Analytical Methods

TECHNICAL NOTE

Using the L/O ratio to determine blend composition in biodiesel by EASI-MS corroborated by GC-FID and GC-MS†

Vanderlea de Souza,[a] Michele Schantz,[b] Vinicius L. Mateus,[a] Rosana M. Alberici,*[ac] Eliane C. P. do Rego,[a] Werickson F. Rocha,[a] Janaina M. Rodrigues[a] and Marcos N. Eberlin[c]

Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is applied to the analysis of biodiesel blends prepared from soybean and animal fat biodiesel. A correlation was established between the ratio of abundance of linoleic acid (L) and oleic acid (O) methyl ester ions with the biodiesel blend composition. To compare these results from the EASI-MS technique, the L/O ratio of these blends was also determined using both gas chromatography with flame ionization detection (GC-FID) and gas chromatography with mass spectrometry (GC-MS). Both these classical techniques confirmed that the ratio between the mass fraction of FAME from L and O as measured by EASI-MS is indeed correlated with the blend composition of soybean–animal fat biodiesel.

1. Introduction

Biodiesel is an attractive renewable fuel alternative which provides less harmful emissions when compared with the conventional fossil-based diesel fuel. Biodiesel is however not a well defined matrix in terms of the chemical composition, being composed of a variable mixture of fatty acid methyl esters (FAME), produced mainly by the transesterification of vegetable oils, animal fats, or used cooking oils[1] with short chain alcohols such as methanol or ethanol.[2] Owing to the many possible feedstocks, the FAME composition of biodiesels may therefore vary substantially in terms of the carbon length, substitution, and degree of unsaturation. With the increased interest and use of biodiesel as an alternative to petroleum-based fuels, the successful commercialization and market acceptance of biodiesel depend on knowing the fuel properties and quality.[3–8]

Waste greases, such as used cooking oil and animal fats, are attractive biodiesel feedstocks mainly due to their low cost and environmental benefits.[9,10] Since the biodiesel obtained exclusively from animal fat may only marginally meet the EN 14214 or ASTM D6751 standards specifications, waste animal fat is normally mixed with soybean oil or other vegetable oils to produce a biodiesel of acceptable quality.[11–17] There are other studies using biodiesel blends with the same goals.[5,18–20] In Brazil, a most common biodiesel blend currently used is composed of *ca.* 70% soybean and 30% animal fat.[21]

To characterize and quantitate the FAME composition of biodiesel,[22] gas chromatography with flame ionization detection (GC-FID) and high-performance liquid chromatography (HPLC) are the two most commonly used techniques.[3,23–25] Direct infusion electrospray ionization mass spectrometry (ESI-MS) in both the positive and negative ion modes has also allowed fast fingerprinting and quality control of biodiesels[26,27] providing biodiesel fingerprints with characteristic FAME and free fatty acid (FFA) profiles and detection of major residual contaminants as well as oxidation.[28] These FFA and FAME profiles provide natural chemotaxonomic markers for the parent animal fat or vegetable oil used for the biodiesel production. Recently, a series of direct desorption/ionization techniques performed in open atmosphere for mass spectrometry analysis[29–33] have been developed. These techniques allow fast and simple biodiesel typification and quality control requiring no or little sample preparation, pre-separation, or derivatization procedures. Among the set of such techniques, easy ambient sonic-spray ionization mass spectrometry (EASI-MS),[34] which is based on the gentle sonic-spray ionization (SSI),[35,36] has been shown to be advantageous for biodiesel analysis since it is free from thermal, electrical, and/or oxidative interferences providing a bipolar stream of charged droplets while offering a much simplified workflow for the undisturbed sample and delivering unprecedented ease of use. We have extensively applied EASI(±)-MS for both oil and biodiesel characterization.[37–43]

[a]National Institute of Metrology, Quality and Technology, 25250-020 Duque de Caxias, RJ, Brazil

[b]National Institute of Standards and Technology, Analytical Chemistry Division, 100 Bureau Drive, Gaithersburg, MD20899, USA

