DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS ARSENIC, ANTIMONY, SELENIUM AND MERCURY DETERMINATION IN PLANTS BY ENERGY DISPERSIVE X RAY FLUORESCENCE SPECTROMETRY

LORENA CORNEJO-PONCE, JORGE ACARAPI-CARTES and MARÍA ARENAS-HERRERA

SUMMARY

The objective of the present work was to develop, implement and validate an analytical methodology, based on the energy dispersive X-ray fluorescence spectrometry (EDXRF), technique for the direct, fast and simultaneous determination of arsenic (As), antimony (Sb), selenium (Se) and mercury (Hg) in samples of wild plants present in the region of Arica and Parinacota, located at the heart of the Atacama Desert. The method was optimized and validated in order to achieve the lowest detection limits. The validation plan of the proposed methodology considered the parameters of linearity, sensitivity, detection limits, accuracy, precision and matrix effect. Detection limits of 2.3, 3.6, 19.8 and 1.6mg·kg^{-1} (dry basis) were determined for As, Se, Sb and Hg, respectively. Concentrations of these elements were measured in plants of different species growing in the region. It is concluded that, relative to conventional methods for determining these elements in plant tissues, the proposed methodology is a suitable analytical alternative that does not require steps such as sample dissolution or analyte transformation in hydride/volatile metal vapors for separation and/or extraction from the matrix prior to instrumental analysis.

The presence, accumulation and dynamics of metallic chemical elements and metalloids in the environment are currently a subject of great relevance and an object of abundant research. This interest is due to the fact that some of these elements may be potentially dangerous, given their toxicity and ability to transfer from the surrounding environment to living organisms, accumulating through the food chain (bioaccumulation and biomagnification; Khana *et al*., 2010; Cornejo and Acarapi, 2011; Bundschuh *et al*., 2012; Hu *et al*., 2014). Plants in particular are able to increase their contents of chemical elements due to the incorporation from the environment, in which case the presence of certain metal/metalloid elements will depend on both natural factors (bedrock/horizon D composition and/or local geothermal activity) and anthropogenic factors (e.g. agriculture and mining). Among the metals/metalloids currently being studied with greater interest are arsenic (As), antimony (Sb), selenium (Se) and mercury (Hg).

Arsenic is widely distributed in the environment, which is why different international regulations (INN,

KEYWORDS / Antimony / Arsenic / EDXRF / Mercury / Multielemental Analysis / Selenium / Vegetation /

Received: 03/10/2017. Modified: 06/01/2018. Accepted: 06/04/2018.

Lorena Cornejo-Ponce. Chemist, Master in Analytical Chemistry and Doctor of Science, University of Campinas, Brasil. Professor, Universidad de Tarapacá (UTA), Chile. Address: Laboratory of Environmental Research for Arid Zones (LIMZA), UTA. General Velásquez 1775, Arica, Chile. e-mail: lorenacp@uta.cl

Jorge Acarapi-Cartes. Chemist and Master in Chemistry, University of Tarapacá, Chile. Master in Engineering in Quality Systems and Productivity, Instituto Tecnológico de Monterrey, Mexico. Research Assistant, LIMZA-UTA, Chile.

María Arenas-Herrera. Chemist and Master in Environmental Sciences, University of Tarapacá, UTA, Chile. Research Assistant, LIMZA-UTA, Chile.

2005; WHO, 2011) consider the safe levels of human exposure to this element, recognizing its toxicity to humans (Hughes, 2011). In the case of plants, As is generally biotransformed into less toxic organic species; however, there are cases where accumulation of high levels of inorganic As have been identified, such as in rice (D'Amato *et al*., 2004; Sanz *et al.*, 2005; Matos-Reyes *et al.*, 2007).

Antimony is an element about which little is known, yet it is considered as a primary environmental pollutant by the USA Environmental Protection Agency and the European Union, thus acquiring increasing interest as a global pollutant. The biological function of this element is unknown, but it may be toxic at high concentrations.

