Analysis of metals and phosphorus in biodiesel B100 from different feedstock using a Flow Blurring[®] multinebulizer in Inductively Coupled Plasma-Optical Emission Spectrometry

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Abstract

A simple and fast method for determining the content of Na, K, Ca, Mg, P, and 20 heavy metals in biodiesel samples with Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) using a two-nozzle Flow Blurring[®] multi-nebulizer prototype and on-line internal standard calibration, is proposed. The biodiesel samples were produced from different feedstock such as sunflower, corn, soybean and grape seed oils, via a base catalyst transesterification. The analysis was carried out without any sample pretreatment. The standards and samples were introduced through one of the multi-nebulizer nozzles, while the aqueous solution containing Yttrium as an internal standard was introduced through the second nozzle. Thus, the spectral interferences were compensated and the formation of carbon deposits on the ICP torch was prevented. The determination coefficients (R^2) were greater than 0.99 for the studied analytes, in the range 0.21 - 14.75 mg Kg⁻¹. Short-term and long-term precisions were estimated as relative standard deviation. These were acceptable, their values being lower than 10%. The LOQ for major components such as Ca, K, Mg, Na, and P, were within a range between 4.9 ng g⁻¹ for Mg (279.553 nm) and 531.1 ng g⁻¹ for Na (588.995 nm), and for the other 20 minor components they were within a range between 1.1 ng g⁻¹ for Ba (455.403 nm) and 2913.9 ng g⁻¹ for Pb (220.353 nm). Recovery values ranged between 95% and 106 %.

Keywords: Multi-nebulizer, ICP OES, Inorganic Elemental Analysis, Biodiesel.

1. Introduction

As a result of the decline in oil world reserves and global warming, biodiesel has taken an increasing importance as an alternative fuel over the last years. One of the most relevant aspects of biodiesel is the fact that it is obtained from biomass feedstock and is therefore considered a renewable energy source. Emissions from combustion of this biofuel are essentially free of aromatic compounds, heavy metals such as Cd, Co, Cu, Pb, V and Ni and other pollutants such as NO_x and SO_x that affect the air quality [1].

According to the ASTM D6751-08 specification, biodiesel is defined as a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, and it is designated B100 [2]. Usually, biodiesel is obtained from vegetable oils by means of a base-catalyzed transesterification owing to the high efficiency and the low cost of this process.

During biodiesel production, contamination may occur with metals such as Na, K, Ca, and Mg, as well as sulfur or phosphorus compounds, which could arise from raw materials or from chemical reagents used. These contaminants could affect the stability and some properties of the biofuels [3, 4]. Furthermore, environmental pollution may be caused if contaminants are at high concentrations and also, undesirable compounds could be formed during the combustion, thus causing engine malfunction.

Governments and agencies around the world regulate the quality of biofuels. The European Standard EN 14214 [5], the American Standard ASTM D 6751 [2] and other Standards such as the Brazilian norm RANP 07/08 [6] or the Argentine norm IRAM 6515-1:2006 [7], describe the requirements and control methods for the most common biodiesel (B 100). These Standards establish the maximum limits for the content of Na plus K as 5 mg Kg⁻¹ and Ca plus Mg as 5 mg Kg⁻¹, as well as a maximum limit of 10 mg Kg⁻¹ for P. Moreover, the maximum limit established for the content of S is 10 mg Kg⁻¹ according to EN 14214, and 500 mg Kg⁻¹ according to ASTM D 6751. The above-mentioned regulations suggest the use of FAAS and/or ICP OES for determining the content of Na, K, Ca, Mg, S and P. In addition, the European standards EN 14107, 14108 and 14109 [8-10] require the use of xylene to dissolve biodiesel samples and recommend the determination of P content by ICP OES, and Na and K content by atomic absorption spectrometry (AAS). The ICP OES has the advantage of being a multielemental and highly sensitive analytical

73 technique. However, interferences and operational instability may occur when organic
74 samples are directly introduced into the plasma. Spectral interferences such as stray
75 radiation, overlapping lines, and poor resolution of lines may be observed [11], and non-
76 spectral interferences such as differences in nebulization, sample transport and
77 desolvation between calibration standards and samples, carbon deposits formation at the
78 injector tube of the ICP torch may be present. Other drawbacks for biodiesel analysis by
79 ICP OES, are the instability of analyte solutions when prepared in an organic medium, and
80 the need to use organometallic standards and hazardous organic solvents, which require
81 special safety conditions.

