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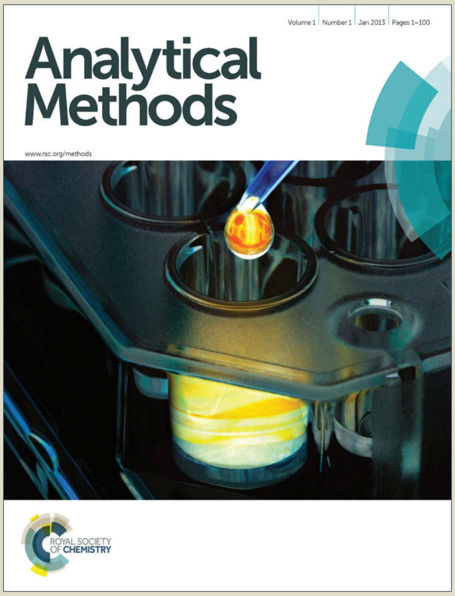

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Determination of Cd and Cr in tannin samples by high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample sequential analysis

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Abstract

A fast and reliable analytical method for the sequential determination of Cd and Cr in tannin samples by high-resolution continuum source graphite furnace atomic absorption spectrometry with direct solid sampling from the same sample aliquot (HR-CS SS-GF AAS) is proposed. A pyrolysis temperature of 400°C and atomisation temperature of 1500°C were used for the determination of Cd; no losses of Cr were observed at these temperatures. After the atomisation of Cd, the wavelength was changed and Cr was atomised at 2500°C. Several studies with different modifiers were performed, but no chemical modifier was needed. Calibration was achieved using aqueous standards. The linearity of the response, performed evaluating the absorbance value relative to the sample mass, was investigated as well. Relative standard deviation (RSD) values lower than 15% were found, which are acceptable for direct analysis. The parameters of merit were: characteristic mass of 0.3 pg and 2.2 pg for Cd and Cr, respectively; limit of detection (LOD) of 0.5 μg kg^{-1} and 17 μg kg^{-1} for Cd and Cr,

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respectively; limit of quantification (LOQ) of 2 µg kg^{-1} and 57 µg kg^{-1} for Cd and Cr, respectively. The accuracy was validated by analysing three certified reference materials (tea, pine needles and bush branches). The results were not statistically different from the certified values at a 95% confidence level. Therefore, calibration using aqueous standards is suitable for this application. This method is simple, fast and suitable for routine analysis.

Keywords: Tannin; HR-CS SS-GF AAS; Sequential analysis; Cadmium; Chromium

1. Introduction

Tannins are a class of polyphenolic compounds found manly in the bark, wood and seeds of several species of trees.[1] Their main function in plants is as a defence against insect attack, as reported by War *et al.*[2] They are classified, according to chemical structure, as condensed or hydrolysable tannins.[3,4] Condensed tannins are formed by flavonoid units and are present in black wattle, quebracho and pine tree.[3,4] Hydrolysable tannins (tara and chestnut) are a mixture of phenols, esters of sugars and other complex structures.[3,4]

The ability of tannins in chelate metals ions has been shown in several studies to be due to the presence of –OH groups in the ortho position.[5,6] The form of the metal chelate depends on the pH value and the nature of the metal ion.[6] This characteristic allows tannins to be used in corrosion studies, surface preparation and water treatment.[5,7,8] The use of tannins as coagulants in the treatment of drinking water may replace the utilisation of aluminium sulphate, thereby decreasing human exposure to aluminium.[8]

The ability to tannins to interact with some enzymes and proteins can inhibit the activities of bacteria (bactericidal) and viruses (antiviral), which makes these compounds suitable for industrial, medical and pharmaceutical applications.[4,9,10] Some authors have also

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reported antioxidant and anticancer activities of tannins.[11,12] Tannins are also used in wine production (oenological tannin), as they improve the colour and oxidation stability of wine.[13]

According to a review by Butler *et al.*[14] flame atomic absorption spectrometry (F AAS), electrothermal atomic absorption spectrometry (ET AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), laser-induced breakdown spectroscopy (LIBS), X-ray spectrometry and secondary ion mass spectrometry (SIMS) are the most commonly used techniques to detect metals in plants, soils and related materials. HR-CS AAS has emerged as a very promising technique in this scenario because it provides a great advantage for direct sampling analysis (SS). The entire spectral environment of the analytical line becomes visible, enabling the detection of spectral interferences.[15] The initial procedures of an analytical method are responsible for the accuracy of the chemical information. The sample preparation phases required for the determination of metals in biological samples through spectroanalytical techniques include digestion (wet and microwave-assisted) and assisted extraction.[14,16]

