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Metals and metalloids determination in bioethanol through

ICP-OES

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Abstract

A new method to carry out the elemental determination of metals in bioethanol through ICP-OES has been developed. The procedure is based on the use of a heated Torch Integrated Sample Introduction System (hTISIS) to directly introduce the vaporized sample into the plasma. Two injection modes (continuous liquid aspiration and air-segmented flow injection analysis) have been evaluated. In a first step, the matrix effects caused by several ethanol-water mixtures were removed by operating the hTISIS at 400°C in segmented injection. Meanwhile, the results also proved that the system could be operated in continuous mode at 200°C with the complete interferences removal. Finally, twenty-eight real samples with bioethanol contents between 55% and 100% were analyzed with the methods previously developed. Regarding validation, recoveries from 80% to 120% were obtained for 18 analytes and the concentrations found with the proposed method were in agreement with those encountered with a preconcentration method, taken as a reference procedure. Limits of detection went from 3 ng mL⁻¹ for manganese to about 500 ng mL⁻¹ for calcium. This allowed to quantify Cr, Fe, Mg, Mn and Zn in segmented flow injection and Al, Cd, Cr, Cu, K, Mg, Mn, Na and Zn in continuous sample aspiration mode in bioethanol samples.

1. Introduction

Bioethanol is known as the alcoholic product obtained from the fermentation of carbohydrates present in a wide renewable feedstock (e.g. sugar cane, corn and switchgrass) using various types of microorganisms [1,2]. Among the possible species present in bioethanol, metals deserve special attention. Metals and metalloids can be present in the raw material [3,4,5,6]. Moreover, bioethanol may be contaminated with metals during its synthesis [4,5,7] and/or its storage and transport in metallic containers [5,6,7,8]. Finally, some metallic species can be incorporated as additives to promote the combustion process [9].

The quantification of metals and metalloids in bioethanol shows many difficulties as they are generally present at low concentrations ($\mu\text{g L}^{-1}$). Besides, commercially available bioethanol contains up to 7% of water and 300 different organic compounds. Both facts may cause a degradation in the accuracy of the determination if the matrix nature is not considered. An additional difficulty is that there are limited certified reference materials that hampers the method validation procedure [1,2].

Several analytical techniques have been proposed to determine metals in ethanol fuel [10], such as electrothermal atomic absorption spectroscopy (ETAAS), [5,11,12,13,14,15,16] microwave inductively plasma optical emission spectrometry (MIP-OES) [6] or voltammetry [17,18,19,20,21]. Flame absorption atomic spectroscopy (FAAS) in turn has been widely employed for this purpose [4,22,23,24,25,26]. However, a preconcentration step is necessary due to the high limits of detection provided by this technique [24,25,26]. Therefore, inductively

coupled plasma optical emission spectroscopy (ICP-OES) [27,28] and mass spectrometry (ICP-MS) [11,28,29,30] are highly appropriate to perform bioethanol analysis due to their low limits of detection and wide dynamic range. Unfortunately, ICP techniques suffer from non spectral interferences caused by the ethanolic matrices that preclude the accuracy of the determinations.

In order to remove or to mitigate non spectral interferences caused by ethanol in ICP-OES, samples should be diluted employing water [30,31]. An obvious limitation of this methodology is that LODs and sensitivity are severely degraded. Matrix matching has also been applied, although the matrix uses to be unknown [11,28,30]. Finally, the use of internal standardization has been recommended [28,30]. The most important drawback of this methodology is the selection of a suitable internal standard.

Additionally, alternative sample introduction systems can be proposed to remove the matrix effects. In previous works, a high temperature Torch Integrated Sample Introduction System (hTISIS) has been described. Basically, this device consists of a temperature programmable single-pass spray chamber. The hTISIS has been specially designed to work at liquid flow rates below roughly $100 \mu\text{L min}^{-1}$. So far, this sample introduction system has been applied to the analysis of aqueous [32] as well as organic [33] samples through ICP-OES. Higher sensitivities, lower limits of detection and absence of interferences are among the benefits of the hTISIS over conventional sample introduction systems.

It should be noted that most of the studies previously mentioned have been developed for ethanol fuel analysis. The main goal of this work was thus to test the suitability of the hTISIS to perform metal determination in bioethanol samples through

ICP-OES. In order to accomplish this, the matrix effects induced by the presence of variable ethanol contents were studied at different hTISIS spray chamber temperatures under continuous and air-segmented sample introduction modes.

2. Experimental

2.1. Solutions and samples

Ethanol-water mixtures were prepared using analytical grade ethanol 96% (Panreac, Spain) and ultrapure water ($R < 18.2 \text{ M}\Omega$) obtained with a Millipore water purification system (El Paso, TX, USA). Blanks containing variable ethanol concentrations were also prepared. Multielemental solutions were obtained from a stock solution (Merck IV, Darmstadt, Germany) and filtered with a $0.45 \text{ }\mu\text{m}$ PTFE filter pore size (Filabet, Barcelona, Spain).

Twenty-eight bioethanol real samples containing water concentrations from 0 to 45% were analyzed. These samples were 1:1 (v/v) diluted with ultrapure water ($R < 18.2 \text{ M}\Omega$) and the standards used to obtain the calibration line were prepared in a 1:1 (v/v) ethanol/water matrix. Recovery studies were performed in which the diluted samples were spiked with the multielemental stock solution. In this case, non spiked samples were taken as blanks.

