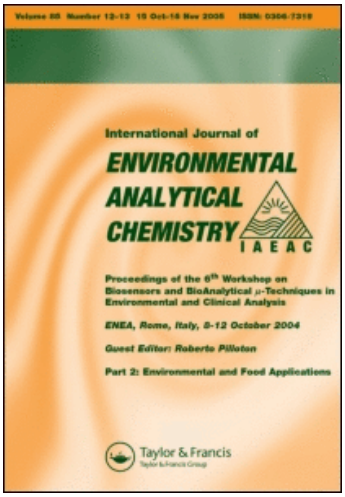

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Determination of Cd and Pb in medicinal plants using solid sampling flame atomic absorption spectrometry

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A procedure for trace determination of Cd and Pb in medicinal samples using solid sampling flame atomic absorption spectrometry (SS-F AAS) was developed for trace determination of Cd and Pb in medicinal plants. Powdered samples were mixed with high purity graphite and introduced as pellets (up to 100 mg) into a quartz device positioned above the burner and heated by air–acetylene flame. Oxygen flowing through the quartz device assure the complete combustion of sample. Combustion was started in 2 s after sample introduction and the produced vapours were subsequently dispersed into the flame. Calibration was performed using different masses of solid certified reference material (for Pb) or with reference solutions applied directly on graphite pellets (for Cd). Integrated absorbance mode was used and signals were integrated in less than 10 s. A deuterium background corrector was used throughout. Characteristic masses were 58 and 790 pg for Cd and Pb, respectively. Accuracy was checked using certified reference materials and the percent relative error was between −7 and 2% for Cd and between −2 and 3% for Pb. Relative standard deviations (repeatability) were always below 10% ($n = 5$). Limits of detection were 0.012 and 0.10 $\mu g\,g^{-1}$ for Cd and Pb, respectively. The proposed procedure by SS-F AAS is simple and may be applied to conventional atomic spectrometers allowing the determination of Cd or Pb in 40 test samples by hour (excluding the steps for sample preparation).

Keywords: solid sampling; flame atomic absorption spectrometry; medicinal plants; cadmium determination; lead determination

1. Introduction

The use of plants in popular medicine has continuously increased in many countries. It is estimated that there is a growth of 10–20% of the commercialised products every year. Moreover, about 20 billions of dollars are expected to be involved in the market of medicinal plants and related products [1]. However, depending on the origin and kind of plant inorganic contaminants may be present and some of them at levels that could cause risks for human health [2]. In this aspect, cadmium and lead are often found as trace

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contaminants in medicinal plants and due to their potential toxicity, these elements are commonly included in routine analysis for plant matrices [3].

Nowadays, graphite furnace atomic absorption spectrometry (GF AAS) is one of the best techniques to perform solid sampling (SS) analysis taking into account the special features that makes its application easier [4–7]. Solid sampling offers a number of important advantages over conventional solution related to the lower risk of contamination, better limit of detection (LOD), and the minimum (or unnecessary) use of corrosive or hazardous reagents [8–11]. In general, analysis of solids using atomic spectrometric techniques can be performed by two main procedures involving the sample introduction as slurries or directly into the atomiser as powders. Using SS procedures with graphite furnace, in some cases, it has been demonstrated improvements in analytical performance mixing the ground samples with graphite powder [12,13].

Concerning to the requirements for routine analysis of plants, techniques allowing suitable LODs and high throughput are of great interest [14]. In spite of the widespread use of flame atomic absorption spectrometry (F AAS), this technique often presents several drawbacks related to relatively poor sensitivity and unsuitable achievable LODs. The presence of Cd and Pb in biological matrices is frequently in the microgram per gram range or below, and the use of F AAS could only be performed using special devices to improve the LODs [15,16], a preconcentration step is often required [17].

Few successful approaches were described in literature for introduction of solids by F AAS using previous combustion and sample vapours introduction [18,19] or direct introduction of powdered biological samples into the flame [20,21]. Although the LOD achieved using these procedures had been considered suitable for plant analysis better LODs might be expected if higher sample amount could be introduced into the atomiser. For these works the maximum sample mass was about 2 mg and it was limited by the design of devices used for sample introduction or vapourisation. Recently, a new device for SS-F AAS was proposed [22] where the solid samples were introduced using paper capsules into a quartz cell heated by the flame of the spectrometer. This procedure allowed the introduction of sample masses up to 7 mg, improving the LODs about 4-fold for Cd.

