of them is carried out with an aqueous solution of HCl to avoid the formation of a stable emulsion in those cases in which the sample is basic, that is, when the biodiesel sample is not properly purified during the production. The detailed procedure is described in Appendix B.

Several experiments were carried out by adding glycerin to samples of biodiesel that were previously washed several times, in order to make sure that no free glycerin was left on the sample.

Table 3 shows the results. It can be seen that there is a very good agreement between the amount of glycerin added to the sample and the experimental determination. Table 2 shows the values that correspond to the interlaboratory test, in which the free glycerin determination by the method described in this work corresponds exactly with the mean value of the other nine laboratories, as determined by the EN 14105.

The ASTM D-6584 renders the repeatability of this determination to be $r = 0.001$ wt %, while the EN 14105 establishes:

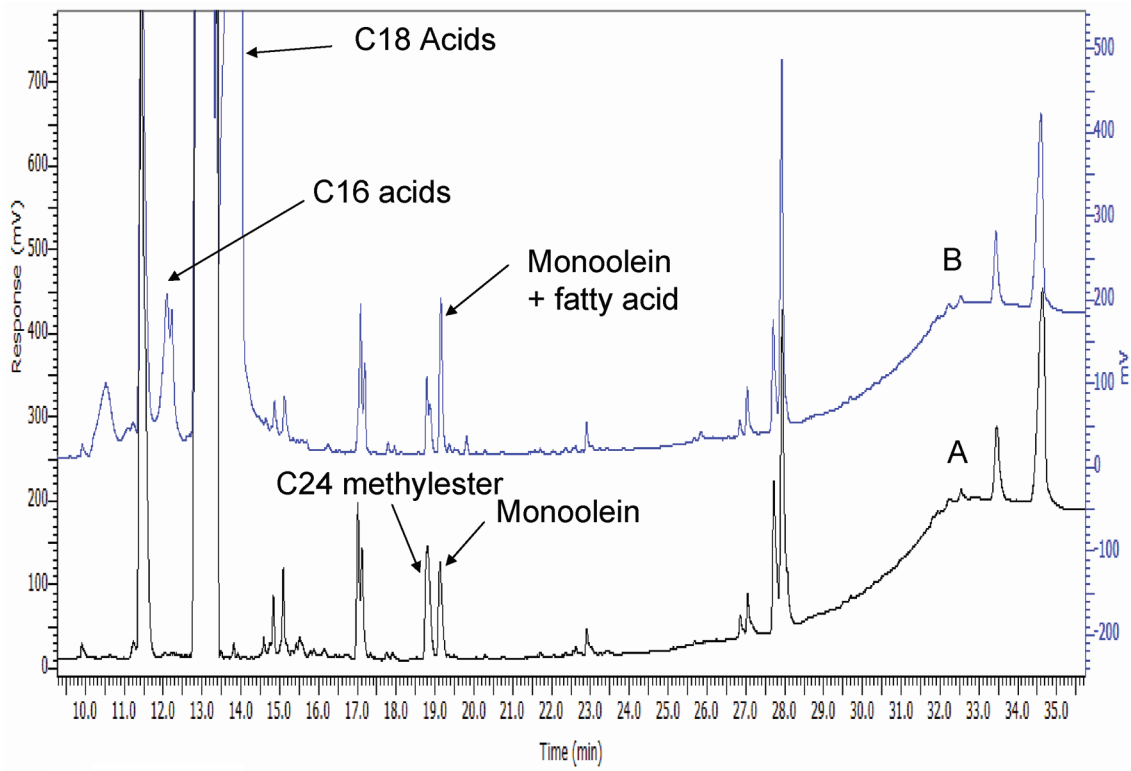

Figure 2. GC analysis of a biodiesel sample before (A) and after (B) addition of a mixture of fatty acids.