[c]ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, 13083-970 Campinas, SP, Brazil. E-mail: rmalberici@hotmail.com; Fax: +55-19-35213049; Tel: +55-19-35213049

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ay02180d

Herein we tested therefore the application of EASI(+)-MS as a screening tool to identify and to quickly establish the L/O ratio from blends of soybean oil and animal fat biodiesel and determine their blend composition. To corroborate these results, classical quantitative techniques, such as GC methods, using both FID and MS detection, were used to quantitate the FAME contents of the blends and determine the L/O ratio. The L/O ratio as rapidly and directly measured by EASI(+)-MS, and as corroborated by the GC analysis, is shown to determine biodiesel blend soybean/animal fat composition.

2.   Materials and methods

a.   Chemicals and samples

Ampoules of standard reference materials (SRMs) for soybean biodiesel (SRM 2772) and animal fat biodiesel (SRM 2773) were used for the study. The soybean biodiesel was obtained from a farmer-owned cooperative. The animal fat biodiesel was obtained from a bioenergy firm and consists of approximately 85% animal fat (70% choice white grease and 15% edible pork lard) and 15% of soybean oil. FAME from palmitic, stearic, oleic, linoleic and linolenic acids used to prepare calibration solutions were purchased from Nu-Chek Prep (Elysian, MN) and were of >99% purity. Tridecanoic methyl ester was purchased from SigmaAldrich (Milwaukee, WI) and used as an internal standard prepared in chloroform. All solvents were of HPLC-grade.

b.   Preparation of blends

The biodiesel blends were prepared gravimetrically from the soybean biodiesel (SRM 2772) and animal fat biodiesel (SRM 2773) in the ratios shown in Table 1. These ratios were chosen to span the range from pure soy-based biodiesel to pure animal-based biodiesel.

For EASI-MS analysis, the pure soybean biodiesel, animal fat biodiesel and soybean/animal fat biodiesel blends (Table 1) were analyzed with no dilutions.

c.   Sample preparation for GC analysis

An aliquot (0.5 mL, exact mass known) from an ampoule of SRM 2772 was added to a 100 mL amber bottle with a Teflon-lined cap which contained 75 mL iso-octane (exact mass known). The same procedure was performed for SRM 2773. Each biodiesel blend was prepared according to Table 1, in a 100 mL amber bottle with a Teflon-lined cap which

contained 75 mL iso-octane (exact mass known). All the samples were prepared in triplicate.

From each blend, one aliquot (1.5 mL, exact mass known) was taken and transferred to a 10 mL vial with a Teflon-lined cap, and 1.5 mL (exact mass known) of the internal standard solution, consisting of tridecanoic acid methyl ester in chloroform, was gravimetrically added. This mixture was stirred for 10 min and divided in three vials for GC analysis. All samples were prepared in triplicate.

Three calibration solutions were prepared for the soybean biodiesel as well for animal fat biodiesel, so as to closely mimic the mass fraction of the individual FAME in the two biodiesel samples. Average response factors were used for quantitation through a single point calibration approach.[44]

d.   GC instrumentation

GC analysis was performed on an Agilent apparatus, model 6890N and 5875B, equipped with an FID and quadrupole MS, respectively, in a CP-WAX 52CB analytical column (30 m × 0.32 mm I.D. × 0.25 μm film thickness) (Varian, USA). For the FID analysis, the column was held isothermally at 120 °C for 2 min and then temperature programmed at 8 °C per min to 200 °C for 2 min and at 5 °C per min to 230 °C for 10 min. The injection port was maintained at 250 °C, and the FID was maintained at 250 °C. All injections were done in the split mode (0.2 μL) with helium as a carrier gas at a constant flow rate of 1.5 mL min^{-1}. The split valve was open to a 100 : 1 split. For the MS analysis, everything was the same with the exception that the transfer line was maintained at 230 °C, and the source was maintained at 230 °C. The MS was operated in the scan mode in the m/z 50 to 400 range.