82 In the last years, a number of researchers have focused their efforts on the development
83 of new methods for the elemental analysis of biodiesel. The proposed method by Dos
84 Santos et al. [12] for the determination of Ca, P, Mg, K and Na in biodiesel obtained from
85 different raw materials by ICP OES with axially-viewed plasma, was based in the dilution
86 of sample in ethanol. De Jesus et al. [13] developed a method for determining Na and K by
87 flame atomic absorption spectrometry (FAAS) in biodiesel obtained from different sources
88 and vegetable oils, by using water-in-oil microemulsion. Paiva de Oliveira et al. [14]
89 developed a method for Na determination in biodiesel fuel by flame atomic emission
90 spectroscopy (FAES), using dry decomposition as sample preparation. Henrique Lyra et
91 al. [15] proposed a procedure for determining Na, K, Ca and Mg in biodiesel samples by
92 FAAS and the preliminary treatment of the sample was prepared as a microemulsion
93 without surfactant. Lobo et al. [16] proposed a method for determining Ni and Cd in
94 biodiesel samples by graphite furnace atomic absorption spectrometry (GFASS) and
95 samples were prepared as microemulsions. Amais et al. [17] developed a method for the
96 determination of Ca, Mg and Zn in biodiesel samples by FAAS involving the
97 microemulsification of samples and discrete aspiration for sample introduction. De Souza
98 et al. [18] evaluated two micronebulizers, PFA and Miramist, using a method for elemental
99 determination by ICP OES in lubricant and residual fuel oils diluted in xylene. This
100 investigation allowed the improvement in the signal-to-background for Ag, Al, Ba, Cr, Cu,
101 Fe, Mn, Si, Ti and V using an oxygen flow that reduced spectral interferences from carbon
102 emission bands. PFA micronebulizer enabled the authors to obtain higher sensitivity, while
103 the limits of detection (LOD) obtained for both micronebulizers were comparable. The
104 above-mentioned methods suppose some disadvantages from an analytical and
105 environmental point of view such as the requirement of organic solvents and complicated
106 procedures for the pretreatment of biodiesel sample such as dilution, emulsification and
107 decomposition of the sample.

108 On the other hand, the use of a multiple nebulizer for simultaneous introduction of different
109 liquid samples/reagents into plasmas, which allows the application of “in chamber” sample
110 preparation procedures, has been proposed recently [19]. The initial four-nozzle prototype,
111 based on the Flow Focusing[®] nebulization principle has been followed by more an
112 advanced prototype based on the Flow Blurring[®] nebulization technology (Flow Blurring[®]
113 multinebulizer (FBMN)). It has been applied to various “in-chamber” sample preparation
114 procedures such as on-line standard addition calibration for organic sample analysis using
115 aqueous calibration standards [20], for the correction of matrix effects caused by high
116 dissolved salts [21], as well as for volatile forms generation by in-chamber derivatization
117 [22].

118 In this work, an evolution in the FBMN design is presented. The prototype described,
119 incorporates two nebulization nozzles, as opposed to three in previous designs. This
120 allows higher nebulization gas pressure to be used for the same flow rate, aiming to obtain
121 better operational characteristics and to generate finer aerosols. Another advantage of the
122 current prototype is that it can be used in commercial spectrometers without the need of
123 external peristaltic pump or nebulization gas supply.

124 In view of the above, the aim of this work is to propose an alternative strategy for ICP
125 OES analysis of trace elements in biodiesel obtained from different feedstock, based on
126 the implementation of a new evolution of the Flow Blurring[®] multinebulizer (FBMN) in the
127 sample introduction system. The FBMN allows the simultaneous nebulization of non-
128 pretreated biodiesel samples and water in order to decrease the Swan band emission
129 background, and to prevent accumulation of carbon deposits on the ICP torch injector. In
130 comparison with previously published applications of earlier FBMN prototypes to the
131 analysis of biodiesel samples by in-chamber standard addition calibration (aiming for high
132 analysis accuracy), the methodology proposed in this work is a simple procedure that
133 achieves significantly higher sample throughput, while maintaining the above mentioned
134 benefits of simultaneous nebulization of organic sample and water.

