Reversed-phase High-performance Liquid Chromatographic Analysis of Biodiesel Obtained from Sunflower Oil

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The paper describes the analysis of biodiesel (BD) obtained by transesterification of sunflower oil with methanol. The system proposed is the reversed-phase high-performance liquid chromatography (RP-HPLC) operating on a C_{18} (250 x 4.6 mm) column with UV detection at 205 nm and gradient elution in both nonaqueous (NARP) and aqueous-organic (ARP) modes. The former separates the main classes of compounds from BD, while the latter allows the separation of individual components belonging to the same class. The method enables separation of fatty acid methyl esters (FAME), monoacylglycerols (MG), diacylglycerols (DG) and triacylglycerols (TG). The results obtained reveal that the proposed approach is suitable for monitoring the process of biodiesel synthesis.

Keywords: biodiesel, RP-HPLC, sunflower, transesterification, acylglycerols

The day-by-day depletion of oil fields requires alternative fuels to fulfill the energy demand of the world. Among the candidates, biodiesel (BD) is the best available alternative that meets this need. Biodiesel is a biofuel based on the fatty acid monoalkyl esters derived from triglycerides (TG) by transesterification with a low molecular weight alcohol in presence of a suitable catalyst [1]. Usually, the reaction is carried out with methanol and the obtained esters are called the fatty acid methyl esters (FAME). Either alone or blended with petrodiesel, the biodiesel is a viable source of energy being renewable, biodegradable, nontoxic, essentially free of sulfur and aromatics, and with no contribution to the greenhouse effect [2, 3].

The interest in biodiesel production, performance, cost, byproducts and greenhouse emissions increased considerably in the last decade, the research being concretized in several books [4-6] and many reviews [2, 3, 7-21]. The interest in biodiesel was also present in our group being reflected by the published papers [22, 23] and patents [24-27]. At the same time, the fuel quality is a main issue to successfully commercialization of biodiesel, and the analysis of this product has been subjected to numerous publications, which have been summarized in excellent reviews [28-30].

During the triglyceride transesterification, which is a reversible and stepped reaction, FAME together with byproducts like monoglycerides (MG), diglycerides (DG), glycerol (GL), unreacted raw material and residual catalyst do result. The byproducts impair the quality of biodiesel by lowering the automotive performance and damaging the engine. Therefore, their amount in DB has to be precisely known and monitored. If one takes into consideration that the parent triglyceride contains mixtures of saturated and unsaturated fatty acids with different number of carbon atoms, the biodiesel analysis is a very difficult task. As a prerequisite, the analyst should be able to quantify within a short period of time, with high accuracy and confidence, and at an acceptable cost all the components of biodiesel.

Among the used approaches, the best results are given by the hyphenated techniques such as liquid chromatography-gas chromatography (LC-GC) [31], gas chromatography-mass spectrometry (GC-MS) [28, 32], and liquid chromatography-mass spectrometry (LC-MS) [28, 33, 34]. However, they are not widely applied because of expensive equipment, excessive cost of maintenance, and highly expertised personnel required for handling the equipment. As a result, the current standard method for BD analysis in Europe and USA is gas chromatography with flame ionization detection (GC-FID) [35, 36], even if it is cumbersome because MG and DG require time-consuming procedures of derivatization.

An ascending technique in BD analysis is the high-performance liquid chromatography (HPLC). It does not require derivatization, the sample being directly injected into chromatograph. The elution is either isocratic or in gradient and the detection is done by UV, refractive index, evaporative light scattering, and mass spectrometry [33, 34, 37, 38]. Previously, we used HPLC to analyze ethoxylated nonionic surfactants [39, 40], to determine their thermodynamic properties [41-43], the hydrophile-lipophile balance [44], the hydrophobibicity parameters [45] and the partition of these surfactants between two non-miscible phases [46, 47].

This work presents our results obtained with the aid of reversed-phase high-performance liquid chromatography (RP-HPLC) in the analysis of BD prepared by transesterification of edible sunflower oil with methanol. Both nonaqueous and aqueous RPLC have been used. The method is expedient in analyzing the main classes of compounds present into biodiesel.