Another important aspect is that the atomisation process in flames is affected by constituents of the sample. Some constituents may change the concentration of C_2 or H radicals that could influence the analyte reduction in the vapour phase [23]. In the case of analysis using SS is possible to make the addition of solid substances (e.g., graphite) to the samples in view of minimising differences of atomisation behaviour between samples with different compositions.

In this work, a new approach for Cd and Pb determination by SS-F AAS in medicinal plants is described in view of achieving a procedure suitable to analyte determination in agreement to the required LODs for environmental or toxicological studies. Pellets of ground plants were prepared with the addition of high purity graphite powder. The proposed procedure was developed to allow the introduction of higher sample masses, up to 50 mg, and also to perform the calibration based on reference solutions or using certified solid reference materials. Different ratios of sample and graphite powder and the influence of operational parameters of sample introduction system were evaluated. In addition, the proposed procedure could reduce the amount of generated residues of laboratory because the digestion step would be not necessary.

2. Experimental

2.1 *Instrumentation*

A Model Vario 6 FL flame atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) was used for all measurements using the proposed system for solid introduction. The spectrometer was equipped with a deuterium background (BG) correction system and an air/acetylene burner (10.2 and 1.7 L min^{-1} for air and acetylene flow rates, respectively). Hollow cathode lamps for Cd and Pb were operated at 4 and 8 mA, respectively. The selected wavelengths were 228.8 and 283.3 nm for Cd and Pb, respectively, and spectral bandwidth was 0.5 nm for both analytes. Pellets were weighed using a Model M2P microbalance (Sartorius, Göttingen, Germany) with a 1 μg resolution and weighing range from 0.1 μg to 2 g.

Commercial medicinal plants were dried at 60°C for 3 h using a conventional oven and further ground using a cryogenic mill Model 6750 (Spex, New Jersey, USA) to obtain samples with particle size smaller than 80 μm. Plant samples (with and without graphite addition) were pressed (5 ton by 1 min) as pellets with 5 mm of diameter using a manual hydraulic press (15 ton, Specac, Orpington, UK).

For comparison of results, medicinal samples were digested in closed quartz vessels using a Model Multiwave 3000 high pressure microwave oven (80 bar, 280°C, Anton Paar, Graz, Austria). Digests were analysed by inductively coupled plasma mass spectrometry (ICP–MS) using a Model Elan DRC II (PerkinElmer, Thornhill, Canada). Measurements were performed using operational parameters and instrumental settings according to the manufacturer recommendations.

2.2 *Reagents*

Nitric acid (Merck, Darmstadt, Germany), used for digestion of samples, was purified by sub-boiling system (Milestone, Model Duopur, Sorisole, Italy). Reference stock solutions, with a concentration of 1000 mg L^{-1} for Cd and Pb (Titrisol nr. 9960.0001 and 9969.0001, respectively), were purchased from Merck (Darmstadt, Germany). Deionised water from a Milli-Q water purification system (18 MΩ cm, Millipore, Bedford, USA) was used throughout. All glassware was soaked in 3 mol L^{-1} nitric acid for at least 24 h and rinsed three times with water before use. Graphite powder (>99.99% of purity) and palladium nitrate solution (10 g L^{-1}) were purchased from Aldrich (Saint Louis, USA) and Merck (Darmstadt, Germany), respectively.

2.3 *Samples*

The following certified reference materials were used for Cd calibration: Olive leaves (IRMM BCR 62) from Bureau Community of Reference and, Spinach leaves and Pine needles (NIST SRM 1570a and 1575a, respectively) from National Institute of Standards and Technology (Gaithersburg, USA). For Pb, Apple leaves, Peach leaves, and Spinach leaves (NIST SRM 1515, 1547, and 1570, respectively) were used. Commercial medicinal plants, *Casearia sylvestris*, *Peumus boldus*, *Pfaffia sp.*, and *Phyllanthus niruri*, were obtained from food shops. Samples were ground using a cryogenic mill and a pre-cooling time of 5 min was selected followed by 2 min for grinding. This procedure was repeated two times with 1 min of re-cooling each time.

