Biodiesel Typification and Quality Control by Direct Infusion Electrospray Ionization Mass Spectrometry Fingerprinting

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Received May 28, 2007. Revised Manuscript Received August 9, 2007

Direct infusion electrospray ionization mass spectrometry (ESI-MS) of aqueous/methanolic extracts of biodiesel samples is shown to permit simple and fast fingerprinting typification, identification of the alcohol used in the trans-esterification process, monitoring of degradation and adulteration, and screening of residual glycerin and mono-, di-, and tri-glycerides. As proof-of-principle cases, ESI-MS fingerprints in both the positive and the negative ion modes were acquired for biodiesel samples derived from soybean oil, sunflower oil, canola oil, olive oil, castor oil, jatropha curcas oil, palm oil, lard, and tallow. In the negative ion mode, characteristic sets of ions corresponding to de-protonated fatty acids were detected for each type of biodiesel, thus allowing their reliable typification via these taxonomic markers. Biodiesel degradation was also monitored by ESI(−)-MS with the detection of degradation markers, mainly oxidized fatty acids. When using ESI(+)-MS, the main diagnostic ions detected were the protonated fatty esters, which reveal therefore both the oil source and the alcohol (methanol or ethanol, or other heavier alcohols) used for trans-esterification. Residual glycerin and mono-, di-, and tri-glycerides are also detected, which makes ESI-MS also applicable for laboratory and on-site screening of biodiesel quality.

Introduction

Biodiesel, an alternative and promising diesel fuel,[1,2] is made from many renewable sources such as vegetable oils and animal fats. Owing to a conjunction of adversities associated with the continuous use of crude oil, there has been recently a renewed and intense focus on biodiesel as a commercially viable, less environmentally harmful and renewable energy source.

Biodiesel[3] is produced via trans-esterification with light alcohols (mainly methanol) of natural tri-glycerides present in vegetable oils or animal fats in the presence of a catalyst.[4–6] There are currently many oil sources being used or evaluated for biodiesel production. In Brazil, soybean oil is currently the main source used for biodiesel production, but other sources, such as sunflower, palm, castor, and jatropha curcas oils, as well as several animal fats are starting to be increasingly used or seriously evaluated. Although methanol is usually regarded as the best light alcohol for biodiesel production, ethanol from sugar cane may also become important particularly in Brazil owing to its nontoxicity and ready availability.[5]

Owing to a number of convenient analytical features, direct infusion electrospray ionization mass spectrometry (ESI-MS)[7,8] has been increasingly used for simple and fast fingerprinting characterization and quality control of samples of different origins and classes, such as those of beer,[9] wine,[10,11] propolis,[12,13]

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10.1021/ef7003078 CCC: $37.00
Published on Web 11/03/2007

whisky,[14] crude petroleum oils,[15] vegetable oils,[16,17] plant extracts,[18,19] rum and cachaça (a typical Brazilian alcoholic spirit),[20,21] perfumes,[22] and transgenic and natural soybeans.[23]

Herein we show that direct infusion ESI-MS of aqueous/methanolic extracts provides simple and very fast fingerprinting typification and quality control of biodiesels from different oil sources.

Experimental Section

Chemical Reagents and Samples. HPLC-grade methanol was purchased from Merck (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a Milli-Q (Millipore) purification unit. Biodiesel samples were prepared according to an optimized procedure[24] using different oil sources (soybean, sunflower, canola, olive, castor, jatropha curcas, palm, and lard).

General Experiment Procedures. ESI-MS fingerprints were obtained either in the negative or in the positive ion modes in a Q-TOF mass spectrometer (Micromass, Manchester, U.K.). Typical ESI-MS conditions were as follow: source temperature 100 °C, desolvation temperature 100 °C, capillary voltage ±3.0 kV, and cone voltage ±40 V.

Biodiesel samples (100 μL) were transferred to a flask containing 200 μL of a 1:1 methanol/water solution. After centrifugation (5 min), 100 μL of the hydroalcoholic phase was taken and diluted to 1 mL of total volume with a 1:1 methanol/water solution containing 0.1% of either ammonium hydroxide or formic acid. This resulting solution was then directly infused using a syringe pump (Harvard Apparatus) at a flow rate of 10 μL·min^{-1}.

All the ESI-MS data were analyzed by using the MassLynx 3.5 software (Waters, Manchester, U.K.). Mass spectra were accumulated over 60 s to generate a final data ranging from *m/z* 50 to *m/z* 1000.

