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Simultaneous and direct determination of iron and nickel in biological solid samples by high-resolution continuum source graphite furnace atomic absorption spectrometry

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ABSTRACT

The simultaneous and direct determination of nickel and iron in plants and lichens has been investigated using high-resolution continuum source graphite furnace atomic absorption spectrometry. The primary resonance line for nickel at 232.003 nm and the adjacent secondary line for iron at 232.036 nm have been used for this purpose. The optimization of the experimental conditions was performed using a pine needles certified reference material (SRM 1575a). The influence of pyrolysis and atomization temperatures, the amount of solid sample introduced into the graphite furnace and the use of aqueous or solid standard for calibration were studied. The spectral interferences caused by absorption of the concomitants of the solid sample were detected and corrected using a least square algorithm. Aliquots of 0.1–1 mg of the solid samples were weighed onto the solid sampling platforms and analyzed directly, without addition of any reagents. The limits of detection were 25 $\mu g\ kg^{-1}$ for nickel and 0.40 $mg\ kg^{-1}$ for iron and the precision, expressed as the relative standard deviation, ranged from 7% to 12%. The proposed method was used to determine both metals in different bioindicator samples with successful results.

1. Introduction

Biological species such as oysters, barnacles, lichens and also different tree species can incorporate and accumulate metals from the environment. The metal content in these samples shows the cumulative effect due to the exposure to environmental pollution, so it can be considered as a useful and interesting parameter in contaminations studies. For this reason, these species have been used to investigate contamination processes in soil, water or air and in phytoremediation studies [1–7]. The analytical methodologies usually employed to determine metals in these bioindicator species are based on atomic spectrometric techniques with previous wet digestion of the solid sample [3–7]. This step for sample treatment is time-consuming, so it is considered as an important inconvenience mainly when a large number of samples must be analyzed. Moreover, the digestion process may cause the sample contamination and/or loss of the analytes.

The methodologies based on direct analysis of solid samples, without previous digestion of the sample, are efficient and reliable alternatives. They are in agreement with the Green Analytical Chemistry principles because they reduce drastically or even do not use toxic or corrosive chemicals for sample pretreatment resulting in both, economic and environmental benefits [8]. These analytical strategies are simple, increase the speed of analysis and reduce the risk of sample contamination and loss of analytes. Other important benefit of these direct methods is the increase of sensitivity because the samples are not diluted and usually the amount of sample needed for the analysis is also reduced [9–12]. As well as these advantages, the methodologies based on direct analysis of solid samples present some problems. The most important issues are the difficulties of sample manipulation and introduction in the measurement instrument, achieving a correct calibration and the poor precision of the obtained results [9–12].

Atomic spectrometric techniques have been suited to solid sampling analysis. Among them, graphite furnace atomic absorption spectrometry (GF AAS) offers high sensitivity and selectivity at a reasonable cost. Different devices to introduce the solid samples automatically into the graphite tube are commercially available nowadays for the spectrophotometers of GF AAS. If an appropriate background correction and an adequate control of the atomization and vaporization processes of the analyte are performed, a straightforward method of calibration based on the use of aqueous standards can be employed successfully in GF AAS with solid sampling analysis [9–12]. Conventional line source graphite furnace atomic absorption spectrometry (LS GF AAS) shows limitations for these purposes, because it cannot resolve some of the

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spectral interferences caused by the matrix of the sample [12]. However, high-resolution continuum source atomic absorption spectrometry (HR-CS AAS) is a useful and interesting tool in solid sampling analysis owing to the superior potential for background corrections [12,13]. The array detector employed in commercial HR-CS atomic absorption spectrometers permits the simultaneous monitoring of two hundred "wavelength sectors" (pixels). Typically only three pixels are used to obtain the atomic absorption measurements. The other pixels are used to correct unwanted spectroscopic events such as lamp flicker noise, temporal changes in the transmittance of the gas phase or any continuous background absorption. The spectral neighborhood of the analytical line becomes visible at high resolution, so spectral interferences caused by absorption of the concomitants as well as molecular absorption with rotational fine structures can be detected [12,14–16]. Thus, it is possible to correct complex, high and even rapidly changing backgrounds, since they are monitored simultaneously with the atomic absorption lines. Moreover, if the analyte and concomitant absorption cannot be separated in wavelength or in time by optimizing the temperature program, the system offers the possibility to measure and store reference spectrum that can be subtracted from the sample spectrum using a least-squares algorithm [12,14–19]. Therefore, most of problems found in solid sampling analysis have been solved using HR-CS AAS. The only disadvantage of this methodology is the imprecision of the results, with RSD values between 10 and 20%, owing to the inhomogeneity of most solid samples and the small sample mass employed for the analysis [9–12].

