# Simultaneous Determination of Methanol and Ethanol Residues in Biodiesel by a Simple Headspace Single-Drop Microextraction and Gas Chromatography with Flame Ionization Detection

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**Abstract**

A simple sample preparation by headspace single-drop microextraction (HS-SDME) and gas chromatography-flame ionization detection (GC-FID) methods were developed for the simultaneous determination of methanol and ethanol residues in biodiesel. The analytes were manually extracted from a mixed standard or sample solutions into a suspended microdrop of ultrapure water containing isobutanol as an internal standard. After extraction, the extracted analytes were injected into GC-FID and separated under the high polarity capillary column (30 m x 0.25 mm i.d. x 0.25 μm) and oven temperature program. Optimization of parameters for the GC-FID and the extraction procedure was carried out, such as oven temperature programs, volumes of microdrop, incubation and extraction times, and temperatures of incubation and extraction. Under the optimum conditions, the order of elution was methanol, ethanol and isobutanol, respectively, with the analysis time of 16.7 min per chromatogram. Linear calibration graphs were in the range of $1.0x10^{-4}$ - $1.0x10^{-2}$ %v/v for both analytes with the limit of detections of $2.4x10^{-5}$ and $7.5x10^{-6}$ %v/v for methanol and ethanol, respectively. A sample throughput of about 2.5 $h^{-1}$ was achieved. The proposed methods were successfully applied to real biodiesel samples. Acceptable recoveries and relative standard deviations were achieved, which were in the ranges of 94±4 - 106±4 % and 1.7 - 8.3 %, respectively, by spiking all samples with mixed standard solution of methanol and ethanol. The developed method had advantages of simple extraction and operation, no organic solvent used for extraction, low consumption of extraction solvent and material, inexpensive of extraction techniques, and acceptable accuracy and precision.

**Keywords**: Methanol, Ethanol, Biodiesel, Single-drop microextraction, Gas chromatography

## Introduction

Biodiesel is a renewable alternative fuel created from vegetable oils (e.g. soybean, jatropha, and palm oils), animal fats, and recycled/waste cooking oils by chemical process. The chemical process called the transesterification, involves the reaction of triglyceride molecules found in natural oils with short chain alcohol (especially methanol or ethanol) under a base catalysis, and then refining the mixture to create two products of 'mono-alkyl ester of long chain fatty acids' or 'fatty acid alkyl esters' and glycerin. Fatty acid alkyl esters are the chemical name for 'biodiesel' which can be easily burned in a diesel engine, while a glycerin is a valuable byproduct usually used in soaps and other personal care products (Fernando, Karra, Hernandez, & Jha, 2007, pp. 844-851). Biodiesel fuel can be used in any diesel engine in pure form (100% biodiesel referred to as B100) or blended with petroleum diesel at any level (such as 5% biodiesel, 95% petrodiesel labeled as B5). Biodiesel has reduced exhaust emissions, lower toxicity and biodegradable as compared to petroleum diesel fuel. Blends of

biodiesel will significantly reduce carcinogenic emissions and gases that may contribute to global warming (Demirbas, 2009, pp. 14-34). In 2005, Thailand began a campaign to promote biodiesel product. Until in 2008, the government adopted a policy requiring replacing all regular petroleum diesel with B2 and also B5 biodiesels. Due to compulsory use of B100 biodiesel for B2 and B5 biodiesel productions, thus in 2009 to until now, the B100 production is expected to grow significantly in Thailand (Kumar, Salam, Shrestha, & Ackom, 2013, pp. 1577-1597). Thus, the quality control of biodiesel (B100) is necessary, including blended biodiesel productions. Biodiesel quality is governed by the ASTM standard D6751 quality parameters and the European biodiesel standard of EN 14214 (Paraschivescu, Alley, French, Hernandez, & Armbrust, 2008, pp. 5901-5905).