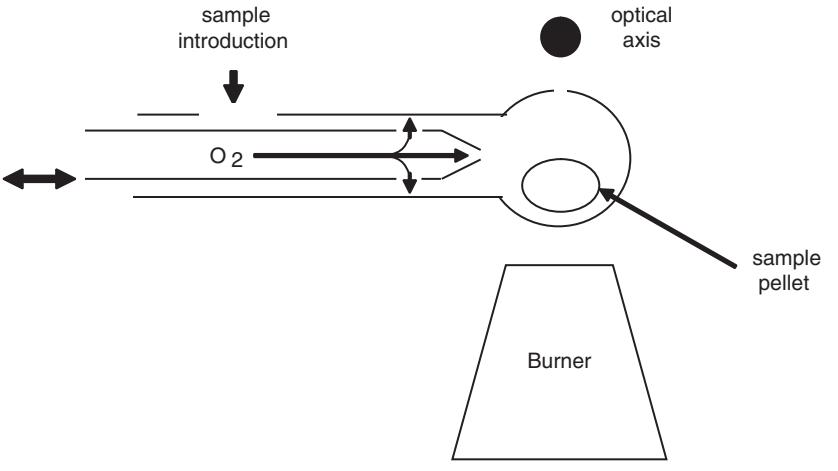

Figure 1. Scheme of the system used for solid introduction and further determination of Cd and Pb by SS-F AAS.

2.4 *Proposed system used for pellets introduction*

The device for SS-F AAS used in this study is schematically shown in Figure 1 (more details can be found in [22]). In this system the respective quartz cell was positioned between the burner and the optical axis of spectrometer. The end of the cell that is in contact with flame has an enlargement where the sample is burned (diameter of 1.4 cm). A slit in the upper part of the extremity of the quartz cell is positioned in parallel with the burner slit. A manually movable hollow quartz piston, through which oxygen is passing (maximum flow rate of 3 L min^{-1}), was fitted to the quartz cell and it was used to thrust the pellets up to the externally heated area of the quartz device (Figure 1). The piston design allows another auxiliary oxygen flow that is supplied just before its tip. This additional oxygen flow was necessary to avoid an eventual return of the combustion fumes across the internal walls of the quartz cell. Initially, the piston is mechanically moved back for pellet introduction step and then moved into its original position when the pellet is pushed up to the quartz cell extremity. When the pellet (up to 100 mg containing graphite and up to 50 mg of ground sample) reaches the end of the quartz cell it quickly burns and the combustion products are driven through the slit and enter into the flame. As described in the previous work, the optical beam was set at 4 mm above the quartz cell [22]. Using these conditions the analytical signals for Cd and Pb were measured in integrated absorbance within 10 s. Calibration was performed by using solid certified reference materials and reference solutions. In the last case, from 10 to 20 μL of reference solutions (concentration between 20 and 200 μg L^{-1}) were added on the graphite pellet before the introduction into the quartz cell.

2.5 *Pellets preparation*

Graphite powder was mixed with ground medicinal plants to produce a mixture with maximum mass of 100 mg with a percent composition of sample and graphite mass of 25 and 75% or 50 and 50%, for Cd and Pb, respectively. This mixture was transferred to the

hydraulic press to prepare the respective pellets. Finally, pellets were kept into closed polypropylene vials up to analysis by SS-F AAS (maximum period of 6 h).

2.6 *Sample digestion in high pressure closed quartz vessels*

Ground commercial medicinal plants were weighed (500 mg) and transferred to quartz vessels followed by the addition of concentrated nitric acid (5 mL), hydrogen peroxide (2 mL), and hydrochloric acid (0.5 mL). Microwave oven was operated according to recommended conditions by manufacturer (ramp of 10 min and holding time of 20 min at 1400 W). After cooling, digests were transferred to polypropylene vessels and diluted with water up to 25 mL for subsequent analysis by ICP–MS.