e.   EASI-MS

Spectra were acquired in the positive ion mode using a single quadrupole mass spectrometer (Shimadzu) equipped with a homemade EASI source.[34] Note that the EASI source can be constructed from a few common laboratory parts since it uses solely a simple Swagelok T-element with appropriate ferrules and tubing for the gas flow and a fused-silica capillary at the sonic spray exit.[34] A tiny droplet of the biodiesel sample (2 μL) was dropped directly onto the paper surface. The main experimental parameters were as follows: a methanol flow rate of 20 μL min^{-1}, nebulizing gas (N$_2$) 3 L min^{-1}, and paper-entrance angle of $ca.$ 30°. Mass spectra were accumulated over 1 min and scanned along the m/z 100 to 500 range. The biodiesel analyses were performed in triplicate.

f.   Statistical analyses

The computer program Matlab version R2007b and program "R-package" were used to analyze data.

3.   Results and discussion

a.   Screening by EASI-MS

Fig. 1 shows typical EASI(+)-MS data acquired from biodiesel blends prepared gravimetrically from soybean biodiesel (SRM

Table 1   Composition of biodiesel blends

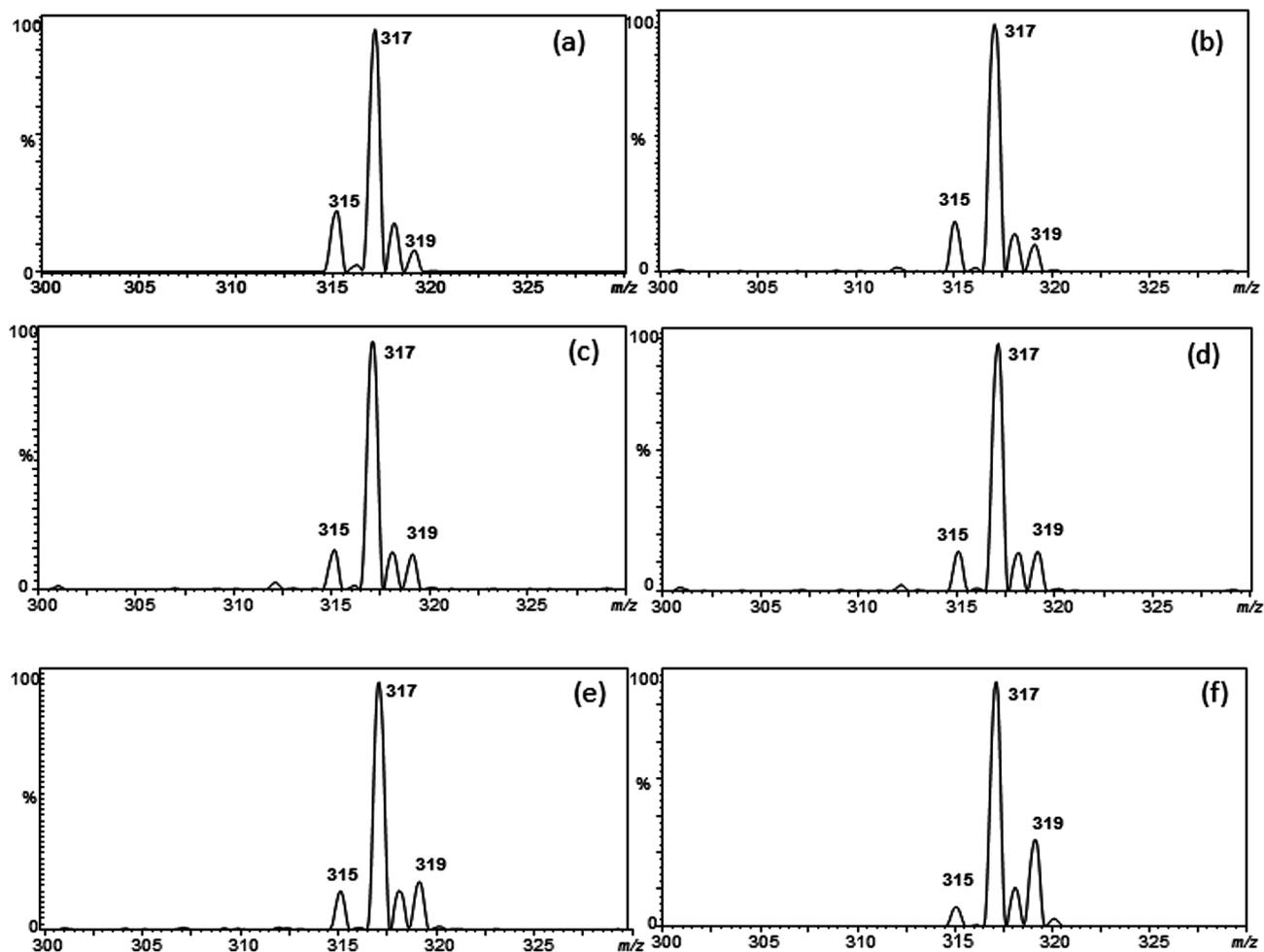| Blends	Soybean (% m m^{-1})	Animal fat (% m m^{-1})
B1	90	10
B2	50	50
B3	40	60
B4	10	90

