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# Determination of esters in glycerol phase after transesterification of vegetable oil

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ABSTRACT

In biodiesel production, glycerol is formed as a side product and it is contained in the glycerol phase. This phase contains (besides glycerol): water, soaps, alcohol, traces of catalyst and glycerides and the remaining esters. In this paper, a new method for the determination of esters in the glycerol phase is introduced. The determination enables the minimization of the losses of biodiesel within the production process. It is based on the gradient RP-LC method (water and acetonitrile) with refractometric detection. The analysis is easy and the samples do not need any treatment (only dilution by water) and has a low detection limit. The results of this method were compared with the results of two other published methods: isocratic HPLC and GC. The disadvantage of these two methods is that they need extensive treatment of the sample, which takes many hours, and they are able to determine only the sum of esters. The new method is reliable, much faster and able to differentiate esters of almost each higher fatty acid (e.g. linoleic, linolenic, strearic alkyl ester) in the glycerol phase.



## 1. Introduction

A mixture of alkylesters of higher fatty acids (biodiesel) and glycerol is formed within transesterification of vegetable oils. Glycerol is a side product and it is contained in the glycerol phase (GP). The GP consists of glycerol and many other chemical substances such as water, organic and inorganic salts, esters (biodiesel) and alcohol, traces of glycerides, pigments and carotenoids. The exact composition of the GP depends on the method of transesterification and the separation conditions of biodiesel production.

The GP contains usually a significant amount of esters with a concentration range of 5–15 wt.% for the GP formed by methanolysis [1] and 20–40 wt.% for the GP formed by ethanolysis of oil [2]. The esters in the GP increase when the oil with a high acid number (with a high content of free acids) is used for the basic catalyst transesterification. The dependence of methylesters remaining in the GP on the acid number of oil was described [3]. Esters contained in the GP are losses of the main product (biodiesel) and furthermore increase the amount of the GP; therefore it is important to know this amount.

Many methods of the determination of ester in pure biodiesel were described. Some of them are based on GC-FID: the standard method EN 14103, other methods using capillary GC-FID [4] or a similar method employing a HP-1 wide-bore column [5]. A HPLC method was described for quantifying fatty acid ethyl, isopropyl and 2-butyl esters, the process involves the use of an ELSD detector [6]. Other reverse-phase HPLC method using gradient elution with UV and mass spectrometric detection was described for the monitoring of methlyesters and glycerides in biodiesel [7,8]. Supercritical fluid chromatography was also used to determine fatty acid methyl esters, fatty acids and glycerol in biodiesel samples [9]. Another analytical determination of methyl ester content is based on FTIR-ATR and FTNIR spectroscopies [10]. Santori published determination of mono-, di- and triglycerides, methylesters, methanol and glycerol content in both phases after filtration of samples. Gradient RP-LC with UV detection was used [11]. This paper describes an easy determination method of the remaining esters in the glycerol phase only with dilution of sample.

## 2. Materials and methods

### 2.1. Used chemicals

Cold pressed rapeseed oil was kindly provided by firm RPN Slatiňany, CZ. The oil had an acid number of 0.59 mg KOH/g and water content of 0.04%. Acetonitrile (purity min 99%), standards of methylesters of the following acids: palmitic, oleic, linoleic, linolenic and stearic (all of purity min 99%), and phosphoric acid (85 wt.%) were obtained from Lach:Ner, Czech Republic, Demineralized water (purity 1.2 μS/cm).

### 2.2. HPLC gradient analysis

The sample of the GP was diluted approximately five times by water prior to analysis.

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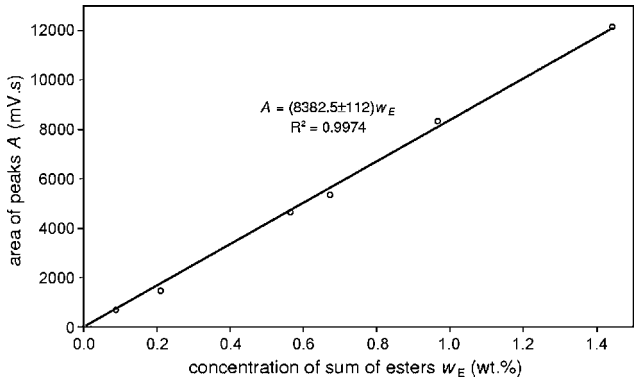


**Fig. 1.** Identification of peaks in gradient HPLC chromatogram for standards and sample.

A high pressure liquid chromatograph (LCP 4000.1, Ecom limited) with a linear low pressure gradient elution (Gradient programmer GP4, Ecom limited) and a refractometric detector (RIDK 102, Laboratory Instrument Prague, Czech Republic) was used to determine the ester concentration in the sample. Determination was performed on the Biospher SI C18 column (150 mm × 3.3 mm) with particle size of 7 μm produced by Labio, Prague. The determination was performed at laboratory temperature with an inject volume of 20 μl (6-port two position valve was used) with the flow rate 0.5 ml/min. The linear gradient was performed with water and acetonitrile (ACN): 0–2 min, 60% ACN; 2–6 min change ACN to 100%; 6–22 min 100% ANC. A clarity 2.0 data station (DataApex, Prague, Czech Republic) was used for the acquisition and processing of data from the refractometric detector.

