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Characterization of fatty acid methyl esters in biodiesel using high-performance liquid chromatography

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ABSTRACT

This study presents a new method for characterizing the composition of a biodiesel that is composed of fatty acid methyl esters (FAMEs) with high-performance liquid chromatography (HPLC). The chromatographic peaks of methyl palmitate (MP) and methyl oleate (MO), which are the main FAMEs, usually overlap chromatographically in the HPLC analysis. HPLC shows poor performance for the separation of MP and MO, even by applying a gradient elution program, and is difficult to use for the accurate quantification of MP and MO. A mathematical method has been developed to estimate the individual masses of MP and MO from their overlapping peaks in the HPLC chromatogram with refractive index (RI) and ultraviolet (UV) detectors in series. As a result, the individual masses of MP and MO in the artificial mixtures can be quantified accurately. Furthermore, FAME composition and the yield of the biodiesel that was obtained from the transesterification of soybean oil were quantified for verification in this study. Therefore, the present approach can provide useful information to precisely estimate the FAME composition in biodiesels with the employment of HPLC.

1. Introduction

Biodiesel composed of fatty acid methyl esters (FAMEs) is a product of the transesterification of triglycerides with methanol. Biodiesel has received a great deal of attention because of its potential advantages in energy conservation and air pollution reduction compared to fossil diesel fuel [1,2]. Recently, some studies attempted to investigate the application of heterogeneous catalysts for the synthesis of biodiesels [3,4]. The properties of a biodiesel are strongly dependent on the FAME composition of the vegetable oils. The iodine value and oxidation stability of the biodiesel included in the specifications of biodiesel standards, such as ASTM D6751 and EN 14214, are related to the number of the unsaturated carbon–carbon double bonds [5,6]. A reliable analytical technique is essential to quantify the individual FAMEs of the biodiesel product from the transesterification of vegetable oils.

Gas chromatography (GC) is the most widely used method for the quantification of FAMEs; however, gas chromatography is often considered to be labor intensive to perform [7,8]. Comprehensive two-dimensional gas chromatography (GC × GC) has recently been developed to efficiently quantify FAMEs [9–11]. Reversed-phase high-performance liquid chromatography (HPLC) equipped with various detectors has also been employed to determine the compounds that are generated during the production of biodiesel. One general advantage of HPLC compared to GC is to reduce analysis time because the time- and reagent-consuming derivatization is not necessary [12]. The detection techniques associated with the HPLC system include refractive index (RI), ultraviolet (UV), fluorescence, evaporative light scattering, and mass spectrometric detection. The application of RI or UV detection is relatively common and lower in cost than other detector systems. Some previous studies using the HPLC system with RI or UV detention for the quantification of FAMEs are summarized in Table 1.

One frequent problem that occurs during HPLC analysis of the quantification of FAMEs is poor chromatographic separation between methyl palmitate (MP) and methyl oleate (MO) [8,14,16–18,21,23]. MP and MO chromatographic peaks overlap in HPLC analysis and cause difficulties in quantification. According to previous literatures as well as our experiments using the gradient elution method to separate MP and MO in HPLC, the separation and quantification of MO and MP has been very challenging. Palmitic and oleic acids are the main fatty acid components of many oils and fats, including canola, corn, cottonseed, rapeseed, safflower, soybean, sunflower, tallow oils, and other oils. A reliable method is still required for the proper

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Table 1
Previous literatures using the HPLC system with UV or RI detection to quantify FAMEs.

Authors	Detector[a]	Mobile phase conditions
Noureddini et al. [13]	RI	Acetone:acetonitrile at 50:50
Saka and Kusdiana [14]		Methanol
Colucci et al. [15]		Tetrahydrofuran
Meher et al. [16]		Methanol
Holčapek et al. [17]	UV_{205}	Gradient elution: (1) 2-propanol and hexane. (2) Acetonitrile, 2-propanol, hexane, and water
Komers et al. [18]		Gradient elution: (1) acetonitrile:water (80:20). (2) Acetonitrile and hexane:2-propanol (40:50)
Jeong et al. [19]		Acetonitrile:acetone:water (48:48:4)
Shibasaki-Kitakawa et al. [20]		Gradient elution of acetonitrile, 2-propanol, and ultra-pure water
Juanéda and Sébédio [21]	UV_{210}	Hexane:acetonitrile (100:2)
Talukder et al. [22]		Gradient elution of methanol, isopropanol, and hexane
Türkan and Kalay [8]		Gradient elution of acetonitrile and acetone
Nightingale et al. [23]	UV_{220}	Hexane:isopropanol of 98:2

[a] The subscripts denote the wavelength (nm) of UV detection.

separation of MP and MO in the biodiesel samples when HPLC is applied.