Fig. 1 Typical EASI(+)-MS for (a) 100% soybean biodiesel, (b) B1, (c) B2, (d) B3, (e) B4 and (f) 100% animal fat biodiesel.
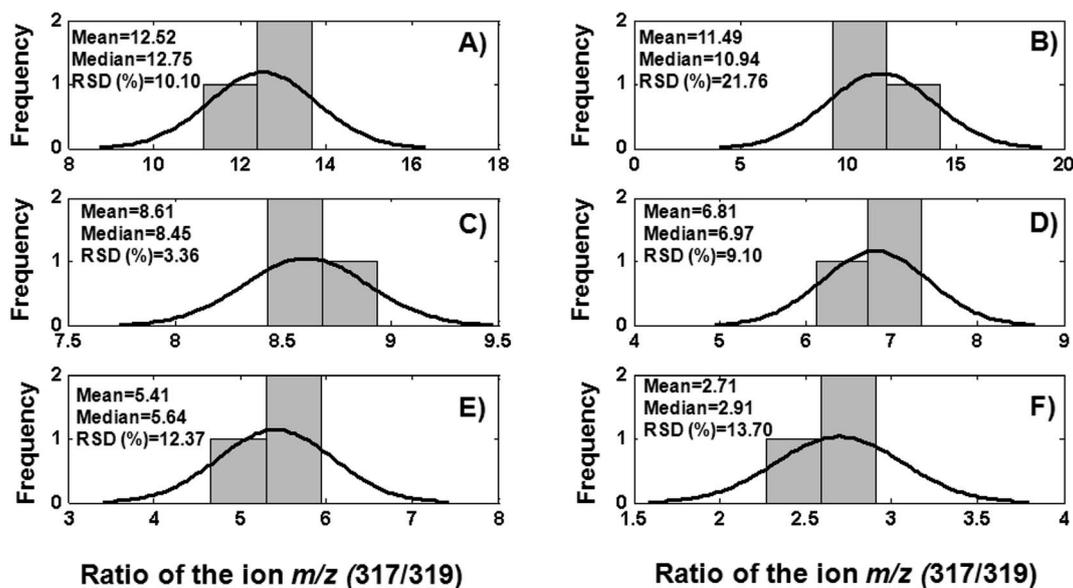

Fig. 2 Histograms showing a visual impression of the distribution of data for the linoleic/oleic methyl ester ratio in biodiesel blends and pure biodiesel obtained by EASI(+)-MS. (A) Soybean, (B) B1, (C) B2, (D) B3, (E) B4 and (F) animal fat.