In HR-CS AAS, the simultaneous monitoring of the spectral environment at both sides of the analytical line allows the simultaneous determination of more than one element, if the absorption lines of the other elements appear within the spectral interval that reaches the detector. This potential for multi-element determination is an important advantage compare to conventional LS AAS. However, the currently available detectors do not permit the easy implementation of this important characteristic. The instrument provides an exceptional resolution as every pixel monitors a range of 1–2 pm, so the detector only allows the simultaneous monitoring of 0.4–2.0 nm of spectrum, depending on the wavelength range [15,20–24]. Although the other lines are almost inevitably less sensitive, they can be used for the determination if the sensitivity ratios between the lines are compatible with the concentration ratio of the analytes in the samples. Another condition to achieve the simultaneous determination is the selection of adequate temperatures for the pyrolysis and atomization stages. It is necessary to establish a common temperature program that allows the simultaneous determination of all elements without too much deterioration of the analytical results [15,20–25].

The aim of this work has been the development of a fast and simple methodology for the simultaneous determination of nickel and iron in bioindicator species by HR-CS AAS using solid sampling analysis. The metal determination can be performed directly without any pretreatment step of the solid sample. The bioindicator species chosen for this purpose were lichen and pine samples. In general, iron is found as a minority element whereas nickel is found as a trace element in these samples. In order to achieve an "optimal" temperature program, the pyrolysis and atomization temperatures were studied at the chosen absorption lines for each element. The presence of spectral interferences at the selected wavelengths was also studied and the detected interferences were corrected. The figures of merit were estimated and the proposed methodology was validated using a standard reference material of pine needles. Lichen and pine samples were collected and analyzed using the developed analytical strategy. In order to compare the obtained results, the samples were digested using a microwave oven and the obtained solutions were analyzed using a conventional LS AA spectrometer.

2. Experimental

2.1. Instrumentation

The absorbance measurements for solid sampling analysis were carried out using a ContrAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with both flame and graphite furnace atomizers. The optical system comprises a 300 W xenon short-arc lamp operating in "hot-spot" mode as the continuous radiation source for the wavelength range from 185 nm to 900 nm and a compact high-resolution double monochromator consisting of a prism pre-monochromator and an echelle grating monochromator. The detector is a linear charge-coupled device (CCD) array with 588 pixels, 200 of which are used for analytical purposes displaying the vicinity of the analytical line at high resolution, while the rest are used for internal functions. The primary resonance line for Ni at 232.003 nm, and the adjacent secondary line for Fe at 232.036 nm were used for the simultaneous determination of the two elements. The integrated absorbance values of three pixels (the central pixel plus the adjacent ones, CP $\pm$ 1) for each analyte were added up to obtain the peak volume selected absorbance (PVSA) which was selected as the measurement mode [26].