Results and Discussion

Typification. Figure 1 shows typical ESI(−)-MS fingerprints (in the negative ion mode) of the aqueous/methanolic extracts of the nine representative biodiesel samples. Note the characteristic sets of diagnostic anions with distinctive relative intensities. Table 1 shows the main fatty acids, as well as the *m/z* values of their de-protonated molecules $[M - H]^-$ detected by ESI(−)-MS. The identities of these species were further confirmed by verifying the experimental and calculated *m/z* for the $[M - H]^-$ ions (deviations were less than 10 ppm). Note that similar results for vegetable oils were obtained previously by Wu and co-workers[16] and by us.[17]

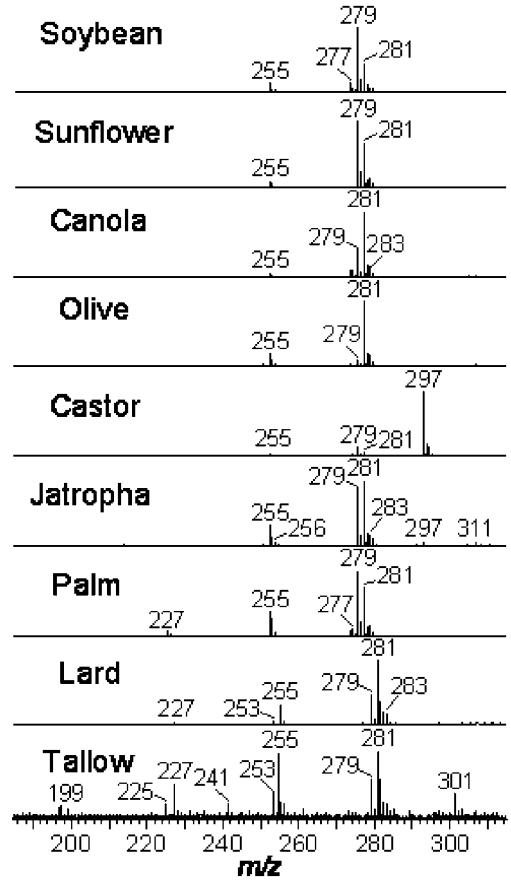

Figure 1. Typical ESI(−)-MS fingerprints for the aqueous/methanolic extracts of biodiesels.

Both the soybean and sunflower biodiesels display similar ESI(−)-MS profiles for the ions of *m/z* 255 (P), 279 (L), and 281 (O), but that of soybean biodiesel is characterized by a considerably more abundant ion of *m/z* 277 (Ln). Canola biodiesel is characterized mainly by the greater abundance of the ion of *m/z* 281 (O) as compared to that of *m/z* 279 (L), whereas this ratio is greatly in favor of the ion of *m/z* 281 (O) for olive biodiesel. These characteristic relative abundances of linoleic and oleic acids (Table 1) have also been determined for canola and olive oils by chromatographic analysis.[25,26] Castor biodiesel provides a unique ESI(−)-MS that displays a very abundant and characteristic ion of *m/z* 297 (R), that is, the deprotonated form of the hydroxylated ricinoleic acid which is so special for castor oil. The last four biodiesel samples (jatropha, palm, lard, and tallow) display ESI(−)-MS with abundant ions of *m/z* 279 (L) and *m/z* 281 (O), and most particularly the ion of *m/z* 255 (P), but the *m/z* 277/279/281/283 ratios allow their distinction. Lard, palm, and tallow biodiesel are also characterized by detectable ions of *m/z* 227 (M). The two biodiesels from aminal fats (lard and tallow) are very clearly distinghished from each other. The ESI(−)-MS for tallow biodiesel is particularly characteristic, displaying greater diversity of *m/z* diagnostic anions corresponding to low mass

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Table 1. Main Fatty Acids and the Relative Abundances of Their Deprotonated Molecules Detected in the ESI-MS of Biodiesel Samples

elemental composition	FA[a]	CN/DB[b] abbreviation	$[M - H]^-$ *m/z*	soybean	sunflower	cannola	olive	castor	jatropha	palm	lard	tallow
$C_{12}H_{24}O_2$	L	C12:0	199	0	0	0	0	0	0	0	0	5
$C_{14}H_{26}O_2$	Mo	C14:1	225	0	0	0	0	0	0	0	0	15
$C_{14}H_{28}O_2$	M	C14:0	227	0	0	0	0	0	0	5	5	50
$C_{15}H_{30}O_2$	Pe	C15:0	241	0	0	0	0	0	0	0	0	15
$C_{16}H_{30}O_2$	Po	C16:1	253	0	0	0	3	0	3	0	10	30
$C_{16}H_{32}O_2$	P	C16:0	255	18	10	7	12	3	30	45	35	100
$C_{18}H_{30}O_2$	Ln	C18:3	277	16	0.5	13	2	3	0	10	3	1
$C_{18}H_{32}O_2$	L	C18:2	279	100	100	48	10	15	90	100	50	55
$C_{18}H_{34}O_2$	O	C18:1	281	47	68	100	100	5	100	69	100	100
$C_{18}H_{36}O_2$	S	C18:0	283	5	5	5	5	0	10	7	18	15
$C_{18}H_{34}O_3$	R	C18:1–OH	297	0	0	0	0	100	0	0	0	0
$C_{20}H_{30}O_2$	E	C20:5	301	0	0	0	0	0	0	0	0	35
$C_{20}H_{40}O_2$	A	C20:0	311	0.5	0.5	2	1	0	2	3	3	3