As reported by Norderberg *et al.*[17] and Langard,[18] Cd and Cr can cause several diseases when ingested. Thus, a reliable analytic technique is necessary to determine Cd and Cr concentrations in products before consumption. Some studies in the literature report on the determination of metals in different samples containing tannin. Ross *et al.*[19] used electrospray ionisation mass spectrometry (ESI-MS) to characterise metal tannin complexes and dissolved tannins in organic matter. Schmidt *et al.*[20] determined the Al(III) and Fe(III) concentrations in soils with a high amount of polyphenols by ICP-OES. High performance liquid chromatography (HPLC) was used to assess for the presence of tannin/metal complexes in aqueous samples.[20]

Direct solid sampling (SS) in HR-CS AAS has become an interesting technique because of the possibility of sequential analysis by HR-CS AAS.[21,22] Its continuum spectrum

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(190 nm to 900 nm) and rapid selection of the correct wavelength by a high-resolution double monochromator make HR-CS AAS ideal for sequential analysis.[21-23] The possibility of direct solid sampling in HR-CS GF AAS is other advantage since it eliminates many steps involved in sample preparation, avoiding the risk of contamination and reducing the preparation time.[24,25] In many cases, calibration with aqueous standards is also possible in the direct solid sampling technique.[24]

According to the literature, some cases of sequential determination of analytes by HR-CS GF AAS have been reported.[21,26-29] Analytes with significantly different volatilities and near absorption lines, localised within the spectral range of the CCD detector, can be determined simultaneously with two different atomisation temperatures.[26-28] When the absorption lines are relatively well-separated, two different atomisation temperatures are applied in sequential determination, since the analytes have different volatilities. In this case, the wavelength line changes between each atomisation cycle and atomisation starts with more volatile before less volatile analytes.[21,26,29]

Due to the use of tannins in drinking water treatment and in the production of pharmaceuticals and wines, the presence of heavy metals can be potentially dangerous to human health. In this scenario, a fast and reliable analytical method to determine Cd and Cr in tannin samples by HR-CS SS-GF AAS with direct solid sampling and sequential atomisation from the same sample aliquot was proposed. To the best of our knowledge, the determination of Cd and Cr in solid tannin samples, especially by sequential determination with HR-CS SS-GF AAS, has not been published up to now.

2. Experimental

2.1. Instrumentation

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All measurements were carried out using a Model contra 700 high-resolution continuum source atomic absorption spectrometer (Analytic Jena AG, Jena, Germany), equipped with a transversely heated graphite tube atomiser. The contra 700 is equipped with a xenon short-arc lamp with a nominal power of 300 W operating in hot-spot mode. The high-resolution double monochromator has a prism pre-monochromator, a high-resolution echelle monochromator and a linear charge coupled device (CCD) array detector with 588 pixels, 200 of which are used for analytical purposes. The analytical lines at 228.802 nm for Cd and 357.869 nm for Cr were used. Atomic absorption was measured using the centre pixel (CP) and the two adjacent pixels (CP±1), corresponding to a spectral interval of 3.9 pm for Cd and 6.0 pm for Cr. The experiments were carried out using the same SS platforms (Analytik Jena Partno.407-152.023) and SS tubes without a dosing orifice (Analytik Jena Partno.407-A81.303).

An M2P microbalance (Sartorius, Göttingen, Germany) was used for weighing the tannin samples directly onto the SS platforms, which was introduced into the graphite tube using a pair of pre-adjusted tweezers from the SSA 6 manual solid sampling accessories (Analytik Jena AG). The sample mass was transmitted to the instrument's computer to calculate the integrated absorbance normalised to a sample mass of 0.25 mg after each measurement. The aqueous standards were injected manually onto the platform using a micropipette. Argon with a purity of 99.996% (White Martins, São Paulo, Brazil) was used as the purge gas with a flow rate of 2.0 L min^{-1} during all stages, except during atomisation, when the flow was stopped for the determination of Cd and Cr. The optimum parameters for the graphite furnace temperature for both elements are shown in Table I.