Experimental part

Equipment and materials

The chromatographic system used was a VARIAN ProStar equipment consisting of the 240 solvent delivery unit, the 335 photodiode array detector, the thermostatic column oven, the 410 autosampler and the Galaxy Chromatography Data System version 6.6. The column was the VARIAN ChromoSep HPLC SS (250 x 4,6 mm), which includes the holder with ChromoSep guard column Intersil

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Item No.	HPLC System	Solvent A	Solvent B	Solvent C	Gradient
1.	Non-aqueous reversed phase (NARP)	Methanol	IPA/Hexane (5:4, vol.)	-	From 100% A to 50% A in 15 min.
2.	NARP	Methanol/ Acetonitrile (1:4, vol.)	IPA/Hexane (5:4, vol.)	-	From 100% A to 34% A in 25 min., followed by isocratic elution with 34% A for 5 min.
3.	Aqueous reversed phase (ARP) – NARP	Water	Acetonitrile	IPA/Hexane (5:4, vol.)	From 30% A + 70% B to 100% B in 10 min, to 50% B + 50% C, in 10 min, and isocratic elution with 50% B + 50% C for 5 min.

Table 1
ELUENTS AND GRADIENTS USED TO ANALYZE THE PRODUCTS OF SUNFLOWER OIL TRANSESTERIFICATION WITH METHANOL

5 ODS-2. The working temperature of the column was of 45 °C. The detector wavelength was set at 205 nm, and the flow rate at 1 mL/min. The elution was done with organic-aqueous solvent mixtures specific to ARP [33, 34], and with nonaqueous solvent mixtures in the case of NARP [33, 34, 48].

Reagents and materials

The used reagents were as following: *n*-Hexane, ≥ 98% (Carlo Erba), methanol Chromasolv for HPLC, ≥ 99.9%, (Sigma-Aldrich), acetonitrile Chromasolv for HPLC, ≥ 99.8% (Sigma-Aldrich) and isopropanol (IPA) Chromasolv for HPLC, ≥99.8%, (Sigma-Aldrich), and reagent grade anhydrous glycerol (Chimopar S.A., Bucharest). The water employed was obtained with the aid of a Millipore Simplicity UV system.

Biodiesel samples

Biodiesel samples were provided by the Laboratory of Bioresources Valorization, National Institute of Research and Development for Chemistry and Petrochemistry (ICECHIM) Bucharest. They were obtained by transesterification of edible sunflower oil with methanol in presence of organic bases as catalysts.

The identification of transesterification products was done with the aid of FAME, MG and DG samples obtained by molecular distillation of the transesterification products. The unreacted triglycerides were identified with sunflower oil, and the glycerol with anhydrous glycerol.

Solutions for analysis had a concentration of 0.4 g/100 mL being prepared with isopropanol/*n*-hexane (5:4, vol.). They were filtered through 0.45 μm Acrodisc filters to remove particulate matter. The injected volume was of 10 μL.

Results and discussions

It is well-known that the fatty matter of oleaginous plants contains mixtures of triglycerides derived from saturated, mono- and polyunsaturated fatty acids. According to the "Lexicon of Lipid Nutrition", the fatty acid composition of edible sunflower oil is very complex and fluctuant. Expressed as percentage mass-fraction of total fatty acids, it has the following values: 5.4-5.9 palmitic (C16:0), 0.2 palmitoleic (C16:1 n-7), 3.5-4.5 stearic (C18:0), 19.6-43.5 oleic (C18:1 n-9), 39.8-65.7 linoleic (C18:2 n-6) and 0.2 linolenic (C18:3 n-3) [49]. A very recent source gives a more precise composition of the European sunflower oil, which has the following percentage content of fatty acids: 6.4 palmitic, 0.1 palmitoleic, 2.9 stearic, 17.7 oleic and 72.9 linoleic [50]. Taking into account the complex composition of sunflower oil, the resulted biodiesel arises serious problems to the analytical chemist because both the major and the minor components of biodiesel have to be identified and quantitatively determined within a reasonable time window and at low cost.

Table 1 presents the composition and the gradient profile of the HPLC systems tested in this work. The first two operate under the nonaqueous reversed phase mode, whereas the third combines the aqueous with the nonaqueous elution. The chromatograms obtained show that one analysis takes in between 20 and 40 minutes, and depends on the analyte and the elution system used.

