Simple TLC-Screening of Acylglycerol Levels in Biodiesel as an Alternative to GC Determination

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Abstract

Thin layer chromatography (TLC) stained with hot acidic *p*-anisaldehyde, is an interesting, fast, and low-cost technique to monitor main lipid contaminants such as triacylglicerols, diacylglycerols, and monoacylglycerols in biodiesel. These acylglycerols are detectable by the proposed planar chromatographic method, provided the content of the contaminants exceeds the limits recommended by the international norms applicable to biodiesel quality/specification, namely 0.25% in mass for total combined glycerin. The TLC data are confirmed by gas chromatography of the methyl esters of soy oil.

Introduction

Biodiesel may be defined as mono-methyl or -ethyl esters of the common fatty acids found in the triacylglycerols of vegetable (oils) and animal (fat) sources through a previous transesterification carried out with the help of a basic catalyst as sodium or potassium methoxides (1). Technically, native tri-esters are converted in methyl or ethyl esters to be used as fuels to be blended with diesel oil or even totally replacing them.

For the purpose of biodiesel quality monitoring/specification, a set of 28 laboratory analyses are necessary. Amongst them, the content of residual-free and combined glycerin are of utmost importance. Combined glycerin refers to residual unreacted triAcylGlycerols (TAGs), diAcylglycerols (DAGs), and mono-Acylglycerols (MAGs). Their official monitoring is carried out by an expensive gas chromatographic procedure according to norms D 6584 and prEN 14105, including the application of two internal standards. The actual limits for combined glycerin, according to European, U.S., and Brazilian norms, are 0.25% on a mass base.

Considering the relative high costs of analytical procedure, such as gas chromatography, and the need of a simpler and screening procedure appropriate to the farm environment, a planar, simple, non-expensive, and fast chromatographic procedure such as thin layer chromatography (TLC) was developed to monitor the quality/specification of biodiesel concerning the occurrence of residual TAGs, DAGs, and MAGs.

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Materials and Methods

Biodiesel synthesis

Fully specified biodiesel was synthesized through transesterification of refined and degummed soy oil with 1% sodium methylate (BASF; stock solution = 30 g%) at 45°C for 60 min using a 100% excess of methanol as referred to the stoichiometric amount. The upper phase of reaction was twice washed with warm water for the complete removal of soaps and salts. This sample was designed "specified biodiesel", and calculated amounts of diolein and monoolein were added to it in order to obtain a "non-specified biodiesel," namely exceeding the upper limits determined in European, U.S., and Brazilian norms as 0.25% of combined glycerin. An intermediate sample, herein designed as "borderline sample," was also prepared; and the content of TAGs, DAGs, and MAGs were roughly adjusted to approach the levels determined in the international norms.

Chromatographic analyses

Merck SG-60 chromatoplates (Whitehouse Station, NJ) were used as stationary phase for TLC realization, and a mixture of toluene–chloroform–acetone (7:2:1, v/v/v) was utilized as the mobile phase. Samples of biodiesel and acylglycerol standards (monoolein, MAG; diolein isomers, DAGs; triolein, TAG) were normalized to 50 mg/mL in isopropanol. One or two microliters were then applied to the origin of a 10 × 10 cm chromatoplate. One percent hot *p*-anisaldehyde in methanol–sulphuric acid (9:1, v/v) was used as color developer through a fine spray (2). Plates developed with acid *p*-anisaldehyde were warmed for 3–5 min in a hot Cimarec plate for the rose-violet color full development, and pictures were then taken with a Sony Cyber-shot 5 megapixel camera

Table I. Quantitation of Biodiesel Contaminants by GC as %/Mass

Sample / compound	TAGs	DAGs	MAGs
SP*	0.12	0.10	0.39
BL†	0.12	0.15	0.78
NSP‡	0.13	0.25	1.30

* Specified biodiesel; † "Borderline" biodiesel; ‡ Non-specified biodiesel.