135

136 **2. Experimental**

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138 **2. 1. Reagent and standards**

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140 Pro-analysis grade chemicals and ultra-pure water ($18.3 \Omega \text{ cm}^{-1}$) were used. Nitric
141 Acid (Suprapur[®] 65%) and Yttrium monoelemental standard (Certipur[®] 1000 mg L⁻¹) were
142 purchased by Merck (Darmstadt, Germany). Yttrium internal standard solution of 1 mg L⁻¹

143 was prepared by diluting appropriate amounts of 1000 mg L⁻¹ Yttrium internal standard
144 solution in 1% nitric acid.

145 Conostan S-21 multielement standard solution containing 500 µg g⁻¹ of Ag, Al, B,
146 Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Ti, V, Zn blended in 75 cSt
147 blank oil, Conostan K standard solution containing 1000 µg g⁻¹ of K blended in 75 cSt
148 blank oil and Conostan Base Oil of 75 cSt, all purchased by SCP Science (Baie d'Urfé,
149 Canada) were used. Certified biodiesel matrix blank (used as blank for calibration) and a
150 5-element biodiesel certified reference material (NIST SRM: 1085b) containing 9.9 µg g⁻¹
151 (±1%) of Ca, Mg and P, and 10.0 µg g⁻¹ (±1%) of K and Na were purchased from Spex
152 CertiPrep (Metuchen, USA). Organometallic calibration solutions were prepared by diluting
153 appropriate amounts of Conostan S-21 and Conostan K in Spex CertiPrep biodiesel matrix
154 blank in the range of 0.2 to 14.7 µg g⁻¹.

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156 **2. 2. Samples**

157

158 Fourteen samples of biodiesel were obtained from sunflower, corn, soybean and
159 grape seed oils, in the FIA Laboratory (INQUISUR-UNS-CONICET), while other samples
160 were purchased from companies in Argentina.

161 The optimized synthesis process, based on base-catalyzed transesterification to
162 obtain biodiesel from different vegetable oils, and the methods applied to biodiesel quality
163 characteristics assessment such as density, kinematic viscosity, acid index and oxidative
164 stability (according to the Argentine Standard IRAM 6515:1 – 2006) were published in a
165 previous work [23]. These procedures were also used in this work for sample synthesis
166 and assessment.

167

168 **2. 3. Instrumental**

169

170 An axial view inductively coupled plasma optical emission spectrometer Agilent 720
171 ES (Agilent Inc., Melbourne, Australia) was used. The instrumental operation parameters
172 are summarized in Table 1. The following wavelengths were selected to measure the
173 emission intensities of the analytes: 328.068 nm for Ag, 396.152 nm for Al, 249.772 nm for
174 B, 455.403 nm for Ba, 393.366 nm and 396.847 nm for Ca, 226.502 nm for Cd, 267.716
175 nm for Cr, 324.754 nm for Cu, 238.204 nm for Fe, 766.491 nm and 769.897 nm for K,
176 279.553 nm and 280.270 nm for Mg, 257.610 nm for Mn, 202.032 nm for Mo, 588.995 nm
177 and 589.592 nm for Na, 216.555 nm for Ni, 213.618 nm for P, 220.353 nm for Pb, 288.158

178 nm for Si, 283.998 nm for Sn, 336.122 nm for Ti, 292.401 nm for V, and 213.857 nm for
 179 Zn. The internal standard (Y) was measured at 360.074 nm. Peak height was used as
 180 signal for quantification. A vortex mixer ZX³ Vortex (Velp Scientifica, Monza, Italy) was
 181 used to homogenize all the organometallic standard solutions and samples.

182

183 **Table 1.** Instrumental parameters for ICP OES instrument.

<i>Parameter</i>	<i>Value</i>
<i>Radiofrequency power</i>	1.35 KW
<i>Plasma gas flow rate</i>	15 L min ⁻¹
<i>Auxiliary gas flow rate</i>	1.5 L min ⁻¹
<i>Nebulizing/carrier gas flow rate</i>	0.7 L min ⁻¹
<i>Peak processing</i>	peak height
<i>Background correction</i>	2 points
<i>Number of replicates</i>	5
<i>Replicate read time</i>	1 s
<i>Sample flow rate</i>	0.2 mL min ⁻¹
<i>Water (with IS) flow rate</i>	0.5 mL min ⁻¹
<i>Nebulizer type</i>	two-nozzles Flow Blurring [®] multinebulizer prototype
<i>Spray chamber</i>	single-pass cyclonic chamber (50 mL)