The HR-CS AAS instrument is also equipped with a transversely heated graphite tube atomizer and solid sampling autosampler. The experiments were carried out using pyrolytically coated graphite tubes for solid sampling (SS) without dosing hole (Analytik Jena Part No. 407-A81.303, Jena, Germany) and SS platforms (Analytik Jena Part No. 407-152.023, Jena, Germany). The samples were accurately weighed onto the SS platforms with a precision of 1 μg using the microbalance integrated in the instrument and automatically inserted into the graphite tube using a pre-adjusted pair of tweezers. Both, the microbalance and the tweezers are part of the SSA 600 solid sampling accessory (Analytik Jena, Jena, Germany), so all these operations were controlled by the computer except the deposition of the sample, which was carried out manually. To carry out the complete measuring procedure, the empty SS platform was first transported to the microbalance for taring, using the pair of tweezers. After, an appropriate amount of the sample, a mass between 0.1 and 1.0 mg, was deposited onto the platform and weighed. The platform was then transferred to the graphite furnace and subsequently subjected to the temperature program. Aqueous standards solutions were injected manually onto the SS platform using micropipettes with disposable tips. In this case, 20 μL was selected as an appropriate volume to perform the measurement. For the measurements of solid sample, the PVSA value was normalized for a sample mass of 1 mg, as it is impossible and unnecessary to weigh and introduce the same sample mass into the graphite furnace. Argon 99.9992% (Carburos Metalicos, Madrid, Spain) was used as purge and protective gas. The optimized graphite furnace temperature program used for the simultaneous determination of Ni and Fe is shown in Table 1.

A Perkin Elmer-Anton PAAR Multiwave microwave sample preparation system (Graz, Austria), using perfluoroalkoxy (PFA) model MF 100 digestion vessels was used to carry out the wet digestion of the solid samples. The nickel and iron determination in previously digested samples was performed using a Unicam Solaar M serie Zeeman spectrometer (Cambridge, United Kingdom), a conventional LS AAS instrument, equipped with a flame and a GF95 graphite furnace as atomizers and a FS95 autosampler. The determination of iron in the digested samples using the LS AAS was performed at a wavelength of 248.3 nm with an air-acetylene

flame. The nickel concentration using the LS AAS was determined at 232.0 nm with a graphite furnace atomizer, applying the temperature program shown in Table 2.

2.2. Reagents and standard solutions

All reagents used in this work were at least of analytical grade and used as received without further purification. Ultrapure water with resistivity not less than 18.2 MΩ cm obtained from an Ultra Clear™ TWF UV EDI water purification system (Siemens, Barsbüttel, Germany) was used throughout. The nitric acid used to prepare standard solutions was trace analysis grade (Fluka, St. Gallen, Switzerland). Nickel and iron standard solutions were prepared from commercially available stock solutions for atomic absorption spectrometry of 1.000 g L^{-1} for nickel (Scharlau, Barcelona, Spain) and 1.001 g L^{-1} for iron (Fluka, St. Gallen, Switzerland). The standard solutions were prepared daily by adequate dilution of the commercial stock solutions with 0.5% (v/v) nitric acid.

2.3. Samples and sample preparation

The certified reference material (CRM), SRM 1575a pine needles (National Institute of Standards and Technology, NIST, Gaithersburg, USA), was used for optimization and validation purposes. This sample was available as fine powder, with a particle size of 100 μm or less.

Samples of pine (*Pinus sylvestris*) and epiphytic lichen specimens (*Evernia* sp.) growing on the bark of the trees were collected in the recreational area of the Barranca Valley located at Sierra de Guadarrama at Norwest of the Community of Madrid (Spain). This area is located sufficiently away from principal sources of contamination of this zone. The samples were collected in separated polyethylene bags. The pine needles were carefully separated from stems manually using disposables nitrile gloves, powder-free, to avoid contamination. First, the lichen and the pine needles samples were air-dried in a clean atmosphere between sheets of filter paper and then, they were dried in a furnace for 24 h at 60 °C. The dried samples were ground using a laboratory mill with high resistant stainless steel cutters (model A10 yellow line, Ika, Staufen, Germany) until a very fine powder was obtained, and stored in closed polyethylene containers. The analysis by SS HR-CS GF AAS was carried out without further sample treatment.

Table 1
Temperature program for the simultaneous determination of nickel and iron directly in the solid samples by HR-CS GF AAS.

Stage	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)	Gas flow rate (L min^{-1})
Drying	110	10	30	2.0
Pyrolysis	1200	150	30	2.0
Auto zero	1200	0	5	0
Atomization	2600	1200	9	0
Cleaning	2700	500	8	2.0

Table 2
Temperature program for the determination of nickel in the digested samples by LS GF AAS.