Selenium is a trace element of great interest because it is essential for humans and animals at low concentrations, while it is also an antioxidant present in essential enzymes (Dumont *et al.*, 2006).

Mercury is distributed through different media in the environment, being transformed into different chemical species, among which the organic forms are notable as they have the highest levels of toxicity (Clarkson, 1998; Feng *et al.*, 2008; Zhang *et al.*, 2010).

Currently, the determination of heavy metals in plant samples is based on different standardized and non-standardized test methods using atomic absorption spectrophotometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES or inductively coupled plasma mass spectrometry (ICP-MS) (Vassileva and Hoenig, 2001; Welna and Szymczycha-Madeja, 2014; Galvão *et al.*, 2016; Habte *et al.*, 2016). All of these methods have in common a prior wet or dry digestion step that is used to convert the solid sample state to an aqueous solution (ISO, 2004, 2014). The solution obtained is then combined with suitable reagents that allow analyte transformation, its separation from the aqueous medium, transport towards the atomization system and interaction with the light in the spectrophotometric system, where it is finally registered in, most commonly, a computational support system (ISO, 1984, 2004).

In the case of As, Sb and Se determination, the standard technique considers the previous formation of the corresponding volatile hydride (HG) and its subsequent analysis by atomic absorption spectrophotometry (AAS). This technique promotes the formation of volatile species of AsH_3, SbH_3 and SeH_2, using borohydride as a reductant (Ranesh and Riyazuddin, 2005). An important aspect to consider is that within determination frameworks of these elements by AAS, they all behave as mutual chemical interferers (Petrick and Krivan, 1987).

In a similar way, the determination of Hg has been carried out using an atomic cold vapor generation, coupled with techniques such as atomic absorption spectrophotometry (CV-AAS) (APHA, 2005; De Jesus *et al.*, 2013).

The methods referred above for the quantitative determination of the elements under study are characterized by having a sequential character that significantly increases the analysis time, increases the complexity of the test, generates the possibility of cross contamination during the preparation of patterns and samples, generates risks for the operator because of the handling of dangerous substances (mineral and organic acids), and generates and emits chemical residues to the environment in the form of vapors, particles or liquids. Similarly, these methods require the destruction of the sample prior to its analysis, which is a disadvantage when limited amounts of a sample are available, as is the case in the exploration of recently discovered plant species that have not been widely studied or are protected by local regulations (Marguí *et al.*, 2009; De la Calle *et al.*, 2013).

The energy dispersive X-ray fluorescence spectrometry (EDXRF) technique is a non-destructive analysis technique that has been greatly developed in recent years. It has been applied successfully as an analytical tool in diverse areas, such as the study of new materials, archaeology, environmental monitoring and control, biological tissues, aerospace technology, and even the study of the chemical compositions of other planets (Carvalho *et al.*, 2007; Fortes *et al.*, 2009). The EDXRF technique meets the desired characteristics for the analysis of plant specimens, including the possibility of performing analysis directly on solid samples; the multi-element capacity; the possibility of performing qualitative, semiquantitative and quantitative determinations; a wide dynamic (linear) range; high performance (number of analyzed samples/time) and low cost per determination.

The objective of the present work was to develop, optimize and validate an analytical methodology, based on the EDXRF technique, for the simultaneous determination of elemental As, Sb, Se and Hg in plant samples. Similarly, the results related to the validation of the method are presented, as it is considered a fundamental aspect for the reliability of the results obtained from the direct analysis of plants via EDXRF.

Materials and Methods

Chemicals and reagents

All the reagents used were analytical grade. The following reagents were used: arsenic III oxide (As_2O_3; Sigma-Aldrich), selenium black powder (Se; Merck), antimony III oxide (Sb_2O_3; Merck), mercury II oxide (HgO; Merck) and cellulose microcrystalline powder ($(C_6H_{10}O_5)n$; Merck).