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185

186 **2. 4. Standards and samples introduction system**

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188 The sample introduction system used in this work consists in assembling the new
 189 two-nozzle Flow Blurring[®] prototype (Figure 1) and one commercial single-pass cyclonic
 190 spray chamber of 50 mL (Model Tracy, Glass Expansion Ptr. Ltd, Melbourne, Australia).
 191 The FBMN prototype consists in two nebulization nozzles (Flow Blurring[®] technology
 192 nebulization units) with separate liquid inlets and a common nebulization gas inlet, all of
 193 them set into a cylindrical PTFE body (Figure 1). The hydrodynamic principles, in which
 194 the Flow Blurring[®] nebulization units are based, have been exhaustively discussed in
 195 previous works [20-24]. The dimensions and physical characteristics of this multinebulizer
 196 prototype permit its direct connection to different commercial spray chambers, commonly
 197 used in ICP OES instruments. The advantage of this prototype compared with previously
 198 described multinebulizers of three or four nozzles [19-23] consists, on one hand, in the
 199 ease of operation, as no additional peristaltic pump is needed (ICP OES instrument with a
 200 three channel pump is sufficient). On the other hand, the nebulization gas flow is divided
 201 only into two streams which lead to higher nebulization pressure (no external gas supply
 202 needed) and, hence to better aerosol characteristics. F-4040-A, id. 0.25 mm (Ismatec,

203 Switzerland) peristaltic pump tubes for organic solutions and Tygon[®] R-3607, id. 0.51 mm
204 (Ismatec, Switzerland) peristaltic pump tubes for aqueous solutions were employed to
205 pump all liquids throughout the system. Also, a Vulkan[®] direct injection nebulizer (Glass
206 Expansion, West Melbourne, Australia) and a MicroMist[®] nebulizer (Glass Expansion,
207 West Melbourne, Australia) have been used for comparison.

208
209 **Figure 1.** Side and front views of the two-nozzle Flow Blurring[®] multinebulizer.

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211

212 2. 5. Procedure

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214 External calibration with on-line addition of internal standard was used as calibration
215 method in order to compensate interferences in the analysis of biodiesel samples. Seven
216 organometallic standard solutions at 0.21, 0.54, 1.01, 2.96, 5.76, 12.46, 14.75 mg Kg⁻¹
217 were used. The calibration procedure consisted in the continuous introduction of the
218 aqueous solution (containing 1 mg L⁻¹ Yttrium as internal standard) into the spray chamber
219 through one of the two nebulization nozzles, while the organometallic standard solutions
220 were consecutively introduced through the other nebulization nozzle. After calibration, the
221 organic samples were introduced in the same way as the organometallic standard
222 solutions.

223

224 3. Results and discussion

225

226 3. 1. Biodiesel samples

227

228 A number of additive-free biodiesel samples (Table 2) from different feedstock,
 229 were obtained by means of a methanolysis reaction using sodium hydroxide as catalyst.
 230 The conversion percentages of triglycerides in fatty acid alkyl esters were between 91 and
 231 96 %. The quality of the biodiesel samples was evaluated according to the requirements
 232 and methods established by the Argentine standard IRAM 6515:1 – 2006 [7]. Physical-
 233 chemical parameters such as density, kinematic viscosity, acid index and oxidative stability
 234 were measured for the biodiesel samples obtained at laboratory scale and commercial
 235 samples, and the results are shown in Table 2. These results are in accordance to the
 236 limits assessed in the Argentine Standard mentioned above [7], except for the kinematic
 237 viscosity values in samples S1 and S3, which were higher.

238

239 **Table 2.** Physical-chemical parameters of the different biodiesel samples studied.