Solution physical properties were measured. The viscosity was obtained with an Ostwald viscometer employing ultrapure water as the reference solvent. In order to measure the density, 5 mL of each standard were weighed. Finally, the surface tension was calculated by determining the weight of 30 drops provided by a peristaltic pump

under controlled conditions using ultrapure water as reference solvent. The obtained data are summarized in Table 1 for eight representative situations: three water-ethanol mixtures and five real samples having variable ethanol contents. It was verified that density and surface tension decreased with the ethanol content whereas the viscosity depended on the water content. Interestingly, these physical properties significantly differed as a function of the real sample considered. Therefore, changes in the performance of the system were expected.

2.2. Instrumentation

Drop size distributions of the aerosols generated by the nebulizer (*i.e.*, primary aerosols) were measured by means of a laser diffraction instrument (model 2600c, Malvern Instruments, Malvern Worcestershire, UK).

An Optima 4300 DV Perkin-Elmer ICP-OES spectrometer (Überlingen, Germany) was employed to axially take the intensities. The spectrometer was equipped with the hTISIS equipped with a 9 cm³ single-pass spray chamber. The operating conditions are gathered in Table 2. In the present work, a glass pneumatic concentric nebulizer, TR-50-C0.5 (Meinhard Glass Products, Santa Ana, USA) was employed. Strictly speaking this is not considered as a 'micro nebulizer', however it was able to work in a stable fashion, thus leading to satisfactory analytical figures of merit, when liquid flow rates on the order of tens of microliters per minute were selected.

The solutions were delivered to the nebulizer by means of a peristaltic pump (Perimax 16 antiplus, Spetec) and a 0.19-mm id flared end PVC-based tubing (Glass Expansion, Melbourne, Australia) was employed. In the segmented flow injection

methodology the peristaltic pump continuously aspirated air. A given sample volume was measured with an automatic pipette (Eppendorf, Hamburg, Germany). Then, the nozzle was adapted to the flared end tubing and the solution was aspirated by means of the peristaltic pump. The sample plug was driven to the nebulizer thus avoiding sample dispersion, as the carrier stream was simply air.

3. Results and discussion

3.1. Drop size distribution

Figure 1 plots the median of the volume drop size distribution (D_{50}) for the primary aerosols and the ethanol solutions considered in Table 1. It was verified that the higher the ethanol content the lower the D_{50} mainly because of the reduction in surface tension. Also interesting was the fact that the bioethanol samples provided aerosols with different D_{50} values. This was clearly due to a modification in the ethanol content and anticipated the appearance of a matrix effect. Finally, it was verified that the aerosols obtained for plain water, led to D_{50} values much higher (*i.e.*, 38.4 μm) than those generated when ethanol was present.

3.2. Effect of the sample pre-treatment

The complexity of the matrix made it necessary to evaluate the stability of the samples. Several treatments were evaluated for an ethanol/water multielemental solution: (i) Sample sonication for 10 minutes just before its analysis; (ii) continuous

sample sonication even when it was introduced into the spectrometer; (iii) sample analysis at room temperature without sonication; (iv) manual sample stirring for one minute just before its analysis; (v) sample cooling at 4°C with no additional treatment; and, (vi) addition of 1% nitric acid to the sample with no additional treatment.

In order to carry out these experiments, the prepared solution was separated in six different aliquots and the above mentioned treatments were applied. The sample analyses were carried out just after the sample preparation, one hour later and three hours later. From the signal data, it was clearly observed that the sample treatment had a marked influence on the obtained sensitivity. Thus, for instance, when the sample was continuously sonicated, treatment (ii), the signal was about twice that obtained when nitric acid was added, treatment (vi). However, this was likely due to the increase in the sample temperature observed in the former case that promoted the aerosol evaporation inside the spray chamber with a concomitant increase in the analyte transport efficiency. The sample temperature increased by 8.2°C when the treatment (i) was applied although the sample volume did not change during this process indicating that solvent evaporation did not take place when the sample was sonicated for 10 minutes. In the case of treatment (i), the sensitivity was around 20% higher than for the four remaining treatments that, indeed, provided similar sensitivities among them. As a result, sample sonication for 10 min prior to the analysis was advisable to improve the sensitivity of the determinations.

3.3. Effect of hTISIS temperature on sensitivity and matrix effects in segmented flow injection

Obviously, the introduction of a small sample volume in an air carrier gas stream yielded transient signals or peaks. Figure 2 shows the peaks registered for the matrices studied and the hTISIS operated at room temperature (Figure 2.a), 200°C (Figure 2.b), 350°C (Figure 2.c) and 400°C (Figure 2.d). It was observed that the improvement in terms of peak height when hTISIS was heated with respect to room temperature depended on the matrix considered. Thus, for water the peak height steeply increased with the chamber temperature. Meanwhile, for 30% and 50% ethanol solutions, the sensitivity reached a plateau at 200°C whereas for 96% ethanol the sensitivity reached a maximum at 200°C and then slowly decreased with temperature. Globally speaking, Figure 2 suggests that the optimum hTISIS temperature was 200°C. These trends were common for all the studied emission lines.

The plasma thermal state was also investigated. To this end, the Ar emission intensity and Mg ionic to atomic net intensity ratio ($MgII/MgI$) were obtained. At room temperature, the Mg ratio was highly affected by the matrix. In fact, a decrease in $MgII/MgI$ was observed as the ethanol content rose. As regards Ar 420.069 nm intensity, it was found that a positive peak was recorded under all the circumstances evaluated. In other words, the plasma appeared not to be degraded as the sample plug reached it. This fact revealed the beneficial role of moderate water loads on the plasma thermal conductivity. At room temperature (Figure 2.a), the argon peak for water was lower and wider than for ethanol matrices. In fact, for 0% ethanol, a plateau was reached likely indicating that a fraction of the aerosol initially impacted against the chamber wall and then evaporated. This trend was partially observed for 30% ethanol as a peak overlapped with the flat top signal. Finally, 50% and 96% ethanol matrices afforded sharp peaks (Figure 2.a). For ethanol-water mixtures, the solvent evaporation

from the chamber wall was apparently less intense likely because of the increase in the aerosol transport yield (*i.e.*, reduction of the droplet impact losses). This was in concordance with the lower boiling point of the mixtures and the finer primary aerosols as compared to plain water (Figure 1).