Figure 1 illustrates the chromatograms obtained by using the HPLC system No.1 from table 1. The analyzed samples were biodiesel (A), monoglycerols obtained by molecular distillation of the transesterification products (B) and purified FAME (C). Samples B and C were used to identify the MG and FAME in biodiesel. The first peak appearing in all the chromatograms corresponds to the unretained component of the mixtures, which leave the column together with the front of the eluent. In the chromatogram that corresponds to the purified sample of FAME there are three main peaks at 3.7, 4.2 and 5.4 min. (fig. 1C). They can be assigned to fatty acid methyl esters having different length and unsaturation of the fatty acid hydrocarbon

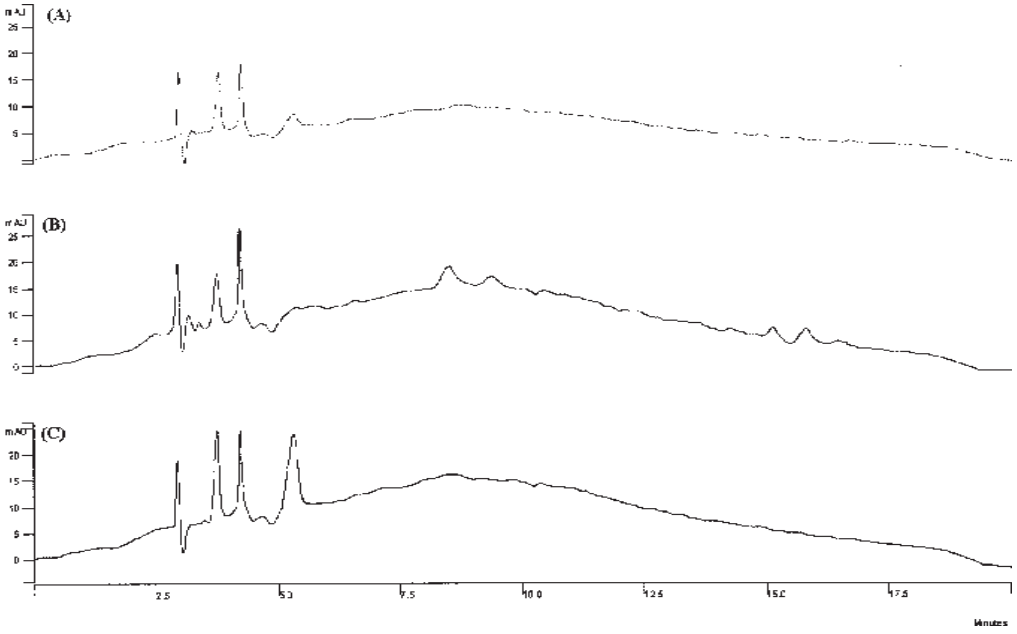

Fig.1. HPLC separation by using the chromatographic system No. 1 of the following samples: Biodiesel EMM9 (A), monoglycerols obtained by molecular distillation of the transesterification products (B), and purified fatty acids methyl esters (C)