(New York, NY). Alternatively and with less sensitivity, plates were equilibrated with iodine vapors for 10 min followed by a spray with fluorescein (50 mg%) for inspection under a long wave chamber. Gas chromatography (GC) of biodiesel was performed in a Select Biodiesel column according to EN norm 14105: injector from 130°C until 380°C; detector 380°C; column program: 50°C (hold 1 min), 15°C/min until 180°C, 7°C/min until 230°C, and 10°C/min until 370°C (hold 5 min). The chromatographic Select column was purchased from Varian, Inc. (Palo Alto, CA). The whole procedure followed essentially the recommendations of the previously mentioned European norm (3).

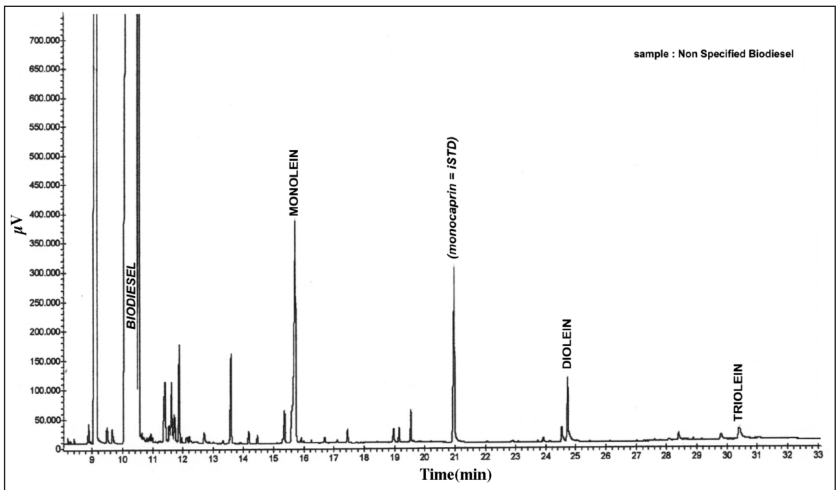

Figure 1. Gas chromatographic profile of non-specified biodiesel.

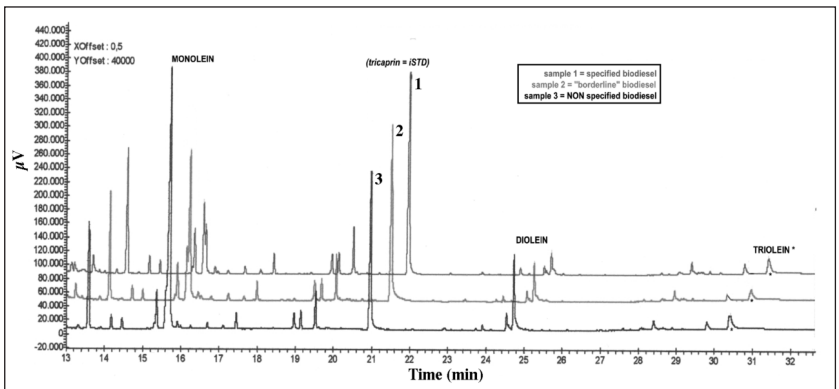

Figure 2. Combined gas chromatographic profiles of specified biodiesel (upper chromatogram), "borderline" biodiesel (middle chromatogram), and non-specified biodiesel (lower chromatogram) with a zoom chromatogram from 13–32 min of retention times.

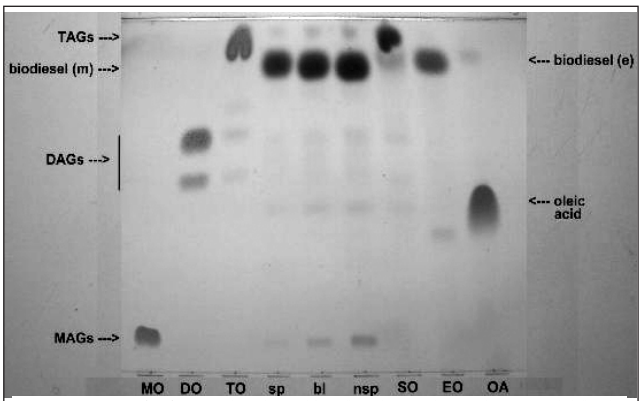

Figure 3. TLC of reference lipids and biodiesel samples with p-anisaldehyde as the detection reagent. Toluene–chloroform–acetone (7:2:1, v/v/v) was utilized as the mobile phase. Abbreviations: MO = monoolein (MAG); DO = dioleins (DAGs); TO = triolein (TAG); sp = specified soy biodiesel; bl = "borderline" soy biodiesel; nsp = non-specified soy biodiesel; SO = soy oil; EO = ethyl oleate; OA = oleic acid.

