PRECISION AND REPEATABILITY IN BIODIESEL ANALYSIS

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Abstract

Intermediate precision and repeatability of fatty acids methyl esters (FAME) from transesterified Camelina sativa and hempseed oils are investigated in this study. The analysis was performed using gas chromatography coupled with a mass spectrometry detector – a technique which offers good response factor and confirmation of compounds identity based on the spectral information. Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated for each fatty acid methyl esters. For FAME from Camelina Sativa oil intermediate precision RSD was between 0.822-4.071% while for repeatability RSD was found between 0.395-2.386%. Concerning FAME from hempseed oil intermediate precision RSD was between 0.491-3.107% while for repeatability RSD was found between 0.509-1.594%.

Key words: gas chromatography, mass spectrometry, fatty acid methyl esters, precision, repeatability

1. Introduction

Fatty acid methyl esters (FAME) has been described by the American Society for testing and Materials (ASTM) as mono alkyl esters of long chain fatty acids. Biodiesel is a mixture of FAME from vegetable oils and it's considered an environmentally friendly alternative to conventional diesel fuel [1-4]. Other studies investigated the possibility to obtain valuable products from biodiesel by using special techniques as supercritical CO_2 fractionation [5], molecular distillation [6-7] etc.

FAME is commercially produced by alkali catalyzed (NaOH, KOH, $NaOCH_3$) transesterification with methanol to form esters and glycerol, which results in a short reaction time. The transesterification reaction is reversible and can never reach 100% completion. The complete process includes the transesterification reaction, separation of the raw ester layer from the glycerol layer and esters

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purification [8]. Other processes for production of biodiesel involve using a heterogenous catalyst [9]. The advantages of these processes are especially based on separation and reusing of catalyst.

Due to FAME destination as a fuel, its characterization has an important part. Gas chromatography (GC) is one of the most widely used commercial analysis technique because it comes with a lot of advantages: is sensitive, precise, rapid and provides reproducible analysis.

In gas chromatography the mobile phase is a carrier gas (an inert gas like helium) and the stationary phase is a layer of polymer on an inert solid support inside a metal tubing which is the chromatographic column. The capillary column contains a stationary phase. The sample is sent through the column by a stream of carrier gas. Components from the sample are separated because some take longer time to pass through the column than others [10]. The resolution of a GC chromatogram is given by the column length, stationary phase polarity and detector type. GC it's able to separate volatile compounds and to provide a good resolution, but it cannot identify them.

Most methods used to characterize biodiesel are using gas chromatography coupled with a flame ionization detector (GC-FID). An alternative to GC-FID for biodiesel analysis is gas chromatography coupled with mass spectrometry (GC-MS) because it offers the advantage of a separation on a GC column with information about molecules structure obtained by MS detection [11]. In mass spectrometry with electron impact ionization the molecules in the gas phase are bombarded with high energy electrons and form radical cations. These cations are instable and decompose in the detector. The rate of fragmentation usually depends on the molecule's ability to stabilize the positive charge. The resulting fragments are separated by their mass to charge ratio (m/z) in an electric field [12].

A combined GC and MS equipment can be successfully used to analyze complex organic and biochemical mixtures. Spectra compounds are collected by the mass spectrometer as they exit the chromatographic column which identifies and quantifies the compounds according to their mass to charge ratio (m/z). The amount of compound can be determined by integrating the peaks in the total ion count chromatogram (TIC) [13].

Different authors investigated the performance of FID vs. MS in quantifying FAME separated by GC. Koza et al. [14] compared FID response factors (RF) of FAME with those obtained using EI in quadrupole (QP). Seven saturated and unsaturated C15-C17 FAME were evaluated. They showed that good response factor can be obtained for both FID and MS. Dodds et al. [13] conducted a comparative study of GC-FID and GC-MS methods and they founded that GC-MS offers two important advantages: the ability to confirm the identity of analytes based on spectral information and the ability to separate peaks from a noisy background.

The aim of the present work is to validate the analytical method based on GC-MS techniques for FAME obtained by transesterification from vegetable oils. Two kind of oil with different content of FAME are investigated (*Camelina Sativa* oil and hempseed oil) and the final product composition are analyzed in terms of precision and repeatability of measurements.