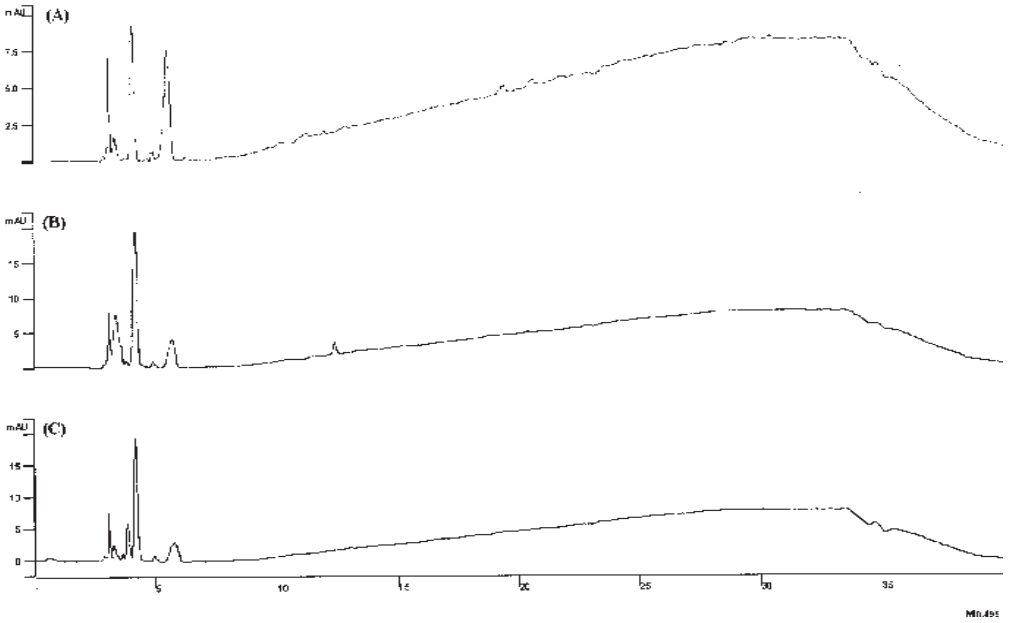

Fig. 2. HPLC analysis of Biodiesel EMMT1 (A), monoglycerols obtained by molecular distillation of the transesterification products (B), and purified fatty acids methyl esters (C) with the aid of the chromatographic system No. 2

chains, which results in various hydrophobicities. The chromatogram of the biodiesel sample (fig. 1 A) is similar to that of the FAME (fig. 1 C) and confirms the formation of fatty acid methyl esters by the transesterification of the sunflower oil. By comparing the chromatogram of the MG sample (fig. 1 B) with those of row (Figure 1 A) and purified (fig. 1 C) FAME, one may observe that the peak at 3.7 min. is smaller than that at 4.2 min., and the peak at 5.4 min. almost vanishes. This result shows that a small amount of esters from the BD is carried over during the molecular distillation process and is found out into the monoglycerols. In the same 1 B chromatogram, there are two small peaks eluting in front of the peak at 3.7 min. They can be safely ascribed to monoglycerols. The presence of peaks at 3.7 and 4.2 min. in all the chromatograms of Figure 1 proves possible co-elution of some esters with monoglycerols. As Figure 1 B shows, at higher elution times, namely into the 8 – 11.4 and 14 – 17 min. retention windows there are two series of eluted peaks. Since the chromatographic method employed by us is similar to that originally proposed by Holcapek *et al*., these peaks can be assigned to di- and triglycerols, respectively [33, 34]. The cluster appearance of these peaks denotes that they contain many compounds with different number of carbon atoms and double bonds. On the basis of the acquired data it is hard to step further with speculations about the structure of the samples presented in figure 1, and we have to do extra work to elucidate them. However, the information acquired by comparison the peak areas corresponding to FAME and TG recommends it as a simple, expedient and efficient means to monitor the transesterification process.

Figures 2 and 3 illustrate the chromatograms obtained with the aid of the second NARP system listed in table 1. The chromatograms in figure 2 present the EMMT1 biodiesel sample (A), and those of monolgycerols obtained by molecular distillation (B) and of FAME (C). The chromatograms in Figure 3 belong to biodiesel EMM10 (A), original sunflower oil (B) and di- and triglycerol residues separated by molecular distillation (C). These chromatograms show that the separation proceeds according to the classes of compounds present into the sample. The shape of the peaks and the elution order is similar to that previously reported [48]. The monoglycerols appear within the 3.0 – 3.8 min. time window (fig. 2 B), FAME at 3.8-6.5 min. (fig. 2 C), diglycerols at 7.8-15 min (fig. 3 C), and triglycerols at 15-26 min. (fig. 3 B and C). One may notice the presence of mono-, di- and triglycerols in the BD samples.