When the hTISIS wall was heated, the impact of ethanol on the plasma state was lower than at room temperature. For instance, at 400°C, the Mg ratio and Ar signals were similar for all the ethanol solutions. Under these circumstances, the totality of the solvent was delivered as a vapor. As a consequence, the plasma energy was not consumed in the solvent vaporization. For the selected RF power the plasma was immune to the discrete introduction of ethanol. All these results are in agreement with previous published work revealing that the plasma electron number density [34,35] and excitation temperature [36] increase in presence of ethanol.

The ethanol – water proportion has a direct influence on sensitivity. In fact, Rocha et al. [28] reported that for some elements 7% of water in hydrated fuel ethanol induced a 30% signal decrease with respect to anhydrous fuel ethanol. Therefore, the influence of the hTISIS temperature in terms of matrix effects was evaluated. The results shown in Figure 2.a reveal that when hTISIS was operated at room temperature, the peak height registered for water was around 20 times lower than that measured for ethanol. In contrast, this parameter became similar irrespectively of the matrix considered as the TISIS temperature was increased up to 400°C, (Figure 2.d). These findings suggested that heating the chamber wall mitigated non-spectral interferences.

In order to evaluate the effect of hTISIS temperature on matrix effects in quantitative terms, the relative intensity was calculated for each analyte according to the following mathematical relationship:

$$I_r = \frac{\text{Intensity for matrix}_i \text{ at } T_j}{\text{Intensity for 96\% ethanol at } T_j} \quad (1)$$

A value of this parameter close to the unity indicated that matrix effects were corrected for. The relative intensities for four analytes (Mn II 257.610 nm, Al I 396.153 nm, Co II 238.892 nm and Mg II 280.271 nm) are shown in Figure 3. As it is observed, the higher the temperature, the higher (and closer to the unity) the relative intensity. In other words, matrix effects caused by ethanol were removed when the hTISIS was heated. For Mn, Al and Co, the influence of the ethanol content on the peak height disappeared at 400°C. However, Mg 280.271, as well as additional easily ionized elements, showed different behaviors. For these analytes the matrix effects could be removed at temperatures lower than 400°C (Figure 3.d). These trends can be explained by taking into account that, at high chamber temperatures and low injected sample volumes, the totality of the solution evaporated in aerosol phase inside the chamber. The direct consequence was that the analyte transport efficiency approached to 100% regardless of the sample matrix. As the plasma conditions were kept at a rather robust level, sensitivity did not depend on the ethanol content.

Effect of hTISIS temperature on sensitivity and matrix effects in continuous aspiration mode

The work under discrete air-segmented flow injection shows some benefits mainly related with the sample consumed volume. However, many routine methods are based on the continuous sample aspiration. The hTISIS suitability for the analysis of ethanol-water mixtures was also tested when the sample was continuously delivered

to the nebulizer at low liquid flow rates (*c.a.*, 25 $\mu\text{L min}^{-1}$). The nebulization gas flow rate (Q_g) was optimized at room temperature. In order to reach this goal, emission intensities for pure ethanol and water were taken at Q_g values between 0.2 and 0.5 L min^{-1} . The results revealed that sensitivity for ethanol reached a maximum at 0.4 L min^{-1} and it decreased at higher flow rates. Meanwhile, the sensitivity for water increased until 0.3 L min^{-1} and then it reached a plateau. On the other hand, the sensitivity improvement when the nebulizer flow rate increased was more pronounced for ethanol than for water. Once the nebulizer gas flow rate was optimized, the hTISIS temperature was modified. It was observed that an increase in this variable led to a growth in the signal with respect to room temperature by a factor going from 4 to 9 and from 1 to 2 for ethanol and water, respectively. The optimum conditions in terms of signal improvement for water were hTISIS temperature: 200°C and nebulizer gas flow rate: 0.3 L min^{-1} whereas the highest signal for pure ethanol was found at 200°C and 0.4 L min^{-1} .

The effect of hTISIS temperature on matrix effects was also evaluated when the hTISIS was working in continuous aspiration. The matrix effects at room temperature were very pronounced for the two Q_g studied. When hTISIS chamber wall temperature increased up to 200°C and the nebulizer gas flow rate was kept at 0.3 L min^{-1} , the matrix effects were virtually removed. In conclusion, the best conditions in terms of sensitivity according to gas flow optimization did not correspond to those in terms of matrix effects mitigation.

Limits of detection

The limits of detection obtained with the hTISIS in segmented flow injection are shown in Table 4. This parameter was calculated according to the $3 s_b / m$ criterion where s_b corresponded to the standard deviation of twenty consecutive measurements of the background and m was the slope of the calibration line. There was not a correlation between LOD and sample injection method. These figures were taken as reference to perform real bioethanol samples analyses.

The information regarding international standards regarding bioethanol analysis is scarce. According to the ASTM D1688 (2012), the maximum allowable level for Cu is 0.1 mg / Kg. This value is above the limit of detection found if the sample is continuously aspirated (see Table 4).