2. Experimental

Reagents

The chemicals used for this study are: *Camelina sativa* oil and hempseed oil from local sources, anhydrous methanol (99.8% purity), potassium hydroxide (90% purity), anhydrous magnesium sulfate (98% purity), n-heptane (99% purity) and acetone (99.5% purity) from Sigma Aldrich (Germany).

Transesterification of Camelina sativa and hempseed oils

Triglycerides from vegetable oils react with methanol in basis catalysis (1% KOH from the oil mass) to form glycerol and FAME. Although the reaction stoichiometry requires a molar ratio of 3:1 alcohol: triglycerides, an excess of alcohol is necessary to achieve a higher reaction conversion (alcohol: triglycerides 6:1 molar ratio).

For the transesterification reaction a high pressure, stainless steel reactor Berghof, SS316TI model (Germany) is used. Since the reaction should take place in liquid phase, a nitrogen atmosphere is required to create enough high pressure inside the reactor for preventing methanol evaporation. The transesterification reaction is performed at 75°C and safe autoclave pressure has been set at 9 bars. After the completion of the reaction, the mixture is cooled at room temperature. The reaction product is two phases state (FAME and glycerol) and the glycerol can be removed via a separatory funnel. Then, FAME is washed several times with distilled water, dried with anhydrous magnesium sulfate and filtered.

Characterization and quality evaluation of FAME from Camelina sativa and hempseed oils

FAME analysis is carried out using an Agilent Technologies gas chromatograph type 7890A equipped with a triple-axis MS detector (Agilent Technology, 5975C type). A ZB-FAME capillary column was used (30m length, 0.25mm internal diameter, 0.20μm film thickness) and helium as carrier gas at 3 mL/min. The GC injector temperature is 250°C and the transfer line temperature was 280°C. The oven temperature is initially set at 50°C, increasing to 160°C with

5°C per minute, the hold time being 1 minute, then the temperature is increased to 190°C with 2°C per minute and a hold time of 5 minutes. In the next ramp, temperature is increased to 206°C with 1°C per minute and a hold time of 5 minutes. In the last ramp, temperature is increased to 230°C with 3°C per minute and a hold time of 10 minutes. The MS detector is operated in EI mode, with an m/z scanning range from 50 to 550. The FAME peaks were identified according to NIST Database and FAME chromatographic standards. The method used is in according with standards SR EN 14103 [15]. The polarity of the column stationary phase plays a critical role in a successful separation of FAME. To improve peak resolution, the polarity of the column stationary phase should be close to the polarity of the fatty acids.

Results for each component were evaluated in terms of SD (standard deviation) and RSD (relative standard deviation), where SD was calculated with eq. 1 and RSD with eq. 2.

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(x-\bar{x})^2}{N-1}} \tag{1}$$

where x = individual data, $\bar{x}$ is the mean of the data, N = is the number of data

$$RSD = \frac{SD}{\bar{x}} \cdot 100 \tag{2}$$

where $\bar{x}$ is the mean of the data.

3. Results and discussions

After FAME layer separation, washing with distilled water, drying with anhydrous magnesium sulfate and filtration, the products are analyzed using GC-MS technique. Method precision is evaluated at two levels: intermediate precision and repeatability. The intermediate precision is evaluated by data measured on different days but in the same conditions and the same operator. The repeatability is evaluated by comparing data from simultaneous injections of the same solution of FAME in n-heptane in the same day, by the same operator.

FAME identified in GC-MS chromatograms for *Camelina sativa* oil sample are presented in Table 1.

Intermediate precision was evaluated by injecting the samples from the same solution of FAME of *Camelina sativa* oil in n-heptane in five consecutive days. Results are presented in terms of SD and RSD in Table 2 to relate the concentration measurements errors. Standard deviation SD is lower than 0.45% for the main compounds identified in transesterification product.