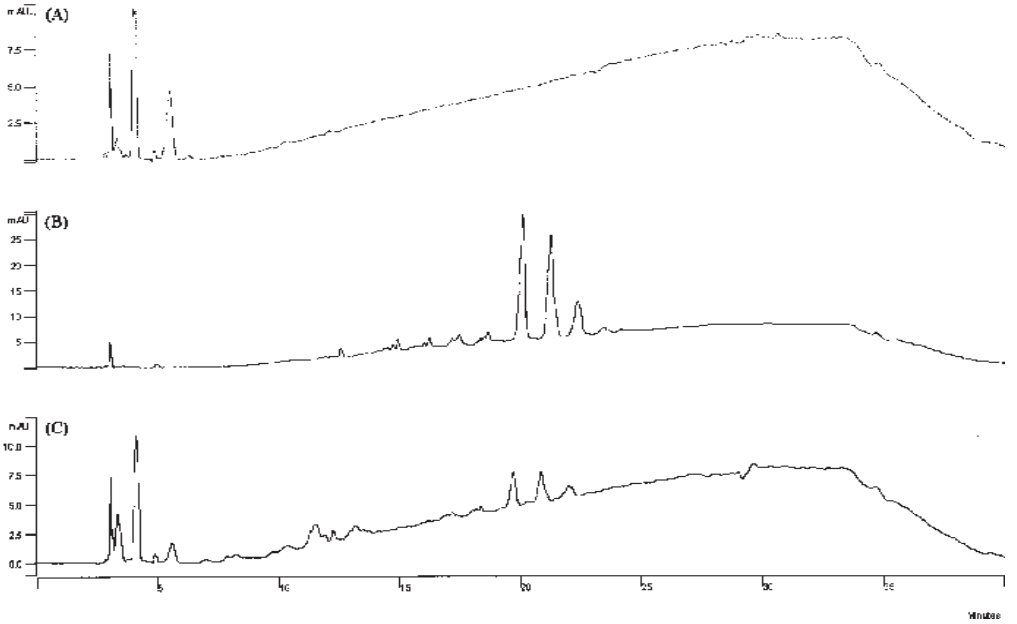

Fig. 3. HPLC separation by using the chromatographic system No. 2 of the biodiesel EMM10 (A), edible sunflower oil (B), and di- and triglycerols separated by molecular distillation (C).

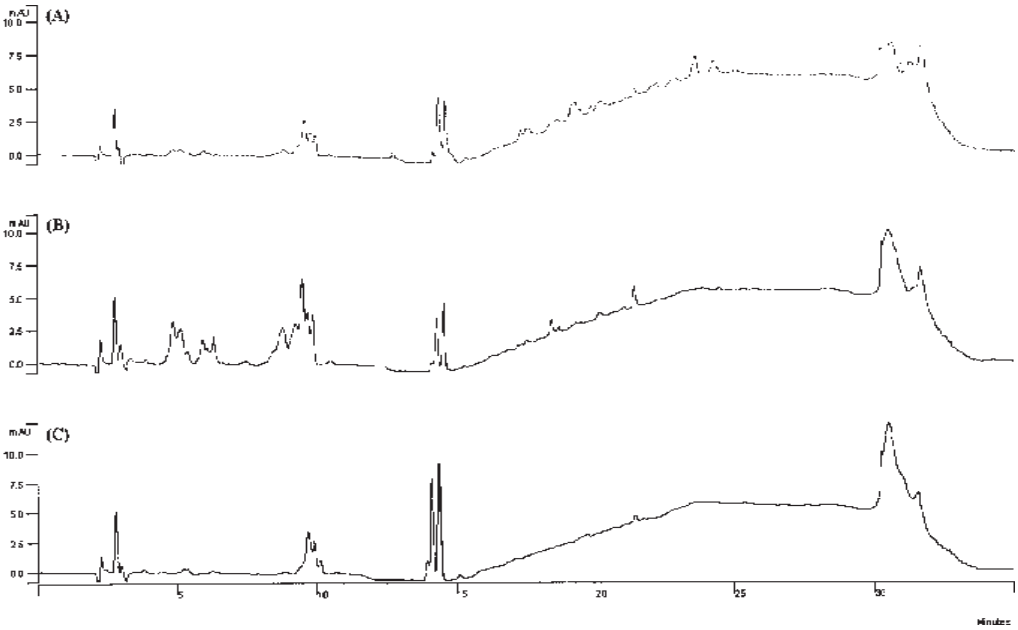

Fig. 4. Chromatograms of biodiesel EMM17 (A), monoglycerols obtained by molecular distillation of the transesterification products (B), and FAME (C) by using the HPLC system No. 3.

To obtain detailed information about the individual components of the transesterification process, a third chromatographic system with elution in two steps has been tested. In the first, the elution is done in gradient with aqueous-organic solvents, whereas in the second, an isocratic non-aqueous reversed phase system was employed.