Methanol, residual alcohol resulting from the transesterification process, is one of the parameter to be control for biodiesel quality. Methanol residue is responsible for metal corrosion (particularly of aluminum), rapid deterioration of the fuel pump and the rubber components of the fuel system, decreasing of the fuel flashpoint of biodiesel, decreasing the cetane number (indicator of ignition quality) and lubricity of diesel fuel. Thus, the ASTM D6751 (test method of ASTM D93) certified B100 must have a flashpoint greater than 130 $^{0}$C by limiting the amount of methanol residue to a very low level (< 0.1 %w/w). Similarly, the EN 14214 (test method of EN 14110) limits a maximum permissible methanol residue in biodiesel at 0.2 %w/w (Fernando, et al., 2007, pp. 844-851; Munari, Cavagnino, & Cadoppi, 2007). Although, no maximum permissible content of ethanol residue are reported, it may affect properties of biodiesel as similar to methanol residue. Therefore, it is necessary to determine contents of methanol and ethanol residues in biodiesel, as well as blended biodiesels.

Several analytical methods have been used for determination of methanol and ethanol in biodiesel and other samples. These methods include gas chromatography (GC) (Fernando, et al., 2007, pp. 844-851; Mittelbach, Roth, & Bergmann, 1996, pp. 431-434; Ruppel, Coodman, & Huybrighs, 2008), high performance liquid chromatography (HPLC) (Chen, et al., 1998, pp. 93-99.), ultraviolet-visible spectroscopy (Dorado, Pinzi, de Haro, Font, & Garcia-Olmo, 2011, pp. 2321-2325), near infrared spectroscopy (Dorado, et al., 2011, pp. 2321-2325) and flow injection analysis (Araujo, Saraiva, Lima, & Korn, 2008, pp. 177-183). According to reviews papers, GC is the most popular method for methanol and ethanol analysis, which requires a proper sample preparation technique to separate analytes from other matrices of sample. A liquid-liquid extraction (LLE) is simple techniques used prior to GC but it has some drawbacks such as high consumption of organic solvent and easy contaminate with non-volatile matrices (Yu, et al., 2010, pp. 5158-5164). A headspace techniques coupled to GC, especially used in the EN 14110 for methanol residue determination, is a solvent-free but it needs an expensive headspace sampler instrument (Ruppel, et al., 2008). Other popular, simple, solvent-free and fast sample preparation technique used prior to GC is a headspace solid-phase microextraction (HS-SPME) but it also has some disadvantages such as expensive cost of fiber and its limited life time (Paraschivescu, et al., 2008, pp. 5901-5905). These drawbacks of those sample preparation techniques can be overcome by an alternative technique of a headspace single-drop microextraction (HS-SDME) which is one mode of a liquid-phase microextraction (LPME) techniques using

a microdrop of extraction solvent (Jeannot, Przyjazny, & Kokosa, 2010, pp. 2326-2336).

A single drop microextraction (SDME) was firstly developed by Jeannot and Cantwell in the 1996 and a HS-SDME is suitable for the analysis of volatile or semi-volatile compounds. The first report of HS-SDME came out in 2001 by Theis and coworkers (Jeannot, et al., 2010, pp. 2326-2336). The principle of HS-SDME is based on the evaporation of volatile compounds into headspace and further extraction into microdrop of extraction solvent at the tip of needle, placing above the sample solution. Thus, a HS-SDME is able to eliminate interferences of non-volatile compounds in the aqueous sample solution such as colloid, pigments, sugars and other high molecular weight compounds. After the extraction was finished, a microdrop was retracted back into the needle of GC microsyringe. Finally, a GC microsyringe was taken out of the sample vial and then injected into various instruments, such as GC, HPLC, inductively-coupled plasma mass spectrometry, capillary electrophoresis, mass spectrometry and electrothermal atomic absorption spectrometry, for further analysis. A HS-SDME has some advantages such as inexpensive, quick and easy operation and low consumption of organic solvent (Sarafraz-Yazdi, & Amiri, 2010, pp. 1-14; Xu, Basheer, & Lee, 2007, pp. 184-192). Due to review papers, a HS-SDME has been successfully applied to the analysis of some volatile organic compounds in various samples such as dialkyl phthalate esters in food simulants (Batlle, & Nerín, 2004, pp. 29-35), some alcohols in beer (Tankeviciute, Kazlauskas, & Vickackaite, 2001, pp. 1674-1677), residual solvents in solid drug product (Yu, et al., 2010, pp. 5158-5164), tributyltin compounds in water and solid samples (Colombini, et al., 2004, pp. 555-560), and hexanal and heptanal in human blood (Li, et al., 2005, pp. 318-326). However, there are no reports on the application of the HS-SDME to the determination of methanol and ethanol residues in biodiesel.