4. Recovery tests

Four bioethanol real samples were spiked with an aqueous multielement standard at 250 ng mL⁻¹. The calibration line was obtained from aqueous standards. Appendix A demonstrates that the obtained recoveries were similar regardless the sample introduction mode employed. In general terms, the recoveries obtained for four spiked samples were between 80% and 120% except for Ag that showed recoveries far from 100% in segmented injection mode and Na and K that provided anomalous recoveries in continuous aspiration.

Alternatively, bioethanol samples were preconcentrated. . Thus, 40 mL of real sample were left to evaporate for 24 h in a hood at 35°C. Once this step was completed, the solid residue was dissolved by adding 8 mL of either ethanol or water.

The solutions were sonicated for 5 min and analyzed through ICP-OES. The obtained results were compared against those found with the hTISIS through an external calibration with aqueous standards. The obtained results revealed that both methodologies (direct analysis and preconcentration) provided similar concentrations. Thus, for instance, for sample B1 the Zn concentration was $642 \pm 28 \text{ ng mL}^{-1}$ and $633 \pm 8 \text{ ng mL}^{-1}$ for direct analysis and preconcentration, respectively. In the case of Ni in sample B7, the respective concentrations were $22 \pm 10 \text{ ng mL}^{-1}$ and $16.4 \pm 0.8 \text{ ng mL}^{-1}$.

5. Analysis of real samples

Although intensity measurements were taken at all the wavelengths indicated in Table 2, the LOD (Table 4) and found concentrations (Table 5 and Table 6) were shown for a selected wavelength per analyte, because the obtained concentrations for a given element were not a function of the emission wavelength.

5.1. hTISIS-ICP-OES-segmented injection

The concentrations obtained in discrete mode are shown in Table 5. Mg and Zn were found in some samples at hundreds of ppb whereas Cr, Fe and Mn were present at trace levels. Concentrations for Ag, Al, B, Ba, Cd, Co, Cu, In, Li and Pb were lower than LOD for all the samples analyzed. On the other hand, the determination of sodium, potassium and calcium was not possible with this methodology because the calibration line reported very bad linearity due to the spectral band widening observed at high

elemental concentrations. As a result, a fraction of the peaks were out of the integration window.

5.2. hTISIS-ICP-OES-continuous injection

The found concentrations through hTISIS-ICP-OES in continuous injection mode are shown in Table 6 whereas the limits of detection are gathered in Table 4. In this case, K, Na, Mg and Zn were found at hundreds of ppb for some samples and Mn, Al, Cd, Cr and Cu at tens of ppb. As in segmented flow injection, a lot of analytes were present at contents below the LOD (Ag, Ba, Ca, Co, Fe, In, Ni and Pb). However, in continuous mode the linearity for sodium, potassium and calcium was acceptable making it possible the determination of these three analytes.

5.3. Comparison between continuous and segmented flow injection

The sensitivity working in segmented flow injection was between 1.5 and 4 times higher than that obtained in continuous injection mode, depending on the analyte. This was expected because the liquid flow rate in continuous mode was around 3 times lower than in segmented injection. However, copper, which also showed anomalies in terms of recovery provided a sensitivity lower in segmented flow.

According to these values of sensitivity, lower LODs were expected in segmented injection than those found in continuous aspiration. However, the blank SDs were higher for the former methodology. As a result, no a clear trend in LODs was noticed. The signal oscillations when the solvent reached the plasma were not

meaningful for the standards with high analyte concentrations but they played an important role when the signal level was low, as in the case of the blank standard.

6. Conclusions

The hTISIS has been employed for the analysis of several bioethanol samples through ICP-OES. This is the first time that direct ICP analysis is employed to provide elemental concentration in this kind of samples, because the existing studies have focused on the analysis of ethanol fuel (a mixture of ethanol and gasoline). The increase in the temperature of a single pass spray chamber while keeping the sample consumed volume at low levels results beneficial from the point of view of sensitivity enhancement and elimination of the matrix effects. As a result, it is possible to perform the analysis of bioethanol samples containing different levels of water with a single set of aqueous standards.

Continuous sample introduction can be used in combination with hTISIS-ICP-OES thus avoiding the need for handling transient signals. However, the obtained results suggest that a more sensitive technique should be employed to obtain information about the presence of further elements in bioethanol. A good approach would be to adapt the hTISIS to an ICP-MS spectrometer. In this case, sample segmentation is highly interesting as the likelihood for soot deposits formation on the sampler cone is less significant than for the sample continuous aspiration mode. These experiments are being currently performed in our laboratories and will be the subject of additional reports.

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References

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- [1] H. Habe, T. Shinbo, T. Yamamoto, S. Sato, H. Shimada, and K. Sakaki, Chemical Analysis of impurities in diverse bioethanol samples, *J. Japan Pet. Inst.*, 56 (2013) 414–422.
- [2] G. M. Walker, *Bioethanol: Science and technology of fuel alcohol*, Ventus Publishing ApS, 2010.
- [3] D. Chiche, C. Diverchy, A. C. Lucquin, F. Porcheron, and F. Defoort, Syntesis gas purification, *Oil Gas Sci. Technol. – Rev. d'IFP Energies Nouv.*, 68 (2013) 707–723.
- [4] E. G. Vieira, I. V Soares, N. L. Dias Filho, N. C. da Silva, E. F. Garcia, A. C. Bastos, S. D. Perujo, T. T. Ferreira, A. H. Rosa, and L. F. Fraceto, Preconcentration and determination of metal ions from fuel ethanol with a new 2,2'-dipyridylamine bonded silica, *J. Colloid Interface Sci.*, 391 (2013) 116–24.
- [5] L. N. Santos, J. A. G. Neto, and N. M. Caldas, Simultaneous determination of Cu and Pb in fuel ethanol by graphite furnace AAS using tungsten permanent modifier with co-injection of Ir, *Fuel*, 99 (2012) 9–12.
- [6] G. L. Donati, R. S. Amais, D. Schiavo, and J.A. Nóbrega, Determination of Cr, Ni, Pb and V in gasoline and ethanol fuel by microwave plasma optical emission spectrometry, *J. Anal. At. Spectrom.*, 28 (2013) 755-759.
- [7] J. C. Dias, L. T. Kubota, P. N. Nesterenko, G. W. Dicinoski, and P. R. Haddad, A new high-performance chelation ion chromatographic system for the direct determination of trace transition metals in fuel ethanol, *Anal. Methods*, 2 (2010) 1565-1570.