Stage	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)	Gas flow rate (L min^{-1})
Drying	130	10	30	0.2
Pyrolysis	1000	150	20	0.2
Atomization	2500	0	3	0
Cleaning	2600	0	3	0.2

For comparison, the CRM and the lichen and pine samples were digested in a microwave sample preparation system. About 100 mg of the sample was accurately weighed into a PFA digestion vessel using a Mettler Toledo model AX205DR microbalance (Greifensee, Switzerland) with a precision of ±0.01 mg. Then, 2.0 mL of concentrated nitric acid was added to the sample and the vessel was closed and placed into the rotor of the microwave oven. A power program (600 W for 7 minutes and then application of 800 W for 15 min) was used with control of maximum temperature (164 °C) and pressure (30 bars). After 18 min of cooling, the vessels were opened carefully and the digests were transferred into volumetric flasks. The digestion vessels were rinsed with ultrapure water, the washes were added into the volumetric flasks and the samples were diluted with ultrapure water to make them up to 25.0 mL.

3. Results and discussion

3.1. Evaluation of possibilities for nickel and iron simultaneous determination.

Although the spectrometers for HR-CS AAS allow monitoring simultaneously the spectral neighborhood of an analytical line, they are not designed for simultaneous multi-element determination. In fact, nowadays the software to control the spectrometer is not designed for this purpose. However, it is possible to determine two or more analytes if the analytical lines are within the wavelength interval that reaches the detector, usually minor than 1 nm, and the obtained signals are processed using spreadsheets or data analysis software. In addition, the sensitivity ratio between the elements to be determined at these wavelengths must correspond roughly to the concentration ratio of the analytes in the sample.

Iron has a secondary line at 232.036 nm close to the primary resonance line of nickel at 232.003 nm as is shown in Fig. 1. The wavelength interval reached by the detector at this nickel analytical line corresponds about ±0.135 nm around 232.003 nm, so the simultaneous monitoring of both lines is possible. Moreover, this iron line is about three orders of magnitude less sensitive than the nickel line. This sensitivity ratio of nickel and iron lines approximately corresponds to the concentration of nickel and iron in lichen and pine samples, where the nickel concentration is about three or four orders of magnitude lower than the iron one according to the sample exposure to environmental pollution.

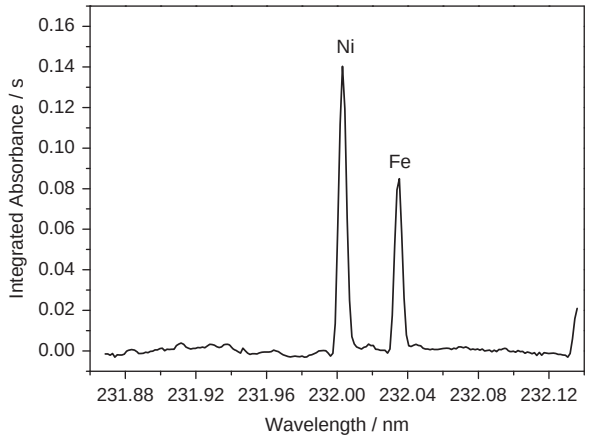

Fig. 1. Wavelength resolved time-integrated absorbance spectrum in the vicinity of the nickel resonance line at 232.003 nm for an aqueous standard containing 0.80 ng of Ni and 24 ng of Fe. Pyrolysis temperature 1200 °C, atomization temperature 2600 °C.