Solid calibration standard solutions

For the elaboration of the calibration standards, cellulose was used to simulate the matrix of the plants that were analyzed with the EDXRF methodology. Suitable amounts of each of the compound mentioned above were added to a 10g portion of this matrix as a source of As, Se, Sb and Hg; each was finely ground (<75µm), carefully mixed and re-powdered in an agate mortar. The resulting solid mixture corresponded to the standard solution of 10000mg·kg^{-1} of each element. From this standard, composed of the four elements of interest, and by successive stages of dilution with cellulose, the calibration standards were prepared, each with a mass of 10g and concentrations in the range of 5 to 300mg·kg^{-1}.

Secondary reference materials

To evaluate the analytical performance of the proposed methodology, two secondary reference materials were used during the optimization stage: COND1 and COND2, both corresponding to samples of chilca (*Tessaria absinthioides*), whose content of As, Se, Sb and Hg is known, as determined by atomic absorption spectrometry (AAS-HG), after acid digestion (Fengxiang *et al.,* 2006). Decomposition of the COND1 and COND2 samples for analysis by AAS was performed by processing 0.1g of solid sample in a 110ml vessel, adding 5ml of concentrated nitric acid (p.a. grade, Merck) and leaving it to rest overnight. The vessel was then closed and digestion performed by heating at 150ºC in a microwave oven (MRS Xpress System, CEM, USA) for 2h.

The AAS technique corresponds to an analytical technique that is a central part of many standardized test methods used to determine As, Sb, Se and Hg in different matrices (USEPA, 1992, 1994; ISO, 1984, 2004, 2014; APHA, 2005, INIA, 2007). The technique is widely available in laboratories around the world and is thus appropriate for comparison.

Sampling sites, samples and samples pretreatment

In order to study the applicability of the proposed methodology, 18 plant samples corresponding to the species *Tessaria absinthioides*, *Typha angustifolia* and *Cyperaceae scirpus* sp. (10 of each) were collected (Table I). These three species of wild plants were selected because they are widely distributed in different ecosystems in the Arica and Parinacota region, northern Chile, an area located in the center of the Atacama Desert. This area has a high level of geothermal activity associated with the vulcanism of the Andes mountain range. These natural phenomena have given rise to soils with high levels of chemicals, leading to high contents of these elements in surface water and groundwater. The chemicals are primarily fixed by plants, and finally transferred and bioaccumulated throughout the entire food chain (Cornejo and Acarapi, 2011; Bundschuh *et al.*, 2012; López *et al.*, 2012).

The samples were collected from six sites located in the interior of the Arica and Parinacota region (Table I) at different altitudes and associated to different ecosystems and with varying levels of chemical elements in the environment. The 'AZ' sector (Azapa Valley) was considered as a 'control' site due to data showing that it has the lowest environmental levels of potentially dangerous chemical elements at the regional level (Cornejo and Acarapi, 2011).

Plant samples (leaves) were collected in 1kg portions, transported to the laboratory and dried at 60 ±3ºC in a forced air oven until constant weight. After cooling to room temperature, the entire sample was pulverised in a rotor beater mill/SK1 (Retsch, Germany), with a final particle size of less than 75µm. The powdered plant samples were stored in polyethylene jars until analyzed in the EDXRF system.

Total elements determination by EDXRF. Preparation of samples

Both for the elaboration of the calibration curves and for the determination of the total content of the elements As, Se, Sb and Hg in plant samples, we proceeded as follows: 4g of each material was weighed within a cylindrical aluminium container (30mm diameter × 8mm height, Spec-Cap® Model 3623, SPEX SamplePrep) and then pressed in a hydraulic press (Bench-Press®, Model 3628, SPEX SamplePrep) at a pressure of 15ton, to obtain a pressed pellet. The pellets were then analyzed in an energy dispersive X ray fluorescence spectrometer (EDX 900-HS; Shimadzu, Japan), equipped with a Rh target X ray tube (5-50kV, 1-1000µA, air cooling with fan, irradiation diameters of 1, 3, 5 and 10mm) as an X ray source and a Si(Li) detector (electron cooling method).