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- [8] P. S. Roldan, I. L. Alcântara, G. R. Castro, J. C. Rocha, C. C. F. Padilha, and P. M. Padilha, Determination of Cu, Ni, and Zn in fuel ethanol by FAAS after enrichment in column packed with 2-aminothiazole-modified silica gel, *Anal. Bioanal. Chem.*, 375 (2003) 574-577.
- [9] T. D. Saint’Pierre, T. D. A. Maranhão, V. L. A. Frescura, and A. J. Curtius, The development of a method for the determination of trace elements in fuel alcohol by electrothermal vaporization-inductively coupled plasma mass spectrometry using external calibration *Spectrochim. Acta - Part B At. Spectrosc.*, 60 (2005) 605-613.
- [10] R. Sánchez, C. Sánchez, C.-P. Lienemann, and J.-L. Todolí, Metal and metalloid determination in biodiesel and bioethanol, *J. Anal. At. Spectrom.*, 30 (2015) 64–101.
- [11] A. P. De Oliveira, J. A. G. Neto, and M. M. C. Ferreira, Uso da análise exploratória de dados na avaliação de modificadores químicos para determinação direta e simultânea de metais em álcool combustível por GFAAS, *Ecletica Quimica*, 31 (2006) 7–12.
- [12] A. P. De Oliveira, M. De Moraes, J. A. Gomes Neto, and E. C. Lima, Direct determination of Al, As, Cu, Fe, Mn, and Ni in fuel ethanol by simultaneous GFAAS using integrated platforms pretreated with W-Rh permanent modifier together with Pd+Mg modifier, *At. Spectrosc.*, 23 (2002) 190-195.
- [13] A. P. De Oliveira, M. De Moraes, J. A. Gomes Neto, and E. C. Lima, Simultaneous determination of Al, As, Cu, Fe, Mn, and Ni in fuel ethanol by GFAAS, *At. Spectrosc.*, 23 (2002) 39-43.

-
- [14] T. Saint’Pierre, R. Q. Aucélio, and A. J. Curtius, Trace elemental determination in alcohol automotive fuel by electrothermal atomic absorption spectrometry, *Microchem. J.*, 75 (2003) 59–67.
- [15] M. B. O. Giacomelli, J. B. B. Da Silva, T. D. Saint’Pierre, A. J. Curtius, and J. B. B. Da Silva, Use of iridium plus rhodium as permanent modifier to determine As, Cd and Pb in acids and ethanol by electrothermal atomic absorption spectrometry, *Microchem. J.*, 77 (2004) 151-156.
- [16] T. D. Saint’Pierre, T. D. A. Maranhão, V. L. Frescura, A. J. Curtius, and R. Q. Aucélio, Determination of Cd and Pb in fuel ethanol by filter furnace electrothermal atomic absorption spectrometry, *Quim. Nova*, 31 (2008) 1626–1630.
- [17] J. Motonaka, H. Konishi, S. Ikeda, and N. Tanaka, Behavior of the Cadmium Ion-Selective Electrode in Alcohol-Water Mixtures, *Bull. Chem. Soc. Jpn.*, 59 (1986) 737 – 740.
- [18] A. I. Kamenev and M. A. Kovalenko, Determination of Lead (II) in Water-Alcohol Media by Stripping Voltammetry at a Modified Carbon-Glass-Ceramic Electrode, *J. Anal. Chem.*, 55 (2000) 594–597.
- [19] E. S. Almeida, E. M. Richter, and R. A. Munoz, On-site fuel electroanalysis - Determination of lead, copper and mercury in fuel bioethanol by anodic stripping voltammetry using screen-printed gold electrodes, *Anal. Chim. Acta*, 837 (2014) 38–43.

- [20] T. F. Tormin, L. C. D. Narciso, E. M. Richter, and R. A. A. Munoz, Batch-injection stripping voltammetry of zinc at a gold electrode: application for fuel bioethanol analysis, *Electrochim. Acta*, 164 (2015) 90–96.
- [21] T. F. Tormin, L. C. D. Narciso, E. M. Richter, and R. A. Munoz, Simultaneous determination of Cu, Pb, Cd, Ni, Co and Zn in bioethanol fuel by adsorptive stripping voltammetry and multivariate linear regression, *Fuel*, 117 (2014) 952–956.
- [22] V. N. Alves, R. Mosquetta, N. M. M. Coelho, J. N. Bianchin, K. C. Di Pietro Roux, E. Martendal, and E. Carasek, Determination of cadmium in alcohol fuel using *Moringa oleifera* seeds as a biosorbent in an on-line system coupled to FAAS, *Talanta*, 80 (2010) 1133–1138.
- [23] C. Pesco, E. A. De Campos, C. Maieru, and M. Costa, Preconcentration of Transition Metal Ions from Ethanol Solution on N-Acyl-N / -Benzoylthiourea - Modified Silica Gel, *Mikrochim. Acta*, 232 (1997) 229–232.
- [24] L. A. de M. Gomes, P. de M. Padilha, J. C. Moreira, N. L. D. Filho, and Y. Gushikem, Determination of Metal Ions in Fuel Ethanol after Preconcentration on 5-Amino-1,3,4-Thiadiazole-2-Thiol Modified Silica Gel, *J. Braz. Chem. Soc.*, 9 (1998) 494–498.
- [25] P. Lessi, N. L. D. Filho, C. Moreira, and J. T. S. Campos, Sorption and preconcentration of metal ions on silica gel modified with 2,5-dimercapto-1,3,4-thiadiazole, *Anal. Chim. Acta*, 327 (1996) 183-190.
- [26] J. N. Bianchin, E. Martendal, R. Mior, V. N. Alves, C. S. T. Araújo, N. M. M. Coelho, and E. Carasek, Development of a flow system for the determination of