3. Results and Discussion

3.1 *Initial developments for the proposed procedure by SS-F AAS*

The basic difference between the proposed system and other flame devices used for direct solid sample introduction into the flames is related to the higher sample amount that is possible to introduce in the system. In this work pellets of samples were mechanically thrust by a quartz piston and pushed towards the hot quartz cell where the combustion process occurs. The high temperature caused by pellets combustion ensures that Cd and Pb are volatilised and the continuous flow of O_2 carries the vapours directly to the flame. Contrarily to previous systems [20,21], where samples were transported directly to the flame as solid aerosols, with the current device problems with memory effects were not observed. In the present work, initial tests were carried out by pellets introduction into the quartz device using only different masses of pressed medicinal plants. However, in spite of several attempts, it was not possible to perform the calibration even using solid certified reference materials or reference solutions. In addition, Cd and Pb signals presented changes in sensitivity for the different kinds of medicinal plants despite the analytical signals have been almost Gaussian and completely integrated. It was assumed that the matrix differences could be responsible for the small sensitivity differences observed.

Addition of graphite powder, used as aid for the combustion process and obtained in a high purity level, was used in view of minimising the matrix differences. With this purpose, further studies were carried out with pellets having different masses of graphite. For the evaluation of operational parameters the percent composition of sample and graphite in pellets was kept at 25:75 and 50:50 for Cd and Pb, respectively (maximum total pellet weight was 100 mg). In addition, as the graphite content in the pellet is a well combustible material it acts supplying additional heat and facilitating the analyte volatilisation. No residues were visually detected until 150 combustions. When the pellet achieves the quartz cell the combustion starts in about 2 s and the burn takes 6–10 s depending on the pellet mass. Probably, particles not completely burned that eventually enter into the flame are combusted completely before passing by the optical axis. Memory effects were always negligible and cleaning step was necessary only after more than 180 combustion runs.

Tests for the optimisation parameters of the device used for SS-F AAS were performed using a ground commercial medicinal plant (*Casearia sylvestris*) with $0.13 \pm 0.01\ \mu g\ g^{-1}$ of Cd and $1.06 \pm 0.13\ \mu g\ g^{-1}$ of Pb. The concentration of Cd and Pb in this sample was previously determined by ICP–MS after sample digestion. The following parameters were

investigated for the proposed system: distance between quartz cell and optical axis, flame composition, O_2 flow rate, and pellets composition.

3.1.1 *Influence of the distance between the quartz cell and the optical axis and flame composition*

The influence of the distance between quartz cell and the optical path on the characteristic mass for Cd and Pb was investigated from 2 to 6 mm. These distances were restricted by the dimensions of the quartz device and the available position of burner. Analysis of variance (ANOVA, $P < 0.05$) was performed and results did not show significant differences between values for characteristic mass obtained in the different positions of the cell in relation to the optical path. However, better repeatability was observed for the distance of 4 mm and this position was chosen for subsequent tests. In this case, the distance between the top of burner and the quartz cell was 2 mm. At this distance the cell is close to the hottest region of the flame allowing better heating of the cell and consequently better volatisation of analytes.

The effect of flame composition on Cd and Pb signals was performed using three mixtures of air and acetylene. The characteristic masses were almost constant for mixtures of air and acetylene of 10.2 and 1.0, 10.2 and 1.7, and 8.5 and 2.0 L min^{-1}, respectively. However, slightly better relative standard deviation was observed for $10.2 + 1.7$ L min^{-1} and this condition was used for subsequent studies.

