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# Novel Aerosol Phase Extraction Method for the Determination of Ca, K, Mg and Na in Biodiesel Through Inductively Coupled Plasma Atomic Emission Spectrometry

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1

12    **Abstract**

13

14    A novel extraction method was developed, optimized and validated for the elemental

15    analysis of organic samples. The method, called aerosol phase extraction (APE), was

16    based on the nebulization of the extracting aqueous solution (0.1 mol $L^{-1}$ nitric acid) on

17    the sample. The extraction was performed at the interface of each generated extractant

18    droplets as they entered in contact with the samples. Afterwards, the phases were

19    allowed to separate and Ca, K, Na and Mg were determined in aqueous phase by means

20    of inductively coupled plasma optical emission spectroscopy (ICP-OES). Measurement

21    of aerosol characteristics demonstrated that a water-in-oil emulsion was generated.

22    Therefore, once the aqueous solution was dispersed into the sample, the phases

23    spontaneously separated. Furthermore, the interfacial specific surface area took values

24    on the order of 1 $m^2$ $mL^{-1}$, hence enhancing the extraction kinetics over a conventional

25    extraction method. The key variables affecting the extraction yield were: the

26    nebulization gas flow rate, liquid flow rate, extraction time, acid concentration,

27    nebulizer tip to sample surface gap and $m_{Organic\ phase}/m_{Aqueous\ phase}$ ratio. Once the

28    optimum conditions were selected, the method was applied and validated for the

29    determination of Ca, K, Na and Mg by ICP-OES in 0.5 mL biodiesel samples with an

30    expanded uncertainty lower than 2%. With the APE method, the extraction time was

31    around 1 minute, whereas conventional methods employed to perform this kind of

32    extraction required from 4 to 50 minutes. Additionally, the APE involved the analytes

33    preconcentration thus lowering the limits of detection down to the ng $mL^{-1}$ level (*i.e.*,

34    LODs based on the 3 $s_b$ criterion were 32, 20, 19 and 24 ng $mL^{-1}$ for Ca, K, Na and Mg,

35    respectively). Furthermore, accuracy when quantification of Ca, K, Na and Mg

36    concentration using APE was not significantly different as compared to that afforded by

37    conventional liquid-liquid extraction. Finally, Ca, K, Na and Mg contents were

38    determined in four real samples in the 0.5-13 mg kg$^{-1}$ range. The obtained results were

39    not statistically different to those encountered with a microwave-based digestion

40    method.

41

**Keywords**

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*ICP-OES; Liquid – liquid Extraction; Aerosol phase extraction; Metals; Biodiesel*

45

**Highlights**

47    A novel rapid aerosol phase extraction (APE) procedure has been applied for the first

48    time to determine metal impurities in biodiesel samples.

49    A water-in-oil emulsion is generated during the extraction process and the phases

50    separation is spontaneously and quickly produced.

51    The method provides high extraction yields with a concomitant analyte

52    preconcentration.

53    As the APE avoids introduction of organic solvents into the plasma, calibration can be

54    performed with a set of plain acidified water standards.

55

**Novelty Statement**

57    The novel Aerosol Phase Extraction method has been applied for the first time to the

58    analysis of organic samples. In this case, the analytes (Na, K, Ca and Mg) are extracted

59    in an aqueous 0.1 mol L$^{-1}$ nitric acid solution. The determination of these metals is

60    finally carried out in inductively coupled plasma optical emission spectrometry by

61    applying external calibration with aqueous standards.

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64 **Introduction**

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66 Biodiesel is a well-established alternative to fossil fuels. Biodiesel quality control

67 involves, among others, the determination of alkaline earth elements (Na, K, Ca, Mg)

68 content.[1] These species are a result of the biodiesel production process and play a very

69 important role because they may modify the efficiency of the biodiesel production as

70 well as its stability. Moreover, the presence of these elements may cause engine damage

71 by corrosion and catalyst poisoning. These elements are present at variable

72 concentrations depending on factors such as the raw materials, production process and

73 the post-production pollution, among others.

74 The quantification of alkaline and alkaline earth elements in biodiesel has

75 several difficulties associated: *(i)* some of them are present at very low concentrations

76 ($\mu g \ L^{-1}$); *(ii)* there are limited certified reference materials for method validation and

77 quality controlling; and, *(iii)* the matrix is complex and its composition depends on the

78 biodiesel origin and treatment.

79 Therefore, it is obvious that sensitive techniques are required to carry out the

80 determination of Na, K, Ca and Mg in this type of samples. The main techniques

81 employed are flame atomic absorption spectrometry (FAAS), graphite furnace atomic

82 absorption spectrometry (ETAAS), inductively coupled plasma optical emission

83 spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).[2]

84 Dilution in an organic solvent (*i.e.* ethanol, xylene or kerosene) has been widely

85 recommended for the determination of Na, Ca, K and Mg by atomic absorption

86 spectrometry or ICP-OES.[3,4,5,6,7,8] Unlike FAAS, the latter technique has multielemental

87 capability, it provides lower limits of detection and wider dynamic ranges than the

88 former. However, the introduction of organic samples into the plasma is a challenging

89  subject as ICP techniques suffer from severe interferences caused by complex organic

90  matrices (e.g., matrix effects, plasma degradation or soot deposition at the injector

91  tip).[9,10,11,12] To circumvent them, several sample preparation approaches have been

92  developed such as acid digestion and sample oxidation or combustion.[2,13,14] However,

93  these methods show some problems caused by the addition of reagents, sample

94  contamination, degradation of limits of detection and volatile compounds losses.