Figure 4 shows the following chromatograms: biodiesel EMM17 (A), MG obtained by molecular distillation of the transesterification products (B) and FAME (C). They were obtained with the aid of the third chromatographic system given in table 1. One may observe that the resolution of the separated compounds is better in comparison with the first two chromatographic systems. The FAME chromatogram in figure 4 (C) contains two peak clusters with retentions within the 9.2-10.1 and 12.8-15.2 min time windows. These data are in accordance with those obtained by [33, 34] in the analysis of transesterification products of the cozla oil with methanol. The peaks corresponding to FAME in [33, 34], appeared inbetween 9.06 and 15.23 min., and the elution of the methyl esters increased in the following order: linolenic (C18:3, n-6), linoleic (C18:2, n-6), oleic (C18:1, n-9) and gadoleic (C20:1, n-9). The FAME peaks in figure 4 C are almost identical to those corresponding to EMM17 (fig. 4 A) and EMM16 (fig. 5 A) samples. This proves that the fatty acids methyl esters are the main components in biodiesel. The peaks corresponding to the fatty acids methyl esters are also present in the chromatogram of the monoglycerol sample (fig. 4 B) and confirm the existence of FAME in this sample. Instead, FAME is not present in the glycerol (fig. 5B) and sunflower oil (fig.5C) chromatograms. One may notice that Figure 4 B shows three groups of peaks in front of those of FAME. The first two are well resolved and the third is very close to the first peak of FAME. Taking into account the results by [33, 34], we inferred that these three groups of peaks correspond to the following monoglycerols: 2- and 1-monolinolenine, 2- and 1-monolinoleine, and 2- and 1-monooleine, respectively. In the chromatograms of EMM16 and EMM17, the peaks of monoglycerols are small denoting trace amounts of such compounds in samples. The chromatogram of FAME presented in figure 4 C shows a small amount of monoglycerol, and those of glycerol (fig. 5 B) and oil (fig. 5C) shows the absence of monoglycerol in these samples. It is also obvious that in both the chromatogram of EMM17 (fig. 4 A) and in that of EMM16 (fig. 5 A) there are two series of peaks appearing at about 20 and respectively 25 min. By comparing them with the

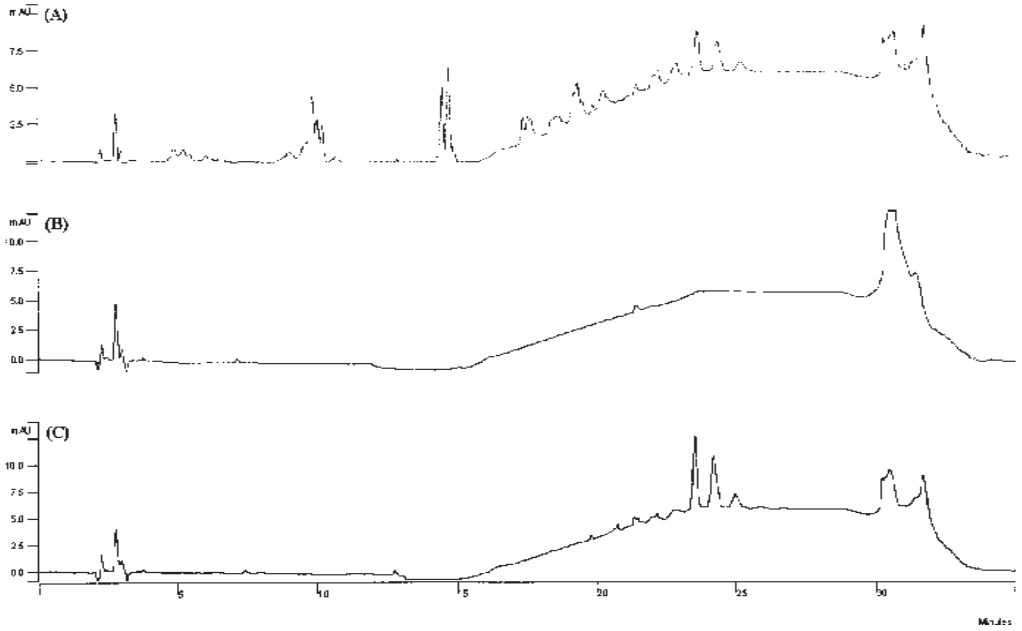

Fig. 5. Chromatograms of the biodiesel EMM16 sample (A), glycerol (B), and edible sunflower oil (C) obtained by using the HPLC system No. 3.

chromatograms of monoglycerol (fig. 4 B) and sunflower oil (fig. 5 C), and taking into account the literature data [33, 34], one may assign these peaks to diglycerols and respectively to triglycerols.