3.1.2 *Effect of O_2 flow rate*

For Cd, the influence of O_2 flow rate in the range of 0.5–3 L min^{-1} was evaluated on the analytical and BG signals using pellets with total masses of 50 mg. As expected, a decrease in sensitivity was observed when relatively higher O_2 flow rates were supplied for the system. On other hand, from 1 to 3 L min^{-1} O_2 the BG decreased about 50 times when compared to 0.5 L min^{-1} (Figure 2(a)). These results could be explained in view of the more complete matrix combustion into the quartz cell, reducing the occurrence of light scattering and molecular absorption. Then, O_2 flow rate of 1 L min^{-1} was chosen as a compromise condition concerning to the characteristic mass ($m_0 = 58$ pg Cd), relative standard deviation (RSD, lower than 10%), and BG (smaller than 0.2 in peak height absorbance). For Pb (Figure 2(b)), the influence of O_2 flow rate was evaluated in the same range of Cd, and as expected, a decrease in sensitivity was observed when higher O_2 flow rates were used. As in the same way for Cd, using higher O_2 flow rates, the BG was significantly decreased. Therefore, O_2 flow rate of 1.5 L min^{-1} was chosen for Pb, regarding to the characteristic mass ($m_0 = 790$ pg Pb), relative standard deviation (RSD lower than 10%), and BG (smaller than 0.2 in peak height absorbance).

Figure 3(a) shows the signal shapes (integrated absorbance and BG in peak height) for Cd determination (absorbance and BG) for 1 L min^{-1} O_2 flow rate. It was possible to observe that analytical signal could be completely integrated in less than 7 s. In addition, the BG signals were within the correction capability of deuterium system (approximately 0.5 in peak height), and no baseline disturbance (e.g. overcorrection) was observed using the chosen conditions.

Similar results were found for lead (Figure 3(b)), but the better O_2 flow rate was 1.5 L min^{-1}. In this condition good sensitivity ($m_0 = 790$ pg Pb), RSD lower than 10%, and BG smaller than 0.2 s were observed. Signal shape for Pb was very similar to those for Cd.

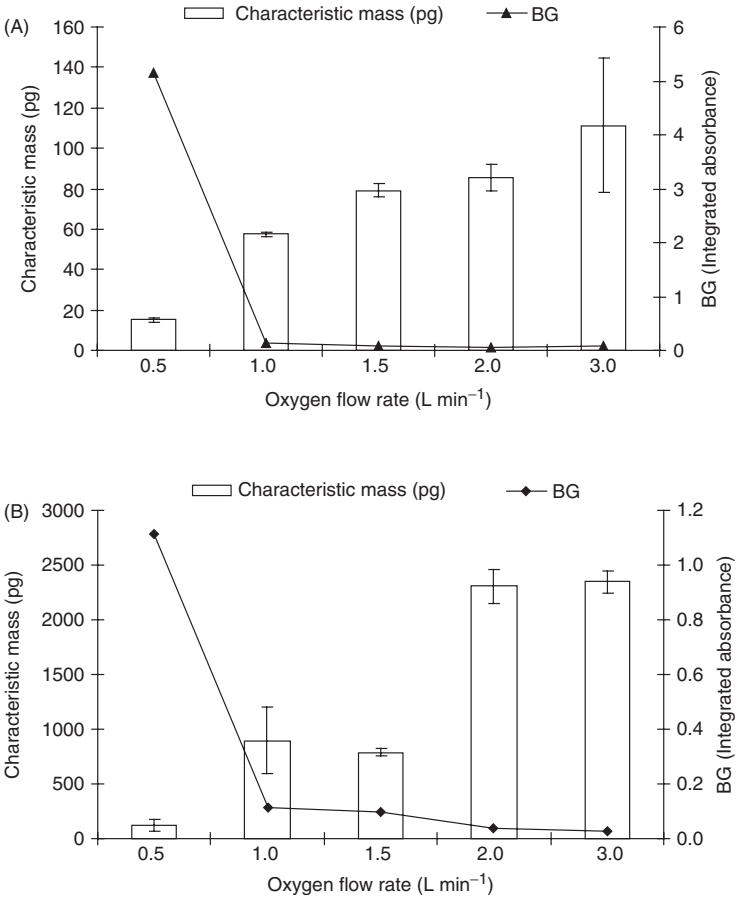

Figure 2. Influence of O_2 flow rate (A) on Cd signal (pellets with 25% of sample and 75% of graphite) and (B) on Pb signal (pellets with 50% of sample and 50% of graphite), in medicinal plant by the proposed procedure by SS-F AAS (pellet mass was from 45 to 51 mg).