The operation parameters of the EDXRF spectrometer were optimized in order to achieve the lowest detection limits. For this, tests were performed using different collimator openings (1 to 10mm), a real integration time of 100 to 1000s and different analysis atmospheres (air, helium and vacuum). The analytical lines considered for the elements As, Se, Sb and Hg were AsK_{α}, SeK_{α}, SbK_{α} and HgL_{α}, respectively. To analyze the intensities (signals) the DXP-700E software v 1.00 Rel. 017 (Shimadzu, Japan) was used. All samples were analyzed in triplicate, with cellulose blanks also being analyzed (zero concentration standards) together with the samples.

The method detection level (MDL) was determined according to a standardized procedure (APHA, 2005), which considers its value to be four times the value of the instrument detection level (IDL), which in turn is the constituent concentration that produces a signal greater than three standard deviations of the mean noise level (using a specific analytical line for each element).

The repeatability of the EDXRF method was calculated by measuring 10 different pellets of vegetable samples at two different concentration levels. The accuracy (expressed as % of standard recovery) was calculated as the difference between the concentration determined by EDXRF (for As, Se, Sb and Hg) and the nominal concentration of the secondary reference materials (MRS) COND1 and COND2. The acceptance criterion was a minimum accuracy within the range of 85-115% of the standard recovery.

In order to evaluate the possible matrix effect (Vessman *et al.*, 2001) due to differences between the composition of calibration standards and the analyzed plant samples, the standard additions technique (Miller and Miller, 2005) was used, consisting of the addition of known and increasing quantities of the analyte to the sample, reading the corresponding instrumental responses and subsequently constructing the standard additions line. The analyte is then quantified by extrapolating the calibration line to the point on the abscissa axis where the answer is zero.

Results and Discussion

Sample preparation and optimised EDXRF parameters

When the pellet is pressed a binder is usually needed in order to

TABLE I
DESCRIPTION OF SAMPLING SITES AND PLANT SAMPLES

Nº	Sample code	Plant sample type	Scientific name	Description site	Location UTM	Altitude (m)
1	AZ1	Chilca	*Tessaria absinthioides*	Azapa valley (km 50)	19K 410733 7944703	1135
2	AZ2	Totora	*Typha angustifolia*	Azapa valley (km 50)	19K 410733 7944703	1135
3	AZ3	Junquillo	*Cyperaceae Scirpus*	Azapa valley (km 50)	19K 410733 7944703	1135
4	LLUT1	Chilca	*Tessaria absinthioides*	Lluta valley (Santa Lucía)	19K 368914 7964248	162
5	LLUT2	Totora	*Typha angustifolia*	Lluta valley (Santa Lucía)	19K 368914 7964248	162
6	LLUT3	Junquillo	*Cyperaceae Scirpus*	Lluta valley (Santa Lucía)	19K 368914 7964248	162
7	CAM1	Chilca	*Tessaria absinthioides*	Camarones town, Camarones valley	19K 409496 7897856	711
8	CAM2	Totora	*Typha angustifolia*	Camarones town, Camarones valley	19K 409496 7897856	711
9	CAM3	Junquillo	*Cyperaceae Scirpus*	Camarones town, Camarones valley	19K 409496 7897856	711
10	TAL1	Chilca	*Tessaria absinthioides*	Taltape, Camarones valley	19K 413516 7898376	802
11	TAL2	Totora	*Typha angustifolia*	Taltape, Camarones valley	19K 413516 7898376	802
12	TAL3	Junquillo	*Cyperaceae Scirpus*	Taltape, Camarones valley	19K 413516 7898376	802
13	ESQ1	Chilca	*Tessaria absinthioides*	Esquiña town, Camarones valley	19K 444136 7906118	2180
14	ESQ2	Totora	*Typha angustifolia*	Esquiña town, Camarones valley	19K 444136 7906118	2180
15	ESQ3	Junquillo	*Cyperaceae Scirpus*	Esquiña town, Camarones valley	19K 444136 7906118	2180
16	ILLA1	Chilca	*Tessaria absinthioides*	Illapata Town, Camarones valley	19K 446266 7905264	2225
17	ILLA2	Totora	*Typha angustifolia*	Illapata Town, Camarones valley	19K 446266 7905264	2225
18	ILLA3	Junquillo	*Cyperaceae Scirpus*	Illapata Town, Camarones valley	19K 446266 7905264	2225