3.2. Optimization of pyrolysis and atomization temperatures.

Another important condition for simultaneous determination using GF AAS is the establishment of a common temperature program for the analytes. The pyrolysis and atomization curves for nickel and iron, obtained using the SRM 1575a pine needles sample, are shown in Fig. 2. In general, the pyrolysis curves obtained for both metals were similar whereas the atomization curves showed different behaviors for each metal. The assayed pyrolysis temperatures for both metals ranged from 600 °C to 1400 °C. As can be seen in Fig. 2, a slight increase in the integrated absorbance values for nickel and iron was shown as pyrolysis temperature increased from 700 °C to 900 °C and 700 °C to 1000 °C, respectively. For temperature values higher than 1200 °C, the integrated absorbance diminished for both analytes. The absorbance values were very similar at pyrolysis temperatures between 900 °C to 1200 °C for nickel and 1000 °C to 1200 °C for iron. The use of a sufficiently high pyrolysis temperature can remove efficiently the bulk of the sample matrix. In addition, the use of chemical modifiers to prevent the loss of the analytes is no needed at these conditions, so the developed methodologies are faster. For these reasons, a pyrolysis temperature of 1200 °C, without using any chemical modifier, was adopted for all future determinations. At this temperature, an efficient removal of most of the matrix components of the sample was obtained and losses of Ni and Fe in the pine needles CRM were not detected when these absorbance signals were compared to the obtained ones for aqueous standard solutions of these metals.

In order to obtain the atomization curves, the atomization temperatures were ranged from 2000 °C to 2800 °C (Fig. 2). For iron, the highest integrated absorbance values were obtained at temperatures higher than 2000 °C and lower than 2500 °C. Very similar values of integrated absorbance were obtained for atomization temperatures equal or higher than 2500 °C. A different behavior was shown for nickel. In this case, a sharp increase in the absorbance values was observed from 2000 °C to 2600 °C. The integrated absorbances for nickel were similar at temperature values higher than 2600 °C. In bioindicators nickel and iron are present as trace and minority elements respectively, so the simultaneous determination could be performed if the sensitivity of the integrated absorbances for both metals, especially for nickel, is high at the atomization temperature used. On the other hand, the lifetime of the SS platforms and the graphite tubes diminished quickly at temperatures higher than 2700 °C. For these reasons, and in order to determine direct and simultaneously both elements with a reasonable cost, an atomization temperature of 2600 °C was adopted as a compromise value.

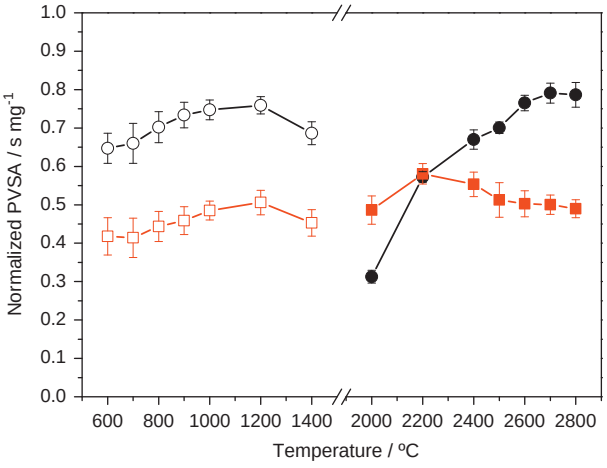

Fig. 2. Pyrolysis (open symbols) and atomization (filled symbols) curves for Ni (circle) and Fe (square) in the SRM 1575a pine needles. Atomization temperature used for the pyrolysis curves: 2600 °C; pyrolysis temperature used for atomization curves: 1200 °C.

3.3. Identification and correction of spectral interferences

The SRM 1575a pine needles sample was analyzed applying the temperature program shown in Table 1. As can be seen in Fig. 3(A), a structured background signal appears in the vicinity of the selected iron and nickel atomic absorption lines. At first attempt, different temperature programs, increasing the time and temperature applied, were tested in order to eliminate this signal, but it was not feasible and the overlap of structured molecular background with the Ni and Fe lines remained. Welz et al. [12] and Lepri et al. [27] have also registered a large number of rotational lines in the vicinity of the Ni absorption lines at 231.096 nm, 232.003 nm and 232.138 nm for sludge and coal samples. The authors associate these rotational lines to a single molecule, SiO. In order to investigate the origin of the interfering molecular spectrum obtained for the SRM 1575a pine needles sample, 30 μg of pure silica were vaporized using the temperature program shown in Table 1. The obtained time-integrated absorbance spectrum is shown in Fig. 3(B). This reference spectrum (Fig. 3B) was subtracted from the pine needles sample spectrum (Fig. 3A) using a least-squares algorithm for background correction. In the obtained spectrum after this subtraction (Fig. 3C), the molecular absorption is corrected to the baseline and only two absorption lines remain at 232.003 nm and 232.036 nm corresponding to Ni and Fe respectively. These results confirm the identity of the interfering diatomic molecule as SiO, and also show the efficiency of this background correction procedure.