3.1.3 *Pellets composition*

In this work, graphite powder was used in order to minimise the effects of the different sample matrices investigated, allowing to increase the sample mass introduced in the atomiser and, consequently, decreasing the LODs. For Cd, the characteristic masses found between different plant species were practically the same using graphite pellets containing 25% of sample. However, for Pb, in spite of reproducible results obtained using pellets with 25% of sample in composition, lower RSD values were found using 50% of sample mass. Then, for subsequent calibration studies the pellets were prepared with the composition of 25 : 75% and 50 : 50% of sample and graphite powder, for Cd and Pb, respectively.

3.2 *Calibration procedures*

Initial attempts were made in order to evaluate the use of reference solutions or solid certified reference materials for calibration. Certified reference materials were used for

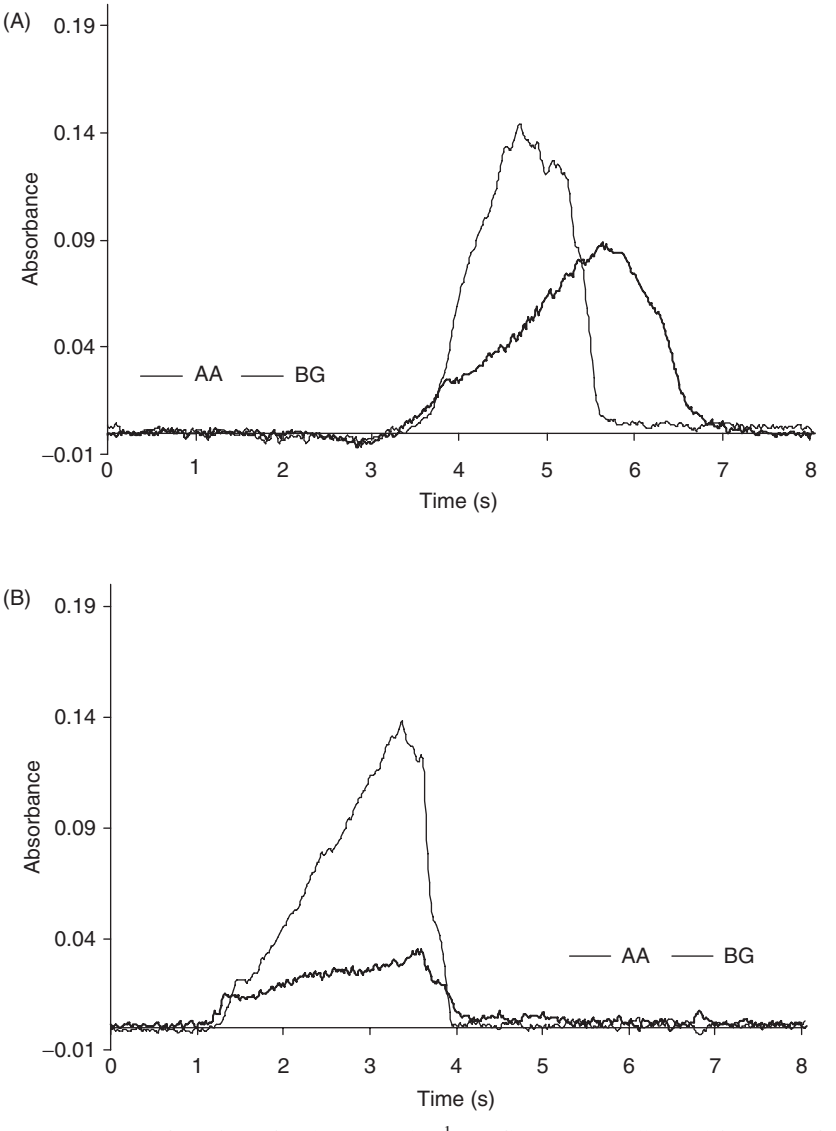

Figure 3. Typical signal for (A) Cd using 1 L min^{-1} O_2 flow rate (pellets with 25% of *Casearia sylvestris* sample and 75% of graphite, total mass of 50.7 mg correspondent to 1.6 ng Cd) and (B) Pb using 1.5 L min^{-1} O_2 flow rate (pellets with 50% of sample and 50% of graphite, total mass of 51.2 mg correspondent to 33.3 ng Pb).

calibration for Cd determination in pellets containing masses up to 100 mg (25% of reference material and 75% of graphite powder). Calibration curves using reference solution and solid reference material were used for Cd determination. It was possible to observe in Figure 4 the good correlation using pellets with the addition of reference solution or solid reference material [$y = 0.0671x + 0.0018$, $R^2 > 0.993$, uncertainty of the slope (standard error) was 0.0014 and the standard deviation of residuals from line ($Sy.x$) was 0.0055].