[a] Fatty acid abbreviations: L, lauric (12:0); Mo, miristicoleic (14:1); M, myristic (14:0); Pe, pentadecilic (15:0); Po, palmitoleic (16:1); P, palmitic (16:0); Ln, linolenic (18:3); L, linoleic (18:2); O, oleic (18:1); S, stearic (18:0); R, ricinoleic (18:1-OH); E, eicosapentaenoic (20:5); A, arachidic (20:0). [b] CN = carbon number. DB = number of double bonds.

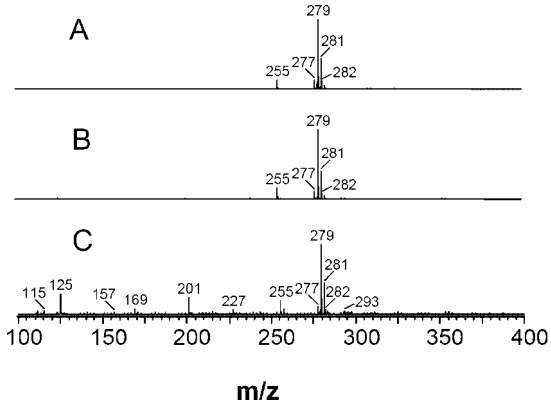

Figure 2. Typical ESI(−)-MS fingerprints obtained for the aqueous/methanolic extracts of soybean biodiesel after heating in the presence of oxygen for (A) 0 h, (B) 4 h, and (C) 8 h.

fatty acids (this is a characteristic feature of animal fats[27]), that is, a series of saturated [*m/z* 199 (L), 227 (M), 241(Pe), 255 (P)] and unsaturated [*m/z* 225 (Mo), 253 (Po), 279 (L), 281 (O), and 301 (E)] fatty acids. As evaluated for triplicates of soybean, sunflower, and castor oil, these results are reproducible with less than 5% discrepancy.

Degradation. ESI(−)-MS fingerprints also permit us to monitor biodiesel degradation. As Figure 2 illustrates, the ESI(−)-MS of the soybean biodiesel extract, after heating in the presence of oxygen for 8 h at 120 °C, shows a set of ions of *m/z* 115, 125, 157, 169, 201, and 227 corresponding to degradation products.[28] These ions constitute therefore degradation markers for biodiesel. A contrasting behavior was observed for the castor biodiesel (spectra not shown). After 8 h of heating at 120 °C in the presence of oxygen, no ion related to oxidation could be detected, and the ESI(−)-MS remained practically unchanged. An extensive investigation of degradation of different types of biodiesels under different conditions as monitored by ESI-MS is underway in our laboratory.

Quality Control. Informative fingerprinting spectra in regard to biodiesel composition are also obtained by ESI(+)-MS. Figure 3 illustrates such spectra for olive biodiesel formed by either methanol or ethanol trans-esterification. For ethanol (Figure 3A), the main ester component, that is, oleic acid ethyl ester, is detected as its protonated molecule $[M + H]^+$ of *m/z* 311 and its fragment formed by ethanol loss of *m/z* 265: $[M - C_2H_5OH + H]^+$. Palmitic acid ethyl ester is also detected as $[M + H]^+$ of *m/z* 285. A major *mono*-glyceride, present due to incomplete transesterification, is also detected as five main ions:

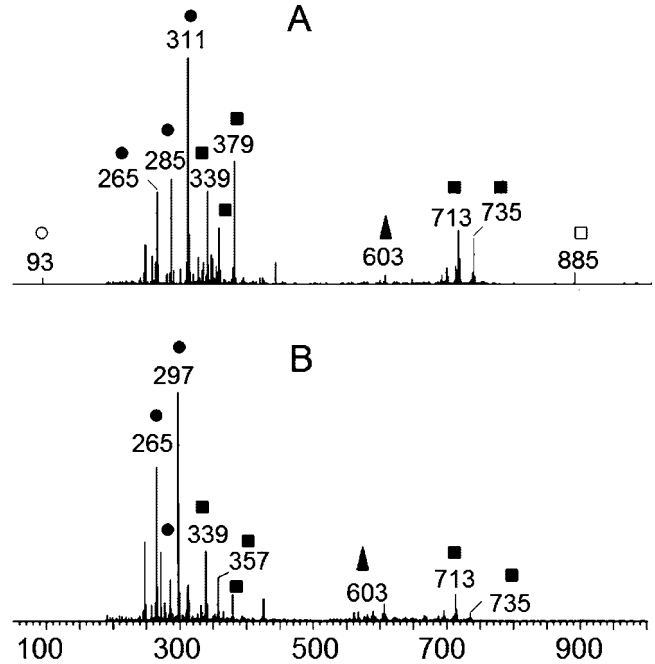

Figure 3. Typical ESI(+)-MS fingerprints in the positive ion mode obtained for the aqueous/methanolic extracts of olive biodiesel prepared using either (A) ethanol or (B) methanol. Ions arising from fatty acid methyl or ethyl esters (●, ◆), the mono-glyceride (■), and the di-glyceride (▼) are marked. The ethanol biodiesel sample was spiked with glycerin and tri-olein (100 ppm), which are detected by the respective protonated molecules of *m/z* 93 (○) and 885 (□).

the protonated molecule $[M + H]^+$ of *m/z* 357, the sodiated molecule $[M + Na]^+$ of *m/z* 379 (due to residual Na^+ from NaOH), and the respective dimers $[M_2 + H]^+$ of *m/z* 713 and $[M_2 + Na]^+$ of *m/z* 735, as well the fragment ion $[M - H_2O + H]^+$ of *m/z* 339. A minor ion of *m/z* 603 is also detected, which corresponds to the fragment formed by water loss from the protonated di-glyceride. Residual glycerin is also detected as $[M + H]^+$ of *m/z* 93. Tri-glycerides were first not detected due to low concentrations in this relatively high quality biodiesel sample, but spiking (Figure 3A) and the detection of the $[M + H]^+$ of *m/z* 885 shows that they can also be detected by ESI(+)-MS fingerprinting.

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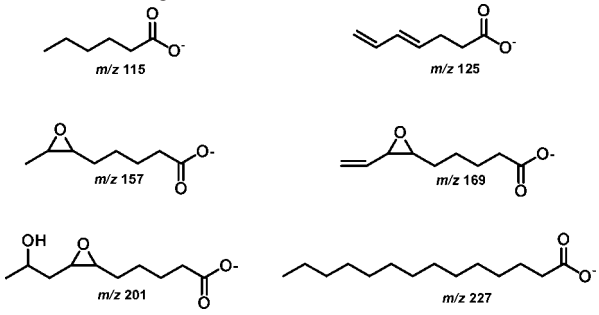
For the methanol biodiesel (Figure 3B), characteristic *m*/*z* shifts occur clearly revealing the nature of the alcohol used for trans-esterification. The main methyl esters are now detected as the ions of *m*/*z* 297 (oleic acid) and 271 (palmitic acid), the two of them shifted 14 *m*/*z* units lower due to ethyl by methyl replacement. Note that the same fragment ion of *m*/*z* 265 is detected due now to methanol loss from the protonated oleic acid methyl ester of *m*/*z* 297. The mono-olein and di-olein set of ions of *m*/*z* 339, 357, 379, 603, 713, and 735 show, as expected, no mass shift.

Conclusion

Direct infusion ESI-MS of aqueous/methanolic extracts uses minimal sample preparation and permits simple and very fast fingerprinting typification and quality control of biodiesel samples. It detects diagnostic, taxonomic marker ions corresponding to major natural fatty acids and esters. A library of ESI-MS fingerprints of biodiesel samples from many different types could therefore be established. This library could then be used to typify biodiesel samples for a variety of purposes including the preparation of reference materials. In addition, ESI-MS fingerprinting also allows fast screening of samples in regard to important parameters of biodiesel quality: (a) it identifies the alcohol used in the trans-esterification process, (b) it detects degradation via characteristic degradation markers, and (c) it permits fast detection and estimates of the amount of residual glycerin and mono-, di-, and tri-glycerides. In the laboratory, ESI-MS fingerprintings can be easily automated particularly when using robotized sample injection systems based on nano-ESI chips, which minimize cross-contamination, carryovers, and ion suppression, thus allowing high throughput analysis.[29] On-site monitoring of biodiesel quality via ESI-MS fingerprinting could also be implemented using miniaturized hand-portable mass spectrometers.[30]

(28) Although other degradation products are likely formed, ESI(−)-MS is expected to selectively detect mainly deprotonated molecules of lighter carboxylic acids formed due to biodiesel oxidation. This oxidation may occur via epoxide formation, and we propose below structures for the detected products which are corroborated in terms of composition by the accurate *m*/*z* measurements. ESI-MS/MS experiments were performed, but these anions showed great resistance towards collision induced dissociation.



Acknowledgment. The authors would like to acknowledge the Brazilian agencies FAPESP, CNPq, and FINEP for financial support.

EF7003078

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