hold the material of the samples or standards. Important characteristics of the binders are: absence of pollutants, stability under the operational conditions and low matrix (for instance C or B, which are not detected by EDXRF). The binders can be either liquid or solid. Cellulose and starch are typical examples of these types of compounds (Reidinger *et al.*, 2012; Takahashi, 2015).

In this study, pellets (Figure 1) of the standards were prepared in a cellulose matrix, with a final composition (pressed pellet) content >90%. This content is similar to that of the organic matter present in the vegetable sample (77.6-93.5mg·kg^{-1} dw, $\overline{X}$= 87.5mg·kg^{-1} and RSD= 7.0). It presents a good compaction capacity, which was the main reason not to require an additional binder.

The operating parameters of the EDXRF spectrometer were optimized to achieve the lowest detection limits and optimum sensitivity. The optimal instrument conditions were: voltage 50 kV, current 300 μA, collimator 10 mm, real integration time 100 s, detector dead time < 1%, atmosphere vacuum (Pressure < 30Pa). The analytical lines considered for the four elements were AsK_{α}, SeK_{α}, SbK_{α} and HgL_{α} (Figure 2).

Validation Parameters

The validation of the proposed EDXRF methodology considered the evaluation of the following parameters: method detection limit (MDL), linearity, accuracy and repeatability. The results of the analytical performance evaluation process of the optimized method are summarized in Table II.

Detection limits and linearity

The instrumental detection limit (IDL), defined as the net minimum intensity (expressed in concentration units) that can be determined in a given analytical context, was determined for the elements As, Se, Sb and Hg, in order to verify if the instrument is sensitive enough to detect each of those elements.

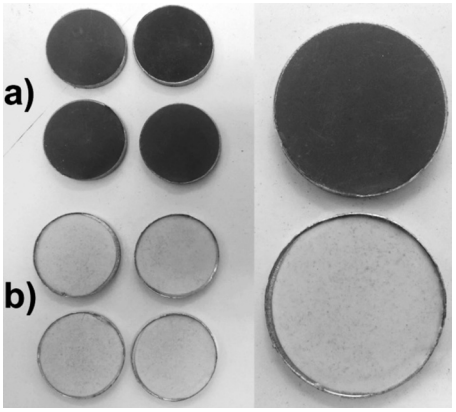

Figure 1. Pressed pelltes of vegetable samples (a) and standards (b), prior to their analysis by EDXRF.