The aim of this research is to develop HS-SDME and GC-FID for extraction and determination of methanol and ethanol residues in biodiesel samples. This proposed method offered simple extraction and operation, less organic solvent, cost-effective, environmental friendly and prolong the life time of GC column. The effects of some parameters for HS-SDME and GC-FID were optimized and the validation of the proposed methods were also carried out.

## Materials and Methods

### Chemicals and Solutions

All chemicals were analytical reagent grades, and used without further purification. Ultrapure water with resistivity 18.2 MΩ.cm (Elgastat maxima, Elga, England) was used to prepare all aqueous solutions.

Stock standard solutions of methanol (10 %v/v) and ethanol (10 %v/v) were prepared by dissolving methanol (99.99 %, density of 0.792 g/mol, Fisher Scientific) and absolute ethanol (>99.5 %, density of 0.790 g/mol, Merck) in biodiesel blank. All stock standard solutions were stored in amber glass bottle and kept at 4 $^{0}$C. All mixed standard solutions were freshly prepared by diluting the stock standard solutions with biodiesel blank. The internal standard (IS) stock solution (10 %v/v) was prepared by dissolving isobutanol (or 2-methylpropan-1-ol, 99.9 %, density of 0.802 g/mol, BDH) in water.

A stock solution of biodiesel blank was prepared using a 100 mL of a commercial biodiesel blend of B3 (referred to 3% biodiesel and 97% petrodiesel), extracted with 50 mL of water for 3 times to remove methanol and ethanol and then dried with anhydrous

sodium sulfate. After that, this blank solution was extracted by HS-SDME and then analyzed by GC-FID to ensure that no signal of methanol and ethanol was observed.

All of biodiesel (B100) and blended biodiesel samples were collected or purchased at a local area of Phitsanulok, Thailand. These sample solutions were mixed thoroughly, accurately weighed (±0.0001 g) and diluted with biodiesel blank solution in a 10 mL volumetric flask before being extracted by HS-SDME and injected into the GC-FID.

**Instruments and Apparatus**

The GC system used in this work was a Varian 3800GC system (USA). It is consisted of a split/splitless injector, a controlled temperature oven and a FID detector. A helium carrier gas (99.999 %, UHP grade) with a flow rate of 1.5 mL/min, a split mode of injector (split ratio of 25:1), a HP-INNOWax fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness of polyethylene glycol, Agilent J&W, USA), and the FID detector gases of hydrogen (99.999 %, UHP grade; at flow rate of 30 mL/min) and air zero (99.99 %; at flow rate of 300 mL/min) were used throughout this work. The chromatographic software version 5 (Varian, USA) was used for recording the chromatograms and evaluating for peak areas and retention times.

A homemade HS-SDME system (Figure 1(a)) consisted of a 10 μL of GC microsyringe (Hamilton, USA), a homemade water bath, a thermometer (0-100 $^{0}$C), a hotplate (C-MAG HS7, IKA, Germany) and a 4 mL screw thread vial with PTFE septum. A thermometer and a GC microsyringe were held with clamp and stand and a 4 mL vial was fitted with a foam sheet. A homemade water bath was designed by using a 250 mL beaker (Duran, Germany), filled with 200 mL water and covered with foam sheet (6.5 cm diameter). A 4 mL screw thread vial was used to contain standard or sample solution and to make a headspace part.