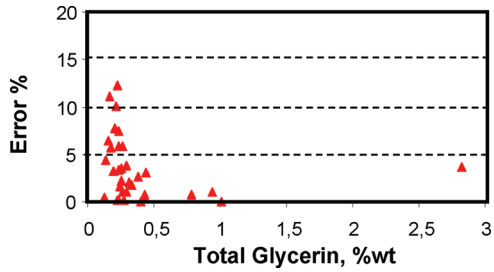

Figure 3. Repeatability for total glycerin determination. Error % computed in reference to the average value of two or three determinations, as a function of total glycerin content.

Table 3. Free Glycerin Determination by the Volumetric Method

glycerin added G_a wt %	glycerin measured G_m wt %	absolute difference wt %	error % $((G_a - G_m)/G_a) \times 100$
0.055	0.056	0.0001	5.4
0.560	0.532	0.028	5.0
0.225	0.190	0.0346	15.4
0.052	0.058	0.006	11.5
0.039	0.051	0.0118	30.2
0.104	0.107	0.0038	3.6
0.059	0.068	0.0091	15.4
0.197	0.199	0.0023	1.17

$r = 0.0538x + 0.0014$. On the other hand, the latter informs that the reproducibility is: $R = 0.5983x + 0.003$. Therefore, from the data shown in Table 3 it can be concluded that the volumetric method does not differ from the chromatographic procedure more than what is expected for two independent determinations by the GC method.

Figure 4 shows results of replicated determinations of free glycerin. It can be observed that the analysis repeatability is very good, typically with a difference of less than 10%.

3.3. ASTM D 6584 and EN 14105 Limitations. 3.3.1. Raw Materials Restrictions. As stated in the introduction, the ASTM D 6584 and the EN14105 are limited to the analysis of biodiesel obtained from rapeseed, sunflower, or soybean oil. In the procedure we present in this work, the raw material used to obtain the biodiesel has no effect on the analysis. In fact, it has been applied to biodiesel obtained with many different raw materials, such as tallow, chicken fat, pork fat, cottonseed, tung, rice, castor oil, coconut, etc.

For example, a modification of the procedure adopted by the EN14103 standard to determine total ester content in biodiesel

Table 4. Repeatability for Glycerin Determination after Being Collected in Water. Total Glycerin Analysis. Error % Computed in Reference to the Average Value

glycerin analysis in water, wt %		
1	2	error %
0.49	0.486	0.41
0.31	0.31	0.00
0.22	0.22	0.00
0.2018	0.2057	0.95
0.6265	0.5884	3.24
0.6165	0.6222	0.46
0.136	0.14	1.43
1.64	1.62	0.62
0.268	0.291	3.95
0.27	0.2863	2.85
0.146	0.152	1.97
1.52	1.48	1.35

Table 5. Influence of the Variables Involved in the Reaction Step on the Total Glycerin Determination

sample number	reaction time (h)	catalyst concentration[a] (vol/vol oil × 100)		
		40	60	80
1	1	0.779	0.777	
2	2.5	0.170		0.162
	1			0.169
	0.5			0.174
3	2.5	0.6265		
	2.5	0.5884		
	1	0.6365		
	0.5	0.6133		0.6165
	0.5			0.6222

[a] Sodium methoxide 40 g/L.

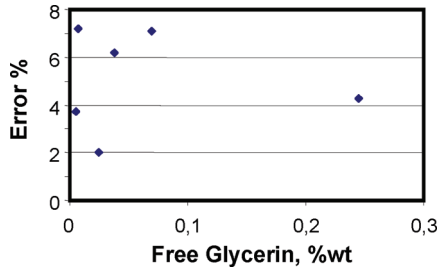

Figure 4. Repeatability for free glycerin determination. Error % computed in reference to the average value of two or three determinations, as a function of free glycerin content.

has been published by Mittelbach et al,[24] in order to analyze samples obtained from tallow. This is an example of the importance of the limitation of the GC methods applicability to biodiesel samples obtained from alternative raw materials.

3.3.2. Detection Limits. ASTM D 6584 and EN 14105 standards have reported the following detection limits: free glycerin, 0.005 to 0.05 wt %; total glycerin, 0.05 to 0.5 wt %.

The method presented here, as described in the appendix, can be applied in the following ranges: free glycerin, 0.0009 to 0.37 wt %; total glycerin, 0.0046 to 5.15 wt %.