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2.2. Reagents and solutions

Analytical grade reagents were used exclusively. Deionised water with a specific resistivity of 18 MΩ cm^{-1} from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for the preparation of standards.

The nitric acid (Merck, Darmstadt, Germany) used to prepare the standard solutions was further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany). All containers and glassware were soaked in 1.4 mol L^{-1} nitric acid for 24 hours and rinsed with deionised water before use.

The Cd and Cr stock standard solutions (1000 mg L^{-1}) were prepared by Specsol (Brazil). The working standards were prepared by serial dilution of the stock solutions with the addition of 0.014 mol L^{-1} nitric acid (Merck, Germany). The chemical modifiers tested were 0.03% (m/v) $Mg(NO_3)_2$ and 0.5% (m/v) $NH_4H_2PO_4$ (Merck, Germany), both in 0.05% (v/v) Triton X-100 (Union Carbide). The use of Triton X-100 was necessary to increase the interaction between sample and the modifier.[30]

2.3. Certified reference materials, samples and sample preparation

The following certified reference materials were used in this work for method development and validation: NCSZC73012 Tea certified reference material (China National Analysis Centre for Iron and Steel, China), SRM 1575a Trace Elements in Pine Needles (National Institute of Standards and Technology, USA**),** DC73349 Bush Branches and Leaves (China National Analysis Centre for Iron and Steel, China).

Eight tannin samples investigated in this work were taken from local industry: pine tannin, black wattle tannin, chestnut tannin, tara tannin, acacia mimosa tannin, quebracho tannin and two oenological tannins.

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Tannin samples were ground in an A-11 Basic micro-mill (IKA-Werke, Germany) and sieved through 150 µm polyester mesh to improve the particle size distribution. The part of the sample that did not pass through the sieve was ground again, until all samples were sieved. After this, the samples were dried at 50°C for three hours in a stove in order to eliminate absorbed humidity and stored in sealed plastic vials until further processing.

The sample mass (0.02 to 0.25mg) was weighted directly on the platform and introduced into the graphite tube by HR-CS SS-GF AAS for the sequential determination of Cd and Cr.

3. Results and discussion

3.1. Evaluation of chemical modifier use and the temperature program

Initially, the use of a chemical modifier was considered due to the high volatility of Cd.[25] A preliminary study of the amount of modifier was performed in order to find the optimum mass that thermally stabilises Cd with no loss of Cr. Initially, the chemical modifiers $NH_4H_2PO_4$ and $Mg(NO_3)_2$were tested based on the literature for Cd and Cr, respectively.[25]

Masses between 1.5 and 6 µg of $Mg(NO_3)_2$ with a pyrolysis temperature of 400°C and atomisation temperatures of 1500°C and 2500°C were investigated for Cd and Cr, respectively (Figure 1a). As can be seen in Figure 1a, the use of magnesium nitrate did not significantly modify the absorbance signal of 150 pg Cr in the aqueous standard. At the same time, the presence of magnesium nitrate decreased the absorbance signal of 10 pg Cd. Regarding the ammonium phosphate modifier (Figure 1b), masses between 2.5 and 10 µg of $NH_4H_2PO_4$ were tested. As shown in Figure 1b, the absorbance signal did not change significantly for Cd and Cr standard solutions, but decreased significantly for the pine tannin samples for both analytes. As consequence of the proposed study, the use of a chemical modifier was not necessary for the determination of Cd and Cr in tannin samples. As reported

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by Duarte *et al.*,[21] the effect of a Pd/Mg modifier was not pronounced in the determination of Cd in biomass samples. Thus, the use of a Pd/Mg chemical modifier was not considered.

Pyrolysis curves for 10 pg of Cd and 150 pg of Cr in aqueous standards and pine tannin samples, without a chemical modifier, are given in Figure 2. As can be seen, Cd was very stable in the aqueous standard and in the pine tannin solid sample until 400ºC (pyrolysis temperature). At temperatures lower than 1500°C, no losses of Cr were detected. The pyrolysis temperature of 400ºC was chosen and used in all further experiments.