As it was mentioned in the introduction section, one of the problems of methodologies based on direct analysis of solid samples is to achieve a correct calibration process. Straightforward methods of calibration based on aqueous standard solutions have been tested to make easy this process. The transient signals for 0.391 mg of the SRM 1575a pine needles sample, after the elimination of the SiO interference, and for 0.600 ng of Ni and 18.8 ng of Fe in aqueous multielemental standard solution were compared in order to evaluate the possibility to perform the calibration process using aqueous standard solutions (Fig. 4). The peak shapes and the total areas of the atomization signals

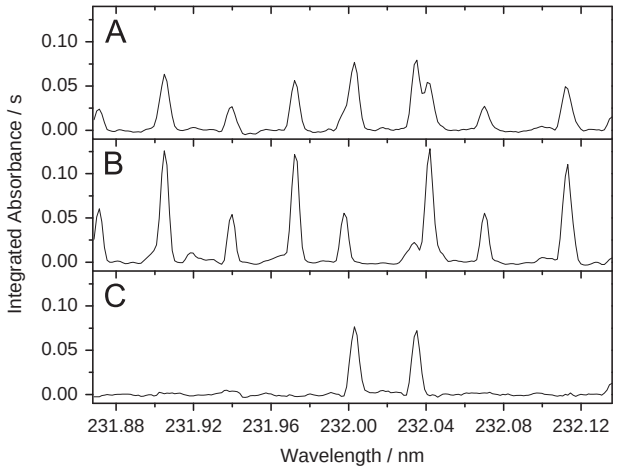

Fig. 3. Wavelength resolved time-integrated absorbance spectra in the vicinity of the nickel resonance line at 232.003 nm obtained according to graphite furnace program in Table 1. (A) Spectrum for certified pine needles (SRM 1575a), (B) reference spectrum of SiO obtained for 30 μg of SiO_2 and (C) the same spectrum as in A after subtraction of the reference spectrum using least-squares background correction.

were very similar at both wavelengths when very similar amounts of both elements in different matrixes were determined using the temperature program shown in Table 1. These results confirm that the least squares background correction procedure to eliminate the spectral interferences due to the matrix of solid samples was adequate and a calibration process against aqueous standard solutions might be feasible [20,21].

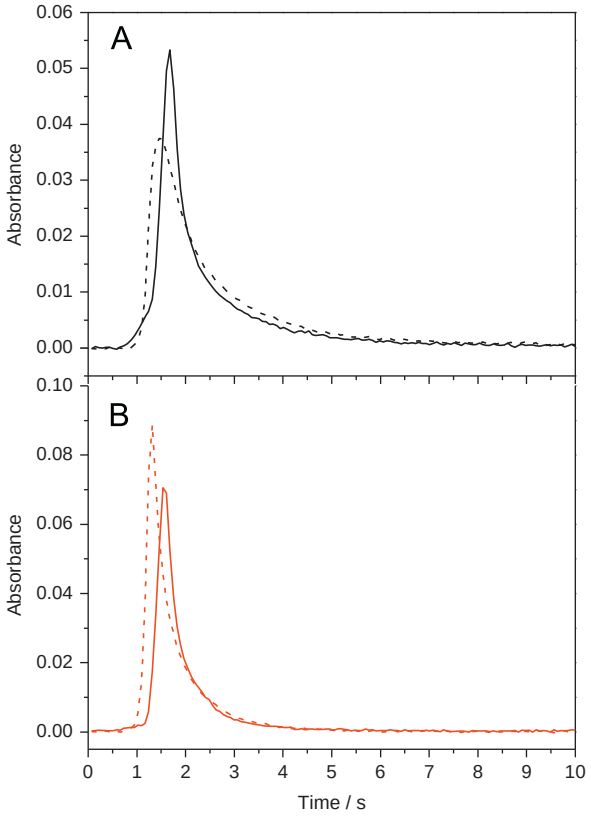

Fig. 4. Overlaid transient signals for 0.391 mg of the SRM 1575a pine needles sample (–) and for 0.600 ng of Ni and 18.8 ng of Fe in aqueous multielemental standard solution (....) at 232.003 nm (A) and 232.036 nm (B). Pyrolysis temperature 1200 °C, atomization temperature 2600 °C.