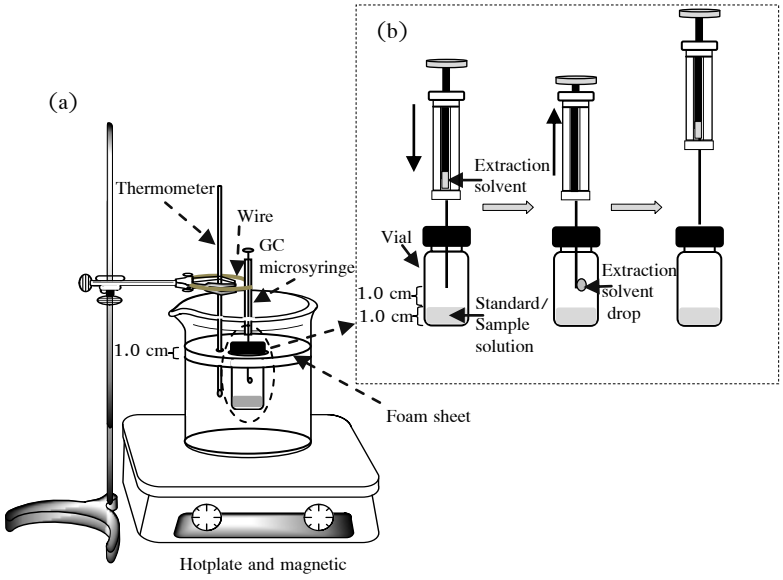


**Figure 1** Schematic diagram of a homemade HS-SDME system: (a) the instrument setup and (b) the operation procedure.

**The operation procedure of HS-SDME and GC-FID systems**

The operation procedures of HS-SDME (Figure 1(b)) and GC-FID systems are described as follows. Firstly, a 1.0 mL of a mixed standard/sample solution was transferred into a 4 mL screw thread vial with a PTFE septum, and a vial was placed into a homemade water bath, which was controlled for a constant temperature. Then, the HS-SDME procedure was started to operate. Analytes in a liquid phase was evaporated into a headspace of a vial under an appropriate incubation time and an incubation temperature. Next, a needle of GC microsyringe, containing a defined micro volume (μL) of water extraction solvent which was added with an internal standard was passed through a vial septum by fixing a needle tip at 1.0 cm (measured by a standard ruler) above a solution surface. By carefully pressing syringe plunger, a microdrop of extraction solvent was then exposed and started to extract the volatile analytes in the headspace into a microdrop liquid phase under an appropriate extraction time and an extraction temperature. After a specified time, a microdrop containing the extracted analytes was retracted back into the GC microsyringe, and then it was further injected (1 μL) into the GC-FID system under the constant conditions of an injector temperature at 220 $^{0}$C, a FID detector temperature at 280 $^{0}$C, and also a selected oven temperature program. Under the selected conditions described above, a chromatogram was obtained within 16.7 min, and the total analysis time for one operation of HS-SDME and GC-FID analyses was 24.2 min, resulting in a sample throughput of approximately 2.5 $h^{-1}$. A calibration graph was constructed by plotting peak area ratio obtained (peak area of analyte ÷ peak area of IS) versus concentration of standard solutions. Concentration of analytes in sample was then evaluated using the calibration graph.

## Results and Discussion

In order to achieve a good separation of methanol and ethanol, including IS, and a short analysis time per chromatogram, the temperature programs of the GC system were investigated. Factors which may affect the extraction procedure of HS-SDME system such as volume of microdrop, incubation and extraction times, and incubation and extraction temperatures were optimized in order to obtain good extraction efficiency, good sensitivity, accurate analytical results, and suitable analysis time. Finally, the selected conditions of the proposed systems were applied to real biodiesel samples.

**Optimization of temperature programs**

Temperature programs of the GC oven were firstly studied by adaptation the recommended conditions from a HP-INNOWax technical guide. Two temperature programs, A (40 $^{0}$C with a holding time of 4.5 min, 40-180 $^{0}$C with a rate of 10 $^{0}$C/min, 180-240 $^{0}$C with a rate of 30 $^{0}$C/min, and 240 $^{0}$C with a holding time of 5 min), and B (40 $^{0}$C with a holding time of 4.5 min, 40-80 $^{0}$C with a rate of 10 $^{0}$C/min, 80-240 $^{0}$C with a rate of 50 $^{0}$C/min, and 240 $^{0}$C with a holding time of 5 min) were investigated in this work, while the following conditions of HS-SDME were kept constant: 5 min and 40 $^{0}$C of incubation time and temperature, 1 μL drop of water extraction solvent containing IS ($3.0\times10^{-1}$ %v/v of isobutanol), 5 min and 40 $^{0}$C of extraction time and temperature. Both the temperature program A and B gave the same elution order of the compounds, i.e., methanol ($1.0\times10^{-2}$ %v/v), ethanol ($1.0\times10^{-2}$ %v/v) and isobutanol (IS), respectively. The program B offered shorter analysis time per chromatogram than

