Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University – Chemistry, Physics, Biology, Geography
Journal homepage: http://www.shu.bg

Determination and discrimination of biodiesel fuels by gas chromatographic and chemometric methods

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Abstract*: Pattern recognition method (PRM) was applied to gas chromatographic (GC) data for a fatty acid methyl esters (FAME) composition of commercial and laboratory synthesized biodiesel fuels from vegetable oils including sunflower, rapeseed, corn and palm oils. Two GC quantitative methods to calculate individual fames were compared: Area % and internal standard. The both methods were applied for analysis of two certified reference materials. The statistical processing of the obtained results demonstrates the accuracy and precision of the two methods and allows them to be compared. For further chemometric investigations of biodiesel fuels by their FAME-profiles any of those methods can be used. PRM results of FAME profiles of samples from different vegetable oils show a successful recognition of biodiesels according to the feedstock. The information obtained can be used for selection of feedstock to produce biodiesels with certain properties, for assessing their interchangeability, for fuel spillage and remedial actions in the environment.*

Keywords*: biodiesel fuel, fatty acid methyl esters, gas chromatography, vegetable oil, animal fat.*

Introduction

Biodiesel is a potentially renewable substitute for diesel oil. A "green" fuel, biodiesel is biodegradable, nontoxic and is essentially free of metals, sulfur, carcinogenic aromatics and generates less greenhouse effect than fossil fuels [1].

Several processes and feedstock have been reported for the production of biodiesel [2]. Transesterification of vegetable oils and animal fats with alcohol (in most cases methanol) is currently one of the most attractive and widely accepted methodologies in the world and in Bulgaria too. Thus, biodiesel is defined as monoalkyl (methyl) esters of long-chain fatty acids.

At present, the dominant feedstocks (about 80 %) are vegetable oils, namely soybean oil in USA, rapeseed and sunflower oil in Europe and palm oil in Southeast Asia. Other feedstock having real or potential commercial interest are animal fats, non-edible and waste oils. Feedstock availability for

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biodiesel production varies according to geography, climate and economic of the countries. Traditional for Bulgaria feedstock are sunflower and rapeseed oils.

Since biodiesel is a mixture of fatty acid methyl esters (FAME), its properties depend on the chemical structure of the individual FAME and their contents (FAME profile). FAME profiles of biodiesel are influenced by the stocks and origin of the oils used [1, 3]. So, FAME profile may be used as an approach for selection of feedstock [3, 4], for investigations [5], for fuel spillage and remedial actions in the environment [6].

Biodiesel FAME profiles can be obtained by chromatographic methods, providing valuable multi-component information. Gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS) [7] and high performance liquid chromatography [8] have been frequently used of FAMEs analysis. However, visual evaluation of chromatograms is difficult and not reliable especially to compare a large number of specimens. The problem was overcome through the development of chemometric methods applying mathematical processing of analytical data. Chemometrics, including pattern recognition method (PRM) has been introduced for estimation of quality characters like aroma, taste adulteration etc., of essential oils [9, 10]. Little data are available on application of PRM for comparison of GC FAME profiles of biodiesel prepared from different feedstock, using neural networks in different models [11, 12] and including the amount of the most commonly esters presented in biodiesel esters.

The targets of this work were to implement GC analysis of biodiesel fuels by using and evaluating various methods for quantification of the contents of individual esters thereof, to obtained FAME profiles by a method with proven precision features and to distinguish between biodiesels from different feedstock by an approach of PRM for comparing the chromatograms based on the retention times and peak areas. To the aim several types of oils, including used in Bulgaria sunflower and rapeseed oils, were utilized for production of biodiesel fuels by transesterification. Only those meeting the requirement of EN 14214 [13] were included in our study.

