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Analysis of oil–biodiesel samples by high performance liquid chromatography using the normal phase column of new generation and the evaporative light scattering detector

Sergey N. Fedosov[a,*], Natalia A. Fernandes[b], Mohd Y. Firdaus[a]

[a] Dept. of Engineering Science, Aarhus University, Science Park, Gustav Wieds Vej 10C, 8000 Aarhus C, Denmark
[b] Universidade do Estado de Santa Catarina, Departamento de Engenharia Ambiental, Lages, SC, Brazil

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ABSTRACT

Conversion of vegetable oil to biodiesel is usually monitored by gas chromatography. This is not always convenient because of (i) an elaborate derivatization of the samples; (ii) inhibition of this process by methanol and water; (iii) low stability of the derivatives under storage. HPLC methods are apparently more convenient, but none of the described variants had won a wide recognition so far. This can be ascribed to the problems of reproducibility (in the case of normal phase chromatography) and limited separation of some analytes (in the case of reverse phase chromatography). Here we report an HPLC procedure suitable for separation of biodiesel, free fatty acids, glycerides, glycerol and lecithin. The normal phase column of new generation (Poroshell 120 HILIC) and the novel gradient were used. The method was tested on both the artificial mixtures and the crude reaction samples. Elution of the analytes was monitored by an evaporative light scattering detector. This method is usually confined to a very limited range of masses, where only a part of the complex calibration curve is used. We have analyzed the light scattering signal within a very broad range of masses, whereupon the calibration curves were produced. The data were approximated by the appropriate equations used afterward to recalculate the signal to the mass in a convenient way. An experimental conversion of rapeseed oil to biodiesel was performed by a liquid lipase formulation. This process was monitored by HPLC to illustrate advantages of the suggested registration method.

1. Introduction

The samples of oil phase, collected under conversion of oil to biodiesel, contain five major groups of analytes: triglycerides (TG), diglycerides (DG), monoglycerides (MG), free fatty acids (FA) and biodiesel (BD). BD is usually represented by either fatty acid methyl ester (FAME) or fatty acid ethyl ester (FAEE). The reaction samples and the final products are most frequently examined by gas chromatography (GC) in its several modifications [1–5]. The GC methods are well established but have a number of disadvantages: (i) partial glycerides and glycerol in biodiesel should be subjected to an elaborate procedure of silylation before analysis; (ii) methanol and water (usual reactants under biodiesel conversion) inhibit this derivatization and should be carefully evaporated; (iii) the silylated glycerides are very unstable under storage and require immediate analysis. Certification of biodiesel by GC method is usually performed in half-automated mode on the products of high purity, where the mentioned issues become of lower importance. On the other hand, they do present a problem if numerous experimental samples of unknown composition should be tested manually.

The alternative methods of TLC [6–8] and HPLC [11–13] are better applicable to assess the reaction progress in crude mixtures. The TLC method provides a reasonable accuracy and is very simple, especially its version on plates [8]. The HPLC method is apparently more precise, but none of its variants described in the literature had won a wide recognition so far. Normal phase chromatography was used in the early publication, where CN-propyl column provided a seemingly good separation of BD, TG, DG, MG and FA fractions [9]. Yet, the method suffered from reproducibility problems, which were independently observed in at least two laboratories during our previous work [10], private communications. This issue will be addressed in the current assay. The more recent publications focused on the reverse phase chromatography of oil–biodiesel mixtures [11–13]. The presented profiles revealed a good potential for separation and a detailed characterization of TG and DG fractions, but the multiple peaks of BD, FA and MG partially overlapped.

Another issue concerns the registration of signal under HPLC. For example, the UV-detectors are relatively cheap and provide a

* Corresponding author. Tel.: +45 87 15 55 21; fax: +45 86 13 65 97.
E-mail address: snf@mb.au.dk (S.N. Fedosov).

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nearly linear dependence of absorbance on the mass of analyte. Yet, the chemical structure of analytes and solvents severely affects the intensity of signal as well as its quality [11]. Other popular methods are based on the analysis of aerosols by evaporative light scattering detectors (ELSD) or charged aerosol detectors (CAD) [14–16]. Both methods provide a universal registration of nonvolatile compounds but show nonlinear calibration curves. Especially ELSD demonstrates a complex shape of the calibration curves, if working within a wide diapason of masses. Therefore, the calibrations are usually confined to a relatively narrow region of measurements, where the data can be approximated by a relatively simple function [9,14–16].

In the current publication we investigate separation of oil–biodiesel mixtures using the normal phase column of new generation (Poroshell 120 HILIC). We also address the problems of reproducibility associated with a previously used method based on the normal phase HPLC. A mathematically convenient approach is suggested to analyze and use the complex calibration curves recorded on an evaporative light scattering detector.