Although has a longer time span, this chromatographic system that combines both aqueous-nonaqueous and nonaqueous gradient elution allows a distinct separation of monoglycerols, FAME, diglycerols and triglycerols. The system performs well in separating the main component classes of the biodiesel, and work is now in progress to identify and characterize them.

Conclusions

The results obtained in this work concerning the separation of the components present into the products obtained by transesterification of sunflower oil with methanol allowed us to conclude that RP-HPLC meets the objectives of the study. The chromatographic systems employed the UV detection at 205 nm and exploit the benefit of gradient elution, which has been done with both aqueous-organic and nonaqueous solvent mixtures. The chromatographic conditions assure an optimal separation of FAME, mono-, di- and triglycerols, which are the main classes of compounds present into the biodiesel obtained by us. The first chromatographic system works with linear gradient of organic solvents and is recommended to monitoring the triglycerol content of FAME. The second system, also applies the gradient elution with organic solvents, and it makes an optimal separation of the transesterification products in the following elution order: MG, FAME, DG and TG. The third system combines the gradient elution with organic and aqueous solvents with isocratic elution with organic solvents. It is capable to separate the individual components present within the monoglycerols. These results prove that HPLC is a suitable method to monitor and optimize the transesterification process of vegetable oils.

References

1.DEMIRBAS, A., KARSILIOGLU, S., Energ. Sources, Part A, **29**, 2007, p.133.
2.MA, F., HANNA, M.A., Bioresour. Technol., **70**, 1999, p. 1.
3.SRIVASTAVA, A., PRASAD, R., Renew. Sustain. Energy Rev., **4**, 2000, p. 111
4.MITTELBACH, M., REMSCHMIDT, C., Biodiesel: The Comprehensive Handbook, Martin Mittelbach Press, Karl-Franzens-Universität Graz, Graz, Austria, 2004
5.KNOTHE, G., VAN GERPEN, J. KRAHL, J., The Biodiesel Handbook, Am. Oil Chem. Soc. Press, Champaign, Illinois, U.S.A., 2005
6.NAG, A., Biofuels Refining and Performance, McGraw Hill, New York, U.S.A., 2008
7.FUKUDA, H., KONDO, A., NODA, H., **92**, 2001, J. Biosci. Bioeng., p. 405
8.DEMIRBAS, A., Energy Convers. Manag., **44**, 2003, p. 2093.
9.KNOTHE, G.H., DUNN, R.O., Industrial Uses of Vegetable Oils, Erhan, S.Z., (Ed.), Am. Oil Chem. Soc. Press, Champaign, Illinois, U.S.A, 2005, p. 42.
10.MEHER, L.C., VIDYA SAGAR, D., NAIK, S.N., Renew. Sustain. Energy Rev., **10**, 2006, 248.
11.AGARWAL, A.K., Prog. Energy Comb. Sci., **33**, 2007, p. 233.
12.MARCHETTI, J.M., MIGUEL, V.U., ERRAZU, A.F., Renew. Sustain. Energy Rev., **11**, 2007, p. 1300.
13.BOZBAS, K., Renew. Sustain. Energy Rev., **12**, 2008, p. 542.
14.NIKIEMA, J., HEITZ, M., Can. J. Civil Eng., **35, 2008, p.** 95.
15.BALAT, M., BALAT, H., Energy Convers. Manag., **49**, 2008, p. 2727.
16.BASHA, S. A., GOPAL, K. R., JEBARAJ, S., Renew. Sustain. Energy Rev., **13**, 2009, p. 1628.
17.ENWEREMADU, C. C., MBARAWA, M. M., Renew. Sustain. Energy Rev., **13**, 2009, p. 2205
18.SINGH, S. P., SINGH, D., Renew. Sustain. Energy Rev., **14**, 2010, p. 200
19.KRALOVA, I., SJOBLOM, J., J. Dispersion Sci. Technol., **31**, 2010, p. 409.
20.JANAUN, J., ELLIS, N., Renew. Sustain. Energy Rev., **14**, 2010, p. 1312.
21.SZULCZYK, K. R., McCARL, B.A., Renew. Sustain. Energy Rev., **14**, 2010, p. 2426.
22.STEPAN, E., VELEA, S., ENĂŞCUŢĂ, C., MARTON, G. I., GÂJIU, C. L., Rev. Chim. (Bucharest), **57**, 2006, p. 693.
23.GAIDĂU, C., NICULESCU, M., STEPAN, E., TALOI, D., FILIPESCU, L., Rev. Chim. (Bucharest), **60**, no. 5, 2009, p. 501.
24.STEPAN, E., ŞERBAN, S., RO Patent 114,603 / 1999.
25.LUPAŞCU, T., DRAGALIN, I., VLAD, P., STEPAN, E., ŞERBAN, S., MD Patent 2,382 / 2004
26.ŢIGĂU, V., ŢIGĂU, L. D., STEPAN, E., ŞERBAN, S., RO Patent 120,907 / 2006
27.STEPAN, E., ŞERBAN, S., VELEA, S., LAŢA, I., RO Patent 121,913 / 2008
28.KNOTHE, G., J. Am. Oil Chem. Soc., **83**, 2006, p. 823
29.KNOTHE, G., Trans. ASAE, **44**, 2001, p. 193.