2772) and animal fat biodiesel (SRM 2773) (Table 1), that is, for pure soybean biodiesel (Fig. 1a), B1–B4 biodiesel blends (Fig. 1b–e) and the pure animal fat biodiesel (Fig. 1f). One beneficial feature of EASI(+)-MS is that it detects each FAME molecule predominantly via a single ion, that is, in the form of [FAME + Na]$^+$ sodium adducts. Hence, the predominance of FAME from linoleic acid (m/z 317) in Fig. 1 and minor ions from those of oleic acid (m/z 319) and linolenic acid (m/z 315) are clearly seen and may reflect the actual composition of the mixture. This feature provides high selectivity and specificity for EASI(+)-MS analysis of biodiesel.[37–43]

As noted in Fig. 1, indeed there is a corresponding increase of the abundance of the ion of m/z 319 in relation to m/z 317 since the animal fat biodiesel content increases in the biodiesel blends. Note also that the relative abundances of the ions of m/z 317 and 319 cannot be directly related to the actual concentrations of the corresponding FAME in the biodiesel sample since their contrasting unsaturation levels affect their ionization efficiency as [FAME + Na]$^+$ ions, as we have recently studied.[43]

Fig. 2 shows a summarized grouping of data for linoleic/oleic methyl ester ratio (m/z 317/319) in biodiesel blends and pure biodiesel obtained by EASI-MS. The pure soybean biodiesel has the highest mean and median datasets whereas animal fat biodiesel has the lowest values. Dispersion within a dataset can be measured or described in several ways including the range, inter-quartile range, standard deviation and coefficient of variation. We chose the coefficient of variation to represent the dispersion of data. The coefficient of variation is a dispersion measurement that is independent of the unit scales, thus allowing the comparison of experimental results involving different samples of biodiesel. Its calculation is crucial for the experiments performed in laboratories because both precision and reliability can be verified. By analyzing the values of coefficient of variation we find that RSD varied from ca. 22% (B1) to as low as 3.4% (B2) for the B1–B4 blends. The B2 blend showed therefore the best precision among all datasets by displaying the lowest coefficient of variation.

To compare these results from the EASI-MS technique, the L/O ratio of these blends was also determined using both GC-FID and GC-MS.

b. Quantitation by GC-FID and GC-MS

A combination of GC-FID and GC-MS was used to quantitate the FAME content in the soybean/animal fat biodiesel blends tested. The GC-FID method was validated according to Inmetro[44] and it includes the studies of selectivity, linearity, accuracy (recoveries), precision (repeatability), limit of detection (LOD) and limit of quantification (LOQ) for each FAME. The only change in the method applied for GC-FID to that using GC-MS was the detection technique. Therefore, the GC-MS method was not validated, but statistically compared with the validated GC-FID method. Validation parameters are shown in Tables S1 and S2.†

To verify the agreement between methods, two variables (linoleic/oleic ratios measured via GC-FID vs. GC-MS, Table S3†)