Experimental

1. Standards and reagents
Certified reference materials: Fatty Acid Methyl Ester (B100), Methyl heptadecanoate (C17:0) were purchased from Spex CertiPrep; F.A.M.E. Mix Standard Rapeseed oil (cat № 07756 F.A.M.E. Mix C14 – C22 (cat № 18917)) – from Supelco; Fatty acid methyl esters –(C14:0), (C16:0), (C16:1), (C18:0), (C18:1), (C18:2), (C18:3), (C20:0), (C20:1), (C22:0), (C22:1), (C24:0), (C24:1) – from Sigma-Aldrich. Reagents of recognized analytical grade were used.

2. Samples
2.1. Known origin
Ten commercial biodiesel fuels, labeled as sample 1 to 10, produced from sunflower (sample (1 to 5) and rapeseed oils (6 to 10); 4 biodiesels – laboratory synthesized by methanol transesterification [6] of commercial oils, namely sample 11 (sunflower), sample 12 (rapeseed oil), sample 13 (corn oil), sample 14 (palm oil).

2.2. Unknown origin
Samples 15 to 18 were commercially available. For samples 15 and 16 we had the information from the producers that the feedstock are rapeseed and sunflower oils respectively, for samples 17 and 18 the raw materials were completely unknown.

3. Gas chromatography
All GC analyses were performed on a GC system Agilent Technologies 7890A equipped with FID, split/splitless injector and Agilent 7693A automated liquid sampler. The fussed silica capillary column HP-INNOWAX, 30m x 0.32mm ID and 0.25μm film thickness was used. Helium was used as a carrier gas, column flow was 1.5 ml/min. Hydrogen and air flows were set to 40 ml/min and 400 ml/min, respectively, makeup gas (nitrogen) 40 ml/min. The injection volume was 1 μl and split ratio was 1:80.

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The temperatures of the injector and the detector were 250 ºC and 300 ºC, respectively. The temperature program of the oven was initial temperature 210 ºC for 9 minutes and then to 230 ºC at 20 ºC/min and hold there for 10 minutes.

ChemStation for GC (Agilent Technologies) was used for instrumental control, data acquisition and data analysis.

GC-MS analyses were carried out using GC system Agilent Technologies 7890A combined with MSD 5975C Inert XL EI/CI, electron impact ionization (70eV) mass range 30 – 500 m/z and the same chromatographic conditions. The components of biodiesels were identified by injection of standards (2.1) and by comparison of mass spectra with those of a NIST MS computer library.

Sample 0.3 µl were injected with split ratio 1:100.

4. Pattern Recognition Method

PRM was performed according to the Package software program "Patreco". The classification program was designed especially for comparing fingerprint chromatographic profiles using pattern recognition approach. It has given excellent results to discriminate tobacco samples in aroma and color by comparing essential oils and polyphenols profiles [14].

FAME profiles of biodiesels were processed using the retention times and area of FAME peaks in the chromatogram.

PRM data were presented as indexes of similarity (I_s, %). The limit of the I_s (I_{so}) was calculated from the experimental data of five repeatable analysis of one sample and was > 96%. Above this value the FAME profiles are undistinguishable.

Results

The chromatogram of FAME obtained from rapeseed oil is shown in Figure 1. Peaks in ascending retention time order are follows: C14:0, C16:0, C17:0 (IS), C18:0, cis9 C18:1, cis9cis12 C18:2, cis9cis12cis15 C18:3, C20:0, cis11 C20:1, C22:0, cis13 C22:1, C24:0, C24:1.