Figure 3 shows the atomisation curves for 10 pg Cd and 150 pg Cr in aqueous standards and pine tannin samples. According to the atomisation curves for Cd (Figure 3), temperatures between 1100°C and 1500°C can be used without a significant decrease in the absorbance signal. For the Cr analyte, temperatures between 2200°C and 2500°C can be used without a decrease in integrated absorbance (Figure 3). The absorbance signals and time- and wavelength-resolved absorbance spectra (Figure 4) were also considered when choosing the proper furnace conditions for Cd and Cr. Figure 4 shows a well-defined peak shape for pine samples for Cd and Cr using the atomisation temperatures of 1500°C and 2500°C, respectively. The absence of background absorption is clear evidence of the background correction made by the equipment (Figure 4). The selected atomisation temperatures were 1500°C for Cd and 2500°C for Cr and were used in all further experiments. The complete temperature program is given in Table I, and as previously mentioned in the literature,[21] the second pyrolysis stage was only made due to the software settings.

3.2. Influence of tannin sample mass

Belarra *et al.*[31] reported complications with the solid sampling method, such as the influence of sample size, homogeneity and problems in sample introduction. Considerable variability in the amount of mass can lead to errors.[32]

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The maximum amount of sample mass that is acceptable for use in solid sampling analysis is reported in the literature.[33] Masses higher than the ideal (critical mass) deviate the integrated absorbance from linearity, which also may decrease the precision.[30] Therefore, the influence of tannin sample mass in the linearity of the response for Cd and Cr was investigated, and the curves were established using pine tannin samples. The response remained linear until 0.25 mg for the determination of Cd (R=0.96) and Cr (R= 0.95) in pine tannin, and 0.3 mg for the determination of Cd (R=0.95) and Cr (R=0.94) in quebracho tannin. Thus, the maximum mass of tannin used in this work was limited to 0.25 mg.

3.3. Figures of merit

Table II gives the figures of merit for the determination of Cd and Cr in tannins by a sequential method. The calibration curves were performed using a blank and aqueous solutions of Cd and Cr with concentrations between 0.0-5.0 µg L^{-1} and 0.0-40 µg L^{-1} for Cd and Cr, respectively. The linear regression equations and the corresponding correlation coefficients are shown in Table II. The limits of detection (LOD) and quantification (LOQ) were calculated (for a sample mass of 0.25 mg) using three and ten times, respectively, the standard deviation of the blank. The measurements of the blank were performed using the "zero mass response" method[33] that consists of running the entire temperature program with an empty platform, several times. For the determination of Cd in all tannin samples, the analytical line of 228.802 nm was used, while for determination of Cr, the analytical line was 357.869 nm (highest sensitivity). In both cases, CP ±1 (centre pixel) was used for analysis.

The characteristic mass (m_o), LOD and LOQ (Table II) are in agreement with the values found in the literature. Török *et al.*[34] used direct solid sampling by ET AAS for the determination of Cd in foodstuffs and found an m_o of 0.7 pg and LOD of 0.279 µg kg^{-1} based on a 5 mg mass of sample. According to dos Santos *et al.,*[27] who used HR-CS SS-GF AAS for

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the determination of Cd in bean and soil samples, an m_0 of 0.7 pg and LOD of 2.0 µg kg^{-1} were found. Duarte *et al.*[21] reported an m_o of 0.4 pg and LOD of 1.1µg kg^{-1} in the determination of Cd in biomass by HR-CS SS-GF AAS. The authors also determined Cr by the same technique, and obtained an m_o of 2.5 pg and LOD of 21 µg kg^{-1}. Lepri *et al.*[35] reported 4.8 pg (m_o) and 50 µg kg^{-1} (LOD) in the determination of Cr in carbon black and activated charcoals by HR-CS SS-GF AAS.

3.4. Analytical results

Due to the lack of a tannin certified reference material (CRM), tea, pine needle and bush branch CRM were used in this work due to the presence of polyphenols in their composition.[36,37] As reported by Wang *et al.*,[36] the main polyphenols present in tea are flavonoids and gallic acid. On the other hand, Park *et al.*[37] described the presence of proanthocyanidins and catechins in pine needles. Therefore, the accuracy of the proposed method was verified by the utilisation of these CRM. The concentration of Cd found in the pine needle CRM was 0.20 ± 0.01 mg kg^{-1} (RSD of 6%) while its certified value is 0.23 ± 0.04 mg kg^{-1}. The tea CRM has a certified value of 62 ± 4 µg kg^{-1}, and the proposed technique gave a value of 64 ± 4 µg kg^{-1} (RSD of 7%). In relation to Cr, the bush branch CRM has a certified value of 2.6 ± 0.2 mg kg^{-1}, while the proposed method found a value of 2.8 ± 0.2 mg kg^{-1} (RSD of 7%). For the tea CRM, the concentration of Cr found was 0.46 ± 0.02 µg kg^{-1} (RSD 3%), while its certified value is 0.45 ± 0.01 µg kg^{-1}. Additionally, the results did not differ significantly at the 95% confidence level when Student's *t*-test was applied, confirming the accuracy of the method. This result supports the possibility of using aqueous standards for calibration.