However, these latter limits are only due to the amount of sodium periodate added in the analysis. As this quantity is increased, the detection upper limits also increase, and it is possible to determine any value of free glycerin or total glycerin content. No calibration is needed to modify the range of application of this procedure. According to the stoichiometry of reaction I, 4.65 g of $NaIO_4$ are needed for each gram of glycerin to be titrated. To guarantee the complete oxidation of glycerin in the sample, 6 g of $NaIO_4$ are used for each gram of glycerin. As indicated in Appendix A, the volume of NaOH used to titrate formic acid, is also used as a guide to know if the $NaIO_4$ added was enough, or if the titration has to be repeated with a lower amount of the collected water, or a higher amount of $NaIO_4$. On the other hand, if the volume of NaOH used is too small, titration has to be repeated using a higher volume of the collected water in order to improve the precision.

4. Conclusions

The procedure presented in this work, designed to determine the free and total glycerin content on biodiesel, is a very good alternative to the instrumental methods used at present. The results obtained with this method present similar or better repeatability and reproducibility, as compared to the GC-method described in the ASTM or EN standards. Another advantage is that it is a cheap method, not requiring any instrument such as GC, IR spectrometer, etc. Since no internal standards are used, and no calibration is needed, the range of applicability of this method has no limits.

Another very important advantage is that the procedure can be applied to any biodiesel, regardless of the raw material used in its production, while the GC-procedure cannot. Additionally, the acidity of the biodiesel sample does not interfere with the analysis.

As a disadvantage, the method does not provide the individual values of the mono-, di-, and try-glycerides, as requested by the EN standard.

Acknowledgment

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Appendix A.

Procedure for Total Glycerin Determination

1. Weigh an exact amount of biodiesel sample between 50 and 100 g, in a flask (250 cc flask is adequate)
2. Reaction. The reaction is carried out in a batch reactor with reflux:
   a. put the flask in water bath (60 to 65 °C) on a magnetic stirrer, and stir vigorously
   b. add 40% (v/v) of sodium methoxide solution (35 g NaOH/L methanol, or 40 g $NaCH_3O$/L methanol) referred to the weighed sample (biodiesel density 0.89 g/mL approximately)
   c. keep the temperature and the stirring during 2.5 h at 60 °C (see the text, shorter times can be used)
3. Glycerin extraction from reacting media by washing:
   a. remove the reflux condenser
   b. add a volume of HCl 5 wt %, equal to the volume used in (step 2b) into the flask, keep the temperature at 60−65 °C with gentle-moderate stirring, approximately 15 min
   c. without cooling, transfer carefully to a separatory funnel
   d. separate and place the aqueous phase in a 200 or 250 mL volumetric flask
   e. return the biodiesel phase to the reaction flask and repeat the washing using a volume of HCl 2.5 wt % equal to half of that in step 3b, keep at 60−65 °C with gentle-moderate stirring, 15 min approximately
   f. separate and collect the aqueous phase into the same volumetric flask as in step 3d
   g. repeat steps e and f but using distilled water instead of HCl 2.5 wt %
   h. rinse the flask with distilled water (20 mL approx.), and add it to the separatory funnel containing the biodiesel phase. Pour the aqueous phase into the volumetric flask containing the water coming from the other two washings
   i. discard the biodiesel phase. Rinse the separatory funnel with distilled water (20 mL approx) and collect it in the same flask
   j. cool the flask at room temperature, fill to the mark with distilled water, stopper, and mix by agitating in order to homogenize all the collected water
4. Analyze the glycerin content in the aqueous phase:
   a. take an aliquot of the flask (80 mL) in a 250 mL Erlenmeyer flask
   b. add 5 drops of phenol red indicator
   c. add NaOH 2 N, to turn to fuchsia (this high concentration is used in order to minimize the volume added to neutralize the glycerin solution)
   d. add HCl 5 wt % to turn to yellow, and then add 0.5 mL of HCl 5 wt % (this volume assures that the final pH is the correct one, in order to facilitate the CO_2 desorption)
   e. boil 3 min (with porous material to prevent violent boiling)
   f. cool to room temperature using a CO_2 trap to avoid the acidification of the solution caused by CO_2 absorption (from the air). The trap is just a short tube with pure NaOH, connected by a hose to the Erlenmeyer
   g. add NaOH 0.1 N until turning point (fuchsia). (in this step, the NaOH solution MUST be the same as that used to carry out the final titration, step j)
   h. add 40 mL of $NaIO_4$ (6 g/100 mL recently prepared), mix and leave 30 min in the darkness, stopper (see text, this time can be shortened)
   i. add 5 mL ethanodiol (ethylene glycol), wash the walls of the Erlenmeyer flask with distilled water, mix, and leave 20 min in the darkness, stopper (see text, this time can be shortened)
   j. titrate with 0.1 N NaOH solution