Chromatographic peaks are well resolved, calibration curves of peak areas (relative or no to IS) against the methyl ester concentration are linear. For C18:1 the calibration curve drawing from 1,0 to 60 % had $r^2 > 0,991$.
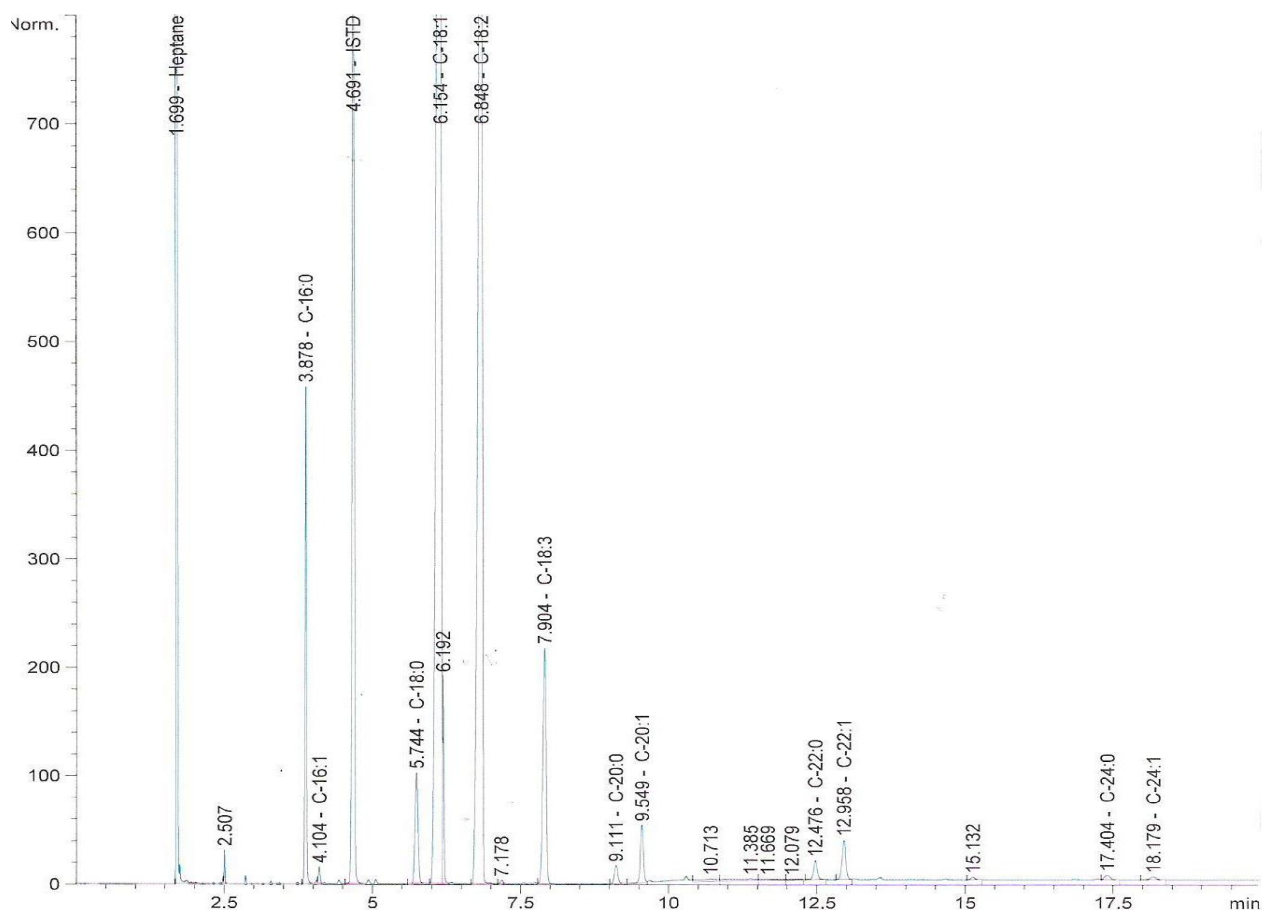
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Figure 1. GC chromatogram of rapeseed oil biodiesel

The total ester content was calculated according to EN 14103 standard [15]. There is no standard method to determine the individual FAMEs in biodiesel.

We compared two quantitative methods to calculate individual FAMEs in biodiesels:

- Area % - reports the area of each FAME peak in the run as a percent of the total area of all peaks in the run.
- Internal Standard Method (ISTD) – a known amount of internal standard C17:0 is added to the samples.