The main objective of this study is to propose a mathematical method for HPLC analysis to precisely quantify the individual amounts of FAMEs, particularly MP and MO. The method was developed based on the distinct characteristics of RI and UV detection and the additivity of the integrated areas for the overlapping peaks. The applicability of the UV wavelength selected at 210 nm is confirmed based on the features of the UV spectra and the calibration curves of the FAMEs. As a result, HPLC analysis that adopted RI detection in cooperation with UV detection is suggested for obtaining accurate injected masses of both MP and MO. The FAME composition and the transesterification yield (Y_{FAME}) during the methanolysis of soybean oil in a batch reactor are determined according to the proposed method. The accuracy of the present method is further confirmed by comparison with the analytical results that were obtained from ^{1}H nuclear magnetic resonance (NMR) spectroscopy.

2. Experimental

2.1. Materials

Refined soybean oil was purchased from Uni-President (Tainan, Taiwan) with a molecular weight of 875 g/mol and a density of 0.917 g/ml. The methanol obtained from Mallinckrodt Chemicals (Phillipsburg, NJ, USA) was ACS certified with the chemical formula of CH_3OH, 32 g/mol molecular weight, 0.79 g/ml density, and 64.7 °C boiling point. Potassium hydroxide (KOH) with a purity of 85% was purchased from Riedel-de Haën (St. Gallen, Switzerland). The standards of all FAMEs, including MP, MO, methyl stearate (MS), methyl linoleate (ML), and methyl linolenate (MLn), were purchased from Fluka (St. Gallen, Switzerland). These FAME standards were prepared at the predetermined concentrations (MP: 2.60–30 mg/ml, MS: 5.06–6.06 mg/ml, MO: 2.58–30 mg/ml, ML: 60.7–61.3 mg/ml, and MLn: 7.14–7.34 mg/ml) by dilution with acetonitrile (99.8% in HPLC grade, Mallinckrodt Chemicals) for HPLC analysis.

2.2. Methanolysis of soybean oil

The methanolysis of the soybean oil was performed in an airtight reactor made of Pyrex glass and equipped with a water jacket to maintain a constant solution temperature at 60 °C. The design of the reactor was based on the criteria for the shape factors of a standard six-blade turbine. The molar ratio of methanol to oil and the homogeneous catalyst (KOH) dosage based on the oil weight were 6 and 1% (w/w), respectively, for conducting the methanolysis reaction based on the optimum transesterification conditions for vegetable oils, as suggested by Freedman *et al.* [24]. The stirring speed was 800 rpm to ensure the proper mixing of two immiscible phases. The samples were taken from the reactor at the desired time intervals during the transesterification process of the oil, and the total sampling volume was within 5% of the solution volume. The samples were immediately quenched by an ice bath to terminate the chemical reactions and then stored overnight to produce the phase separation [16,25]. The stabilized volumes of the upper ester and lower glycerol phases were recorded prior to the following analyses. The ester phase was washed with a saturated salt solution at three times its volume to remove possible residuals of methanol, KOH, and glycerol [26].

2.3. HPLC and ^{1}H NMR analyses

The concentrations of the FAMEs were analyzed in the HPLC system model L-2000 series (Hitachi, Tokyo, Japan) equipped with the silica-based column model STR ODS-II (5 μm, 250 mm × 4.6 mm I.D.) (Shinwa Chemical Industries, Ltd., Tokyo, Japan). The HPLC system has a pump (model L-2130) with an online vacuum degasser, an autosampler (model L-2200) with a variable injection capacity from 0.5 to 500 μL, and a RI detector (model L-2490) in series with a diode array detector (UV detector) (model L-2455). This UV detector can supply the full UV spectrum for every component in the HPLC separation. The acquisition and processing of data were performed using the EZChrom Elite software provided with the HPLC instrument. The column oven model Super co-150 (Enshine, Taipei, Taiwan) was used to keep the column temperature constant at 40 °C. The mobile phase in the HPLC analysis was pure acetonitrile at a flow rate of 1.0 ml/min. The injection volume of the samples in the HPLC analyses was set at 1–100 μL. The analyses of the FAME standards and biodiesel samples were performed in duplicate to confirm the observations.

The NMR spectroscopy of the soybean biodiesel was performed on a Varian Unity INOVA 500 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 500 MHz using $CDCl_3$ as the solvent. The Y_{FAME} value was determined from the signal area ratio of the methoxy protons of the methyl esters at 3.7 ppm to the methylene protons adjacent to the ester group at 2.3 ppm in the ^{1}H NMR spectrum [16,27–29].

3. Results and discussion

3.1. HPLC patterns of soybean oil biodiesel

The HPLC chromatograms using the RI detector for the soybean oil biodiesel is illustrated in Fig. 1(a), and the chromatogram