95  Emulsification has been proposed as an alternative to sample dilution, because

96  the mass of organic solvent that reaches the plasma is minimized.[15] Emulsification

97  involves the addition of an aqueous phase containing an acid and/or surfactant to the

98  sample. The selection of the surfactant and its concentration are the most crucial points.

99  On this subject, it is necessary to consider that the emulsion physical properties may

100  affect the nebulization process,[16] the analyte transport efficiency and the stability of the

101  emulsion. By mixing water, oil and one or more surfactants under controlled

102  experimental conditions, either a cloudy (i.e., emulsion) or a transparent (i.e.,

103  microemulsion) mixture is obtained.[17] To prepare an emulsion or microemulsion, the

104  surfactant is first dissolved in water and then it is added to the sample. The dispersion is

105  prepared by vigorously stirring or by using an ultrasonic bath.[17,18] The major drawback

106  of this methodology is related with the loss of sensitivity and the degradation of LODs

107  as the sample is diluted.

108  Solvent extraction is one of the most versatile and widely used procedures for

109  the removal, separation and concentration of metals.[19,20] In the last years, research

110  efforts have been focused on the development of efficient, fast and miniaturized

111  extraction methods.[20,21] In classical liquid–liquid extraction, small droplets are

112  generated by agitation, which increases the contact surface area and improves the

113  extraction efficiency. However, in some instances, the classical procedure is not

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114 effective enough and presents some disadvantages: laborious manipulation, large

115 volumes of solvents required, high operational costs, possible formation of emulsions,

116 large equipment and long analysis time. One of the means for improving the extraction

117 efficiency is to increase the medium temperature and/or to apply sonication. This

118 methodology has been satisfactorily used for the determination of Ca, K, Mg and Na in

119 biodiesel by ion chromatography.[22] However, complex procedures, large volumes of

120 solvents and long analysis time are still required. In the dispersive liquid-liquid

121 microextraction (DLLME) method, a dispersing agent is added to the extracting one.

122 The extraction efficiency is improved due to the increase in the exchange surface as a

123 substantial number of small droplets of the extracting solvent are dispersed into the

124 liquid sample.[23,24]

125     In this paper, a new liquid-liquid extraction method, based on the concept of

126 aerosol extraction, previously developed in our laboratory,[25] has been applied for the

127 first time to the determination of Ca, K, Mg and Na in organic samples (*c.a.*, biodiesel).

128 The Aerosol Phase Extraction (APE) method is based on the confinement of an aerosol,

129 previously generated from the extraction solution, into the sample. As indicated in

130 previous reports,[26] the extraction has been carried out by using a diluted nitric acid

131 solution, thus eliminating the aforementioned drawbacks associated to the introduction

132 of organic matrices into ICP-OES.

133

134 **Materials and methods**

135

136 *Chemicals and samples*

137 Ultrapure water was supplied by a three-step ion-exchange system Milli-Q, fed by

138 reverse osmosis, Elix 3, both from Millipore (El Paso, TX, USA). 65% $HNO_3$

139 (Suprapur®, Merck KGaA, Darmstadt, Germany) was used to prepare washing

140 solutions and acidify the standards and samples. An ICP multielement standard solution

141 (Merck IV, Merck KGaA, Darmstadt, Germany) containing 1000 mg element per litre

142 was used to prepare the standards by serial dilutions. Stock and standard solutions were

143 prepared in 2 % (v/v) $HNO_3$.

144     To evaluate the feasibility of the developed method four real samples were

145 analyzed. A biodiesel certified reference material (CRM), VHG-B100M5-10-100G,

146 produced by VHG Labs (Manchester, U.S.A.) was also studied. The CRM had a Fatty

147 Acid Methyl Ester matrix (B100) and the certified Na, Ca, Mg, K and P concentrations

148 were $10.0 \pm 0.1$ $\mu g\ g^{-1}$.

149

150 *Aerosol phase extraction procedure*

151 Figure 1 shows the experimental set-up used to perform the APE procedure. 0.5-0.9 g of

152 biodiesel were poured into a 5 mL extraction vial. Then, a $HNO_3$ solution was delivered

153 to a glass pneumatic concentric nebulizer (TR-30-A2, Meinhard®, USA) through a

154 peristaltic pump (Perimax, Spetec, Erding, Germany). The generated droplets were thus

155 directed towards the organic phase. The metal extraction occurred at the interface of

156 each generated droplet, thus giving rise to a large contact surface area. Once the

157 solutions were separated, the Ca, K, Mg and Na concentrations were determined in the

158 aqueous phase through ICP-OES.

159     All the experiments and measurements were carried out at room temperature.

160 Three extractions and ten measurement replicates were performed for all the evaluated

161 conditions.

162

163 *Volume drop size distributions and ICP-OES measurements*

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164　The aerosols generated by the nebulizer were measured by means of a laser Fraunhofer

165　diffraction system (model 2600c, Malvern Instruments, Malvern Wolcestershire, UK).

166　The sizer was equipped with a 63 mm lens focal length, which enabled the system to

167　measure droplets with diameters included within the 1.2 to 118 μm range and provided

168　the aerosol liquid volume fraction for a set of 31 diameter intervals (bands) thus giving

169　rise to the complete volume drop size distribution. The nebulizer tip was set at 30 mm

170　from the lens and at 15 mm from the laser beam center.