Table 1

Main FAME content of *Camelina sativa* oil sample

Compound	Chemical formula	Shortened formula	CAS No.	Molar mass (g/mol)
Methyl palmitate	$C_{17}H_{34}O_2$	C16:0	112-39-0	270.5
Methyl stearate	$C_{19}H_{38}O_2$	C18:0	112-61-8	298.5
Methyl oleate	$C_{19}H_{36}O_2$	C18:1	112-62-9	296.5
Methyl linoleate	$C_{19}H_{34}O_2$	C18:2	112-63-0	294.5
Methyl linolenate	$C_{19}H_{32}O_2$	C18:3	301-00-8	292.5
Methyl eicosenoate	$C_{21}H_{40}O_2$	C20:1	2390-09-2	324.5

Table 2

Intermediate precision results for FAME from *Camelina sativa* oil

Compound		C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	Others
day 1	%	6.616	2.128	20.266	23.842	28.420	14.510	4.219
day 2	%	6.591	2.089	19.913	23.731	28.441	15.32	3.920
day 3	%	6.823	2.057	20.246	24.041	28.655	14.749	3.429
day 4	%	6.646	2.047	20.017	23.678	28.575	15.363	3.674
day 5	%	6.961	1.908	20.266	24.394	29.367	14.400	2.704
average	%	6.727	2.046	20.142	23.937	28.692	14.867	3.589
SD	(%)	0.159	0.083	0.166	0.291	0.390	0.449	0.575
RSD	(%)	2.366	4.071	0.822	1.215	1.358	3.020	16.020

The repeatability was evaluated by comparing data from five simultaneous injections of the same solution of FAME from *Camelina sativa* oil in n-heptane. Results are presented in terms of SD and RSD in Table 3 to relate the measurements errors. Standard deviation SD is lower than 0.35%.

Table 3

Repeatability results for FAME from *Camelina sativa* oil

Compound		C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	Others
injection 1	%	6.766	2.133	20.023	23.514	28.290	15.657	3.617
injection 2	%	7.019	2.241	20.397	23.548	27.558	15.424	3.813
injection 3	%	6.880	2.208	20.461	23.319	27.867	15.760	3.505
injection 4	%	7.034	2.276	20.611	23.474	27.562	15.482	3.561
injection 5	%	6.915	2.214	20.565	23.532	27.490	15.632	3.652
average	%	6.923	2.214	20.411	23.477	27.753	15.591	3.630
SD	%	0.110	0.053	0.233	0.093	0.334	0.136	0.117
RSD	%	1.584	2.386	1.141	0.395	1.202	0.875	3.217

FAME composition from hempseed oil is presented in Table 4.

Table 4

Main FAME content of hempseed oil sample

Compound	Chemical formula	Shortened formula	CAS No.	Molar mass (g/mol)
Methyl palmitate	$C_{17}H_{34}O_2$	C16:0	112-39-0	270.5
Methyl heptadecatrienoate	$C_{18}H_{30}O_2$	C17:3	155273-05-5	278.4
Methyl stearate	$C_{19}H_{38}O_2$	C18:0	112-61-8	298.5
Methyl oleate	$C_{19}H_{36}O_2$	C18:1	112-62-9	296.5
Methyl linoleate	$C_{19}H_{34}O_2$	C18:2	112-63-0	294.5
Methyl linolenate	$C_{19}H_{32}O_2$	C18:3	301-00-8	292.5

Intermediate precision was evaluated by injecting the same solution of FAME from hempseed oil in n-heptane in five days. Results are presented in terms of SD and RSD in Table 5 to relate the measurements errors. Standard deviation SD values are lower than 0.5%.

Table 5

Intermediate precision results for FAME from hempseed oil

Compound		C16:0	C17:3	C18:0	C18:1	C18:2	C18:3	Others
day 1	%	7.082	3.924	3.140	7.350	56.795	20.054	1.655
day 2	%	6.997	3.875	3.088	7.289	56.872	20.472	1.407
day 3	%	7.100	3.951	3.197	7.301	57.509	19.834	1.108
day 4	%	7.043	3.891	3.308	7.370	57.033	20.030	1.325
day 5	%	7.130	3.805	3.311	7.414	56.967	19.974	1.399
average	%	7.070	3.889	3.209	7.345	57.035	20.073	1.379
SD	(%)	0.052	0.056	0.100	0.051	0.280	0.239	0.196
RSD	(%)	0.732	1.427	3.107	0.697	0.491	1.190	14.218

The repeatability was evaluated by comparing data from five simultaneous injections of the same solution of FAME in n-heptane. Results are presented in terms of SD and RSD in Table 6. Standard deviation SD values are lower than 0.3%.