The both quantitative methods were applied for determination of the content of the FAME in F.A.M.E. Mix C14–C22 and F.A.M.E. Mix Rapeseed oil reference materials (2.1). The results obtained are given in Tables 1 and 2. The values concentration are mean from 6 analyses of each material. For comparison data from the Certificate of analysis of the reference mixture are given. In order to estimate the results statistically, the homogeneity of variance was examined using Cochran's test (G-test). The results are shown in Table 3.

Table 1. Results of analysis of F.A.M.E. Mix C14 – C22

FAME	Concentration (w%) by method						From Certificate of Analysis		
	Area %	SD	RSD (%)	ISTD	SD	RSD (%)	Concentration (w%)	SD	RSD
C14:0	4,2	0,02	0,57	3,9	0,02	0,50	4,1	0,06	1,46
C16:0	10,4	0,05	0,47	9,7	0,02	0,17	10,0	0,01	0,10
C18:0	6,3	0,10	1,51	6,1	0,01	0,20	6,0	0,10	1,67
C18:1	34,5	0,47	1,37	34,7	0,34	0,98	35,1	0,45	1,28
C18:2	35,2	0,52	1,48	35,0	0,35	1,01	35,9	0,68	1,89
C18:3	4,2	0,04	0,87	4,8	0,04	0,75	5,0	0,09	1,80
C20:0	2,1	0,02	1,17	1,9	0,02	0,95	2,0	0,03	1,50
C22:0	2,2	0,03	1,19	1,9	0,02	0,95	2,1	0,03	1,43

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Table 2. Results of analysis of F.A.M.E. Mix Rapeseed oil

F A M E	Concentration (w%) by method						From Certificate of Analysis		
	Area (%)	SD	RSD (%)	ISTD	SD	RSD (%)	Concentration (w%)	SD	RSD
C14:0	0,9	0,05	0,50	1,0	0,01	0,49	1,0	0,01	1,00
C16:0	3,9	0,02	0,45	3,9	0,01	0,20	4,0	0,03	0,75
C18:0	2,9	0,05	1,61	2,8	0,02	0,71	3,0	0,02	0,67
C18:1	60,3	0,66	1,10	60,4	0,40	0,66	60,0	0,45	0,75
C18:2	11,9	0,06	0,52	12,0	0,10	0,83	12,0	0,09	0,75
C18:3	4,9	0,04	0,88	4,9	0,03	0,61	5,0	0,04	0,80
C20:0	2,9	0,03	1,10	3,1	0,03	0,98	3,0	0,03	1,00
C20:1	1,0	0,01	0,55	1,0	0,01	0,50	1,0	0,01	1,00
C22:0	3,1	0,03	0,85	2,9	0,02	0,69	3,0	0,02	0,67
C22:1	4,9	0,03	0,61	5,0	0,03	0,60	5,0	0,03	0,60
C24:0	2,9	0,02	0,69	3,1	0,03	0,97	3,0	0,02	0,67

Table 3. G-test and F-test values to the results in Tables 1 and

Results - Table 1		Results - Table 2		
Area %	ISTD	Area %	ISTD	
G – ratio*				G -crit
0.218	0.217	0.309	0.182	0.445
F – ratio**				F – crit (f =5, p=0,05)
1.57		1.03		5.05

*$G – ratio = RSD_{max}^2 / \sum RSD_i^2$ **$F\ ratio = RSD_1^2 / RSD_2^2,\ RSD_1 > RSD_2$

The values concentration is mean from 6 analyses of each material. For comparison data from the Certificate of analysis of the reference mixture are given. In order to estimate the results statistically, the homogeneity of variance was examined using Cochran's test (G-test). The results are shown in Table 3.

In the same table values from F-test are given. In Table 4 data for accuracy and precision of the methods are shown. Values are calculated from the data in Table 2.

The samples used in this study were analyzed by GC under the conditions described and the content of each methyl ester was calculated by the method ISTD. The results are given in Table 5.