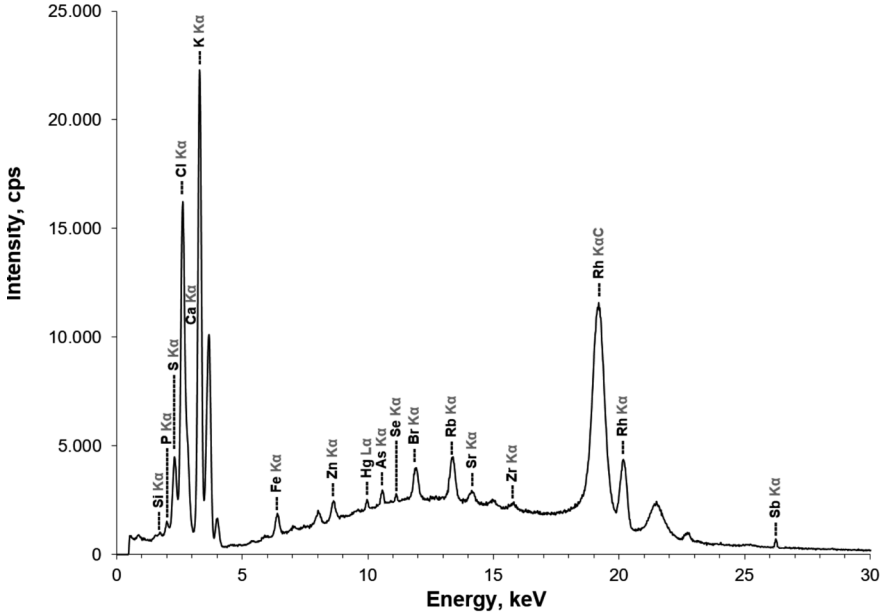

Figure 2. Energy dispersive X-ray fluorescence spectrum of the sample LLUT1 (chilca).

TABLE II
ANALYTICAL PERFORMANCE OF THE EDXRF METHOD

Regression parameters	Arsenic	Selenium	Antimony	Mercury
Linearity range (mg·kg^{-1})	3-300	4-300	20-300	2-300
Sensitivity (kg·mg^{-1}) ×10^{-3}	13.5	6.1	0.1	14.3
IDL[a] (mg·kg^{-1}) dw	0.58	0.90	5.0	0.40
MDL[a] (mg·kg^{-1}) dw	2.3	3.6	19.8	1.6
MDL[b] (mg·kg^{-1}) ww	0.53	0.84	4.55	0.36
R^2 [c]	0.9993	0.9960	0.9991	0.9972
r	0.9996	0.998	0.9995	0.9986
Analytical line	AsKα (10.543 keV)	SeKα (11.224 keV)	SbKα (26.359 keV)	HgLα (9.989 keV)
Accuracy[d]				
		AAS-HG (mgkg^{-1})	EDXRF (mgkg^{-1})	Recovery (%)
Arsenic	$COND_1$[a]	58.4	61.1	95.6
	$COND_2$[a]	155	161	96.3
Selenium	$COND_1$[a]	51.1	53.6	95.3
	$COND_2$[a]	87.2	90.9	95.9
Antimony	$COND_1$[a]	26.0	28,4	91.5
	$COND_2$[a]	33.8	37.6	89.9
Mercury	$COND_1$[a]	8.3	8.6	96.5
	$COND_2$[a]	17.5	18	97.2
Repeatability[e]				
	Arsenic	Selenium	Antimony	Mercury
RSD (%)	0.9-14.7	0.8-17.1	3.4-14.5	2.0-6.2
Max-Min (mg·kg^{-1})[a]	(693-10.9)	(117-3.6)	(271-20.0)	(54.7-1.6)

[a] Results on dry weight basis (dw). [b] Results on wet weight basis (ww). [c] Acceptance criteria: R^2>0.9950. [d] Acceptance criteria: Minimum 85-115%, as recovery of a standard of known concentration. [e] Acceptance criteria: Minimum 80%, i.e. differences not exceeding 20%, as relative standard deviation in the implementation of the method, and between replicates during routine work.

The IDL is calculated using the equation (Marguí *et al.*, 2005):

$$ILD = \frac{4.65}{S_i}\sigma_b$$

where S_i: sensitivity and σ_b: fluctuation of the background noise. The method detection limit (MDL) is determined as 4 times the calculated IDL value (APHA, 2005).