The calibration curve of pure esters in ACN from 0.1 to 1.5 wt.% was used to determine esters in the sample (Fig. 1). It was identified, according to statistical tests, that the intercept of calibration curve is insignificant. The esters were prepared from rapeseed oil according to patent [12]: the reaction temperature 60 °C with 1 wt.% KOH, the molar ratio oil to methanol was 1:6 and the reaction time 90 min. After stopping of the reaction by gas $CO_2$, distillation off of methanol excess and phase separation acceleration by water [13], the ester was washed by water (removing of residual soaps) and dried. The esters purity was 99 wt.% (determined on the GC by standard method EN 14105) with the traces of glycerides (approximately 1 wt.%). The determination for each standard was repeated three times. The resulting concentration of ester in the GP was calculated with Eq. (1):

$$w_{E(\mathrm{GP})} = \frac{A - q}{k} \times \frac{m_{\mathrm{water}} + m_{\mathrm{GP}}}{m_{\mathrm{GP}}} \quad (1)$$

$w_{E(\mathrm{GP})}$ is the weight percent of esters in the GP (wt.%); $A$ is the peak area of esters in chromatogram; $q$ is the intercept of calibration curve; $k$ is the slope of calibration curve; $m_{\mathrm{water}}$ is the amount of the water for dilution of the GP (g); $m_{\mathrm{GP}}$ is the amount of the analyzed sample of the GP (g).

The peaks were identified by 1 wt.% solutions of methylesters standard of the following acids: palmitic, stearic, oleic, linoleic and linolenic in ACN.

### 2.3. Referential comparative determination

The described determination was compared with two other methodical determinations of ester by the GP. The sample of the GP (for these determinations) had to be treated prior to analysis.

#### 2.3.1. Treatment of samples

The sample of the GP ($m_{\mathrm{GP}}$—approx. 20 g) was acidified by concentrated $H_3PO_4$ (2 ml) and diluted by water (10 ml). Soaps were transformed into fatty acids and the GP was distributed into a lighter non-polar phase ($m_{\text{non-polar phase}}$) containing ester, fatty acids and other non-polar substances and a heavier polar phase containing glycerol, water, potassium phosphate and other polar substances. The separation of phases was accelerated by heating (80 °C) and took many hours. After separation, the content of ester in the non-polar phase ($w_{E(\text{non-polar phase})}$) was determined by two chromatographic methods. The concentration of ester in the GP ($w_{E(\mathrm{GP})}$) was calculated according to (2):

$$w_{E(\mathrm{GP})} = w_{E(\text{non-polar phase})} \times \frac{m_{\text{non-polar phase}}}{m_{\mathrm{GP}}} \quad (2)$$

#### 2.3.2. GC analysis

The resulting non-polar phase has similar properties as the ester phase and therefore can be analyzed by the published GC method (Shimadzu GC-2010). Method is the same as the method for determination of glycerides in biodiesel (EN 14105) with the same treatment of the sample and condition of analysis, which takes approx. 1 h. The peak of total ester was identified in the chromatogram of the non-polar phase and its concentration was calculated according to the calibration curve. Then the concentration of ester in the GP was calculated according to (2) [2].

#### 2.3.3. Isocratic HPLC analysis

As the second method, isocratic HPLC analysis with column SGX was used [14]. Hexane/propan-2-ol = 97/3 (v/v) was used as a mobile phase with a flow rate of 0.5 ml/min. Two peaks on the chromatogram of the non-polar phase were observed: the first is the sum of glycerides; the second is the sum of esters. The concentration of ester in the sample was calculated according to calibration curve and with the help of Eq. (2).