- cadmium in fuel alcohol using vermicompost as biosorbent and flame atomic absorption spectrometry, *Talanta*, 78 (2009) 333–6.
- [27] D. R. Wiederin, R. S. Houk, R. K. Winge, and A. P. D'Silva, Introduction of Organic Solvents into Inductively Coupled Plasmas by Ultrasonic Nebulization with Cryogenic Desolvation *Anal. Chem.*, 62 (1990) 1155–1160.
- [28] M. S. Rocha, M. F. Mesko, F. F. Silva, R. C. Sena, M. C. B. Quaresma, T. O. Araújo, and L. A. Reis, Determination of Cu and Fe in fuel ethanol by ICP OES using direct sample introduction by an ultrasonic nebulizer and membrane desolvator, *J. Anal. At. Spectrom.*, 26 (2011) 456–461.
- [29] T. D. Saint'Pierre, L. Tormen, V. L. Frescura, and A. J. Curtius, The direct analysis of fuel ethanol by ICP-MS using a flow injection system coupled to an ultrasonic nebulizer for sample introduction, *J. Anal. At. Spectrom.*, 21 (2006) 1340–1344.
- [30] D. R. Neves, R. S. Amais, J. A. Nóbrega, and J. A. G. Neto, Assessment of Polyatomic Interferences Elimination Using a Collision Reaction Interface (CRI) for Inorganic Analysis of Fuel Ethanol by ICP-QMS, *Anal. Lett.*, 45 (2012) 1111–1121.
- [31] E. McCurdy and D. Potter, Techniques for the Analysis of Organic Chemicals by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), *Agil. Technol.*, 1 (2002) 5988–6190E.
- [32] F. Ardini, M. Grotti, R. Sánchez and J. L. Todolí, *J. Anal. At. Spectrom.*, Improving the analytical performances of ICP-AES by using a high-temperature single-pass spray chamber and segmented-injections micro-sample introduction for the analysis of environmental samples, 27 (2012) 1400–1404.

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- [33] R. Sanchez, J. L. Todoli, C.-P. Lienemann, and J.-M. Mermet, Universal calibration for metal determination in fuels and biofuels by inductively coupled plasma atomic emission spectrometry based on segmented flow injection and a 350 °C heated chamber, *J. Anal. At. Spectrom.*, 27 (2012) 937-945.
- [34] R. I. McCrindle and C. J. Rademeyer, Electron density and hydrogen distribution in an ethanol-loaded inductively coupled plasma, *J. Anal. At. Spectrom.*, 9 (1994) 1087-1091.
- [35] R. I. McCrindle and C. J. Rademeyer, Ethanol loading in an inductively coupled plasma., *Anal. Bioanal. Chem.*, 355 (1996) 264-266.
- [36] R. I. McCrindle and C. J. Rademeyer, Excitation temperature and analytical parameters for an ethanol-loaded inductively coupled plasma atomic emission spectrometer, *J. Anal. At. Spectrom.*, 10 (1995) 399-404.

Table 1. Physical properties for a series of samples with different ethanol content.

Samples	Ethanol content (%, v/v)	Density (g mL ⁻¹)	Surface tension (mN m ⁻¹)	viscosity (mPa s)
Water	0	1.0021 ± 0.0006	72	0.8909
Water/EtOH	50%	0.942 ± 0.003	29.1 ± 1.0	2.131 ± 0.003
EtOH	100	0.814 ± 0.004	21.98 ± 0.09	1.249 ± 0.008
B5	70	0.882 ± 0.003	25.3 ± 0.8	2.038 ± 0.014
B2	93	0.827 ± 0.002	21.3 ± 0.5	1.412 ± 0.004
B7	98	0.825 ± 0.004	22.70 ± 0.14	1.359 ± 0.010
B3	100	0.801 ± 0.002	20.9 ± 0.5	1.240 ± 0.008
B8	55	0.910 ± 0.003	27.5 ± 0.2	2.19 ± 0.03

Table 2. ICP-OES operating conditions.

	hTISIS in segmented injection flow	hTISIS in continuous aspiration
	Plasma: 15	Plasma: 15
Ar flow rates/L min ⁻¹	Auxiliary: 0.2	Auxiliary: 0.2
	Nebulizer: 0.4	Nebulizer: 0.3, 0.4
Volume injected/μL	5	-----
Liquid aspiration conditions	350 rpm (air)	25 μL min ⁻¹

Emission lines /nm	Ag I 328.068	Cr II 267.716	Mg II 280.271
	Al I 396.153	Cu II 213.597	Mg II 279.077
	Ar 420.069	Cu I 324.752	Mn II 257.610
	Ba II 233.527	Cu I 327.393	Na I 589.592
	Ca II 317.933	Fe II 239.562	Ni II 231.604
	Cd I 228.802	Fe II 238.204	Pb II 220.353
	Co II 228.616	In II 230.606	Zn II 206.200
	Co II 238.892	K I 766.490	Zn I 213.857
	Cr II 205.560	Li I 670.784	Zn II 202.548
		Mg I 285.213	
RF power/W	1400		
Number of replicates	5		

Table 4. Limits of detection (ng mL^{-1}) obtained in both Injection methodologies.

