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Unsaturation levels in biodiesel via easy ambient sonic-spray ionization mass spectrometry

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HIGHLIGHTS

- A new ambient mass spectrometry methodology able to evaluate FAME levels in biodiesel.
- A fast, direct and accurate method to quantitate unsaturated FAME in biodiesel.
- Estimating the iodine value in biodiesel by EASI(+)-MS.

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ABSTRACT

Using a non-natural fatty acid methyl ester (FAME) as an internal standard we evaluated the ability of easy ambient sonic-spray ionization mass spectrometry (EASI-MS) to quantitate the unsaturated FAME levels in B100 biodiesel from soybean, canola and *Jatropha curcas*. The ability of EASI(+)-MS to discriminate between saturated versus unsaturated FAME was also evaluated. Calibration curves for the major FAME, those from linolenic, linoleic and oleic acids, showed good linearity with coefficient correlation >0.99 with the EASI(+)-MS method. The spike recoveries ranged from 76% to 127% with good repeatability and reproducibility as indicated by the relative standard deviation (RSD), which ranged from 5.1% to 17.7%. The accuracy of the method was established using a standard reference material (SRM). Real biodiesel samples were also quantitated via EASI(+)-MS, and the unsaturated FAME content was found to correlate adequately with the data obtained from the iodine number. Therefore, EASI(+)-MS was shown to provide fast, direct, linear and accurate quantitation of the unsaturated FAME in biodiesel samples.

1. Introduction

Biodiesel is an advantageous fuel because it is renewable, biodegradable and generally emits fewer pollutants. However, this fuel contains a mixture of saturated and unsaturated fatty acid methyl esters (FAME) with dissimilar physicochemical properties. Saturated FAME display higher freezing points whereas high unsaturation levels (as typically measured using the iodine number) lead to poor oxidative stability. Therefore, the properties of biodiesel are directly affected by the FAME properties and are described by the biodiesel standards as ASTM D6751 [1], EN 14214 [2] and, specifically in Brazil, by ANP [3]. Among the unsaturated fatty acids (FA) found in vegetable oils, oleic acid is most likely the least problematic, whereas highly unsaturated FA containing three or more double bonds are less desirable [4,5]. The EN 14214 has therefore established limits on highly unsaturated FAME as measured using the iodine value (IV). The maximum limit for linolenic acid methyl ester is 12%, whereas the maximum IV is typically set at (120 g kg^{-1}). Currently, IV limits for biodiesel remain among the most discussed topics with respect to the worldwide variations in biodiesel specifications.

Conventionally, the IV is determined in biodiesel by the EN14111 [6]. However, this procedure is time-consuming, requires the use of freshly prepared reagents and most importantly is non-specific, providing only a rough estimate of the total degree of unsaturation. More selective methods based on nuclear magnetic resonance (NMR) spectroscopy have been reported [7]. Gas chromatography (GC) with flame ionization detection [8] and GC coupled with mass spectrometry (GC/MS) have also been widely used to characterize and estimate the unsaturated FAME levels in biodiesel (B100). Direct MS analysis via electrospray ionization

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(ESI-MS) has also been applied to identify the FAME in biodiesel [9,10]. We have recently demonstrated a direct desorption/ionization ambient MS technique [11], easy sonic-spray ionization mass spectrometry (EASI-MS) [12] as a direct, simple, and rapid method for characterizing the FAME in biodiesel at the molecular level and determining their triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG) and free glycerin contaminants [13–17]. EASI-MS is a spray-based voltage and heating-free technique that allows the MS analysis of samples in the open atmosphere with little or no sample preparation. We tested therefore the ability of EASI-MS to determine the composition and to evaluate the unsaturation level of biodiesel samples, focusing on linolenic acid methyl ester. Additionally, the IV for the biodiesel was determined experimentally using the conventional method [6] and estimated based on the EASI-MS results. The method was applied to biodiesel samples obtained from several vegetable oil seeds to evaluate its applicability.