Table 3
Figures of merit for the simultaneous determination of Ni and Fe by SS HR-CS GF AAS using the temperature program in Table 1.

Parameter	Ni (232.003 nm)	Fe (232.036 nm)
Sensitivity (aqueous standards)	0.430 s ng^{-1}	9.10×10^{-3} s ng^{-1}
Sensitivity (CRM, SRM 1575a)	0.408 s ng^{-1}	9.29×10^{-3} s ng^{-1}
R	0.999	0.992
LOD[a]	25 μg kg^{-1}	0.40 mg kg^{-1}
LOQ[a]	83 μg kg^{-1}	1.3 mg kg^{-1}
m_0	16 pg	0.18 ng
RSD ($n=5$)	7%	12%

[a] Value calculated for a sample mass of 1 mg.

3.4. Calibration and figures of merit

The figures of merit for the proposed SS HR-CS GF AAS method are summarized in Table 3. Calibration curves for both analytes were obtained simultaneously, using aqueous standard solutions or the SRM 1575a pine needles sample, for comparison of both calibration modes. A blank and five aqueous standard solutions in the concentration range from 8 to 80 μg L^{-1} (mass range: 0.2–1.5 ng) for Ni and in the concentration range from 0.2 to 2.3 mg L^{-1} (mass range: 5–46 ng) for Fe in 0.5% HNO_3 were employed for aqueous standard calibration. Calibration curves were also obtained using increasing masses of the pine needles CRM. The slope values for both analytes are shown in Table 3. As can be seen in the table, the sensitivities obtained in the aqueous standard calibration are in good agreement with those obtained using the solid CRM. These results indicate the possibility to perform the calibration of the method using aqueous standard solutions.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated as three and ten times, respectively, the standard deviation of ten measurements of the empty SS platform divided by the slope of the calibration curve. Both limits were calculated for the maximum sample mass that has been used in this work. The characteristic mass (m_0), defined as the mass of the analyte that produces a PVSA of 0.0044 s was 0.016 ng for Ni and 0.18 ng for Fe. The characteristics mass for Ni at the primary resonance line is very similar to the literature data [27–29]. For the secondary line used for Fe, we do not found literature data for comparison.

Five replicate measurements of the pine needles CRM with a mass range of 0.37–0.48 mg were performed using the proposed methodology to estimate the precision and accuracy. The precision, expressed as relative standard deviation (RSD), was better for Ni than for Fe. The higher RSD value for Fe than for Ni could be due to the omnipresence of this element in the environment or a more inhomogeneous distribution for Fe than for Ni in the investigated CRM. In fact, the RSD values obtained for methods based on solid sampling analysis are, in general, between 10 and 20% due to the small sample mass used for the analysis and the inhomogeneity of the sample [9–11]. The found values in the simultaneous determination of both analytes using the proposed SS HR-CS GF AAS method are in very good agreement with the certified and informed values for Fe and Ni, respectively, in the SRM 1575a sample (Table 4) according to the Student's t-test at a 95% confidence level.

The sample mass introduced into the graphite furnace was also evaluated. An inefficient matrix removal in the pyrolysis stage and an increase of background signals could be produced if high mass samples are used. If very small mass of sample are used, very low precision in the analysis could be obtained due to the

Table 4
Comparison of the results obtained for the simultaneous determination of Ni and Fe in bioindicator samples by SS HR-CS GF AAS and those obtained after a wet digestion of the samples and analysis by LS AAS.