Table III shows the values for Cd and Cr in tannin samples. The values for Cd were between 2.6 ± 0.3 µg kg^{-1} and 180 ± 25 µg kg^{-1}, while for Cr they were between 0.8 ± 0.1 mg

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kg^{-1} and 4.4 ± 0.3 mg kg^{-1}. The highest concentration of Cr was found in tara tannin (4.4 ± 0.3 mg kg^{-1}) and the lowest concentration was found in quebracho tannin (0.8 ± 0.1 mg kg^{-1}). The highest concentration of Cd was found in pine tannin (180 ± 25 µg kg^{-1}), followed by chestnut tannin (53 ± 8 µg kg^{-1}). Other samples showed very low concentrations of Cd when compared to the pine and chestnut tannins (e.g. black wattle tannin has ~65 fold less Cd than pine tannin). The variability in the metal concentrations in tannins could be attributed to several factors: different tannin extraction methods, dissimilar biological characteristics of the trees, geographical localisation of the plants, soil and air composition, and pollution.[38,39]

Since tannins are frequently used for oenological purposes, as medicines and in drinking water treatment (high amounts), methods to control the concentration of heavy metals are necessary to avoid human exposure. The proposed method avoids extensive manipulation of the sample, and is a fast and reliable procedure to detect Cd and Cr in tannins by sequential analysis.

4. Conclusion

The use of the high-resolution continuum source graphite furnace atomic absorption spectrometry technique with direct solid sampling and sequential atomisation from the same sample aliquot was proposed to determine Cd and Cr levels in tannin samples. The method is accurate and allows the use aqueous standards for calibration. As another advantage, the method does not require the use of a chemical modifier and permits the fast sequential analysis of Cd and Cr. It is expected that this technique can be extended to different analytes. The analysed tannin samples showed different concentrations of Cd and Cr, which could have been influenced by several factors (extraction methods, biological characteristics, geographical localisation, soil and air composition, and pollution). As tannin samples are

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frequently used in drinking water treatment, medicine and the wine industry, monitoring the concentration of harmful metals may be of great value to human safety.

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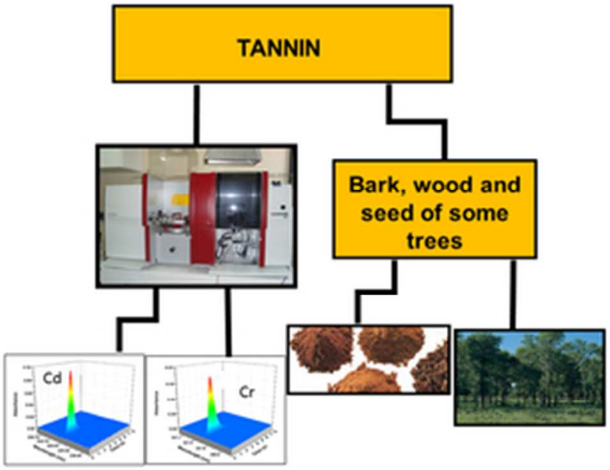

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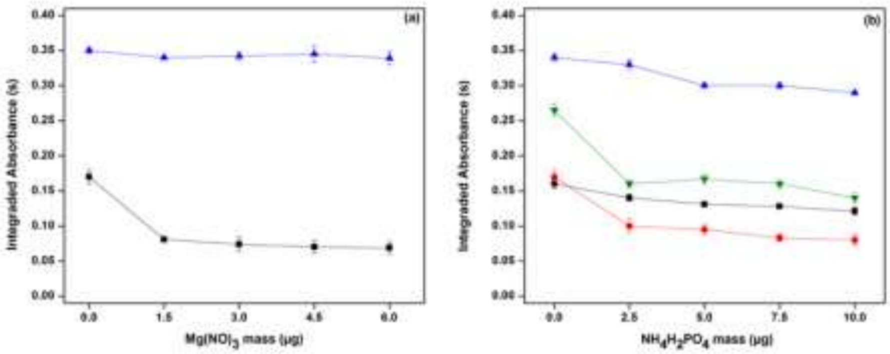