2. Experimental

2.1. Chemical reagents and samples

High-performance liquid chromatography (HPLC)-grade methanol was purchase from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Tetrahydrofuran (anhydrous, 99.9%), linolenic acid methyl ester (C18:3, acyl carbon number/double bonds number), linoleic acid methyl ester (C18:2), oleic acid methyl ester (C18:1), stearic acid methyl ester (C18:0) and palmitic acid methyl ester (C16:0) were obtained from Sigma–Aldrich (St. Louis, US). Liquid paraffin (mineral oil) was from União Química e Farmacêutica Nacional S/A (São Paulo, Brazil). Stock solutions of linolenic acid methyl ester (5 g kg^{-1}), linoleic acid methyl ester (10 g kg^{-1}) and oleic acid methyl ester (50 g kg^{-1}) were prepared using THF. For calibration curves, solutions were made by diluting the stock solutions in a mineral oil:THF (80:20) mixture in the final concentrations of: 0.025, 0.05, 0.10, 0.25, 0.35 and 0.50 mg g^{-1} for linolenic acid methyl ester; 0.05, 0.10, 0.25, 0.50, 0.75 and 1.00 mg g^{-1} for linoleic acid methyl ester and 0.50, 1.00, 2.50, 3.50, 5.00 and 7.50 mg g^{-1} for oleic acid methyl ester. Additionally, 10-heptadecenoic acid methyl ester (C17:1, from Sigma–Aldrich) was used as an internal standard (IS) in all the solutions of the calibration curves at a final concentration of 1.65 mg g^{-1}. For an efficiency ionization study, an equimolar mixture of the palmitic, linolenic, linoleic, oleic and stearic acid methyl ester in a final concentration of 0.2 mM of each standard, was prepared in chloroform. 10-heptadecenoic acid methyl ester (0.8 mM) was used as internal standard. All solutions were stored at 4 °C prior to analysis. The standard reference material 2772 B100 biodiesel (soybased) from NIST-USA was properly diluted in mineral oil:THF (80:20) for EASI-MS analysis according to Table 1.

In addition, three biodiesel samples (soybean, *Jatropha curcas* and canola) were also diluted (Table 1) and used to determinate the unsaturated fatty acid methyl esters content of "real" commercial samples using EASI-MS. The biodiesel samples were obtained from vegetable oil through a transesterification reaction using methanol as the alcohol source.

Table 1
Biodiesel sample dilutions for quantitation of specific FAME.

Biodiesel	C18:1[a]	C18:2[a]	C18:3[a]
Soybean	1:70	1:1000	1:300
Jatropha	1:200	1:1000	1:300
Canola	1:200	1:1000	1:300
SRM 2772	1:100	1:1000	1:300

[a] Acyl carbon number/double bonds number.

2.2. General experimental procedures

Data were acquired in the positive ion mode [EASI(+)-MS] using a single-quadrupole mass spectrometer (Shimadzu LC2010) equipped with a homemade EASI source. A tiny droplet of the sample (2 μL) was placed directly onto the paper surface with a methanol flow rate of 20 μL min^{-1}, N_2 as the nebulizing gas at 3 L min^{-1}, and a paper-entrance angle of ~30°. The mass spectra were acquired over 30 s and scanned over the 300–330 m/z range.

2.3. GC-FID analysis

The GC analysis was performed according to the EN 14103:2011 method [8].

2.4. Iodine value

The IV is the halogen quantity expressed as grams of iodine that reacts with 100 g of material under specified conditions [6]. The biodiesel sample was reacted with excess iodine monochloride solution (Wijs reagent) under controlled conditions. The halogens add quantitatively to the double bonds in the unsaturated fatty acids, in the case of soybean biodiesel, principally oleic, linoleic and linolenic acids. The unreacted halogens were determined via titration with thiosulfate.

2.5. Method performance

During the method development, the following analytical figures of merits were evaluated: linearity, quantification limits for each compound, repeatability, reproducibility, recovery and accuracy. To demonstrate the linearity of the method, calibration curves (6 points) were constructed for the three most prominent esters in biodiesel samples i.e. linolenic acid methyl ester (C18:3), linoleic acid methyl ester (C18:2) and oleic acid methyl ester (C18:1), by plotting the analyte concentration versus the ratio of the absolute intensity of the ester to that of the internal standard (I_{ester}/I_{IS}). The coefficients of determination were calculated to demonstrate the linearity. The repeatability and reproducibility experiments were performed through replicate analyses of the standards in the intra-assay ($N = 12$) and inter-assay ($n = 3$, on three different days) experiments. The recovery was examined at low, medium and high spiking levels of the appropriate stock solutions spiked in a mineral oil:THF (80:20) solution. The accuracy was obtained by analyzing the standard reference material from the NIST. The limit of quantitation (LOQ) of the method for each analyte was obtained based on the lowest concentration at an S/N ratio $\geqslant 10$.

2.6. Statistical calculations

Statistical calculations were performed using the GraphPad InStat 3.10 software (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results and discussion

Fig. 1a presents a typical EASI(+) mass spectrum for the standard reference material B100 biodiesel (soybean-based) from the NIST-USA. The FAME from linoleic acid is the most abundant ion and is detected as its sodiated ion $[FAME + Na]^+$ of m/z 317. The other minor ions derive from the esters of oleic (m/z 319) and linolenic (m/z 315) acids. This characteristic FAME profile not only results from the relative concentration of each FAME in the sample but is also affected by the specific ionization efficiency of each FAME, which is expected to be influenced by its unsaturation level.