The MDL values determined in the present study (Table II) indicate that the EDXRF methodology developed allows the detection of the elements As, Se, Sb and Hg in plant tissues ($mg \cdot kg^{-1}$ levels). The values (dw) obtained for each element were in the following ascending order: Hg (1.6$mg \cdot kg^{-1}$) <As (2.3 $mg \cdot kg^{-1}$) <Se (3.6$mg \cdot kg^{-1}$) <Sb (19.8$mg \cdot kg^{-1}$). Although the detection limits determined are high (especially for Sb and Se) compared to other instrumental techniques (Table IV), the analytical methodology developed has proved useful as a low-cost and quick alternative for determining chemical elements in hyperaccumulative

TABLE III
CHEMICAL COMPOSITION OF THE SAMPLES

Nº	Sample code	Humidity % mm mmg^{-1}	Arsenic ($mg\ kg^{-1}$)	Selenium ($mg\ kg^{-1}$)	Antimony ($mg\ kg^{-1}$)	Mercury ($mg\ kg^{-1}$)
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
1	AZ1	83.5 ±0.7	10.9 ±1.8 (1.6 ±0.3)	7.9 ±1.3 (0.3 ±0.05)	<MDL	2.2 ±0.4 (0.1 ±0.01)
2	AZ2	74.0 ±1.0	18.8 ±4.9 (2.7 ±0.7)	4.2 ±0.69 (0.2 ±0.03)	<MDL	1.9 ±0.5 (0.1 ±0.02)
3	AZ3	71.0 ±0.3	25.9 ±7.5 (2.8 ±0.8)	3.6 ±1.0 (0.2 ±0.02)	20.2 ±4.8 (3.0 ±0.7)	<MDL
4	LLUT1	85.7 ±1.1	63.6 ±9.1 (7.7 ±1.1)	59.9 ±8.60 (1.2 ±0.17)	32.2 ±4.6 (4.2 ±0.6)	11.2 ±1.6 (0.2 ±0.03)
5	LLUT2	76.7 ±0.6	55.4 ±12.9 (6.0 ±1.4)	12.0 ±2.80 (0.8 ±0.19)	20.1 ±4.6 (2.6 ±0.6)	5.2 ±1.2 (0.2 ±0.05)
6	LLUT3	74.6 ±0.5	73.2 ±18.6 (7.1 ±1.8)	3.8 ±0.63 (0.2 ±0.03)	38.7 ±9.9 (4.6 ±1.2)	1.6 ±0.4 (0.1 ±0.02)
7	CAM1	84.1 ±0.8	190 ±30 (5 ±1)	96.3 ±15.3 (1.4 ±0.22)	48.1 ±7.6 (4.8 ±0.8)	32.1 ±5.1 (0.1 ±0.02)
8	CAM2	73.2 ±0.5	316 ±85 (8 ±2)	33.9 ±9.1 (1.1 ±0.29)	40.9 ±11.0 (3.8 ±1.0)	25.0 ±6.7 (0.1 ±0.03)
9	CAM3	70.0 ±0.7	393 ±118 (6 ±2)	3.7 ±0.9 (0.3 ±0.07)	137 ±41.0 (6.5 ±1.9)	2.9 ±0.9 (0.1 ±0.02)
10	TAL1	83.5 ±0.9	172 ±28 (6 ±1)	106 ±17.6 (1.7 ±0.28)	48.1.7±.9 (3.3 ±0.5)	29.6 ±4.9 (0.3 ±0.04)
11	TAL2	74.2 ±0.4	280 ±72 (3 ±1)	29.2 ±7.5 (0.8 ±0.21)	24.3 ±6.3 (2.3 ±0.6)	34.4 ±8.9 (0.4 ±0.1)
12	TAL3	71.0 ±0.6	365 ±106 (2 ±1)	18.7 ±5.4 (0.6 ±0.18)	226 ±65.5 (8.1 ±2.3)	4.8 ±1.4 (0.1 ±0.0)
13	ESQ1	85.4 ±1.2	15.1 ±2.2 (2.2 ±0.3)	8.9 ±1.3 (0.4 ±0.06)	20.1 ±4.7 (2.8 ±0.7)	2.8 ±0.4 (0.1 ±0.01)
14	ESQ2	75.5 ±0.6	31.0 ±7.6 (4.4 ±1.1)	4.1 ±0.9 (0.3 ±0.07)	20.0 ±4.8 (2.9 ±0.7)	1.6 ±0.4 (0.1 ±0.02)
15	ESQ3	72.6 ±1.0	48.9 ±13.4 (6.2 ±1.7)	3.7 ±0.05 (0.1 ±0.00)	21.3 ±4.6 (2.1 ±0.5)	1.7 ±0.4 (0.1 ±0.02)
16	ILLA1	82.2 ±1.0	226 ±40 (12 ±2)	117 ±20.9 (0.9 ±0.16)	59.2 ±10.5 (3.0 ±0.5)	44.1 ±7.8 (0.9 ±0.2)
17	ILLA2	76.9 ±0.7	554 ±99 (13 ±3)	98.8 ±22.9 (1.1 ±0.25)	31.3 ±7.2 (2.1 ±0.5)	54.7 ±12.7 (1.1 ±0.3)
18	ILLA3	73.2 ±0.8	693 ±186 (6 ±2)	7.0 ±1.9 (0.6 ±0.16)	271 ±72.8 (9.2 ±2.5)	6.3 ±1.7 (0.2 ±0.1)
Minimum - Maximum ($mg\ kg^{-1}$)[a]			10.9 - 693	3.6 - 117	20.0 - 271	1.6 - 54.7
Minimum - Maximum ($mg\ kg^{-1}$)[b]			1.8 - 186	1.0 - 20.9	4.8 - 72.8	0.4 - 12.7