Sample	Density 15 °C (g mL⁻¹)	Kinematic Viscosity 40 °C (mm² s⁻¹)	Oxidative Stability 110 °C (h)	Acid Index (mg KOH g⁻¹)
IRAM 6515-1 Limits	0.875 - 0.900	3.5 - 5.0	Min. 6	Max. 0.5
S1 - Sunflower biodiesel	0.918	13.1	0.4	0.4
S2 - Corn biodiesel	0.872	4.2	0.6	0.3
S3 - Grape seeds biodiesel	0.917	12.9	0.4	0.4
S4 - Soybean biodiesel	0.870	3.6	2.2	0.3
S5 - Biodiesel Company 1	0.876	4.7	0.5	0.3
S6 - Biodiesel Company 2	0.869	3.6	2.0	0.6
S7 - Sunflower biodiesel	0.899	4.9	0.4	0.4
S8 - Corn biodiesel	0.885	3.9	0.6	0.3
S9 - Grape seeds biodiesel	0.891	4.7	0.4	0.4
S10 - Soybean biodiesel	0.889	4.4	2.2	0.3
S11 – Sunflower biodiesel	0.908	4.6	0.6	0.3
S12 – Corn biodiesel	0.878	4.1	0.6	0.3
S13 – Grape seeds biodiesel	0.904	4.3	0.2	0.4
S14 – Soybean biodiesel	0.879	3.9	0.4	0.3

240

241

242 3. 2. Sample introduction system selection

243

244 Three different arrangements for the introduction of organic standard solutions and
 245 samples were considered in this work, in order to select the most suitable sample
 246 introduction system for the determination of sodium, potassium, heavy metals, and
 247 phosphorus in samples of biodiesel, obtained from different feedstock, by ICP OES.

248 The first introduction system consisted in a Vulkan[®] direct injection nebulizer, the second
 249 one was provided with a MicroMist[®] microconcentric nebulizer and a cyclonic spray

250 chamber of 50 mL. However, the implementation of these sample introduction systems
251 was not successful owing to the typical issues related to the introduction of organic
252 samples into ICP, such as low plasma stability, formation of carbon deposits on the ICP
253 torch, as well as presence of spectral interferences. Therefore, a third introduction system
254 consisting of a two-nozzle Flow Blurring[®] multinebulizer prototype and a cyclonic spray
255 chamber of 50 mL, was finally used for the inorganic elemental analysis of the different
256 biodiesel samples. The advantages of the FBMN-based introduction systems have been
257 already described [20-23]. In this work a new version of a multinebulizer prototype was
258 used in order to develop a simple, fast and robust method for determination of metals in
259 organic samples without previous treatment, while maintaining the advantage of
260 simultaneously introducing organic and aqueous solutions (increased plasma stability,
261 elimination of spectral interferences caused by Swan bands and elimination of carbon
262 deposition on the ICP torch).

263

264 3. 3. Analytical figures of merit

265

266 The performance of the two-nozzles FBMN-based sample introduction system
267 proposed in this work for the elemental analysis of biodiesel by ICP OES, was evaluated
268 studying different analytical figures of merit: (i) linearity, (ii) precision, (iii) limits of
269 detection, quantification and linear range.

270

271 **(i) Linearity:** The 'best fit' line estimation using the least squares method, was applied to a
272 set of test results in order to confirm the correlation between the measured emission
273 signals and the analyte standard concentrations. Table 3 shows the values obtained for
274 the slope, intercept, and coefficient of determination (R^2) for each of the studied analytes,
275 when a set of standard solutions in a range of 0.21 – 14.75 mg Kg⁻¹ was used. The
276 coefficient of determination values of the calibration curves were higher than 0.998, and
277 the intercept values were close to zero except for Ca 393.366 nm and Ca 396.897 nm,
278 with values of 1.7 and 2.4 cps (ng g⁻¹)⁻¹, respectively. These results indicate a reasonable
279 linearity, and confirm the fact that the analytical calibration curves are able to correlate test
280 results directly or mathematically/statistically proportional to the concentration of the
281 analytes in the biodiesel samples.

282

283 **(ii) Precision:** In this work, short-term (in-run) and long-term (1 hour) precisions were
284 assessed by means of the RSD of several emission signal measurements consecutively

285 taken in a specific period of time. For this purpose, 1 mg Kg⁻¹ multielemental organic
286 standard solution was nebulized into the ICP-based spectrometer using the introduction
287 system proposed in this work at discrete times of 8 min (in-run) and 2 hours during the
288 analysis. The RSD values shown in Table 3 for short-term precision ranged between 0.8%
289 for Cu (324.754 nm) and Fe (238.204 nm), and 5.5% for P (213.618 nm), and the values
290 for long-term precision ranged between 2.6% for Pb (220.353 nm) and 8.8% for P
291 (249.772 nm). Although the proposed method provided a relatively low short- and long-
292 term precisions for the analysis of P at 1 mg Kg⁻¹ concentration level, it had better average
293 RSD values for short- and long-term precisions, 1.9% and 4.1% respectively, than the
294 whole set of emission lines at the same concentration level. This means that the average
295 RSD values were close to 3% RSD, which are quite acceptable especially when organic
296 samples/standards with low analyte concentrations are analyzed, and taking into account
297 that these analyses are commonly affected by carbon deposition on the ICP torch.