171　　　An axially viewed Agilent 720 ICP-OES spectrometer (Santa Clara, USA) was

172　used to determine the intensities for the selected elements. The system was equipped

173　with a 40.68 MHz free-running generator and a polychromator with an echelle grating.

174　A glass pneumatic concentric nebulizer (TR-30-K2, Meinhard®, USA) fitted to a

175　cyclonic spray chamber was used as sample introduction system. Table 1 summarizes

176　the operating conditions.

177

178　**Results and Discussion**

179

180　*Fundamental studies*

181　In the Aerosol Phase Extraction (APE) procedure, the extracting (aqueous) aerosol was

182　pneumatically generated through the interaction between the liquid stream and a high

183　velocity gas stream. For a given pneumatic nebulizer, the experimental variables that

184　influence the aerosol drop size distribution are the gas and liquid flow rates. The

185　efficiency of liquid-liquid extraction, in turn, is precluded by the total liquid interface

186　area. The higher this magnitude, the higher the extraction yield. To accomplish this in

187　the case of the APE method, aerosols as fine as possible must be generated. It was

188　clearly observed that at low gas flow rates (*i.e.*, 0.2 L min$^{-1}$) the proportion of liquid

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189    volume contained in coarse droplets was significantly higher than at higher values of

190    this variable. This was a direct consequence of the increase in the gas kinetic energy.[27]

191    As regards the liquid flow rate, $Q_l$, an increase in this variable led to a drop in the liquid

192    to gas volume ratio thus leading to coarse aerosols.

193        In the APE, the metals extraction took place at the interface of each particular

194    droplet what resulted in a larger contact surface area than in the case of the classical

195    extraction method. The aerosol surface area distribution was thus obtained by applying

196    the following equation to each diameter range:

197

198    
$$A_i = \frac{600 \times V_{AP} \times \overline{\%}}{\overline{D}} \qquad \textbf{Equation 1}$$

199

200    where, $A_i$ is the absolute area for a given band drop diameter ($cm^2$); $V_{AP}$ is the total

201    volume of extracting solution required for a complete run, *e.g.*, 0.5 mL; $\overline{D}$ is the

202    diameter at each band midpoint (μm); and, $\overline{\%}$ is the % aerosol volume at the midpoint

203    of the band provided by the sizer.

204        Figure 2 shows the absolute surface drop size distributions obtained after

205    applying Equation 1 at the three gas flow rates studied. It was first observed that a

206    significant liquid surface was contained in droplets with diameters lower than 1.2 μm (7

207    and about 20% for 0.2 and 0.4 L $min^{-1}$, respectively). When considering the curves for

208    diameters above this value, it was found that they peaked towards lower drop diameters

209    as increased the gas flow rate (Figure 2).

210        By integrating the curves in Figure 2 it was possible to determine the effective

211    liquid surface or liquid-liquid interfacial surface area. The obtained values were 0.33,

212    0.82 and 0.92 $m^2$ per $cm^3$ of liquid at $Q_g$ values of 0.2, 0.4 and 0.6 L $min^{-1}$, respectively

213    It was therefore verified that the higher the gas flow rate, the higher the interfacial area.

214     Taking into account a 0.5 mL total volume of extracting solution, the respective total

215     interfacial surface values were 0.16, 0.41 and 0.45 $m^2$.

216        The effect of the liquid flow rate was also evaluated. Aerosol measurements

217     demonstrated that at 0.3 and 0.5 mL $min^{-1}$, about 25% of the liquid surface was

218     contained in droplets with diameters below 1.2 μm; whereas, at 1.0 mL $min^{-1}$ the

219     surface area contained in droplets with diameters lower than 1.2 μm decreased down to

220     3%. According to these data, the interface surface areas were 0.95, 0.83, 0.41 and 0.28

221     $m^2$ per liquid $cm^3$ at 0.25, 0.50, 0.75 and 1.0 mL $min^{-1}$, respectively.

222        Finally, it was verified that the aerosols generated were composed by droplets

223     whose diameters ranged from 1.2 to around 100 μm. This size range corresponded to a

224     conventional emulsion.[28] In conclusion, the presented method was based on the

225     generation of an emulsion without the need of using a dispersing agent. This fact was

226     advantageous, because, due to the instability of emulsions, in comparison with

227     microemulsions,[29,30] both phases could separate spontaneously.

228

229     *Optimization of the operating variables*

230     A univariate optimization of the variables affecting the extraction efficiency was carried

231     out. The parameters considered were those that affected the characteristics of the

232     generated aerosols at the sample surface (the nebulizer gas and liquid flow rates and

233     nebulizer tip – sample gap) as well as those that influenced the status of the extraction

234     equilibrium (nitric acid concentration and mass phases ratio). In order to perform this

235     study, Na, K, Ca and Mg concentrations were determined in the biodiesel CRM. For a

236     given element, the recovery (R, %) was given by:

237

238                    $$R(\%) = \frac{C_{det}\, m_{ext}}{C_{cert}\, m_{CRM}} x100 \qquad\qquad \textbf{Equation 2}$$

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240 where $C_{det}$ corresponded to the experimentally determined analyte concentration, $m_{ext}$

241 was the mass of extracting solution and $C_{cert}$ and $m_{CRM}$ were the CRM concentration and

242 mass (0.5 g), respectively.

243

244 *Effect of the nebulizer gas flow rate, $Q_g$*

245 Regarding the effect of the gas flow rate ($Q_g$), it was possible to anticipate that the finer

246 the generated aerosols (*i.e.*, the higher the interfacial surface), the better recoveries.

