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Method development for Cd and Hg determination in biodiesel by electrothermal atomic absorption spectrometry with emulsion sample introduction

Pedro R. Aranda*, José A. Gásquez, Roberto A. Olsina, Luis D. Martinez, Raúl A. Gil

Instituto de Química de San Luis, (UNSL–CONICET), Área de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, San Luis, 5700 Argentina

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ABSTRACT

A novel method for analysis of biodiesel by electrothermal atomic absorption spectrometry is described. This analytical strategy involves sample preparation as emulsions for routine and reliable determination of Cd and Hg. Several experimental conditions were investigated, including emulsion stability and composition, furnace temperature program and matrix modification. Different calibration strategies were also evaluated, being the analyte addition method preferred both for Cd and Hg. The accuracy was verified through comparison with an acid digestion in a microwave closed system. The injection repeatability was evaluated as the average relative standard deviation (R.S.D %) for five successive firings and was better than 4.4% for Cd and 5.4% Hg respectively. The detection limits, evaluated by the 3σ concept of calculation ($n=10$), were of 10.2 μg kg^{-1} (0.9 μg L^{-1}) for Hg and 0.3 μg kg^{-1} (0.04 μg L^{-1}) for Cd. This method was successfully applied to the determination of Cd and Hg in biodiesel samples obtained from local vendors.

1. Introduction

Biodiesel is presented as a natural substitute of diesel being a renewable, biodegradable and non-toxic fuel derived from biological sources such as vegetable oil or animal fat [1,2]. The use of biodiesel presents many advantages, such as better lubricity than diesel fuel because of its higher viscosity and a significantly lower emission of carbon monoxide, carbon dioxide, sulfur dioxide and hydrocarbons [3–5]. Among others, As, Cd, Fe, Hg, Mn, P, Pb and V are released into the environment due to combustion of fuel in automobiles and are an important source of atmospheric pollution [6–9].

Cadmium and mercury are highly toxic elements because of their accumulative and persistent nature in the environment and biota [10–12]. As a result, sensitive, accurate and rapid analytical methods are required to monitor Cd and Hg in biological and environmental samples [13–15].

Electrothermal atomic absorption spectrometry (ETAAS) enables excellent sensitivity and has the capability to analyze samples with high organic content. Cd and Hg are considered difficult elements to be determined by ETAAS, principally in complex matrices due to their high volatility [16]. Thus, a systematic optimization of the experimental conditions should be accomplished in order to perform safe and reliable determinations. For this, the use of chemical modifiers, which are part of the stabilized temperature platform furnace (STPF) concept, is needed [16,17].

Methodologies that enable the use of aqueous standards for calibration can be more reliable and also bring about practical advantages. In order to achieve signal matching between the sample and aqueous standards, thoroughly optimized experimental parameters and appropriate sample preparation techniques must be employed.

A unique approach for the analysis of organic liquid samples that are non-miscible with water is the modification of the sample matrix by formation of emulsions. Emulsions are thermodynamically stable systems composed of water, oil and surfactant, in some cases, an alcohol can be added as co-surfactant [18,19]. The dilution and the dispersion of the organic sample in an aqueous media make possible the use of simple aqueous standards for calibration [20]. The use of emulsion as sample preparation has been applied for trace metal determination with the main atomic techniques such as electrothermal atomic absorption spectrometry (ETAAS) [21–23], inductively coupled plasma optical emission spectrometry (ICP OES) [24,25] inductively coupled plasma-mass spectrometry (ICP-MS) [26,27] and cold vapor coupled to atomic absorption spectrometry (CV-AAS) [28] or cold vapor atomic fluorescence spectrometry (CV-AFS) [29].

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* Corresponding author. Tel./fax: +54 266 4425385.
E-mail address: paranda@unsl.edu.ar (P.R. Aranda).