30.MONTEIRO, M. R., AMBROZINA, A. R. P., LIAO, L. M., FERREIRA, A. G., Talanta, **77**, 2008, p. 593
31.LECHNER, M., BAUER-PLANK, C., LORBEER, E., J. High Resolut. Chromatogr., **20**, 1997, p. 581
32.SAWANGKEAW, R., BUNYAKIAT, K., NGAMPRASERTSITH, S., Green Chem., 9, 2007, p. 679
33.HOLCAPEK, M., JANDERA, P., FISCHER, P., PROKES, B., J. Chromatogr. A, **858**, 1999, p. 13
34.HOLCAPEK, M., JANDERA, P., FISCHER, P., Crit. Rev. Anal. Chem., **31**, 2001, p. 53
35.*** Test Method EN 14105:2003: Fat and oil derivatives, Fatty acid methyl esters (FAME), Determination of free and total glycerol and mono-, di-triacylglycerol contents (Reference method)
36.*** ASTM D6751 - 08 Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
37.LIN, T., WOODRUFF, C. L., MCKEON, T. A. J. Chromatogr. A, **782**, 1997, p. 41
38.FOGLIA, T. A., JONES, K. C., PHILLIPS, J. G., Chromatographia, **62**, 2005, p. 115
39.ANGHEL, D. F., BALCAN, M., VOICU, A., ELIAN, M., Rev. Chim. (Bucharest), **38**, 1987, p. 148
40.ANGHEL, D. F., BALCAN, M., VOICU, A., ELIAN, M., J. Chromatogr. A, **668**, 1994, p. 375
41.BALCAN, M., ANGHEL, D. F., VOICU, A., BALCAN, D. C., Colloids Surf. A, **204**, 2002, p. 141
42.BALCAN, M., ANGHEL, D. F., Colloids Surf. A, **221**, 2003, p. 1
43.BALCAN, M., ANGHEL, D. F., DONESCU, D. RAICU, V., Rev. Roum. Chim., **52**, 2007, p. 771
44.BALCAN, M., ANGHEL, D. F., VOICU, A., CORNILESCU, N., Rev. Roum. Chim., **44**, 1999, p. 369
45.BALCAN, M., ANGHEL, D. F., BOBICA, C., Rev. Roum. Chim., **43**, 1998, p. 1105
46.M.BALCAN, D. F. ANGHEL, RAICU, V., Colloid Polym. Sci., **281**, 2003, p. 143
47.M.BALCAN, D. F. ANGHEL, Colloid Polym. Sci., **283**, 2005, p. 982.
48.DI NICOLA, G., PACETTI, M., POLONARA, F., SANTORI, G., STRYJEK, R., J. Chromatogr. A, **1190**, 2008, p. 120
49.BEARE-ROGERS, J., DIEFFENBACHER, A., HOLM, J. V., Pure Appl. Chem., **73**, 2001, p. 685.
50.KRALOVA, I., SJOBLOM, J., J. Dispersion Sci. Technol., **31**, 2010, p. 409

Manuscript received: 30.07.2010