Table 6 shows the similarity indices of 10 samples tested (sample designations are the same as in Table 5). $I_s > 96\%$ was observed between samples 2 and 3, and between samples 7 and 8. Data shows that the samples of biodiesel from the same material do not differ in fatty acid composition. Similar values of a I_s above 96% was observed between samples 15 and 7, 16 and 3, and between samples 2 and 3, which classify the fatty acid composition of sample 15 similar to that of the rapeseed oil, sample 16 similar to that of the sunflower oil. This similarity is confirmed by producer information. $I_s > 96\%$ was found between samples 17, 2, 3 and between 16 and 18, and between samples 7, 8 and 15

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Table 4. Accuracy and precision of the methods (*CV (%) – Coefficient of variation; n = 6; $\bar{x}$ - average values of the results**CI – Confidence Interval; t – Student's test; f = n-1, p = 0,05)

FAME	Concentration (%)	Results (Area %)	Recovery (%)	*CV (%)	**CI	Results (ISTD %)	Recovery (%)	*CV (%)	**CI	
C14:0	1,0	0,9	90,0	0,56	0,9 ± 0,005	1,0	100,0	0,50	1,0 ± 0,005	$CV\ (\%)$
C16:0	4,0	3,9	97,5	0,46	3,9 ± 0,018	3,9	97,5	0,26	3,9 ± 0,008	$= \dfrac{\sqrt{(n}}{\bar{x}}$
C16:1	3,0	2,9	96,7	1,62	2,9 ± 0,049	2,8	93,3	0,71	2,8 ± 0,021	$* 100$
C18:1	60,0	60,3	100,5	1,09	60,3 ± 0,692	60,4	100,7	0,66	60,4 ± 0,419	
C18:2	12,0	11,9	99,2	0,50	11,9 ± 0,063	12,0	100,0	0,83	12,0 ± 0,105	
C18:3	5,0	4,9	98,0	0,92	4,9 ± 0,042	4,9	98,0	0,61	4,9 ± 0,031	
C20:0	3,0	2,9	96,7	1,03	2,9 ± 0,031	3,1	103,3	0,97	3,1 ± 0,031	
C20:1	1,0	1,0	100,0	0,60	1,0 ± 0,006	1,0	100,0	0,60	1,0 ± 0,006	CI
C22:0	3,0	3,1	103,3	0,97	3,1 ± 0,031	2,9	96,7	0,69	2,9 ± 0,021	$= \bar{x}$
C22:1	5,0	4,9	98,0	0,61	4,9 ± 0,031	5,0	100,0	0,60	5,0 ± 0,031	$\pm t(f,p)$
C24:0	3,0	2,9	96,7	0,69	2,9 ± 0,021	3,1	103,3	0,97	3,1 ± 0,031	$* \dfrac{SD}{\sqrt{n}}$