were compared using a regression line and t-test, as recommended by Miller and Miller[45] and Massart et al.[46] To test a significant correlation between these two analyses, we calculate the t-value (t_{cal}) using eqn (1), where t_{cal} is the calculated
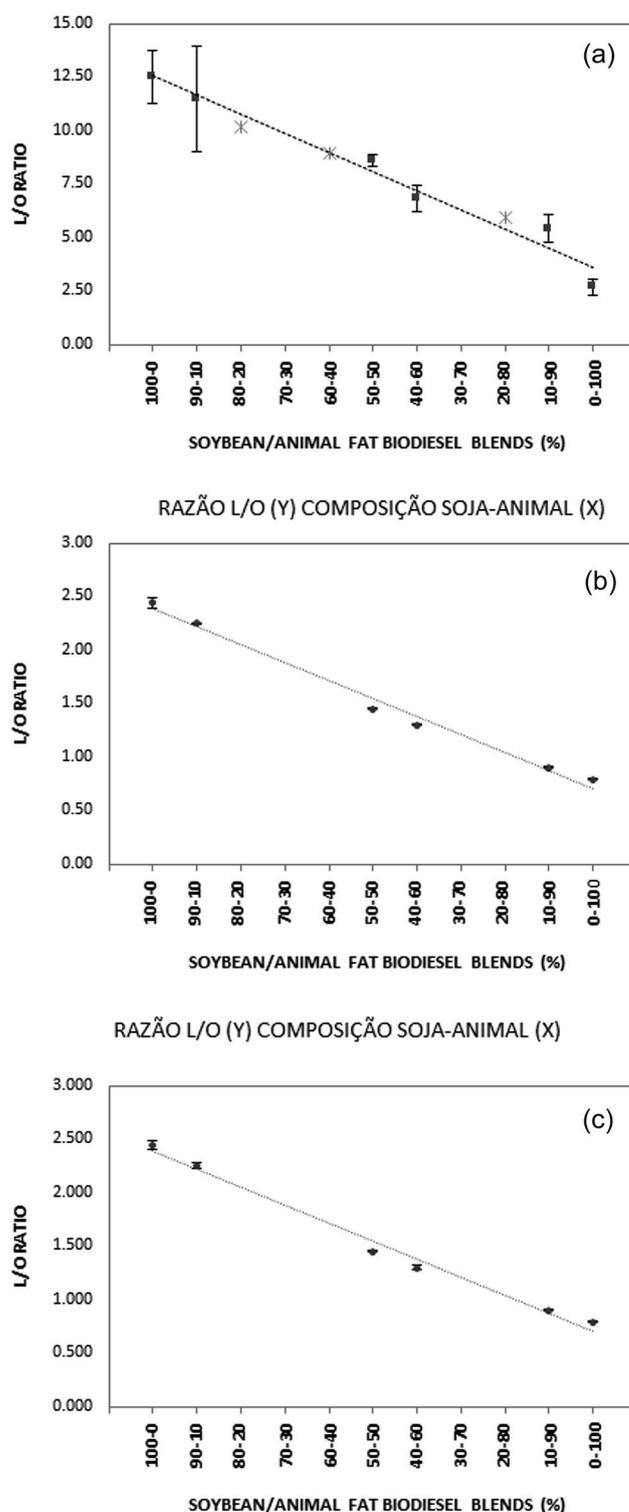

Fig. 3 Correlation between the composition and L/O ratio by EASI–MS (a), GC-FID (b) and GC-MS (c). Symbol (×) in (a) represents real samples.

value, n is the number of data points in the regression line and r is the correlation coefficient.

$$t_{cal} = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \qquad (1)$$

The t_{cal} is compared with the tabulated value (t_{tab}) of the Student's t-distribution with $n-2$ degrees of freedom at the desired significance level (p-level). We used 95% confidence, applying a *two-sided* t-test and four degrees of freedom. The null hypothesis in this case is that there is no correlation between the analysis by GC-FID and GC-MS. If the t_{cal} was found to be greater than the tabulated one, the null hypothesis would be rejected and we conclude, in such a case, that a significant correlation does exist. The values found for the t-test were 14.72, 8.67, 13.19, 16.69 and 5.73 for the FAME of palmitic acid (P), stearic acid (S), oleic acid (O), linoleic acid (L) and linolenic acid (Ln), respectively, whereas the critical value of t_{tab} is 2.78 ($p = 0.05$). According to the results from the regression line and t test, the mass fractions of FAME obtained by GC-FID (b) and GC-MS (c) were statistically equivalent.

Fig. 3 shows a correlation between the ratio of abundance of L and O acid methyl ester ions with the biodiesel blends composition. Note the linear decrease of the L/O ratio with the increase of the amount of animal fat in the blend, and that the soybean/animal fat ratio in biodiesel blends can indeed be directly correlated with the L/O ratios.

c. Real samples by EASI-MS

The EASI methodology was used to determine the real composition of samples prepared by proportions of soybean and fat biodiesel. Blends containing 80 : 20, 60 : 40 and 20 : 80 of soybean : fat biodiesel were analyzed by EASI-MS and the L/O ratios obtained were 10.18, 8.94 and 5.93, respectively. The results were within the expected range, as shown in Fig. 3 (a).