298

299 **(iii) Limits of detection and quantification, and the linear range:** Table 3 shows the
300 limits of detection (LOD) and the limits of quantification (LOQ) for the different analytes
301 studied in this work. The LOD was based on 3 times the standard deviation of 5
302 measurements of a blank standard, and the LOQ was based on 10 times the standard
303 deviation of 5 measurements of a blank standard. Biodiesel matrix blank was used for the
304 determination of these parameters. The obtained LOQ values for the major contaminants
305 such as Ca, K, Mg, Na, and P, ranged between 4.9 ng g⁻¹ for Mg (279.553 nm) and 326.2
306 ng g⁻¹ for Na (589.592 nm).

307 It is important to notice that these LOQ values were well below 5000 ng g⁻¹, which is the
308 maximum allowable limit established by the Argentine Standard IRAM 6515:2006 [7], the
309 European Standard EN 14214 [5], and the American Standard ASTM D 6751 [2] for those
310 contaminants in biodiesel. The obtained LOQ values for the other 20 minor components
311 were within a range between 1.1 ng g⁻¹ for Ba (455.403 nm) and 2913.9 ng g⁻¹ for Pb
312 (220.353 nm).

313 The linear range of an analytical method evaluated by visual inspection is defined as the
314 interval between the lowest concentration in which the quantitative measurements can be
315 made (LOQ) and the concentration in which the calibration curve loses its linearity, which
316 is called limit of linearity (LOL). As can be seen in Table 3, the linear ranges for the
317 different analytes studied by the proposed analytical method were at least two orders of
318 magnitude. The linear range in the case of Ba was wider, and for Pb it was bounded.
319 Therefore, it can be said that the use of the proposed method is limited for the

320 determination of Pb. This is partially due to the low sensitivities of the emission lines of this
321 element in ICP OES, especially at low sample uptake rates required for the organic
322 sample introduction.

323 **Table 3.** Analytical parameters associated with the validation of the proposed method for the inorganic elemental analysis by ICP OES in biodiesel samples.