247 Thus, according to the data shown in Figure 2 the best conditions would correspond to

248 the highest $Q_g$ evaluated (*i.e.*, 0.9 L min$^{-1}$). Figure S1 plots the recovery versus the

249 nebulizer gas flow rate showing an unexpected optimum (100%) at 0.4 L min$^{-1}$. Higher

250 values of this variable induced a drastic drop in the extraction yield. For example, at 0.9

251 L min$^{-1}$ only 20% of the analyte mass initially present in the sample was extracted. This

252 could be explained by an increase in the speed at which the aerosol impacted against the

253 organic phase. Indeed, it was visually observed that above 0.4 L min$^{-1}$, a fraction of the

254 sample was ejected from the sample vial and it was consequently lost. For this reason,

255 0.4 L min$^{-1}$ was taken as the optimum $Q_g$ for the remainder of the study.

256

257 *Effect of the liquid flow rate, $Q_l$*

258 Studies on extraction kinetics were carried out by modifying the liquid flow rate ($Q_l$) set

259 to aspirate 0.5 g of aqueous phase (extracting solution). According to Figure S2, 100%

260 recoveries were obtained at low liquid flow rates (*i.e.*, from 0.1 to 0.5 mL min$^{-1}$). This

261 parameter dropped down to 65% as $Q_l$ was further increased up to 1 mL min$^{-1}$. Because

262 the volume of extracting agent was kept constant, this trend was likely due to the

263 inverse relationship between $Q_l$ and extraction time. Additionally, as mentioned before,

12

264     aerosols became coarse (with lower liquid surface areas) as the liquid flow rate was

265     increased. It was finally verified that a 60 s extraction time was enough to obtain

266     recoveries close to 100% for the four elements tested. This time was from 3 to 50 times

267     shorter than those required in previous works.[22,31]

268

269     *Effect of the distance from nebulizer tip to the sample surface, d*

270     The effect of the nebulizer nozzle sample surface gap (Figure 1) on R was evaluated. It

271     was observed that recoveries were 100% when the distance was set at values of 1.0 or

272     1.5 cm. Higher d values made a fraction of the aerosol to impact against the vertical

273     walls of the biodiesel container and did not efficiently interact with the sample. Under

274     these circumstances, recoveries ranged from 80 to 90%. When the nebulizer was set at

275     closer distances from the sample, the aerosol droplets impacted against the surface of

276     the organic phase at a high speed. This, together with the effect of the high velocity

277     stream, caused the ejection of a significant sample mass. The direct consequence of this

278     phenomenon was that a portion of the sample was lost and did not interact efficiently

279     with the extracting agent. For instance, for a 0.8 cm nebulizer – sample surface distance,

280     the measured recovery was 90%.

281

282     *Effect of $HNO_3$ concentration*

283     Figure S3 shows that the extraction efficiency increased with the acid concentration.

284     The major effect was observed for calcium. For this element, the recovery went from

285     60% to 100% as the acid concentration was increased from 0.05 to 0.1 mol $L^{-1}$. In

286     contrast, magnesium exhibited an around 80% extraction yield for the lowest acid

287     concentration tested. For all the elements, a plateau was reached at a 0.1 mol $L^{-1}$ $HNO_3$

288     concentration.

13

289

290  *Effect of m$_{Organic\ phase}$/m$_{Aqueous\ phase}$ ratio (r)*

291  Values of this parameter from 0.5 to 3 were chosen to evaluate the analyte pre-

292  concentration effect of the APE method. Figure S4 shows that, for the four elements

293  tested, the intensity in the aqueous phase increased almost linearly with the r value. This

294  trend suggested that the analyte extraction efficiency did not vary as a function of the

295  aqueous to organic phase ratios within the r studied range. It was later verified that

296  recoveries were close to 100% for all the evaluated r values. Therefore, a r value of 3

297  was selected because of the expected decrease in the limits of detection.

298      In order to monitor whether the phase separation step was properly performed,

299  once the extraction was completed, the carbon 193.030 nm emission signal was

300  measured in the extracting phase and a clean diluted nitric acid solution. No significant

301  differences were found between both signals, thus confirming the absence of emulsified

302  biodiesel traces in the extracting solution.

303

304  *Optimized parameters*

305  Table 2 summarizes the optimized operating conditions for the new aerosol phase

306  extraction approach. A new test was done to check whether the analyte extraction was

307  complete under the optimized conditions or not. The biodiesel sample initially exposed

308  to the APE procedure under optimum conditions was subjected to a second extraction

309  step. Ca, K, Mg and Na concentrations were again measured in the new aqueous phase

310  and they were not significantly different from those measured for a blank. As a

311  conclusion, a single procedure was sufficient for the complete extraction of the analytes.

312

313  *Phase separation step*

14

314    According to previous studies carried out in our laboratories,[25] an aqueous sample could

315    be nebulized over an organic solution in order to perform the extraction of

316    molybdenum. It was verified that, once the aerosol interacted with the organic phase,

317    the phase separation took place in ten minutes. This could be associated to the fact that

318    too fine aerosols were generated. In fact, the nebulizer employed in ref. 25 yielded

319    aerosols whose maximum diameter was 15 μm. Presumably, the existence of a

320    significant proportion of fine droplets caused a slow phase separation. Based on these

321    results, a nebulizer able to generate aerosols more compatible with the production of an

322    emulsion was chosen. As mentioned before, under optimized conditions, the maximum

323    aerosol drop diameter was close to 100 μm. As a consequence, the phase separation

324    took place instantaneously once the aerosol generation ceased. This involved a

325    significant shortening in the analysis time and, hence, a ten times increasing in the

326    sample throughput from 5 to 50 $h^{-1}$.