Table 5. FAME profiles of biodiesel from known and unknown origin

FAME	COMPOSITION %, Sample № (known origin)														unknown origin			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
C14:0	0,08	0,15	0,05	0,05	0,20	0,06	0,05	0,05	0,06	0,06	0,05	0,05	0,06	1,25	0,06	0,06	0,18	0,06
C16:0	6,40	7,76	5,57	5,04	7,36	5,13	4,53	5,48	5,71	5,20	5,51	4,60	10,9	41,72	5,62	5,64	8,53	6,38
C16:1	0,13	0,15	0,10	0,15	0,23	0,18	0,24	0,51	0,15	0,28	0,08	0,22	0,20	0,23	0,32	0,14	0,22	0,22
C18:0	3,08	3,18	3,74	4,47	3,97	2,06	1,64	2,54	2,94	2,20	3,48	1,78	2,41	5,31	1,55	2,85	3,42	3,05
C18:1	27,3	26,23	30,3	26,5	27,96	56,00	60,8	55,41	50,9	56,12	22,24	55,9	32,4	39,85	55,29	35,80	31,09	52,22
C18:2	62,1	58,26	54,5	61,1	57,20	25,50	18,7	26,29	29,4	23,23	64,60	24,7	48,0	9,50	23,45	48,00	51,08	28,10
C18:3	0,09	1,72	1,30	1,52	0,10	4,87	7,92	5,62	7,50	7,80	0,05	5,25	1,20	0,28	7,62	1,96	0,10	6,71
C20:0	0,22	0,18	0,27	0,17	0,30	0,42	0,50	0,48	0,35	0,50	0,22	0,57	0,50	0,30	0,60	0,30	0,25	0,30
C20:1	0,24	0,09	0,89	0,36	0,24	1,14	1,58	0,98	0,80	1,22	0,15	1,27	0,30	0,28	1,50	0,50	0,30	0,75
C22:0	0,37	0,20	0,69	0,17	0,66	0,48	0,29	0,58	0,43	0,38	0,60	0,40	0,11	0,31	0,30	0,52	0,77	0,38
C22:1	0,08	0,09	0,20	0,26	0,23	0,94	0,38	0,71	0,30	0,45	0,10	0,58	0,10	0,10	0,36	0,31	0,23	0,40
C24:0	0,22	0,05	0,29	0,09	0,05	0,05	0,11	0,05	0,25	0,19	0,30	0,25	2,05	0,10	0,11	0,19	0,27	0,21
C24:1	0,05	0,05	0,05	0,05	0,05	0,15	0,18	0,05	0,08	0,05	0,08	0,45	0,11	0,29	0,05	0,07	0,13	0,08

It is seen that certainly can be said that the sample 17 is from sunflower oil and sample 18 is from rapeseed oil.

Table 6. Similarity indices

	2	3	7	8	13	14	15	16	17	18
2	x	96.36	84.54	87.31	93.69	64.49	87.11	94.64	96.12	89.08
3		x	88.36	90.79	92.55	63.33	90.54	97.69	96.05	91.72
7			x	97.16	85.51	63.66	97.03	89.95	87.23	95.56
8				x	87.90	63.99	97.55	92.45	89.95	97.77
13					x	68.95	87.99	93.77	96.07	89.97
14						x	64.87	64.31	67.18	65.04
15							x	92.42	89.65	97.63
16								x	97.22	93.65
17									x	91.72
18										x

The palm samples show the lowest I_s to all samples (I_s differ between 63% and 68%). The data point to the most distinction of the fatty acid composition of biodiesel from palm oil from all investigated samples.

In corn oil, a relatively greater similarity to the sunflower oil (I_s = 92% -93%) was observed than compared to the rapeseed oil (I_s = 85% -87%).

Discussion

It can be seen (Table 1and 2) that SD for low concentrations of esters are logically less than those for high ones, but RSD does not so greatly depends on the concentration. Data show comparability with the certified values. Table 3. show homogeneity of the data and we can calculate the mean values of RSD to use them for statistical comparison of the two methods by means of F- ratio. Since F-ratio for both methods are less than the critical F at 5 degrees of freedom and significance level 0.05, it follows that the results obtained by each of the two methods are statistically equal. This demonstrates the possibility for further chemometric investigations of biodiesel fuels by their FAME-profiles, obtained by any of those most commonly used methods. The results in Table 4 indicate that accuracy and precision of both methods are very close. The limit of quantification (MQL) was determined experimentally by six analysis of the mixture containing 1% C18:2. Calculated as 3 x SD, MQL is 0,05%.

The PRM method used in this investigation can be very easily applied without using very sophisticated mathematical procedure.

Conclusion

Pattern recognition method is proposed for differentiating biodiesel oils from different feedstock, using their gas chromatograms, obtained by a GC method with established accuracy and precision. Such method allows statistical comparison of chromatograms. A differentiation criteria, index of similarity (I_s, %) is applied. The value of I_s enables biodiesel oils to be classified according to the feedstock. Also, PRM allows differentiation between similar in composition biodiesels like those from sunflower and corn. The information obtained can be used for selection of feedstock to produce biodiesels with certain properties, for assessing their interchangeability, for fuel spillage and remedial actions in the environment.

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