the program A (i.e., 16.7 versus 25.5 min) because of a higher temperature rate of program B actually at 50 $^{0}$C/min of 80-240 $^{0}$C than program A. Both temperature programs provided a good separation for all compounds (with peak resolution; $R \geq 1.5$) and no baseline drift was found. Thus, the program B was chosen for further study, providing retention times ($t_R$) of 3.27±0.01, 3.87±0.01, and 7.27±0.01 min for methanol, ethanol and IS, respectively. Furthermore, this temperature program gave the total GC-FID operation time of 24.2 min (16.7 min per chromatogram plus 7.5 min of cooling time).

**Effect of microdrop volumes of water extraction solvent**

Although, many organic solvents had been successfully applied to extract some alcohols by HS-SDME such as using ethylene glycol to extract methanol in beer (Tankeviciute, et al., 2001, pp. 1674-1677), and using butyl acetate to extract methanol and ethanol in solid drug product (Yu, et al., 2010, pp. 5158-5164). In this work, water was chosen as an extraction solvent to extract methanol and ethanol residues in biodiesel sample because of its general properties and its supported results of preliminary tests than other organic solvents. Generally, water is low cost, available in laboratory, environmental friendly, dissolvable methanol and ethanol, and good solvent for prolongation of life time of the HP-INNOWax GC column. Furthermore, for the preliminary tests, it was resulted that: 1) water microdrop could be hung on and stable at the tip of GC microsyringe in the range of 1 - 3 µL within 10 min at 40 $^{0}$C comparing with butyl acetate, and 2) no background signal of water was found at methanol, ethanol and IS peaks comparing with ethylene glycol and butyl acetate.

According to properties and the stability of a microdrop of water extraction solvent and to increase the sensitivity and extraction efficiency, the volumes of a microdrop of water extraction solvent in the range of 1 - 3 µL were investigated, while the following conditions of HS-SDME were kept constant: 5 min and 40 $^{0}$C of incubation time and temperature, 5 min and 40 $^{0}$C of extraction time and temperature. It is clear that the higher volume of the microdrop, the higher peak area ratio of methanol ($5.0x10^{-3}$ %v/v) and ethanol ($5.0x10^{-3}$ %v/v) as shown in Figure 2. Although, a 3 µL gave the best extraction sensitivity and efficiency, it tended to easy fall down from the needle tip of GC microsyringe and difficult manually injected into GC-FID. Thus, to compromise between good sensitivity, good extraction efficiency and simple operation, a 2 µL drop volume was selected.

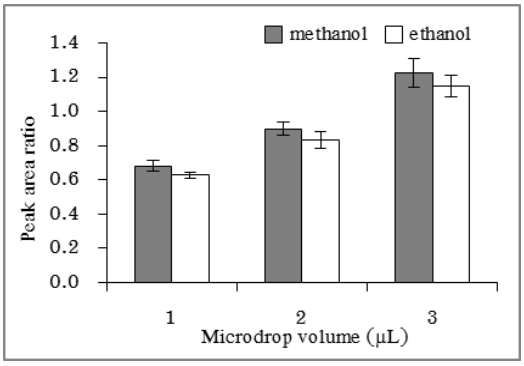


**Figure 2** Effect of microdrop volumes of water extraction solvent on peak area ratio of methanol and erthanol. Error bar was 1S.D. (n = 3).