<i>Element Emission Line</i>	<i>LOD (ng g⁻¹)</i>	<i>LOQ (ng g⁻¹)</i>	<i>Short-term RSD%</i>	<i>Long-term RSD%</i>	<i>Linear range (ng g⁻¹)</i>	<i>Slope (cps (ng g⁻¹)⁻¹)^a</i>	<i>Intercept (cps)^a</i>	<i>R²</i>
<i>Ag 328.068 nm</i>	3.6	10.9	2.2	3.1	10.9 – 14750.0	0.0812 ± 0.0009	-0.012 ± 0.006	0.9994
<i>Al 396.152 nm</i>	26.9	81.4	3.0	5.0	81.4 – 14750.0	0.068 ± 0.001	0.023 ± 0.004	0.9996
<i>B 249.772 nm</i>	10.3	31.1	1.2	3.1	31.1 – 14750.0	0.0453 ± 0.0004	0.002 ± 0.004	0.9996
<i>Ba 455.403 nm</i>	0.4	1.1	0.9	3.5	1.1 – 14750.0	4.5 ± 0.1	0.7 ± 0.4	0.9994
<i>Ca 393.366 nm</i>	10.6	32.0	0.9	3.7	32.0 – 12460.0	5.2 ± 0.1	1.7 ± 0.5	0.9987
<i>Ca 396.847 nm</i>	19.2	58.2	1.0	3.6	58.2 – 12460.0	8.7 ± 0.1	2.4 ± 0.6	0.9993
<i>Cd 226.502 nm</i>	12.7	38.5	1.3	4.8	38.5 – 14750.0	0.056 ± 0.001	0.004 ± 0.004	0.9995
<i>Cr 267.716 nm</i>	8.6	26.1	1.5	4.2	26.1 – 14750.0	0.058 ± 0.001	0.005 ± 0.004	0.9996
<i>Cu 324.754 nm</i>	2.9	8.9	0.8	3.1	8.9 – 14750.0	0.1127 ± 0.0003	0.001 ± 0.002	0.9999
<i>Fe 238.204 nm</i>	42.0	127.2	0.8	3.0	127.2 – 14750.0	0.0514 ± 0.0004	0.006 ± 0.003	0.9997
<i>K 766.491 nm</i>	16.3	49.5	1.2	3.2	49.5 – 14750.0	0.1092 ± 0.0004	-0.006 ± 0.003	0.9999
<i>K 769.897 nm</i>	25.2	76.3	1.2	4.7	76.3 – 14750.0	0.0711 ± 0.0003	0.014 ± 0.002	0.9999
<i>Mg 279.553 nm</i>	1.6	4.9	3.3	5.1	4.9 – 12460.0	1.18 ± 0.01	0.11 ± 0.05	0.999
<i>Mg 280.270 nm</i>	1.7	5.1	1.4	4.0	5.1 – 12460.0	0.291 ± 0.001	0.015 ± 0.005	0.9999
<i>Mn 257.610 nm</i>	2.4	7.3	1.3	3.8	7.3 – 14750.0	0.297 ± 0.002	0.021 ± 0.017	0.9997
<i>Mo 202.032 nm</i>	58.4	176.9	1.4	3.4	176.9 – 14750.0	0.0129 ± 0.0001	0.0006 ± 0.0004	0.9999
<i>Na 589.592 nm</i>	107.6	326.2	0.9	3.4	326.2 – 14750.0	0.276 ± 0.004	0.167 ± 0.031	0.9992
<i>Ni 216.555 nm</i>	90.4	274.1	2.3	3.4	274.1 – 14750.0	0.0086 ± 0.0001	0.0008 ± 0.0003	0.9999
<i>P 213.618 nm</i>	70.8	214.5	5.5	8.8	214.5 – 14750.0	0.00191 ± 0.00001	0.0004 ± 0.0001	0.9999
<i>Pb 220.353 nm</i>	961.6	2913.9	4.1	2.6	2913.9 – 14750.0	0.00170 ± 0.00001	0.0023 ± 0.0001	0.9998
<i>Si 288.158 nm</i>	81.6	247.3	1.7	5.4	247.3 – 14750.0	0.0116 ± 0.0001	0.033± 0.001	0.9995
<i>Sn 283.998 nm</i>	83.0	251.6	2.5	4.0	251.6 – 14750.0	0.00520 ± 0.00004	0.0026 ± 0.0003	0.9997
<i>Ti 336.122 nm</i>	1.5	4.6	2.4	5.1	4.6 – 14750.0	0.245 ± 0.002	0.024 ± 0.015	0.9997
<i>V 292.401 nm</i>	10.7	32.4	1.7	4.2	32.4 – 14750.0	0.0515 ± 0.0003	0.004 ± 0.002	0.9998
<i>Zn 213.857 nm</i>	12.4	37.6	1.2	3.9	37.6 – 14750.0	0.0352 ± 0.0004	0.002 ± 0.003	0.9994

^a ± % RSD

324

3. 5. Analysis of certified reference material

The trueness of the proposed method was assessed by the analysis of 5-element biodiesel certified reference material containing Ca, Mg, P, K and Na. Table 4 shows the results obtained. The certified reference material was not subjected to any treatment prior to analysis. Recovery values ranged between 95 and 106%, which mean that interferences (matrix effects) are not significant. Students's t-test was used in order to assess the null hypothesis, which establishes that the proposed method is not subject to systematic errors. The results in Table 4 show that the calculated $|t|$ values with $n=5$ for all the analytes in the certified reference material, were always lower than the t -statistical critical value obtained from the literature ($t=2.78$) at 95% confidence level and $n - 1=4$ degrees of freedom. Hence the null hypothesis is not rejected and there is no statistical evidence of systematic errors at a significance level of $\alpha = 0.05$.

Table 4. Analysis of 5-element biodiesel certified reference material by ICP OES applying the proposed method.