4. Conclusions

A rapid, simple and precise method based on easy ambient sonic-spray ionization mass spectrometry (EASI-MS), *via* the abundance ratio of the $[FAME + Na]^+$ ions of linoleic acid (L) and oleic acids (O), has been shown to be able to analyse biodiesel and to determine the relative amounts of soybean oil and animal fat used in its preparation. The very good results of the EASI-MS analysis were confirmed *via* comparisons with results provided by GC-FID and GC-MS data. The availability of such technique is important since biodiesel prepared from soybean/animal fat blends are by far the most common type in Brazil as well as a few other countries around the world.

Disclaimer

Certain commercial equipment or instruments are identified in this paper to adequately specify the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology nor does it imply that the equipment or instruments are the best available for the purpose.

References

1 P. R. C. Neto, L. F. S. Rossi, G. F. Zagonel and L. P. Ramos, *Quim. Nova*, 2000, **23**, 531–537.

2 F. Ma and M. A. Hanna, *Bioresour. Technol.*, 1999, **70**, 1–15.

3 G. Knothe, *J. Am. Oil Chem. Soc.*, 2006, **83**, 823–833.

4 M. A. Hanna, L. Isom and J. Campbell, *J. Sci. Ind. Res.*, 2005, **64**, 854–857.

5 B. R. Moser, *Energy Fuels*, 2008, **22**, 4301–4306.

6 M. Canaki and H. Sauli, *J. Ind. Microbiol. Biotechnol.*, 2008, **35**, 431–441.

7 R. Wang, B. Song, W. Zhou, Y. Zhang, D. Hu, P. S. Bhadury and S. Yang, *Appl. Energy*, 2011, **88**, 2064–2070.

8 M. Balat, *Energy Convers. Manage.*, 2011, **52**, 1479–1492.

9 T. W. Charpe and V. K. Rathod, *Waste Manag.*, 2011, **31**, 85–90.

10 M. S. A. Moraes, L. C. Krause, M. E. Cunha, C. S. Faccini, E. W. Menezes, R. C. Veses, M. R. A. Rodrigues and E. B. Caramão, *Energy Fuels*, 2008, **22**, 1949–1954.

11 L. Canoira, M. Rodriguez-Gamero, E. Querol, R. Alcantara, M. Lapuerta and F. Oliva, *Ind. Eng. Chem. Res.*, 2008, **47**, 7997–8004.

12 T. Issariyakyl, M. G. Kulkarni, L. C. Meher, A. K. Dalai and N. N. Bakshi, *Chem. Eng. J.*, 2009, **140**, 77–85.

13 J. M. Dias, M. C. M. Alvim-Ferraz and M. F. Almeida, *Energy Fuels*, 2008, **22**, 3889–3893.

14 S. Taravus, H. Ternmur and A. Yartasi, *Energy Fuels*, 2009, **23**, 4112–4115.

15 G. A. A. Teixeira, A. S. Maia, I. M. G. Santos, A. L. Souza, A. G. Souza and N. Queiroz, *J. Therm. Anal. Calorim.*, 2011, **106**, 563–567.

16 M. Primata, Y. C. Seo and Y. H. Chu, *J. Mater. Cycles Waste Manage.*, 2013, **15**, 223–228.

17 Z. Jurac and V. Zlatar, *Fuel Process. Technol.*, 2013, **106**, 108–113.

18 C. A. Nogueira Jr, F. X. Feitosa, F. A. N. Fernandes, R. S. Santiago and H. B. Sant'Ana, *J. Chem. Eng. Data*, 2010, **55**, 5305–5310.

19 M. Ozcanli and H. Serin, *J. Sci. Ind. Res.*, 2011, **70**, 466–470.

20 S. Lebedevas, A. Vaicekauskas and G. Lebedeva, *Energy Fuels*, 2006, **20**, 2274–2280.

21 http://www.anp.gov.br, accessed 04/10/2013.

22 P. Baptista, P. Felizardo, J. C. Menezes and M. J. N. Correia, *Anal. Chim. Acta*, 2008, **607**, 153–159.

23 A. C. Pinto, L. L. N. Guarieiro, M. J. C. Rezende and J. B. de Andrade, *J. Braz. Chem. Soc.*, 2005, **16**, 1313–1330.