**Effect of incubation and extraction times**

Because of the required short operation time of HS-SDME, increasing both of sensitivity and extraction efficiency of analytes in liquid phase into a headspace, and volatile analytes in headspace into a microdrop, effects of incubation and extraction times in the ranges of 1-15 and 1-10 min, respectively, were optimized, while the following conditions of HS-SDME were kept constant: 2 µL drop of water extraction solvent containing IS ($2.0x10^{-1}$ %v/v of isobutanol), 40 $^{0}$C of both incubation and extraction temperatures. It could be noticed that the higher peak area ratios of methanol ($5.0x10^{-3}$ %v/v) and ethanol ($5.0x10^{-3}$ %v/v) were observed by increasing the incubation time (Figure 3(a)) and extraction time (Figure 3(b)). However, longer operation time and inconvenient operation due to the drop easily fell down at longer extraction time were observed. To compromise between short operation time of HS-SDME, good sensitivity, and good extraction efficiency, the 10 and 5 min of incubation and extraction times, respectively, were selected for all subsequent studies with the total operation time of 15 min for HS-SDME.

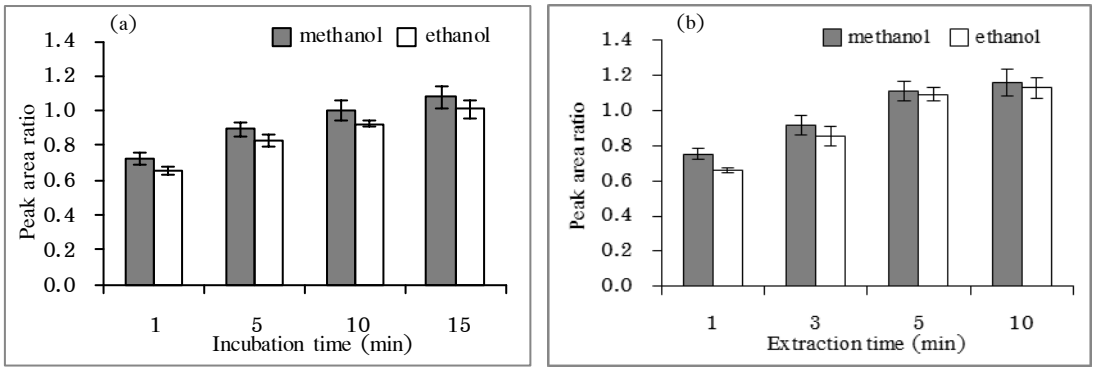


**Figure 3** (a) Effect of incubation time and (b) effect of extraction time on peak area ratio of methanol and ethanol. Error bar was 1S.D. (n = 3).

**Effect of temperature of incubation and extraction**

According to the boiling point of methanol and ethanol are 64.7 and 78.0 $^{0}$C, respectively, and stability of microdrop could be maintained only at extraction temperatures < 60 $^{0}$C. Therefore, the effect of temperature of incubation and extraction in the range of 30±0.5 - 50±0.5 $^{0}$C was studied, while the following conditions of HS-SDME were kept constant: 10 min of incubation time, 2 µL drop of water extraction solvent containing IS ($2.0x10^{-1}$ %v/v of isobutanol), 5 min of extraction time. It was found that the increase of incubation and extraction temperatures slightly increased peak area ratios (Figure 4) of methanol ($5.0x10^{-3}$ %v/v) and ethanol ($5.0x10^{-3}$ %v/v), especially at 40±0.5 - 50±0.5 $^{0}$C. Thus, a 40±0.5 $^{0}$C of both incubation and extraction temperatures was chosen which gave good sensitivity.

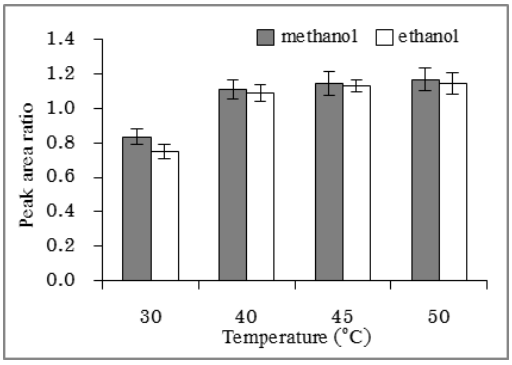


**Figure 4** Effect of incubation and extraction temperatures on peak area ratio of methanol and ethanol. Error bar was 1S.D. (n = 3).