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Petroleum derivates such as automotive fuels, oils and alternative fuels have been analyzed after emulsion sample preparation, as reported in a recent review [20]. The European norms recommend sample dilution with xylene and direct determination by inductively coupled plasma-optical emission spectrometry (ICP-OES) and flame atomic absorption spectrometry (FAAS); calibration is performed with organic standards, diluted in xylene and care must be taken to match the viscosity between the calibration and sample solutions.

In a previous work, we reported the optimization of a method for the determination of total and inorganic Hg in biodiesel with a flow injection-cold vapor generation system coupled to AFS [29].

This paper presents a novel method that involves sample preparation as emulsions for routine and reliable determination of Cd and Hg in biodiesel. The expected advantage of this procedure is the stability and ease of sample handling and that the use of organ metallic standards or toxic solvents is not needed. The composition of emulsion, the stability of the standards and samples, type of calibration along with the instrumental parameters were investigated and optimized. A microwave-assisted digestion was also carried out for comparative purposes. This method was successfully applied to the determination of Cd and Hg in biodiesel samples obtained from local vendors.

2. Experimental

2.1. Instrumentation

The measurements were performed with a Shimadzu Model AA-6800 Atomic Absorption Spectrometer (Tokyo, Japan), equipped with a deuterium background corrector, a EX7- GFA electrothermal atomizer and an ASC-6100 auto sampler. Stabilized platform (L′Vov) graphite tubes from Shimadzu (Tokyo, Japan) were used in all experiments. Cadmium and mercury hollow-cathode lamps from Hamamatsu Photonics (K.K., Japan) were employed as radiation sources. All measurements were performed using integrated absorbance (peak area). The temperature programs for ETAAS analysis are shown in Table 1. The 228.8 nm and 253.7 nm wavelengths were used for Cd and Hg respectively. Background correction was accomplished with deuterium lamp mode.

Microwave digestion was performed with a Milestone START D microwave furnace (Italy) and Milestone hermetically sealed 100 mL internal volume, 1 cm wall thickness polytetrafluoroethylene (PTFE) reactors.

An ultrasonic bath (Astrason Ultrasonic Clear, Farmingdale, NY, USA) was employed for emulsions preparation.

2.2. Reagents

All reagents were of analytical-reagent grade and the presence of Cd and Hg were not detected within the working range. A stock mercury standard (1000 μg mL^{-1}) was prepared from mercury (II) chloride Merck (Darmstadt, Germany) in nitric acid and raise to 1000 mL with ultrapure water. A stock standard solution of cadmium at a concentration of 1000 μg mL^{-1} was prepared from pure cadmium nitrate-4-hydrate Merck (Darmstadt, Germany) in 0.4 mol L^{-1} HCl Merck (Darmstadt, Germany). Nitric acid was provided by Fluka Sigma-Aldrich (Steinheim, Germany). Triton X-100 was obtained from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan) and biodiesel from local producers. Isopropyl alcohol, 99.8%, was from Pharmco Products Inc. (Brookfield, CT, USA).

Table 1
Furnace temperature program for Hg and Cd determination.

Stage	Temperature (°C)	Ramp (s)	Hold (s)	Argon gas flow (L min^{-1})
	Cd/Hg	Cd/Hg	Cd/Hg	Cd/Hg
Drying	140/140	5/5	15/15	1.0
Pyrolysis	900/650	5/5	35/50	1.0
	900/650	–	1/1	0.0 (read)
Atomization	1800/2000	–	4/2	0.0 (read)
Cleaning	2200/2200	–	2/2	1.0

A Pd–Mg mixture (500 μg g^{-1}) was employed as chemical matrix modifier for Hg and Cd determinations by ETAAS. The Pd–Mg solution was prepared by mixing stock standard nitrate solutions of the elements in a high purity nitric acid solution. The presence of Hg and Cd were not detected in the final solutions obtained.

Ultrapure water (18.2 MΩ cm) was obtained from a Mili-Q system from Barnstead EASY pure RF water system (IA, USA).