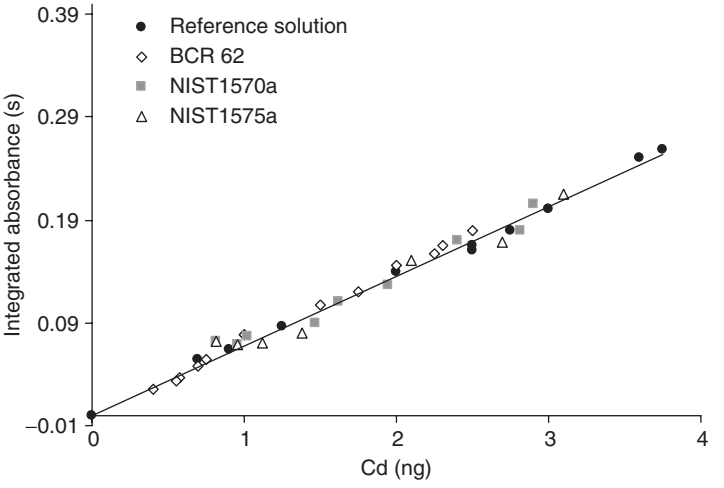

Figure 4. Correlation between integrated absorbance and Cd mass for olive leaves (BCR 062), spinach leaves (NIST 1570a) and pine needles (NIST 1575a) plus graphite pellets and for Cd reference solutions under optimised conditions for the proposed procedure.

Table 1. Results for Cd determination in medicinal plants by SS-F AAS (mean and SD in $\mu g\,g^{-1}$, $n = 5$).

		Calibration method	
Samples	Reference values	A	B
Casearia sylvestris	0.13 ± 0.02*	0.13 ± 0.01	0.14 ± 0.02
Pfaffia sp.	0.10 ± 0.01*	0.10 ± 0.02	0.10 ± 0.01
Phyllanthus niruri	0.15 ± 0.02*	0.14 ± 0.01	0.15 ± 0.02

Note: A: calibration using graphite pellets with certified reference material, and B: graphite pellets with reference solution. The mass of pellets used for both procedures was between 45 and 51 mg.
*Results obtained by ICP–MS after acid digestion.

This result is important in view of more convenient calibration to be possible without use of solid certified reference material. The percent relative error between concentrations for commercial medicinal samples determined by the proposed procedure and those obtained using acid digestion (determined by ICP–MS) was from −7 to 2% for both calibration techniques (Table 1). Moreover, no statistical difference (*t*-test with 95% of confidence level) was found. The relative standard deviation (RSD) was $<10\%$ for all samples ($n = 5$). This data evidenced the feasibility of calibration using reference solutions instead of solid certified reference material.

For lead, the calibration was also performed using reference solutions or solid reference materials in graphite pellet form (Figure 5).

Contrarily to Cd, for Pb the slope of the calibration curves obtained with pellets of certified reference materials and reference solutions was different. Calibration using reference solutions was not possible and further attempts in order to obtain better agreement were not successful. The difference of sensitivity observed for Pb by comparison