Sample	Certified value		[a]SS HR-CS GF AAS		[b]LS AAS	
	Ni (mg kg^{-1})	Fe (mg kg^{-1})	Ni (mg kg^{-1})	Fe (mg kg^{-1})	Ni (mg kg^{-1})	Fe (mg kg^{-1})
SRM 1575a	1.47 ± 0.20	46 ± 2	1.45 ± 0.10	45.1 ± 5.5	1.61 ± 0.16	47.7 ± 4.8
Pine needles	–	–	1.50 ± 0.22	109 ± 11	1.70 ± 0.05	92 ± 10
Lichen	–	–	3.16 ± 0.20	570 ± 78	3.44 ± 0.10	634 ± 20

[a] $n=5$.
[b] $n=3$.

non-representative aliquot of the sample used for the determination. In order to investigate if the proposed methodology for simultaneous determination of Ni and Fe is influenced by the sample mass, seven aliquots of the SRM 1575a pine needles samples with different mass, between 0.1 and 1.0 mg, were introduced into the graphite furnace. The RSDs obtained in this study, 4% for nickel and 14% for iron, are at the same order of magnitude and also similar to the RSD values shown in Table 3.

3.5. Analysis of real samples

The proposed methodology was used to determine Ni and Fe in pine needles and lichen samples from Sierra de Guadarrama (Spain). Five aliquots of each sample, with sample mass between 0.1 and 0.5 mg depending on the expected analyte concentration, were introduced into the graphite furnace for the direct and simultaneous determination of both elements (Table 4). The Fe and Ni contents in the samples are in accordance to the amounts found in pines and lichens sited in low exposed areas to vehicular activity, that are away from city center or highways, lacking of any direct pollution source [4,7]. In these areas the Ni and Fe amounts accumulated in lichens and pine needles are associated to the possibility of dispersion and transport of the metals by the winds from polluted areas. The higher Ni and Fe contents in lichen than in pine show the accumulation ability of lichens for metals.

For validation purposes, the nickel and iron concentration in these samples and in the SRM 1575a pine needles sample, after wet digestion of the samples, were also determined by LS AAS. In this case, three aliquots of about 100 mg of each sample were digested in a microwave oven using concentrated nitric acid as digestion reagent. The digests were transferred into volumetric flasks, made up to volume and then analyzed using a conventional LS AA spectrometer. The obtained results are also shown in Table 4. The Ni and Fe concentrations determined by LS AAS are also in good agreement (95% confidence level) with the certified values in the SRM 1575a pine needles. According to the Student's *t*-test for two independent samples at a 95% confidence level, no significant differences between the obtained data for both analytes in lichen and pine needles samples were observed using the proposed solid sampling method and the conventional wet digestion methodology, indicating the suitability of the SS HR-CS GF AAS method for the direct and simultaneous determination of nickel and iron.

4. Conclusions

In this work a solid sampling method based on HR-CS GF AAS has been developed for the direct and simultaneous determination of nickel and iron in biological samples. This method is faster and simpler than conventional methods based on wet digestion of samples. The simultaneous determination of Ni and Fe is carried out in few minutes using a minimum sample mass and without using additional reagents, except for aqueous calibration standards. These characteristics of the developed method are in agreement with the principles of Green Analytical Chemistry.

One more time, it has been demonstrated that direct solid sampling methodologies are benefited from the advantages introduced by HR-CS GF AAS instrumentation, which allows the simultaneous monitoring of the spectral neighborhood of the analytical line at high resolution. Due to this capability, an adequate identification and correction of background signals and spectral interferences caused by the matrix of solid samples has been performed and the simultaneous determination of Ni and Fe can be carried out despite the overlap of the Fe line with the SiO structured signals.

The significant analytical advantages of the developed method make it a useful and interesting tool for fast evaluation of contamination processes due to nickel, a priority pollutant released mainly in the combustion of coal and heavy fuel oils, and iron, an essential element to living organisms that it is also accumulated in the environment due to the human activities. This methodology could be particularly helpful for routine laboratories dedicated to biomonitoring studies with large number of samples, because time and analytical costs may be significantly reduced.

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