Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTAL INFORMATION

The selectivity was evaluated by separating the FAME with different retention times. Each FAME peak was characterized by comparing EI-MS data to the NIST library. Quantification was done by average response factors through the single point calibration approach, but the linearity of detector was also evaluated for all FAME studied. To evaluate the linearity for GC-FID, successive dilutions of calibration solutions were gravimetrically prepared and new response factor (Rf) were calculated for quantitation. Through the correlation between the mass fraction and analytical results for each calibration solution it was possible to observe the linearity of the GC-FID method for each FAME. **Table S1** summarizes the retention times, working ranges, r^2 values (the square of correlation coefficient) and the regression lines obtained. The angular coefficient close to 1 and the r^2 higher than 0.990 show the good correlation between the mass fraction (theoretical values) and analytical results (experimental values). The linearity of FID detector for each FAME is therefore proved and the single point calibration approach can be applied on the working range.

Table S1-Validation parameters used to access selectivity and linearity for GC-FID

Methyl Ester	Retention Time (min) *	Working range (mg g^{-1})	r^2	Regression line
Palmitic	12.536	32.7 – 197.0	0.999	y = 1.000x – 0.371
Stearic	15.479	13.6 – 95.0	0.999	y = 0.973x + 0.219
Oleic	15.818	58.9 – 365.3	0.997	y = 0.997x – 0.284
Linoleic	16.545	50.4 – 804.6	0.999	y = 0.995x – 0.423
Linolenic	17.556	16.4 – 230.9	0.992	y = 0.985x + 0.702

* The retention time of internal standard (tridecanoic methyl ester) is 8.929 min

The lowest concentrations obtained for each ester were considered the LOQ and the LOD was calculated from the Equation 1. The LOD values were between 4.1 mg g^{-1} for the stearic acid methyl ester and 17.9 mg g^{-1} for the oleic acid methyl ester. The LOQ values were between 13.6 mg g^{-1} for the stearic acid methyl ester and 58.9 mg g^{-1} for the oleic acid methyl ester.

$$LOD = LOQ/3.3 \quad (1)$$

The method precision was evaluated by performing repeatability studies, and by calculating the relative standard deviation (RSD%) of samples analyzed in replicate. All values found are below 5% and it was considered acceptable for the method. In accordance with AOAC recommendations, values of RSD up to 10% were accepted in the range of concentration used in this study. The accuracy was evaluated through recoveries studies throughout the working range for each FAME. All recovery values were between 84% and 112%. Our results are similar to those of Pardo in the validation by GC-MS, in which the accuracy for all the FAME was reported to range between 89.5 and 117.6% with RSD lower than 7.45%, in the range of acceptable values. The accuracy of the method should be within 70 and 120% at all concentrations with RSD< 20%. **Table S2** shows the results for LOD, LOQ, recovery and repeatability of the GC-FID method.

Table S2–Validation parameters used to access LOD, LOQ, recovery and repeatability for GC-FID.

Methyl Ester	LOD (mg g^{-1})	LOQ (mg $g^{-1)}$	Recovery (%)*	Repeatability (RSD %)*
Palmitic	9.9	32.7	96 – 102	0.08 – 0.59
Stearic	4.1	13.6	93 – 106	0.13 – 3.50
Oleic	17.9	58.9	95 – 104	0.12 – 0.90
Linoleic	15.3	50.4	95 – 105	0.13 – 0.77
Linolenic	5.0	16.4	84 – 112	0.14 – 1.98

*minimum and maximum values.

Table S3- Mass fractions(mg g^{-1}) of selected FAME[a] in biodiesel blends by GC-FID and GC-MS.

[a]

	GC-FID						GC-MS					
	P	**S**	**Ln**	**L**	**O**	**L/O**	**P**	**S**	**Ln**	**L**	**O**	**L/O**
<Soybean>	128	53.3	82.2	655	268	**2.44**	120	47.2	69.7	638	250	**2.55**
RSD (%)[b]	1.09	1.32	6.00	1.95	0.86	2.05	0.98	2.22	4.49	1.92	1.11	1.59
<B1>[c]	140	60.1	80.7	647	287	**2.25**	142	63.2	81.6	655	288	**2.27**
RSD (%)	1.73	1.68	1.57	1.68	1.94	0.44	2.17	4.20	3.18	1.68	2.61	1.22
<B2>	184	86.0	61.0	508	350	**1.45**	185	88.7	62.2	522	344	**1.52**
RSD (%)	1.88	2.00	4.45	1.21	1.60	0.42	1.65	3.07	2.18	0.93	1.12	0.39
<B3>	192	90.8	54.9	470	360	**1.30**	192	93.7	57.0	482	355	**1.36**
RSD (%)	1.29	1.29	2.83	1.96	1.33	0.72	1.41	2.69	3.57	1.95	1.60	0.55
<B4>	226	110	42.2	367	408	**0.90**	226	113	41.6	381	404	**0.94**
RSD (%)	3.15	3.25	3.74	3.52	3.22	0.75	3.58	4.00	4.28	3.93	3.64	**0.69**
<Animal>	234	115	37.2	328	413	**0.79**	221	105	27.2	309	389	**0.80**
RSD (%)	0.99	0.92	1.35	0.59	0.94	0.86	1.16	1.16	4.60	1.43	1.11	0.97

The FAME quantified are palmitic acid methyl ester (P), stearic acid methyl ester (S), oleic acid methyl ester (O), linoleic acid methyl ester (L) and linolenic acid methyl ester (Ln)

[b]RSD (%) is the relative standard deviation of measurements.

[c]The mixtures are 90% soy-based and 10% animal fat biodiesel (B1), 50% soy-based and 50% animal fat biodiesel (B2),40% soy-based and 60% animal fat biodiesel (B3), 10% soy-based and 90% animal fat biodiesel (B4).