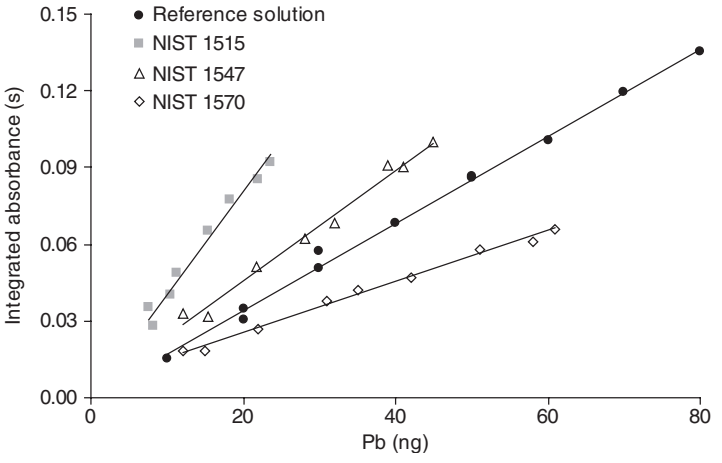

Figure 5. Correlation between integrated absorbance and Pb mass for apple leaves (NIST 1515), peach leaves (NIST 1547), and spinach leaves (NIST 1570) plus graphite pellets and for Pb reference solutions under optimised conditions for the proposed procedure.

Table 2. Results for Pb determination in medicinal plants by SS-F AAS (mean and SD in $\mu g\,g^{-1}$, $n = 5$).

		Calibration method	
Samples	Reference values	A	B
Casearia sylvestris	1.06 ± 0.13*	1.10 ± 0.07	1.53 ± 0.24
Pfaffia sp.	0.32 ± 0.04*	0.34 ± 0.03	0.24 ± 0.05
Peumus boldus	0.45 ± 0.04*	0.44 ± 0.03	1.06 ± 0.15

Note: A: calibration using graphite pellets with certified reference material, and B: graphite pellets with reference solution. The mass of pellets used for both procedures was between 45 and 51 mg.
*Values obtained by ICP–MS after acid digestion.

of the analytical curves for solid samples and reference solutions could not be fully understood evaluating the complete set of experimental data. In addition, as the chemical form of Pb in the samples is unknown, it is very difficult to make conclusive appointments concerning to the mechanism of atomisation of Pb from the different samples. However, Pb determination using solid reference materials was shown to be feasible. Table 2 presents the results for analysis of three medicinal plants. For Pb, the agreement between values found for medicinal plants determined by SS-F AAS and by acid digestion and determination by ICP–MS was considered satisfactory (*t*-test, 95% of confidence level) only for calibration using solid certified reference material (between −2 and 3%).

3.3 *Figures of merit*

The main figures of merit for the proposed procedure are shown in Table 3. The characteristic masses obtained for Cd and Pb were 58 and 790 pg, respectively.

Table 3. Analytical figures of merit for the proposed procedure.

Figure	Cd	Pb
Sample mass (maximum), mg	25	50
Pellet mass (maximum), mg	100	100
LOD ($n = 10$), $\mu g\,g^{-1}$	0.012	0.10
m_0, pg	58	790
RSD, %	<10	<10
Percent relative error, %	−7–2	−2–3
Throughput, h^{-1}	40	40

These values were considered satisfactory when compared with results obtained using other combustion procedures and subsequent F AAS analysis [18,21]. Using the proposed procedure the sensitivity for Cd was 7-fold better than a similar system using paper capsules for solid introduction [20].

The LOD for Cd was $0.012\,\mu g\,g^{-1}$ applying a sample mass of 25 mg (pellet mass = 100 mg). For Pb the LOD was $0.10\,\mu g\,g^{-1}$ using 50 mg of sample. The results show that is possible to perform the determination of Cd and Pb with suitable LODs for plant analysis using conventional spectrometers with flame atomisers. In addition, it was achieved a sample throughput of 40 samples per analyte per hour, excluding the pellet preparation step, and with suitable accuracy, repeatability and LODs. Based on the relatively high throughput and the nonnecessity to use more expensive techniques to obtain low LODs, it is possible to propose the SS-F AAS procedure for the determination of Cd and Pb in medicinal plants. Moreover, the proposed procedure does not require a digestion step avoiding the use of toxic reagents and consequently causing a reduction of the effluents that could be generated by the laboratory. It is important to point out that this aspect is in agreement to the recommendations of green chemistry. On the other hand, the proposed procedure allows a relatively high throughput for the determination of Cd and Pb in medicinal plants that could help to a fast quality control of these matrices for environmental assessment.

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