**Calibration data of analytes by HS-SDME and GC-FID**

The calibration graphs were constructed from seven concentrations ($1.0x10^{-4}$, $2.5x10^{-4}$, $5.0x10^{-4}$, $7.5x10^{-4}$, $1.0x10^{-3}$, $5.0x10^{-3}$, and $1.0x10^{-2}$ %v/v) of each methanol and ethanol. The calibration results are summarized in Table 1. Precisions (percentage relative standard deviation; %RSD) were obtained from triplicate injections of standard solution containing methanol and ethanol in the concentration range of $1.0x\ 10^{-4}$ – $1.0x10^{-2}$ %v/v. The limit of detection (LOD) was calculated from three times standard deviation of the blank signals ($SD_b$); $3SD_b$/slope, while the limit of quantitation (LOQ) was calculated from ten times standard deviation of the blank signals; $10SD_b$/slope.

**Table 1** Calibration data of methanol and ethanol determination by HS-SDME and GC-FID systems.

| Analyte | Range (%v/v) | Linear equation (y = ax + b) | $R^2$ | %RSD (n=3) | LOD (%v/v) | LOQ (%v/v) |
|---|---|---|---|---|---|---|
| Methanol | $1.0x10^{-4}$ - $1.0x10^{-2}$ | y = 503.21x - 0.0414 | 0.9995 | 1.7-7.5 | $2.4x10^{-5}$ | $7.9x10^{-5}$ |
| Ethanol | $1.0x10^{-4}$ - $1.0x10^{-2}$ | y = 512.67x - 0.0265 | 0.9993 | 1.9-6.9 | $7.5x10^{-6}$ | $2.5x10^{-5}$ |

**Application to real biodiesel samples**

The optimum conditions of HS-SDME and GC-FID systems were applied to determine methanol and ethanol residues in real biodiesel samples (B100 and blended biodiesel) collected from community in Phitsanulok province. These biodiesel samples were produced from different sources including palm oil, soybean oil, jatropha oil, and waste cooking oil. Each sample solution was diluted different folds (2-500 folds) with biodiesel blank before analysis and analyzed in triplicate. Results obtained are summarized in Table 2. The typical chromatograms of sample are shown in Figure 5. Concentrations found of methanol and ethanol in samples were represented in percentage volume by volume (%v/v) by determining with a calibration graph of each analyte and then converted to percentage weight by weight (%w/w). From the results, methanol residues found in most of samples were less than a maximum permissible limit (0.2 %w/w). Ethanol residues were also found in some biodiesel samples which may be produced by the transesterification reaction using ethanol. For method validation in this work, a mixed standard solution of methanol and ethanol was spiked into all samples at two different concentration levels ($5x10^{-4}$ and $70x10^{-4}$ %v/v) and recoveries and precision (RSD) in the ranges of 94±4 to 106±4 % and 1.7 to 8.3 %, respectively, were obtained.

**Table 2** Contents of methanol and ethanol residues in real biodiesel samples, as determined by HS-SDME and GC-FID systems