Results

Gas chromatographic analysis

The chromatographic profile of non specified biodiesel can be seen in Figure 1. The mixture of methyl esters (linoleic, oleic, palmitic, etc.) is the major peak at tR = 10.2. The contaminant peaks of monolein, diolein, and triolein are clearly detected at tR(s) = 15.7, 24.0, and 30.5 min., respectively, with dominance for monolein, which is the most common cause of biodiesel non-specification, taking into account the progressive reaction, TAGs → DAGs → MAGs → biodiesel. The peak at 21.0 min is monocaprin, added as the internal standard.

Table I shows the concentration of contaminant TAGs, DAGs, and MAGs for the three biodiesel samples as determined by GC using monocaprin as the internal standard.

Figure 3 displays the TLC plate using *p*-anisaldehyde as the detection reagent. All analytes that are relevant to the study show a reasonable separation. Triolein and soy oil standards presented the highest Rf values, 0.88 and 0.89 respectively, followed in decreasing order of migration by ethyl oleate (Rf = 0.83), DAGs [Rf(s) = 0.62 and 0.50], and oleic acid (Rf = 0.45). Clearly distinguishable are the biodiesel mixture of methyl esters with Rf = 0.83. As compared to the lane of specified biodiesel (sb), the contaminants in the lane for non-specified biodiesel (nsb), namely residual TGAs, DAGs, and MAGs, are then clearly detectable.

Figures 4 and 5 show that iodine vapors even followed with fluorescein are less sensitive than *p*-anisaldehyde (Figure 6) as the detection reagent. The estimation detection limits for TAGs, DAGs, and MAGs are at least in the range of 1–2 µL of 50 mg/mL solutions or 50–100 µg of lipid compounds.

TLC with visual detection after application of a detection reagent, as well as detection with a flame ionization detector developed to TLC plates, are sometimes employed to check the purity of biodiesel samples (4–6), but the official methods consider only quantitative determination by GC (i.e., EN 14105) or high-performance liquid chromatography (7). TLC separation, followed by a sensitive chromogenic reagent like *p*-anisaldehyde, as proposed in the present work, offers the following advantages for the preliminary screening of contaminant or residual acylglycerols that limit biodiesel quality: a) complex instrumentation and sample preparation are not required, b) no risk of contaminating the stationary phase with successive sample components because a new separation layer is used each time, c) possibility of inspection of at least 10 different

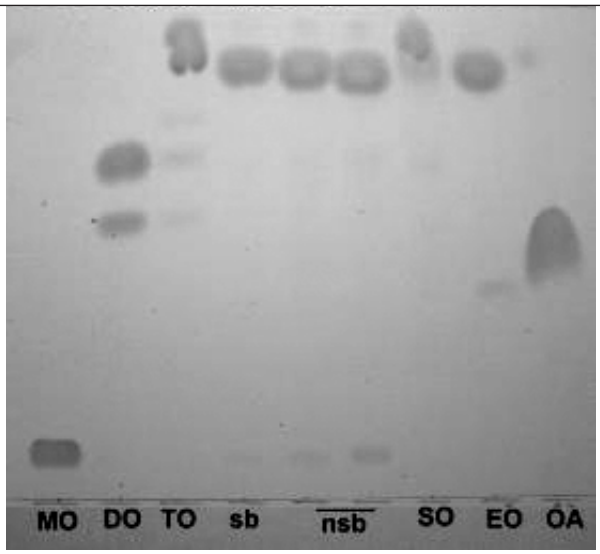

Figure 4. TLC of biodiesel samples and lipid standards after treatment with iodine vapors as chromogenic reagent.