2.3. Sample treatment

2.3.1. Emulsion formation

Oil-in-water emulsions were prepared using a specific sequence in order to guarantee their stability. Aliquots of biodiesel were accurately weighed and 0.25 mL of concentrated nitric acid was added. This mixture was kept for 15 min, followed by addition of Triton X-100. Afterward a required amount of isopropilyc alcohol was added as co-surfactant.

This mixture was then placed in an ultrasonic bath for 15 min and after that a desired volume of ultra pure water was added. Finally, 10 μL aliquots of the resulting solution, were directly injected into the graphite tube by means of the auto sampler and submitted to the optimized temperature programs shown in Table 1. Calibration was performed against aqueous standards acidified with 1% nitric acid solution and aqueous standards prepared as emulsions. The analyte addition method was also evaluated for calibration. Blank solutions were prepared and analyzed in parallel to the samples and calibration solutions.

2.3.2. Microwave-assisted digestion procedure

After preparation 0.5 g of each sample was accurately weighed and introduced into the PTFE reactors. Then, 7.0 mL of concentrated nitric acid and 1.0 mL of hydrogen peroxide were added and the reactors were closed. The samples were digested applying the following microwave program: 200 °C (ramp, 10 min) and 200 °C (step, 20 min) being 1000 W the maximum power applied. The vessels were then removed from the oven and cooled at 20 °C. After that, they were opened and vented and completely clear solutions were obtained. The concentration was obtained directly from calibration graphs after correction of the ETAAS signal with appropriate reagent blanks.

3. Results and discussion

3.1. Graphite furnace temperature programs

A study of the best conditions for graphite furnace determination was carried out by optimization of the temperature program; i.e. ramp and hold time, drying, pyrolysis and atomization temperatures. For this purpose, the biodiesel samples were prepared as emulsions following the recommended procedure and were spiked with aqueous standards of Cd and Hg. Suitable amounts of Pd–Mg modifier were co-injected with the extract directly into the graphite furnace.

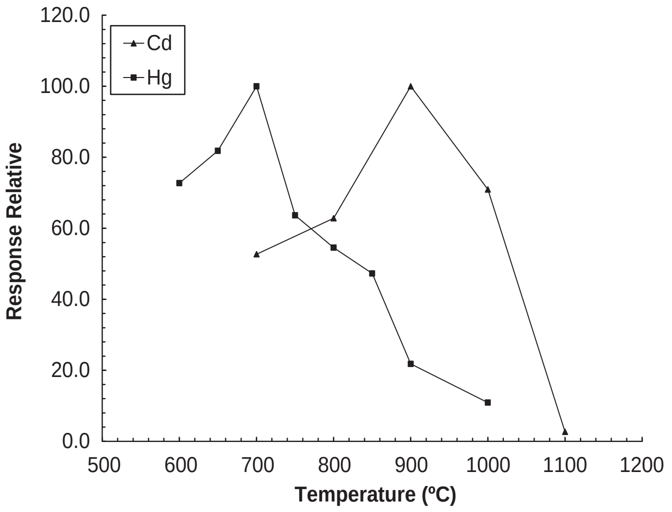

Fig. 1. Pyrolysis curve for Cd and Hg with Pd–Mg modifier co-injected.

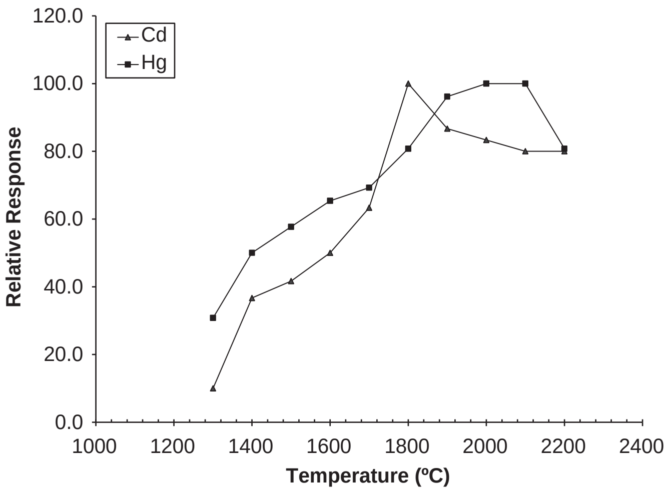

Fig. 2. Atomization curve for Cd and Hg with Pd–Mg modifier co-injected.