Figure 1: (a) Influence of the $Mg(NO_3)_2$ chemical modifier mass on the integrated absorbance signal for 10 pg Cd (black square) and 150 pg Cr (blue triangle). (b) Influence of the $NH_4H_2PO_4$ chemical modifier mass on the integrated absorbance signal for 10 pg of Cd (black square), 150 pg of Cr (blue triangle), pine Cd (red circle) and pine Cr (inverted green triangle). The integrated absorbance was normalised to 0.25 mg of pine tannin. T_{pyr} = 400°C and T_{atom} = 1500°C for Cd; T_{pyr} = 1500°C and T_{atom} = 2500°C for Cr.
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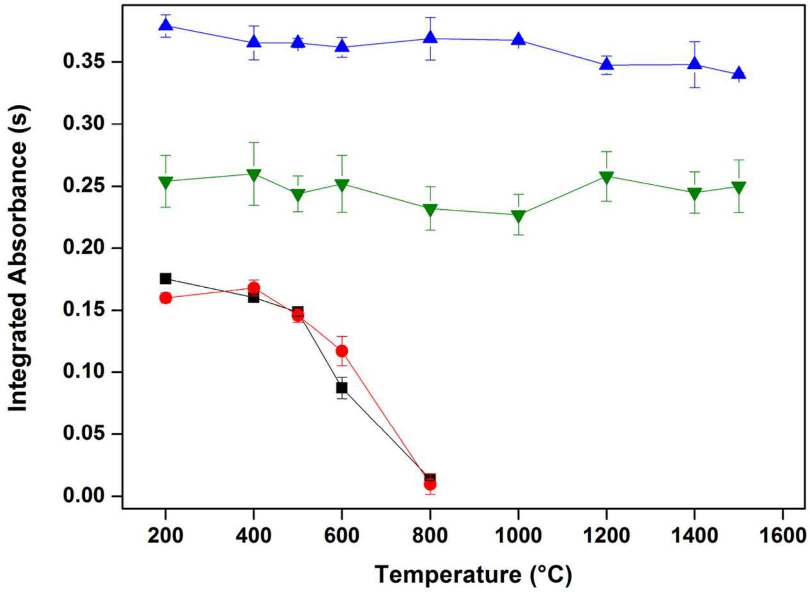

Figure 2: Pyrolysis curves for Cd (T_{atom}=1500°C) and Cr (T_{atom} = 2500°C); 10 pg Cd (black square) and 150 pg Cr (blue triangle), both in aqueous solution; integrated absorbance signal for Cd (red circle) and Cr (inverted green triangle), both normalised to 0.25 mg pine tannin.
124x87mm (300 x 300 DPI)

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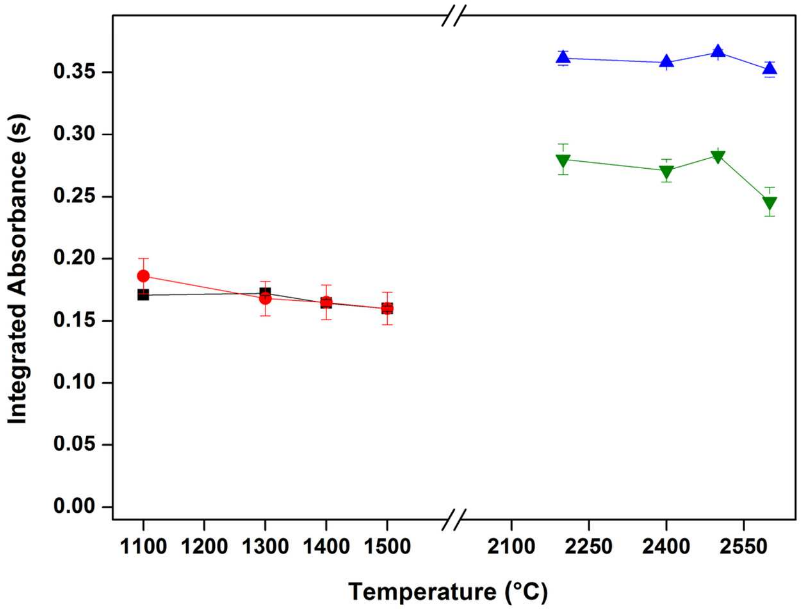