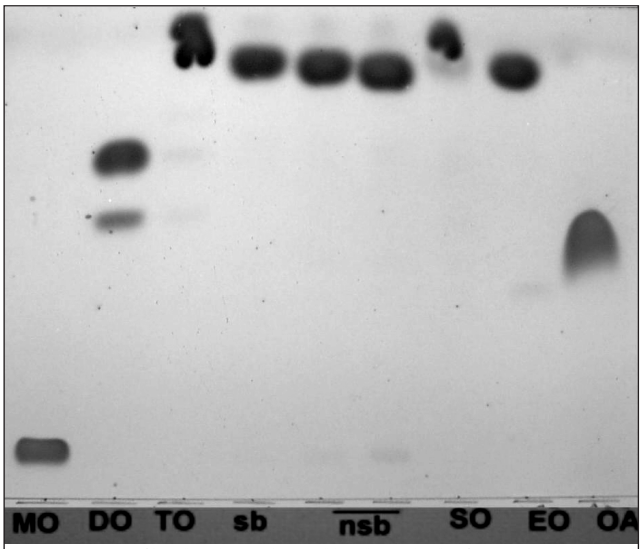

Figure 5. TLC of biodiesel samples and lipid standards after sequential treatment with iodine vapors, fluorescein nebulization as chromogenic reagents, and UV light at 365 nm.

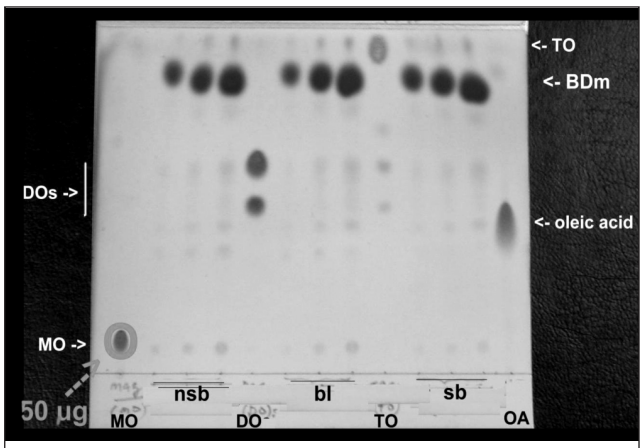

Figure 6. TLC of biodiesel samples and lipid standards after treatment with iodine vapors as chromogenic reagent.

samples of biodiesel per plate, d) markedly reduced costs per chromatographic analysis.

Discussion

Due to the progressive popularization of biodiesel as an alternative fuel for diesel engines, less expensive analytical tools should be made available close to biodiesel production facilities, especially at small farms in rural areas. As compared with the official methods recommended to monitor biodiesel specification with respect to the degree of contamination with residual TAGs, DAGs, and MAGs, namely GC and high-performance liquid chromatography, TLC is an attractive option mainly due to the low equipments and operation costs. Most of the current sources for biodiesel production (soy, sunflower, canola, palm, *Jatropha curcas*, castor oil) have unsaturated fatty acids as predominant compounds, and these are easily detectable with hot *p*-anisaldeyde in the presence concentrated acid sulphuric acid giving deep rose- to wine-colored spots. Figures 3 and 6 show clearly how this chromogenic reagent allows the detection of unsaturated glycerides and free fatty acids with significant sensitivity as low as 0.05 mg/sample (Figures 3 and 6).

Conclusion

TLC, using acid anisaldehyde and plate heating, is a simple, sensitive, less expensive, and fast detection reagent for analyzing simultaneously up to six samples of biodiesel besides the acylglycerol standards regarding the quality/specification resulting from TAGs, DAGs, and MAGs contaminants. The screening procedure is especially useful in rural areas with restricted access to more sophisticated and expensive GC or high-performance liquid chromatography systems. MAGs, the most often seen combined glycerin contaminant of biodiesel, turns clearly detectable provided the contamination level surpass the limit from EM 14105 and BR ANP 7, namely, 0.25% mass for total glycerin or 0.8 % mass for MAG (EM 14214), the case of present non-specified sample BDm-SO-ns.

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