327

328    *Aerosol phase extraction versus conventional extraction*

329    An extraction procedure adapted from a method recently validated for Na, K, Mg and

330    Ca determination in biodiesel samples was taken for comparison purposes.[22] To lower

331    the sample and reagent consumption, the extraction procedure was miniaturized. 250

332    mg of each sample were directly weighed into a centrifuge tube with a precision of 0.1

333    mg. Then, 500 $\pm$ 0.1 mg of ultrapure water were mixed with 1.25 μL of a 1 mol $L^{-1}$

334    $HNO_3$ solution. The mixture was shaken for 1 min in a vortex apparatus (Selecta,

335    Barcelona, Spain). Afterwards, the tube was partially immersed in a thermostatic bath

336    set at 85 ◦C for 30 min. This tube was then placed for 15 min in an ultrasonic bath

337    (Selecta, Barcelona, Spain) operating at a 50 kHz frequency. After that, it was

15

338     centrifuged at 1000 rpm for 5 min. The aqueous phase was separated and diluted with

339     $0.625 \pm 0.0001$ g of ultrapure water.

340     Table S1 gathers the Na, K, Ca and Mg concentrations found for the two

341     extraction procedures in the biodiesel CRM. Both methods provided similar results.

342     However, the aerosol extraction procedure proved to be slightly more reproducible than

343     the reference method. Certainly, the main advantage of the APE over the reference

344     procedure was the significantly shortening in analysis time from 51 min in the case of

345     the former procedure to 1 min for the aerosol phase extraction methodology.

346

347     *Method validation*

348     A complete in-house validation and uncertainty estimation according to IUPAC[32] and

349     EURACHEM[33] guidelines was carried out.

350

351     *Linearity and working range*

352     Calibration was performed using five mass fraction levels covering from 0 to 6 mg kg$^{-1}$.

353     To counter potential memory effects, five replicates for each calibration solution were

354     measured in a random order. Coefficients of determination ($R^2$) in the 0.9987-0.9996

355     range were obtained for five different calibration curves, and no outlying measurements,

356     i.e. > 3 times the standard error of the calibration function, were found.

357

358     *Limit of detection and limit of quantification*

359     Ten independent replicate analyses of the Element Blank Oil 75 Viscosity standard

360     (Conostan®, Ponca City, USA) were carried out under repeatability conditions. This

361     standard was selected because it could be considered as a matrix similar to that of

362     biodiesel. The LOD and LOQ were estimated as 3 or 10 times the total standard

16

363  deviation ($s_T$), respectively. Equation 3 was applied to calculate $s_T$. Total standard

364  deviation includes the standard deviation of all the measurements ($s_M$) and the standard

365  deviation coming from the CRM ($s_{Sd}$ = 0.015 mg kg$^{-1}$). The LOD and LOQ values were

366  around 0.06 and 0.18 mg kg$^{-1}$ (Table 3). The major contribution came from the blank

367  standard.

368

369
$$s_T = \sqrt{s_M^2 + s_{Sd}^2} \qquad \textbf{Equation 3}$$

370

371      In order to compare the LODs with previous works, they were estimated

372  according to the $3s_b$ criteria, where $s_b$ was the standard deviation of ten background

373  consecutive measurements. In the present study, the blank corresponded to the biodiesel

374  CRM after performing two consecutive APE procedures in order to ensure that the

375  analytes were quantitatively removed. As Table 3 shows, LODs for the APE method in

376  combination with an ICP-OES spectrometer were from 2 to 20 times lower than those

377  found in previous works. Even considering the contribution of the standard deviation

378  coming from the CRM (Equation 3), LODs found in the case of the new APE were

379  lower than those encountered in the already described methods (see Table 3).

380  Obviously, the use of a sensitive technique such as ICP-OES promoted the reduction in

381  LODs over other techniques considered in Table 3 such as ion chromatography or flame

382  photometry.

383

384  *Trueness*

385  Trueness was evaluated by measuring the Ca, K, Mg and Na concentration for a

386  biodiesel CRM. Three replicate samples were analyzed on two different days. The

387  evaluation of the measurement results on a certified reference material was done by

17

388 comparison of the difference between the certified and measured values ($\Delta_m$) with the

389 combined uncertainty of certified and measured value ($U_\Delta$). The absolute difference

390 between the mean measured value and the certified value were calculated according to

391 Equation 4. The expanded uncertainty $U_\Delta$, corresponding to a confidence level of

392 approximately 95 %, was obtained by multiplication of $u_\Delta$ by a coverage factor (k),

393 usually equal to 2. The uncertainty $u_\Delta$ was calculated, based on Equation 5, from the

394 uncertainty of the certified value and the standard deviation of the measurement result.

395 To evaluate the method performance, $\Delta_m$ was compared with $U_\Delta$. As $\Delta_m$ was lower than

396 $U_\Delta$ for all the elements, it was concluded that there were no statistically significant

397 differences between the values obtained experimentally and the certified concentrations

398 (Table S2).