Element	Continuous sample aspiration	Air-segmented flow injection analysis
Ag I 328.068 nm	22	72
Al I 396.153 nm	20	62
Ba II 233.527 nm	162	8
Ca II 317.933 nm	488	476

Cd I 228.802 nm	18	24
Co II 228.616 nm	24	13
Cr II 267.716 nm	12	4
Cu II 213.597 nm	62	520
Fe II 238.204 nm	18	28
In II 230.606 nm	142	558
Mg II 280.271 nm	8	62
Mn II 257.610 nm	3	3
Ni II 231.604 nm	16	5
Pb II 220.353 nm	64	52
Zn II 206.200 nm	218	42

Table 5. Found concentrations (in ng mL⁻¹) in bioethanol real samples through hTISIS-ICP-OES in segmented flow injection. The confidence level* has been obtained for n=3 and $\alpha=0.05$.

Sample	Cr	Fe	Mg	Mn	Zn
B1	15.6 ± 1.0	< 28	< 62	4.6 ± 1.0	516 ± 128
B2	32 ± 6	< 28	< 62	4.4 ± 0.6	44 ± 28
B3	16 ± 2	< 28	< 62	3.0 ± 0.4	< 42
B4	14.6 ± 0.8	< 28	< 62	3.0 ± 0.8	52 ± 28

B5	28 ± 18	28 ± 12	418 ± 34	12 ± 6	< 42
B6	13.8 ± 1.8	< 28	< 62	3.8 ± 1.2	< 42
B7	13.8 ± 0.4	< 28	< 62	4.2 ± 0.6	134 ± 32
B8	13.6 ± 1.6	28 ± 10	< 62	4.6 ± 1.8	< 42
B9	18 ± 4	< 28	< 62	3.0 ± 1.0	< 42
B10	15.4 ± 1.6	< 28	< 62	4.2 ± 1.8	< 42
B11	16 ± 2	32 ± 8	< 62	4.0 ± 1.4	< 42
B12	14.2 ± 0.8	< 28	< 62	3.6 ± 0.4	< 42
B13	13.2 ± 1.0	< 28	< 62	3.6 ± 1.0	< 42
B14	14.4 ± 1.4	32 ± 12	< 62	5 ± 2	< 42
B15	13.6 ± 1.0	< 28	< 62	3.2 ± 0.4	< 42
B16	13.6 ± 0.8	32 ± 16	< 62	3.6 ± 0.6	< 42
B17	< 4	< 28	< 62	< 3	< 42
B18	< 4	< 28	< 62	< 3	< 42
B19	< 4	< 28	< 62	< 3	< 42
B20	< 4	< 28	< 62	< 3	< 42
B21	< 4	< 28	< 62	< 3	< 42
B22	< 4	< 28	< 62	< 3	< 42
B23	< 4	< 28	< 62	< 3	< 42
B24	< 4	< 28	< 62	< 3	< 42
B25	< 4	< 28	< 62	< 3	< 42
B26	< 4	< 28	< 62	< 3	< 42
B27	11 ± 5	< 28	< 62	< 3	< 42
B28	< 4	157 ± 26	61 ± 31	7.4 ± 1.7	< 42

* Obtained according to $t_{s/\sqrt{n}}$ ($\alpha=0.05$, $n=3$)

Table 6. Found concentrations (in ng mL^{-1}) in bioethanol real samples through hTISIS-ICP-OES in continuous injection. The confidence level* has been obtained for $n=3$ and $\alpha=0.05$.

Sample	Al	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
B1	56 ± 4	< 488	20 ± 6	< 12	< 62	< 18	50 ± 2	< 8	< 3	528 ± 58	< 16	642 ± 28
B2	40 ± 6	< 488	18 ± 6	< 12	< 62	< 18	74 ± 4	< 8	< 3	1238 ± 36	< 16	< 218