| Sample No. | Biodiesel sources | Added ($x10^{-4}$ %v/v) | Concentration found, % relative standard deviation and % recovery (n = 3) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Methanol | | | Ethanol | | |
| | | | $x10^{-4}$ %v/v ($x10^{-4}$ %w/w)[c] | %RSD | %Rec | $x10^{-4}$ %v/v ($x10^{-4}$ %w/w) | %RSD | %Rec |
| 1 | B3[a] | 0 | 8.6±0.2 (10.2±0.2) | 2.3 | - | 11.5±0.3 (13.6±0.3) | 2.6 | - |
| 2 | | 5 | 13.4±0.3 | 2.2 | 96±6 | 16.6±0.3 | 1.8 | 102±6 |
| 3 | | 70 | 77.6±3.2 | 4.1 | 99±4 | 85.1±3.4 | 4.0 | 105±5 |
| 4 | B5[a] | 0 | 1.2±0.1 (1.4±0.1) | 8.3 | - | 2.0±0.1 (2.4±0.1) | 5.0 | - |
| 5 | | 5 | 6.4±0.2 | 3.1 | 104±4 | 7.3±0.2 | 2.7 | 106±4 |
| 6 | | 70 | 68.0±1.7 | 2.5 | 95±3 | 74.0±1.5 | 2.0 | 103±2 |
| 7 | Palm oil[b] | 0 | 7.4±0.4 (671±3) | 5.4 | - | ND[d] | - | - |
| 8 | | 5 | 12.1±0.2 | 1.7 | 94±4 | 5.1±0.2 | 4.0 | 102±4 |
| 9 | | 70 | 76.5±1.4 | 1.8 | 99±2 | 70.1±2.0 | 2.9 | 100±3 |
| 10 | Soybean oil[b] | 0 | ND | - | - | ND | - | - |
| 11 | | 5 | 4.8±0.2 | 4.2 | 96±4 | 5.1±0.3 | 5.9 | 102±6 |
| 12 | | 70 | 68.1±1.3 | 1.9 | 97±2 | 70.7±2.5 | 3.5 | 101±4 |
| 13 | Jatropha oil[b] | 0 | ND | - | - | 7.3±0.3 (3310±120) | 4.1 | - |
| 14 | | 5 | 4.8±0.2 | 4.2 | 96±4 | 12.5±0.3 | 2.4 | 104±6 |
| 15 | | 70 | 67.2±2.2 | 3.3 | 96±3 | 75.7±2.7 | 3.6 | 98±4 |
| 16 | Waste cooking oil[b] | 0 | ND | - | - | ND | - | - |
| 17 | | 5 | 4.8±0.3 | 6.2 | 96±6 | 5±0.2 | 4.0 | 100±4 |
| 18 | | 70 | 66.5±1.4 | 2.1 | 95±2 | 68.3±2.1 | 3.1 | 98±3 |

[a] B3 and B5 - referred to 3% biodiesel blended with 97% petroleum diesel and 5% biodiesel blended with 95% petroleum diesel, respectively.

[b] Referred to B100 - a 100% biodiesel with different sources.

[c] Average value ± standard deviation of triplicate results ($\bar{x}$ ± SD).

[d] ND - not detected (or < LOD).

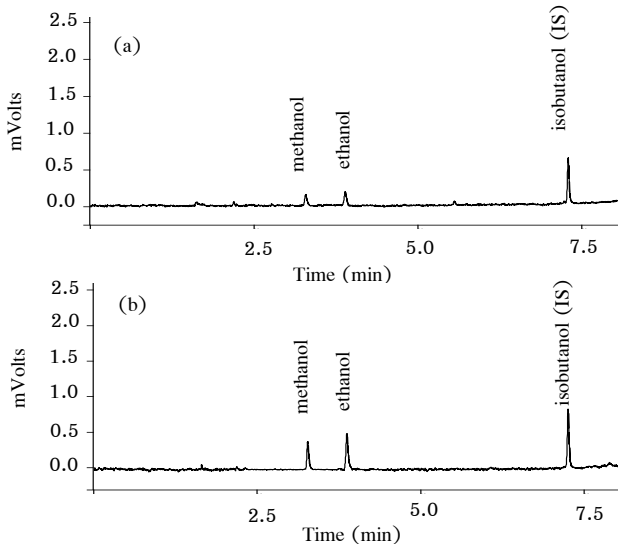


**Figure 5** GC chromatograms by HS-SDME and GC-FID systems for the determination of methanol and ethanol residues of: (a) biodiesel B3 sample (S) and (b) S + a mixed standard solution of $5.0x10^{-4}$ %v/v of each analyte. IS of $2.0x10^{-1}$ %v/v was added into the extraction solvent.

## Conclusion

A homemade HS-SDME and GC-FID were developed for the simultaneous determination of methanol and ethanol residues. A HS-SDME was a useful sample preparation and separation techniques for volatile analytes from different of sample matrices. This method offered simple extraction and operation, no organic solvent used (ultrapure water being used as extraction solvent), low consumption of extraction solvent and material, inexpensive of extraction techniques, environmental friendly, good separation techniques for prolongation of life time of GC column, and acceptable accuracy and precision. The proposed method was successfully applied to real biodiesel samples.

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