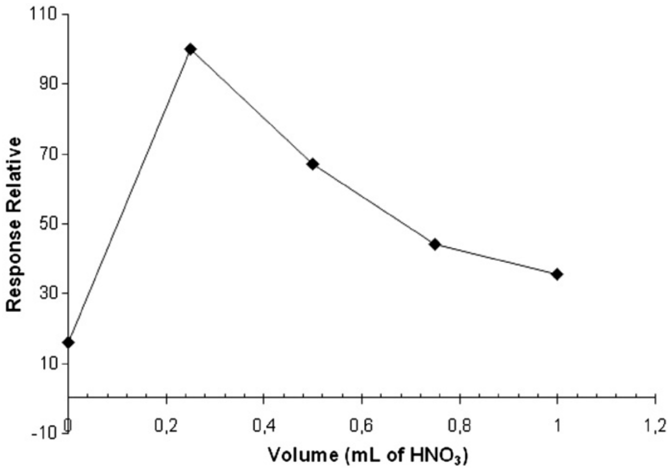

Fig. 3. Effect of the volume HNO_3 on the formation of emulsions.

The drying temperature was selected at 140 °C, with a hold time of 15 s, which was enough to take out the solvent completely avoiding sputtering. Pyrolysis temperatures lower than 600 °C could not be used, since matrix components were not eliminated efficiently and background was considerable, leading in some cases to over-correction and as a consequence, erroneous absorption values for the analytes. The ramp time for the pyrolysis stage was carefully adjusted to allow gradual elimination of the matrix, avoiding any analyte loss by a sudden increase in temperature. Then, pyrolysis temperatures of 900 °C and 650 °C were used for Cd and Hg respectively. The atomization steps were studied within the range of 1300–2200 °C. The optimum atomization temperatures were selected at 1800 °C (2 s) and 2000 °C (4 s) for Cd and Hg respectively. Figs. 1 and 2 show the pyrolysis and atomization profiles for Cd and Hg.

3.2. *Study of the emulsion stability and conditions*

Four criteria were used to choose the components and their relative quantity in the emulsion: (i) the emulsion should contain only the components necessary to stabilize the sample, in other words, sample, surfactant and water; (ii) the emulsion should be stable for a reasonable period and not produce coalescence or creaming; (iii) the emulsion should present low viscosity to allow correct sampling and (iv) analytes should present the same noise-to-signal ratio as the standards during the ETAAS measurement.

The oil-in-water emulsion was chosen mainly due to its characteristics of long-term stability and low viscosity. After choosing the type of appropriate emulsion, the next step was to select the emulsifier. It should be taken into account that it is better to use emulsifiers without foaming properties in order to avoid heterogeneous distribution of the oil drops in the final emulsion. Furthermore, for analytical purposes, other factors should be considered such as surfactant solubility and the background generated during the ETAAS measurement. Non-ionic emulsifiers such as Triton X-100 and Triton X-114 satisfy these requirements and are inexpensive and readily available in most analytical laboratories.

Beyond the use of a surfactant, the use of a co-surfactant is usually required to stabilize the emulsion through the formation of a three component system. Then, different amounts of isopropyl alcohol were introduced and the stability of the emulsion was evaluated. Thus, increasing volumes of this agent (0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 mL) were added and it was experimentally corroborated that the greatest stability was achieved with the addition of 1.5 mL of isopropyl alcohol.