24 N. Sang, R. Liu, Y. Chen, C. Chang and R. Lin, *J. Taiwan Inst. Chem. Eng.*, 2012, **43**, 354–359.

25 S. Chattopadhyay, S. Das and R. Sen, *Appl. Energy*, 2011, **88**, 5188–5192.

26 R. R. Catharino, H. M. S. Milagre, S. A. Saraiva, C. M. Garcia, R. Augusti, R. C. L. Pereira, M. J. R. Guimarães, J. M. Rodrigues, V. Souza, U. Schuchardt and M. N. Eberlin, *Energy Fuels*, 2007, **21**, 3698–3701.

27 I. Eide and K. Zahlsen, *Energy Fuels*, 2007, **21**, 3702–3708.

28 A. T. Godoy, G. G. Pereira, L. L. Ferreira, I. B. S. Cunha, D. Barrera-Arellano, R. J. Daroda, M. N. Eberlin and R. M. Alberici, *Energy Fuels*, 2013, **27**, 7455–7459.

29 R. M. Alberici, R. C. Simas, G. B. Sanvido, W. Romão, P. M. Lalli, M. Benassi, I. B. S. Cunha and M. N. Eberlin, *Anal. Bioanal. Chem.*, 2010, **398**, 265–294.

30 A. H. Glenn, A. S. Galhena and F. M. Fernandez, *Trends Anal. Chem.*, 2011, **83**, 4508–4538.

31 H. Chen, G. Gamez and R. Zenobi, *J. Am. Soc. Mass Spectrom.*, 2009, **20**, 1947–1963.

32 D. R. Ifa, C. Wu, Z. Ouyang and R. G. Cooks, *Analyst*, 2010, **135**, 669–681.

33 D. J. Weston, *Analyst*, 2010, **135**, 661–668.

34 R. Haddad, R. Sparrapan and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2901–2905.

35 A. Hirabayashi, M. Sakairi and H. Koizumi, *Anal. Chem.*, 1995, **67**, 2878–2882.

36 A. Hirabayashi, Y. Hirabayashi, M. Sakairi and H. Koizumi, *Rapid Commun. Mass Spectrom.*, 1996, **10**, 1703–1705.

37 P. V. Abdelnur, L. S. Eberlin, G. F. de Sá, V. Souza and M. N. Eberlin, *Anal. Chem.*, 2008, **80**, 7882–7886.

38 L. S. Eberlin, P. V. Abdelnur, A. Passero, G. F. de Sá, R. J. Daroda, V. Souza and M. N. Eberlin, *Analyst*, 2009, **134**, 1652–1657.

39 R. M. Alberici, R. C. Simas, V. Souza, G. F. de Sá, R. J. Daroda and M. N. Eberlin, *Anal. Chim. Acta*, 2010, **659**, 15–22.

40 R. M. Alberici, R. C. Simas, P. V. Abdelnur, M. N. Eberlin, V. Souza, G. F. de Sá and R. J. Daroda, *Energy Fuels*, 2010, **24**, 6522–6526.

41 R. M. Alberici, V. Souza, G. F. de Sá, R. J. Daroda, S. R. Morelli and M. N. Eberlin, *BioEnergy Res.*, 2012, **5**, 1002–1008.

42 I. B. S. Cunha, A. M. A. P. Fernandes, D. U. Tega, R. C. Simas, H. L. Nascimento, G. F. de Sá, R. J. Daroda, M. N. Eberlin and R. M. Alberici, *Energy Fuels*, 2012, **26**, 7018–7022.

43 R. M. Alberici, V. Souza, L. V. Gonçalves, V. S. Cunha, M. N. Eberlin and R. J. Daroda, *J. ASTM Int.*, 2012, **9**, 1–9.

44 DOC-CGCRE-008, Orientação sobre Validação de Métodos Analíticos, 2011, rev. 04, Inmetro.

45 J. N. Miller and J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson, Harlow, 6th edn, 2010.

46 D. L. Massart, *Handbook of Chemometrics and Qualimetrics, Part A*, Elsevier, 1997.