399

400 $$\Delta_m = |c_m - c_{CRM}| \quad \textbf{Equation 4}$$

401

402 $$u_\Delta = \sqrt{s_m^2 + u_{CRM}^2} \quad \textbf{Equation 5}$$

403

404 where $c_m$ is the mean measured value, $c_{CRM}$ is the certified value, $s_m$ is the standard

405 deviation and $u_{CRM}$ is the uncertainty of the certified value.

406

407 *Repeatability and intermediate precision*

408 Three sub-samples of a CRM sample were measured on 5 different days, using 5

409 different calibration curves. One-way ANOVA was used to estimate the repeatability

410 and intermediate precision as within-group and between-group standard deviation,

411 respectively. The repeatability of the method was lower than 0.8 % for all the elements,

412 whereas the intermediate precision was lower than 0.11%.

18

413

414    *Uncertainty estimation*

415    Calibration solutions preparation has a contribution to the final measurement

416    uncertainty through the purity of the standards and the weighing steps. Furthermore, the

417    uncertainty of the purity of the standards has also been assessed to estimate the total

418    uncertainty contribution of the calibration. The relative standard uncertainty of the

419    calibration resulted in 0.6% by taking into account the uncertainty contribution of the

420    preparation of the calibration solution, all additional contributions coming from the

421    stock solution preparation and the uncertainty of the purity of the standards.

422    The relative standard uncertainty contributions related to the repeatability and

423    intermediate precision were obtained applying one-way ANOVA to the 15

424    measurements (equations 6 and 7):

425
$$u_{rep} = \frac{100}{c_{CRM}} \sqrt{\frac{RSD_{rep}^2}{n_{rep}}} \qquad \textbf{Equation 6}$$

426
$$u_{ip} = \frac{100}{c_{CRM}} \sqrt{\frac{RSD_{ip}^2}{n_{days}}} \qquad \textbf{Equation 7}$$

427

428    where $c_{CRM}$ is the mean value of concentration measured for each element, $RSD_{rep}$ is the

429    repeatability, $n_{rep}$ is the number of replicates, $RSD_{ip}$ is the intermediate precision, and

430    $n_{days}$ is the number of days. Moreover, the relative standard uncertainty related to the

431    trueness contribution was estimated by applying Equation 8.

432

433
$$u_t = \frac{100 \times u_\Delta}{c_{CRM}} \qquad \textbf{Equation 8}$$

434

435    The contributions of the calibration, repeatability, intermediate precision and trueness

436    were taken into account for the calculation of the expanded uncertainty (*U*) of the

437    measurements.[34]

438

439    $$U = k \cdot \sqrt{u_{cal}^2 + u_{rep}^2 + u_{ip}^2 + u_t^2}$$                **Equation 9**

440

441    where *U* is the expanded relative uncertainty, *k* is the coverage factor (k=2), $u_{cal}$ is the

442    relative standard uncertainty of calibration, $u_{rep}$ is the relative standard uncertainty of

443    repeatability, $u_{ip}$ is the relative standard uncertainty of intermediate precision, and, $u_t$ is

444    the relative standard uncertainty of trueness. The coverage factor applied was 2

445    corresponding to the 95 % confidence level.[34] Table S3 shows the final expanded

446    relative uncertainty for each element and the contribution of the different uncertainty

447    sources. For all the elements, the expanded relative uncertainty was around 1.5-1.9%.

448    The major sources of uncertainty contribution were related to the calibration and

449    trueness assessment and they represented around 47-56%   and 36-46% of the

450    uncertainty, respectively.

451

452    *Analysis of real samples*

453    With the aim of validating the aerosol phase extraction methodology, the biodiesel

454    certified reference material and four real samples including three biodiesels and a waste

455    cooking oil were analyzed. Concentration values were also calculated by sample acid

456    digestion and subsequent determination by ICP-OES. The samples were treated using

457    the microwave digestion system Start D (Milestone, Sorisole, Italy). Approximately 250

458    mg of the sample, weighed with a precision of ±0.1 mg, were transferred to a

459    microwave digestion vessel and then 7 mL of 65% $HNO_3$ and 1 mL of 30% $H_2O_2$ were

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460    added. The sample was digested at 200 °C for 30 min. The digests were transferred to

461    graduated glass flasks and diluted to 10 mL with Milli-Q water.

462            To evaluate whether the difference between the concentrations provided by the

463    two tested methods was significantly different, the statistical t-test was applied. As

464    Table 4 shows, no significant statistical differences were observed between the values

465    obtained        by        the        APE        and        by        the        digestion        procedure.

466

467     **Conclusions**

468     The new aerosol phase extraction (APE) method could be considered a valuable sample

469     preparation method. From the point of view of green chemistry, the use of organic

470     solvents has been avoided and waste generation has been minimized. Moreover, the

471     developed method reduced significantly the time required for a quantitative extraction.