Concentrated nitric acid was added to biodiesel sample before the emulsion formation. This acid is usually added to help the conversion of organ metallic species into inorganic ones, providing a better matching with the standards. An additional aspect is that the droplets formed in acid emulsions are thought to be smaller than those formed in aqueous media, helping to the stabilization of the system. The effect of the amount of nitric acid was studied. The results obtained indicated that with a biodiesel to HNO_3 ratio of 4:1, the most stable emulsion could be formed (Fig. 3).

3.3. *Calibration strategy*

Cd and Hd were determined in case samples after calibration with aqueous standards, aqueous standards added with Triton X-100 and isopropyl alcohol and with the analyte addition method. The accuracy of each strategy was evaluated as the analyte percent recovery when compared with a determination of Cd and Hg in the same samples, but after a total acid digestion (Section 2.3.2).

When aqueous standards were used for calibration, recoveries lower than 90% were obtained when compared with the amount of Cd and Hg found after the microwave-assisted digestion. Better

Table 2
Determination of Cd and Hg in soya biodiesel by the proposed method.

Sample	Calibration curve (analyte addition)	Emulsion($\mu g\ kg^{-1}$)
Hg in Soya biodiesel	$y=0.0075x+0.014$ $R^2=0.9999$	23.2 ± 0.2
Cd in Soya biodiesel	$y=0.0788x+0.03$ $R^2=0.9983$	4.83 ± 0.25

recoveries were obtained using standards added with Triton X-100 and isopropyl alcohol; but quantitative determinations were achieved only with the analyte addition method. A reasonable explanation for these observations is that the organic matter coming from the sample is mainly responsible for the thermal behavior of the sample in the pyrolysis stage. So, when the organic constituents are not present in the furnace (as in the aqueous standards), the amount of heat generated is rather high and the analytes are volatilized even in the presence of the chemical modifier.

3.4. Analytical performance

Cd and Hg were determined in all samples by the analyte addition method. The calibrations covered concentration ranges from 0.1 to 5.0 $\mu g\ L^{-1}$ for Cd, and 1.0 to 15.0 $\mu g\ L^{-1}$ for Hg in the emulsions (base value+added value). The injection repeatability was evaluated as the average relative standard deviation (R.S.D %) for five successive firings and was better than 4.4% for Cd and 5.4% Hg. The detection limits, evaluated by the 3σ concept of calculation ($n=10$), were of 10.2 $\mu g\ kg^{-1}$(0.9 $\mu g\ L^{-1}$) for Hg and 0.3 $\mu g\ kg^{-1}$(0.04 $\mu g\ L^{-1}$) for Cd. The accuracy was verified, by comparing with a total microwave-assisted acid digestion and the results are presented in Table 2.

3.5. Application to real samples

Real biodiesel samples were obtained from local producers and the amounts of Cd and Hg were determined by ETAAS after applying the recommended sample treatment, being the analyte addition method the calibration strategy employed. The values obtained are shown in Table 2.

4. Conclusions

This method for Cd and Hg determination in biodiesel samples joins the excellent sensitivity attainable by ETAAS and the advantages of the emulsion sample preparation technique. Emulsions were easily prepared by dilution of the acidified sample in an aqueous media with Triton X-100 as an emulsifier. The use of isopropyl alcohol as cosurfactant is also recommended. This simple and fast method enables the use of aqueous standards for calibrations being suitable for environmental analysis or monitoring of these elements at different levels in biodiesel. Also avoiding the use of organic standards for calibration and thereby the disadvantages that they generate, such as being an important source of atmospheric pollution, and being relatively expensive, might also show a sensitivity different from that of the metal-organic compounds present in the biodiesel.

The disadvantage in sampling the non-aqueous samples in ETAAS into the graphite furnace was overcome and the corresponding analyte losses were eliminated. The method is cheap and fast, since it does not require time consuming digestion process. The procedure has the potential to be extended to other fuel samples, being suitable for routine application.

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