Calculation

$$\%G = V_{NaOH} \times 0.0921 \times N_{NaOH} \times (100/80) \times 100/w_{sample}$$

where %GL = g of glycerin/100 g of sample, V_{NaOH} = volume titrated of NaOH (mL), N_{NaOH} = normality of NaOH solution, w_{sample} = amount of weighed sample (g), 200/80 = aliquot used in the titration; flask volume (e.g., 200 or 250 mL)/ analyzed volume taken in step 4a (e.g., 80 mL).

Notes

If the volume of 0.1 N NaOH used in the titration is greater than 56 mL, the analysis has to be repeated with a lower amount of the collected water or a higher amount of $NaIO_4$ (see Detection Limits). In fact, using the solution of $NaIO_4$ (6 g/100 mL), the volume of 0.1 N NaOH solution used in the final titration should be less than 1.4 times the volume of $NaIO_4$ solution added to the sample. If this is not the case, repeat the titration using a smaller amount of sample, or a larger amount of $NaIO_4$ solution.

Appendix B.

Procedure for Free Glycerin Determination

1. Weigh an exact amount of biodiesel sample between 50 and 100 g, in a 250 cc flask
2. Extraction of glycerin from the biodiesel by washing:
   a. put the flask in a water bath (60 to 65 °C) and stir
   b. add 20 mL of HCl 5 wt %, stir approximately 15 min
   c. transfer carefully to a separatory funnel
   d. separate and place the aqueous phase in a 250 mL Erlenmeyer
   e. put the biodiesel phase in the flask and repeat the washing using 20 mL of HCl 2.5 wt %
   f. separate and collect the aqueous phase into the Erlenmeyer
   g. repeat steps e and f but using distilled water instead of HCl 2.5 wt %
   h. rinse the flask with distilled water (20 mL approx.), and add it to the separatory funnel containing the biodiesel phase. Transfer the aqueous phase to the Erlenmeyer containing the previous washings
3. Analyze the glycerin content in the aqueous phase:
   a. add 5 drops of phenol red indicator
   b. add NaOH 2 N, to turn to fuchsia
   c. add HCl 5 wt % to turn to yellow, and then add 0.5 mL of HCl 5 wt %
   d. boil 3 min (with porous material to prevent violent boiling)
   e. cool with CO_2 trap
   f. add NaOH 0.1 N until turning point
   g. add 15 mL of $NaIO_4$ (6 g/100 mL recently prepared), mix and leave 30 min in the darkness
   h. add 2 mL ethanodiol (ethylene glycol), wash the walls of the Erlenmeyer with distilled water, mix, and leave 20 min in the darkness
   j. titrate with 0.1 N NaOH

Calculation

$$\%G = V_{NaOH} \times 0.0921 \times N_{NaOH} \times 100/w_{sample}$$

where %GL = g of glycerin/100 g of sample, V_{NaOH} = volume titrated of NaOH (mL), N_{NaOH} = normality of NaOH solution, w_{sample} = amount of weighed sample (g).

Notes

If the volume of 0.1 N NaOH titrated is greater than 20 mL, the analysis has to be repeated with a lower amount of weighed sample or a higher amount of $NaIO_4$ (see Detection Limits).

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