Element Emission Line	Certified value ($\mu\text{g g}^{-1}$)^a	Found value ($\mu\text{g g}^{-1}$)^b	Recovery (%)	Calculated t values
Ca 393.366 nm	9.9 $\pm$ 0.1	9.9 $\pm$ 0.2	100	0.39
Ca 396.847 nm	9.9 $\pm$ 0.1	9.9 $\pm$ 0.2	100	0.08
K 766.491 nm	10.0 $\pm$ 0.1	9.5 $\pm$ 0.2	95	2.52
K 769.897 nm	10.0 $\pm$ 0.1	9.5 $\pm$ 0.2	95	2.49
Mg 279.553 nm	9.9 $\pm$ 0.1	9.7 $\pm$ 0.3	98	0.88
Mg 280.270 nm	9.9 $\pm$ 0.1	9.6 $\pm$ 0.2	97	1.43
Na 589.592 nm	10.0 $\pm$ 0.1	9.7 $\pm$ 0.2	97	2.68
P 213.618 nm	9.9 $\pm$ 0.1	10.1 $\pm$ 0.3	102	1.41

^a $\pm$ 1% Uncertainty

^b $\pm$ % RSD

^c t -statistical critical value $t=2.78$ ($n-1=4$, $\alpha=0.05$)

3. 6. Analysis of real samples

All lab-made and commercial biodiesel samples analyzed in this work were directly introduced into the plasma without any sample pretreatment.

Table S1a and S1b in the supplementary material (see Appendix A) showed the results obtained from a set of 14 samples of biodiesel from different feedstock. The names of the different analyzed samples showed in Tables S1a and S1b were previously revealed in Table 1. In general, concentration values for most major and minor analytes in almost every biodiesel sample were below the LOQs except for samples S5, S13 and S14. The

354 accuracy of the proposed method for the determination of minor elements (heavy metals)
355 in biodiesel samples was not validated since no CRM was available for such a study.
356 However, in view of the good accuracy obtained for the major CMR analysis, the results
357 for the minor elements in this work could be considered very useful from the point of view
358 of qualitative analysis.

359 It was expected that major elements such as Na, K, Ca and Mg, minor elements such as
360 Ag, Al, Cu, Cd, Si, Sn, Zn, and others, were to be found in very low concentrations or in
361 concentrations below LOQ in biodiesel samples produced from refined and non-refined
362 vegetable oils. However, it is not unusual that such elements may be present in this kind of
363 organic matrixes because they may come from the reagents and materials used in the
364 transesterification process. The presence of P in biodiesel samples from vegetable oils is
365 entirely understandable because of the phospholipids present in the oils. The Argentine
366 Standard IRAM 6515:1 – 2006 [7], the European Standard EN 14214 [5], and the
367 American Standard ASTM D 6751 [2], establish a maximum limit of $5 \mu\text{g g}^{-1}$ for Na plus K,
368 and $10 \mu\text{g g}^{-1}$ for P in biodiesel, but these Standards do not establish maximum limits for
369 Ca, Mg, and trace elements such as heavy metals. Table S1a demonstrates that only the
370 concentration value for Na in sample S5 is not in accordance with the established
371 maximum limits for this element. The particularly high concentration value for Na in sample
372 S5, suggests that the removal of catalyst in the biodiesel refining step was not efficient.

373

374 **4. Conclusions**

375

376 The use of the new two-nozzle Flow Blurring[®] multinebulizer prototype has proven to
377 be a useful tool for the analysis of phosphorus, alkaline and heavy metals in biodiesel
378 samples obtained from different feedstock, by means of ICP OES using the on-line internal
379 standard calibration method. The implementation of this device has allowed the
380 simultaneous introduction of aqueous internal standard solution and organic
381 standard/sample solutions as an effective strategy to avoid the formation of carbon
382 deposits and to reduce spectral interferences. The consequent loss of precision and
383 sensitivity caused by injector clogging has also been prevented. Short-term and long-term
384 precision values ranged between 0.8 and 8.8 % RSD, this being quite acceptable.
385 Student's t-test permitted assessing that the proposed method does not show evidence of
386 systematic errors for the analysis of major contaminating elements such as Na, K, Ca, Mg
387 and P. This test has also permitted verifying that the difference between the experimental
388 values and the true values for major contaminating elements, are not statistically

389 significant at a 95% confidence level. The new FBMN prototype has enabled the direct
390 introduction of organic standard solutions and biodiesel samples without any pretreatment,
391 which makes the method proposed in this work an easy, fast, and relatively inexpensive
392 alternative for elemental analysis of biodiesel.

393

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395

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407

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