2. Material and methods

2.1. Materials

All salts and solutions were purchased from Sigma–Aldrich. Lipid standards of high purity (methyl oleate, ethyl oleate, triolein, diolein, monoolein, oleic acid, phosphatidylcholine) were from Sigma–Aldrich. Rapeseed oil of known specification was from either a Danish supermarket (refined oil, TG = 97%) or Emmelev A/S (crude oil, TG = 90%). Crude fish and squid oils, as well as biodiesel from fish oil, were provided by Novozymes, Denmark. Biodiesel (96% FAME) was prepared on a preparative scale from rapeseed oil by the enzymatic reaction with Novozym 435 (Novozymes, Denmark) as described earlier [13]. Olein-based preparations of MG, DG and FA (major components of 95%, 80% and 99%, respectively) were from Danisco (Denmark). A mixture of FA (76%) and partial glycerides was prepared by the enzymatic hydrolysis of rapeseed oil. The immobilized and liquid formulations of the enzymes (Novozym 435 and Callera Trans L) were kindly provided by Novozymes (Denmark).

2.2. Methods

2.2.1. HPLC analysis

Two technical setups were used in our work. The first HPLC system was represented by Hitachi D-7000 (Hitachi Ltd., Japan) equipped with a Penomenex Luna 5u CN column (250 mm × 4.6 mm, particle size of 5 μm, pores of 100 Å, chromatography at 30 °C). The separation procedure generally resembled the method described by Foglia and Jones [9]. In short, the two solvents A (hexane + 0.4% acetic acid) and B (methyl tert-butyl ether + 0.4% acetic acid) were mixed according to the following gradient: 0–5 min (B = 0%), 18 min (B = 80%), 20 min (B = 80%), 22 min (B = 0%), 30 min (B = 0%). The flow was maintained at 1 mL/min. The analyte samples (0.2–20 mg) were dissolved in 1 mL of either heptane or iso-octane (both with 0.4% acetic acid) and injected to HPLC in the volume of 1–10 μL.

The second system (Agilent 1260 Infinity, Agilent Technologies, USA) was equipped with Poroshell 120 HILIC column (150 mm × 3 mm, particle size of 2.7 μm, pores of 120 Å, chromatography at 30 °C). The particular technical setup of the Agilent system was adjusted to the normal phase chromatography, where seals and valves of the pump were resistant toward hexane as the main solvent. Other details are described in the main text.

2.2.2. Quantification of the analytes by ELSD

Analytes eluted from both HPLC systems were monitored on the evaporative light scattering detector PL-ELS 2100 (Polymer Laboratories Ltd., USA) connected to a computer via the interfaces provided by the HPLC manufacturers. The setup of ELSD corresponded to nebulization at 50 °C, evaporation at 40 °C and the compressed air flow of 1.5 mL/min. The signal was recorded at a rate of 1 Hz. The calibration standards included both the compounds of high purity (based on oleic acid) and natural products of the known specifications. They were applied to HPLC both individually and in the mixtures. The data were independently collected by three operators. As no systematic deviation was found, all the data were pooled.

2.2.3. Enzymatic preparation of biodiesel

Synthesis of biodiesel (BD) on a preparative scale was conducted as described earlier by reacting 1 L of rapeseed oil with methanol in the presence of Novozym 435 [10]. An analytical reaction on a smaller scale was conducted using a liquid formulation Callera Trans L (modified lipase from *Thermomyces lanuginosus*). Conversion was performed by adding 0.3 mL catalyst and 0.5 mL water to 15 mL of rapeseed oil (35 °C), whereupon methanol was added in three portions of 0.9 mL (start), 0.9 mL (3 h), 0.9 mL (8 h). Presence of water in the mixture was obligatory to preserve activity of the enzyme. The samples of 200 μL were collected at time intervals and immediately centrifuged (1 min, 15,000 rpm) to precipitate water–glycerol phase with the enzyme. Approximately 150 μL of the upper oil–biodiesel phase was collected, frozen and used for the following analysis.

2.2.4. Determination of free fatty acids

Fatty acids in partially hydrolyzed oil–biodiesel samples were measured by the micro-titration method using absorbance and/or fluorescence signals of pyranine as described elsewhere [17].

2.2.5. Nonlinear regression analysis

The approximation of nonlinear curves was done by the computer program KyPlot 5 (KyensLab Inc., Japan) using quasi-Newton method of least squares.

3. Results and discussion

3.1. Problems of reproducibility using CN-propyl column

At the first step, the separation of oil–biodiesel mixtures generally followed the method from literature [9], see also Section 2.2.1. This procedure employed the normal phase CN-propyl column (Penomenex Luna 5u CN), where the mixtures of highly purified compounds were successfully separated [9]. Our column was periodically used for examination of raw oil and biodiesel samples. This analysis was interchanged with the test runs of artificial mixtures used as a quality control. They typically contained biodiesel derived from refined rapeseed oil, refined rapeseed oil, diolein, monoolein and oleic acid. The initially observed test runs of such mixtures (see an example in Fig. 1A) resembled the profiles from literature [9], though the registered noise was a little excessive. We concluded that the noise was caused by a delayed elution of polar lipids, because phosphatidylcholine (PC, a major component of lecithin) was not eluted by the gradient from Ref. [9], as was established in a separate test (not shown). Repeated analytical injections of crude oil and biodiesel (containing 1–2% of lecithin according to the specifications) gradually contaminated the column making the noise unacceptable. This is visible from the profile in Fig. 1B, where the analytes (identical to those in Fig. 1A) were separated. The column was washed with the mixture of 66% 2-propanol, 33% methanol and 0.4% acetic acid, capable to elute PC. This treatment removed the