B3	42 ± 8	< 488	22 ± 6	< 12	< 62	< 18	62 ± 4	< 8	< 3	848 ± 80	< 16	< 218
B4	48 ± 6	< 488	18 ± 4	12 ± 1	< 62	< 18	37.4 ± 1.8	< 8	< 3	194 ± 52	< 16	< 218
B5	< 20	< 488	< 18	< 12	< 62	< 18	430 ± 10	762 ± 46	14.2 ± 0.8	1506 ± 48	< 16	< 218
B6	40 ± 10	< 488	20 ± 10	< 12	< 62	< 18	74 ± 10	< 8	< 3	< 400	< 16	< 218
B7	24 ± 4	< 488	< 18	< 12	< 62	< 18	86 ± 6	< 8	< 3	< 400	22 ± 10	< 218
B8	< 20	< 488	< 18	< 12	< 62	< 18	96 ± 6	< 8	< 3	< 400	< 16	< 218
B9	46 ± 4	< 488	22 ± 6	20 ± 3	< 62	< 18	146 ± 8	< 8	< 3	952 ± 62	< 16	< 218
B10	46 ± 10	< 488	< 18	< 12	< 62	< 18	138 ± 14	< 8	< 3	924 ± 46	< 16	< 218
B11	50 ± 6	< 488	18 ± 6	12 ± 4	< 62	< 18	61 ± 4	< 8	< 3	694 ± 56	< 16	< 218
B12	48 ± 6	< 488	22 ± 6	< 12	< 62	< 18	57 ± 3	< 8	< 3	594 ± 72	< 16	< 218
B13	58 ± 4	< 488	22 ± 6	12 ± 2	< 62	< 18	58 ± 6	< 8	< 3	502 ± 64	< 16	< 218
B14	58 ± 4	< 488	< 18	< 12	< 62	< 18	60 ± 3	< 8	< 3	730 ± 24	< 16	< 218
B15	56 ± 4	< 488	< 18	< 12	64 ± 4	< 18	31 ± 2	< 8	< 3	420 ± 42	< 16	< 218
B16	54 ± 8	< 488	20 ± 3	< 12	62 ± 14	< 18	38 ± 4	< 8	< 3	558 ± 78	< 16	< 218
B17	< 20	< 488	19 ± 4	< 12	< 62	< 18	< 4	< 8	< 3	773 ± 86	< 16	< 218
B18	62 ± 5	< 488	< 18	< 12	< 62	< 18	< 4	< 8	< 3	659 ± 76	< 16	< 218
B19	62 ± 8	< 488	< 18	< 12	75 ± 10	< 18	641 ± 55	< 8	< 3	1200 ± 44	< 16	< 218
B20	< 20	< 488	< 18	< 12	74 ± 11	< 18	< 4	63 ± 5	< 3	1160 ± 56	< 16	< 218
B21	64 ± 4	< 488	< 18	< 12	79 ± 11	< 18	255 ± 25	< 8	< 3	767 ± 45	< 16	< 218
B22	65 ± 13	< 488	< 18	< 12	68 ± 19	< 18	174 ± 58	< 8	< 3	887 ± 52	< 16	< 218
B23	68 ± 6	93 ± 36	< 18	< 12	< 62	< 18	991 ± 28	20.4 ± 1.6	< 3	1312 ± 28	< 16	< 218
B24	< 20	< 488	< 18	< 12	73 ± 37	< 18	< 4	< 8	< 3	689 ± 24	< 16	< 218
B25	< 20	< 488	< 18	< 12	70 ± 22	< 18	< 4	< 8	< 3	588 ± 114	< 16	< 218
B26	< 20	< 488	< 18	< 12	< 62	< 18	783 ± 36	< 8	< 3	1048 ± 58	< 16	< 218
B27	< 20	169 ± 64	< 18	< 12	82 ± 18	< 18	507 ± 35	18 ± 3	< 3	1225 ± 31	< 16	< 218

B28	< 20	244 ± 45	< 18	< 12	< 62	164 ± 22	< 4	38 ± 11	10 ± 6	1470 ± 47	57 ± 5	< 218
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* Obtained according to $t_{s/\sqrt{n}}$ ($\alpha=0.05$, $n=3$)

Figure 1. D₅₀ of primary aerosols for solutions containing different percentage in ethanol. Liquid flow rate: 200 $\mu\text{L min}^{-1}$; nebulizer gas flow rate: 0.5 L min^{-1} . Error bars obtained according to $t_{s/\sqrt{n}}$ ($\alpha=0.05$, $n=3$).

Figure 2. Peaks for Mn 257.610 (orange) and Ar 420.069 (blue) and magnesium ratio in the maximum of the peak (red triangles) for several water-ethanol mixtures at: (a) room temperature; (b) 200°C; (c) 350°C and (d) 400°C. Error bars for Mg II/ Mg I were obtained according to error propagation method.

Figure 3. Relative intensity under discrete sample injection versus the hTISIS temperature. (a) Mn II 257.610 nm; (b) Al I 396.153 nm; (c) Co II 238.892 nm; (d) Mg II 280.271 nm. Error bars obtained by means error propagation method taking into account the maximum RSD for each signal measured.

Appendix A. Maximum and minimum found recoveries (%) for seven spiked samples of bioethanol (B1, B2, B3, B4, B17, B23 and B25). The samples were spiked with 250 ng mL⁻¹ of an aqueous standard.

Element emission line	Segmented flow injection	Continuous sample aspiration
Ag I 328.068 nm	56.0 – 147.1	81.7 – 110.3
Al I 396.153 nm	93.3 – 105.1	92.6 – 119.8
Ba II 233.527 nm	91.7 – 115.0	94.2 - 112.8
Ca II 317.933 nm	95.7 – 114.8	90.9 - 109.6
Cd I 228.802 nm	74.3 – 101.8	93.8 – 111.4
Co II 228.616 nm	93.4 – 121.7	94.5 – 113.9
Cr II 267.716 nm	86.5 – 118.0	92.4 – 112.5
Cu I 213.597 nm	85.5 – 119.7	87.2 – 107.7
Fe II 238.204 nm	80.3 – 116.0	87.8 – 112.2
Fe II 239.562 nm	113.9 – 123.0	88.3 – 111.9
In II 230.606 nm	96.7 - 118.1	79.7 – 112.6
K I 766.490 nm	n.a.	48.9 – 118.6
Mg II 280.271 nm	88.0 - 98.8	98.0 - 122.4
Mn II 257.610 nm	92.9 – 119.4	93.7 – 114.0
Na I 589.592 nm	76.9 - 101.0	47.3 - 184.4
Ni II 231.604 nm	90.8 – 115.8	94.9 - 111.5
Pb II 220.353 nm	92.5 – 108.0	95.6 – 110.0
Zn II 206.200 nm	85.1 – 122.8	96.6 – 112.6

Highlights

- The aerosol heating leads to an increase in the ICP-OES sensitivity and a mitigation of the matrix effects caused by ethanol
- External calibration with aqueous standards can be performed by applying the high temperature torch integrated sample introduction system with accurate results
- Sample continuous introduction is compared against segmented flow injection