Table 6

Repeatability results for FAME from hempseed oil

Compound		C16:0	C17:3	C18:0	C18:1	C18:2	C18:3	others
injection 1	%	7.075	3.864	3.098	7.547	56.699	20.121	1.596
injection 2	%	7.009	3.867	3.176	7.589	56.475	20.527	1.357
injection 3	%	7.086	3.926	3.097	7.388	57.219	20.023	1.261
injection 4	%	7.054	3.899	3.103	7.300	57.008	20.104	1.532
injection 5	%	7.107	3.876	3.091	7.414	56.969	19.975	1.568
average	%	7.066	3.886	3.113	7.448	56.874	20.150	1.463
SD	(%)	0.037	0.026	0.035	0.119	0.290	0.219	0.146
RSD	(%)	0.527	0.670	1.140	1.594	0.509	1.087	9.993

Generally, SD for repeatability must be smaller or equal to SD for intermediate precision. In case of FAME obtained from transesterified *Camelina Sativa* oil (Tables 2 and 3), all the fatty esters are in good agreement with this theory, except C18:1 which has a sensitive higher repeatability SD. Concerning FAME obtained from hempseed oil (Tables 5 and 6), also C18:1 registered a higher repeatability SD compared with the other compounds. In Figure 1a) is presented RSD for each component in both oils. There are differences less than 1.5% for measurements performed for the same sample in the same conditions in different days. In Figure 1b), RSD differences for measurements performed in the same day are less than 1%.

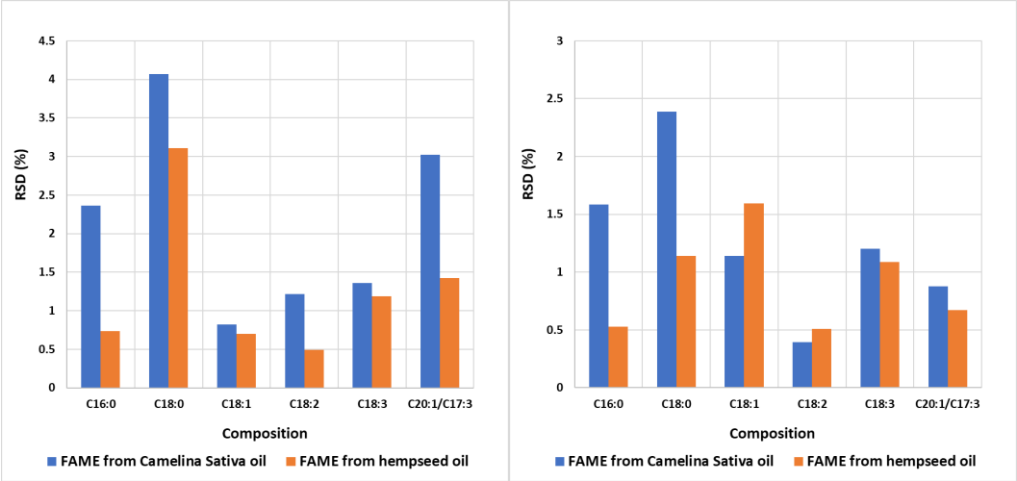

Fig.1. RSD for FAME from *Camelina sativa* oil vs. hempseed oil a) intermediate precision b) repeatability

3. Conclusions

In this study intermediate precision (analysis performed in five consecutively days) and repeatability (five injections of the same sample, in the same conditions and in the same day) of FAME (also known as biodiesel) obtained from transesterified *Camelina Sativa* and hempseed oils were investigated using a gas chromatograph and mass spectrometer detector (GC-MS). Standard deviation (SD) and relative standard deviation (RSD) were calculated for each ester from the FAME mixture. For FAME from *Camelina Sativa* oil intermediate precision RSD is between 0.822-4.071% while for repeatability RSD was found between 0.395-2.386%. Concerning FAME from hempseed oil intermediate precision RSD was between 0.491-3.107% while for repeatability RSD was found between 0.509-1.594%. RSD for repeatability is smaller than RSD for intermediate precision for both types of biodiesel, except C18:1 fatty ester which in both cases is higher.

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