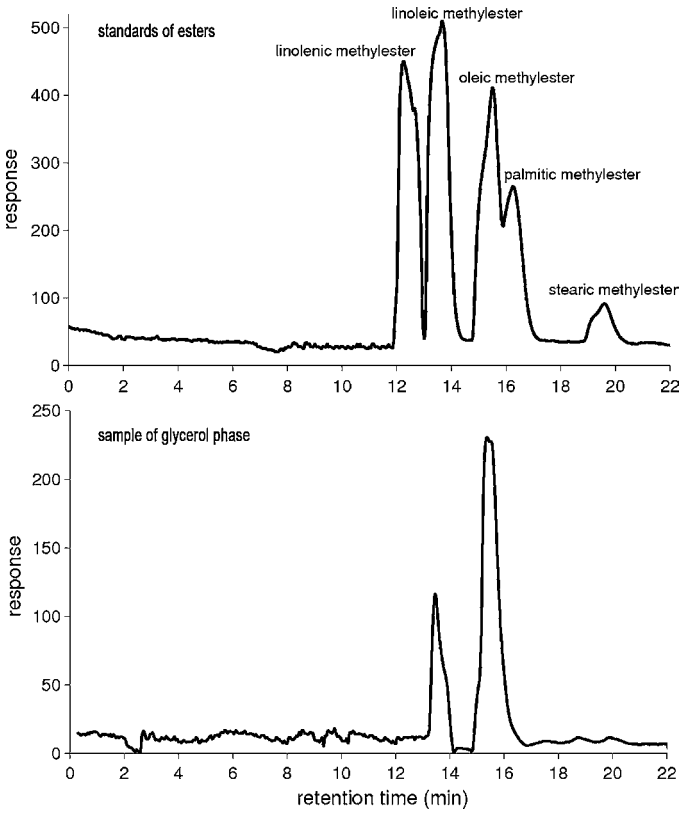


**Fig. 2.** Calibration curve for gradient HPLC for determination of esters concentration in GP.

**Table 1**
Detection and quantitation limits for all determined esters.

| Ester type | Detection limit (wt.%) | Quantitation limit (wt.%) |
|---|---|---|
| Linoleic ester | 0.0064 | 0.021 |
| Linolenic ester | 0.0037 | 0.012 |
| Oleic ester | 0.0066 | 0.022 |
| Palmitic ester | 0.0071 | 0.024 |
| Stearic ester | 0.0056 | 0.019 |

**Table 2**
Comparison of results determined by all three methods.

| Methods | Gradient HPLC | GC | Isocratic HPLC |
|---|---|---|---|
| Concentration of esters in the GP in different samples (wt.%) | 5.79 | 5.25 | 5.89 |
| | 15.11 | 13.54 | 13.99 |
| | 7.41 | 7.55 | 7.54 |
| | 9.1 | 8.18 | 8.37 |
| | 13.52 | 12.91 | 13.37 |

## 3. Results and discussion

### 3.1. Determination of esters—gradient HPLC

The GP contained many chemical substances: polar such as water, alcohol, glycerol, soaps and apolar esters. Therefore, the gradient elution of separation of the esters from other substances had to be used: the first elution agent was distilled water, which eluted all polar substances. The second was acetonitrile, which was used for the elution of esters.

At first, the base line was measured. This base line chromatogram was subtracted from the measured chromatogram (with sample) and the result is depicted in Fig. 2. Five peaks were observed and identified by the noticed standards (pure methylesters of the following acids: palmitic, stearic, oleic, linoleic and linolenic), all esters are eluted in the retention time from 12 to 22 min. The esters were eluted in the following order: linoleic ester (12.3 min), linolenic ester (13.7 min), oleic ester (15.6 min), palmitic ester (16.3 min) and stearic ester (19.7 min). The order of ester elution depends on the Equivalent Carbon Number (ECN) of the ester molecule; the lower ECN, the lower retention time. Therefore the peaks of oleic and palmitic esters are difficult to differentiate, because of the same ECN. Nevertheless, for the purpose of this analysis, it is sufficient to determine the total amount of esters. The detection and quantitation limits for all determined methylesters are stated in Table 1. The GP with ethyl esters was also tested with the similar results.

For the determination of ester quantity in the GP, the mixture of esters was used as standard. This mixture was prepared by transesterification from the same oil as the analyzed GP, because of the same composition of bonded fatty acids in the esters. The total time of analysis is less than 30 min.

### 3.2. Comparison of results of GC and HPLC method

The GC and isocratic HPLC were used as the comparative methods, the results of these methods are considered reliable. Five samples of the GP (prepared from rape seed oil by methanolysis) were used for comparison and they are stated in Table 2. The analyses were repeated seven times and the relative standard deviation was 0.46%. The table shows that all three methods led to practically identical results. The differences can be caused by the treatment of samples for GC and isocratic HPLC.

The results from all three methods were tested by the statistical method ANOVA in the program QC Expert, which proved that all three methods led to practically the same result with a 95% significance level.

## 4. Conclusion

A new method for ester determination in the side product of biodiesel production by gradient HPLC was tested and compared with two other chromatography methods. It was verified and proved by statistical tests that all methods give almost the same results. The advantages of the new method are: the total time of analysis is many times shorter (it does not require any complicated and long time consumption treatment of the sample) and it needs a 20 times lower amount of the sample than the two comparing methods. The gradient HPLC method is reliable, fast (complete analysis takes approx. 30 min) and environmentally friendly with high detection limit. In contrast to the published methods, the developed analysis enables to determine esters in the GP only with dilution of the sample by water using refractometric detector.

The method was tested for the GP containing methyl and ethyl esters, but is also suitable for other low molecular esters.

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