472     Fundamental studies showed that the extraction procedure is based on an emulsion

473     formation, thus phase separation is spontaneously produced. These advantages have

474     been exploited in the determination of Ca, K, Mg and Na in biodiesel samples. The

475     combination of the APE with ICP-OES provides a simple, reliable, accurate and

476     reproducible method whose results are deemed to be of low uncertainty. In fact, the

477     expanded uncertainty values are in the order of 2%. In face of the results, the APE

478     method together with ICP-OES determination could be proposed as an alternative

479     method to the conventional and regulated ones, where the dilution with an organic

480     solvent is recommended.[3,4,5]

481              Compared with the previously studied method,[25] the new APE procedure shows

482     several differences: *(i)* the phase separation took place quickly and spontaneously. As a

483     consequence, the analysis time was shortened. This was due to the use of a nebulizer

484     able to generate droplets whose diameters matched perfectly within the values

485     theoretically required to generate emulsions (i.e., $< 100$ μm). In fact, the whole sample

486     preparation took place in around 65 and 630 s for the APE and the previously developed

487     method,[25] respectively; *(ii)* the analyte was extracted in aqueous phase, thus avoiding

488     the problems associated to the introduction of organic solutions into the ICP; *(iii)*

489     sample losses caused by re-nebulization were minimized as on the one hand the

490     extracting solution was nebulized over the sample and, on the other hand, the nebulizer

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491    tip to sample surface was optimized; and, *(iv)* it was not necessary to add complexing

492    agents in order to promote the analyte extraction.

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**Table 1. Operating conditions of the ICP-OES detector**.

| Variable | Value |
| --- | --- |
| Liquid flow rate, $Q_l$/ mL min$^{-1}$ | 0.8 |
| Nebulizer gas flow rate, $Q_g$/L min$^{-1}$ | 0.7 |
| Outer plasma gas flow rate, L min$^{-1}$ | 15 |
| Intermediate plasma gas flow rate, L min$^{-1}$ | 1.5 |
| Rf power, kW | 1.4 |
| Integration time, ms | 25 |
| Sampling time, s | 1 |
| Elements and Wavelengths, nm | Ca 396.847 |
| | K 766.491 |
| | Mg 279.553 |
| | Na 589.592 |

**Table 2. Optimized operating conditions of the aerosol phase extraction procedure.**

| Variable | Value |
|---|---|
| Nebulizer gas flow rate, $Q_g$, L min$^{-1}$ | 0.4 |
| Nebulizer liquid flow rate, $Q_l$, mL min$^{-1}$ | 0.3 |
| Extraction time, s | 60 |
| $HNO_3$, mol L$^{-1}$ | 0.1 |
| Nebulizer tip to sample surface gap (d), cm | 1.5 |
| $m_{Organic\ phase}$, g | 0.9 |
| $r = m_{Organic\ phase}/m_{Aqueous\ phase}$ | 3 |

**Table 3. Limit of detection (mg kg$^{-1}$) obtained for Ca, K, Mg and Na by applying different extraction and determination procedures.**

| | APE* | APE[#] | APE[&] | Liquid extraction + Flame photometry[#,31] | Liquid extraction + Line source flame atomic absorption spectrometry[#,31] | Conventional liquid-liquid extraction + Ion chromatography[#,22] |
|---|---|---|---|---|---|---|
| *Ca* | 0.06 | 0.03 | 0.017 | -- | 0.15 | 0.23 |
| *K* | 0.05 | 0.02 | 0.008 | 0.18 | 0.21 | 0.42 |
| *Mg* | 0.05 | 0.02 | 0.015 | -- | 0.03 | 0.36 |
| *Na* | 0.05 | 0.02 | 0.014 | 0.36 | 0.04 | 0.11 |

*Calculated considering the standard deviation obtained by applying Equation 3.

[#] Calculated considering only the standard deviation of all blank signal measurements.

[&] Calculated considering the standard deviation of the biodiesel sample initially exposed to the APE procedure subjected to a second extraction.

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**Table 4. Ca, K, Mg and Na concentration (mg kg$^{-1}$) obtained by APE and acid digestion methods. Calculated t values, s values, the critical t value ($\alpha$=0.05) and equations employed to calculate t and s.**

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| | Concentration (Mean ± s) (mg kg⁻¹) | | | | | | | | Statistical analysis | | | | | | | |
| | Ca | | K | | Mg | | Na | | Ca | | K | | Mg | | Na | |
| | APE | Digestion | APE | Digestion | APE | Digestion | APE | Digestion | t* | s# | t* | s# | t* | s# | t* | s# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biodiesel A | 4.16 ± 0.02 | 4.3 ± 0.3 | 2.07 ± 0.04 | 2.31 ± 0.17 | 5.805 ± 0.006 | 5.85 ± 0.06 | 2.538 ± 0.011 | 2.60 ± 0.08 | 0.9 | 0.2 | 2.43 | 0.12 | 1.34 | 0.04 | 1.38 | 0.06 |
| Biodiesel B | 3.65 ± 0.03 | 3.71 ± 0.2 | 2.089 ± 0.007 | 2.11 ± 0.03 | 5.76 ± 0.02 | 5.67 ± 0.12 | 3.005 ± 0.014 | 2.94 ± 0.08 | 0.49 | 0.16 | 1.61 | 0.02 | 1.20 | 0.08 | 1.29 | 0.06 |
| Biodiesel C | 1.03 ± 0.02 | 1.05 ± 0.10 | 2.724 ± 0.004 | 2.74 ± 0.09 | 6.56 ± 0.03 | 6.49 ± 0.05 | 2.536 ± 0.009 | 2.55 ± 0.02 | 0.38 | 0.07 | 0.38 | 0.06 | 2.22 | 0.04 | 1.164 | 0.017 |
| Waste oil | 12.83 ± 0.08 | 12.9 ± 0.5 | 7.787 ± 0.016 | 7.71 ± 0.05 | 6.64 ± 0.02 | 6.68 ± 0.02 | 13.207 ± 0.010 | 13.43 ± 0.18 | 0.1 | 0.3 | 2.58 | 0.04 | 2.49 | 0.02 | 2.20 | 0.13 |