Figure 3: Atomisation curve for Cd (T_{pyr} = 400°C) and Cr (T_{pyr} = 400°C and 1500°C); 10 pg Cd (black square) and 150 pg Cr (blue triangle), both in aqueous solution; integrated absorbance signal for Cd (red circle) and Cr (inverted green triangle), both normalised to 0.25 mg pine tannin.
124x87mm (300 x 300 DPI)

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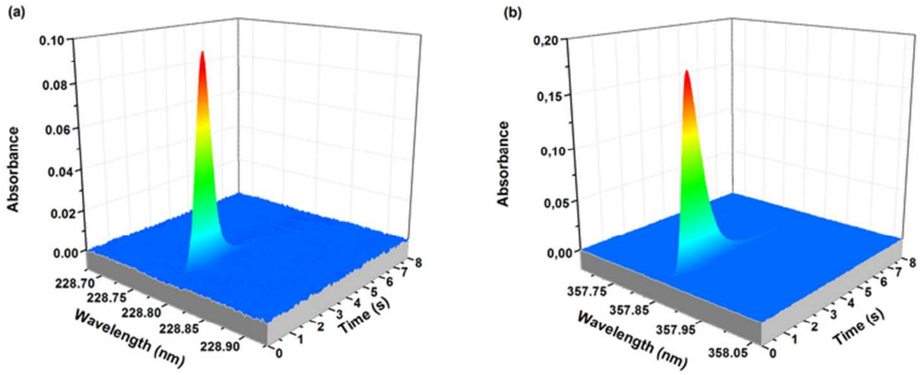

Figure 4: Time and wavelength resolved absorbance signals for (a) Cd (T_{atom} = 1500°C) and (b) Cr (T_{atom} = 2500°C) in 0.25 mg pine tannin.
70x30mm (300 x 300 DPI)

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Table I : Graphite furnace temperature program for the determination of Cd and Cr in tannin samples by HR-CS SS-GF AAS

Stage	Temperature (°C)	Ramp (°C s^{-1})	Hold time	Gas flow rate (L min^{-1})
Drying 1	90	10	20	2
Drying 2	120	20	40	2
Pyrolysis	400	300	40	2
Atomization [a]	1500	3000	8	0
		Cooling and change of wavelength		
Pyrolysis	1500	500	1	2
Atomization [b]	2500	3000	7	0
Cleaning	2650	500	8	2

[a] Atomization of Cd [b] Atomization of Cr

Table II: Figures of merit obtained of the sequential determination of Cd and Cr in tannin samples using HR-CS SS-GF AAS

Parameter	Cd (CP ±1) λ=228.802 nm	Cr (CP±1) λ=357.869 nm
LOD* (µg/kg)	0.5	17
LOQ* (µg/kg)	2	57
m_0 (pg)	0.31	2.2
Linear regression equation	$A_{int} = 0.01316\ m\,(pg) + 0.00201$	$A_{int} = 0.00194\ m\,(pg) + 0.01346$
Correlation coefficient	0.99952	0.99781

* LOD and LOQ calculated for 0.25mg of sample for Cd and Cr

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Table III: Results for Cd and Cr in tannin samples using HR-CS SS-GF AAS and calibrations against aqueous solutions

Sample	Cd (µg kg^{-1})		Cr (mg kg^{-1})	
	Found value	RSD (%)	Found value	RSD (%)
Pine Tannin	180 ± 25	14	3.1 ± 0.3	9
Black wattle tannin	2.6 ± 0.3	10	3.2 ± 0.2	6
Quebracho tannin	3.5 ± 0.2	7	0.8 ±0.1	13
Acacia Mimosa Tannin	8.0 ± 0.8	10	1.8 ± 0.1	5
Chestnut tannin	53 ± 8	15	2.4 ± 0.2	8
Oenological tannin	3.8 ± 0.4	10	3.1 ± 0.2	6
Oenological tannin 2	21 ± 2	9	3.0 ± 0.4	13
Tara tannin	3.7 ± 0.4	11	4.4 ± 0.3	7

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