$*t = \dfrac{(\bar{x}_1 - \bar{x}_2)}{s\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$; $\#s^2 = \dfrac{(n_1-1)s_1^2+(n_2-1)s_2^2}{(n_1+n_2-2)}$; The F-test demonstrated that the variances of both methods procedures were statistically comparable. Critical t value ($\alpha=0.05$): 2.78.
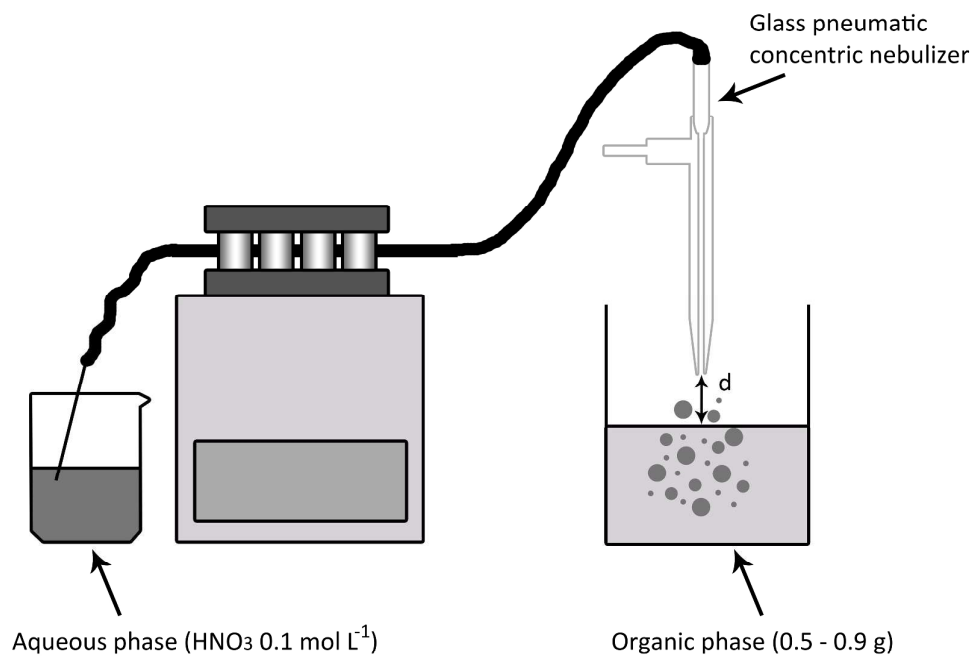
**Figure 1.** Scheme of the experimental setup employed to perform the Aerosol Phase Extraction, APE.
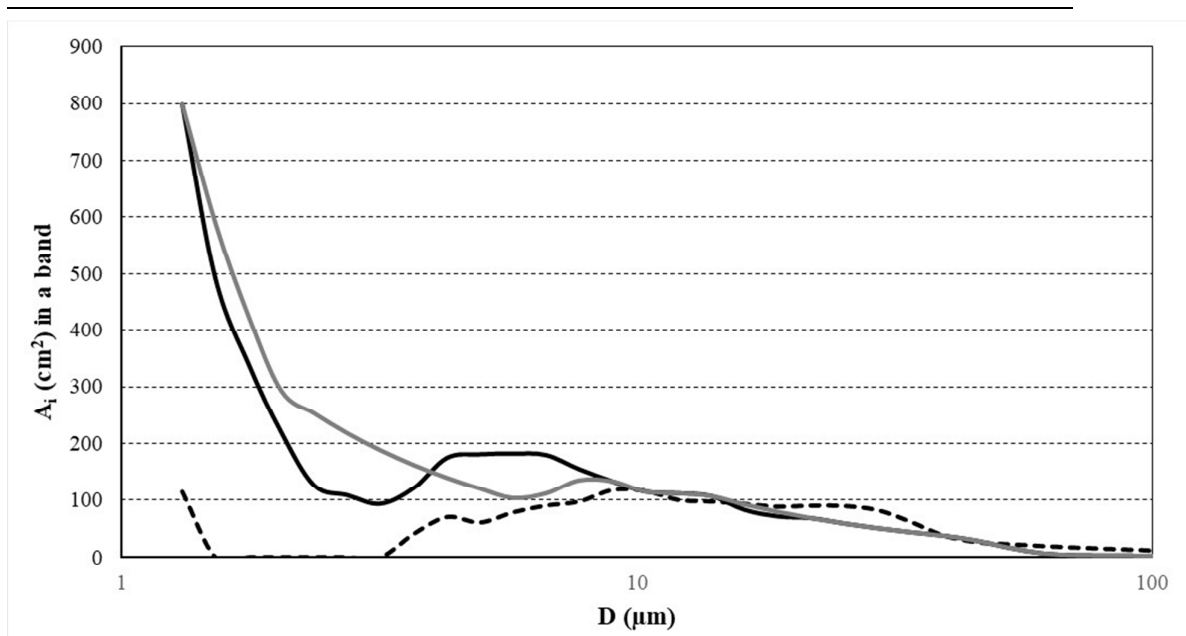
**Figure 2.** Absolute surface drop size distribution curves in band for the aerosols

generated by a concentric nebulizer under different gas flow rates. Liquid flow rate 0.5

mL min$^{-1}$; gas flow rate: 0.2 L min$^{-1}$ for dotted line; 0.4 L min$^{-1}$ for black line; and, 0.6

L min$^{-1}$ for grey line.