For sample codes, see Table I.
The means without parentheses are expressed on dry weight basis (dw). The means in parentheses are expressed on wet weight basis (ww). The results for each of the 18 samples correspond to an average and standard deviation (SD) obtained out of 10 determinations.

TABLE IV
ANALYTICAL PERFORMANCE OF THE DIFFERENT METHODS EMPLOYED TO DETERMINE ARSENIC, SELENIUM, ANTIMONY AND MERCURY IN ENVIRONMENTAL SAMPLES

Element	Method	Calibration range	MDL ($\mu g\ l^{-1}$)	MDL ($\mu g\ kg^{-1}$)	RSD[e] (%)	Analysis time[f] (hours)	References
Arsenic	HG-AFS	0-0.9$\mu g\ l^{-1}$ (0-0.18$\mu g\ kg^{-1}$)[e]	0.016	0.0032[a]	< 2 (50$\mu g\ l^{-1}$)	7	Frank and Krachler (2006)
	HG-AAS	0-20$\mu g\ kg^{-1}$	<5	1.25[a]	<20 (10$\mu g\ l^{-1}$)	6	SISS (2017)
	HG-ICP-AES	2-200$\mu g\ l^{-1}$ (0.4-40$\mu g\ kg^{-1}$)[e]	-	0.54	0.6 (200ug l^{-1})	3	Ilander and Väisänen (2011)
Selenium	HG-AFS	0-5$\mu g\ kg^{-1}$	0.28	0.056[a]	3.1	12	Niedzielski and Siepak (2003)
	HG-AAS	0-20$\mu g\ kg^{-1}$	0.038	0.0076[a]	12 (4.3$\mu g\ l^{-1}$)	6	APHA *et al.* (2017)
	HG-ICP-AES	0-40$\mu g\ kg^{-1}$	0.40	0.08[a]	1.9	3	Martinez *et al.* (1997)
	HG-ICP-MS	0-1$\mu g\ kg^{-1}$	0.030	0.006[a]	12.3	3	Niedzielski and Siepak (2003)
Antimony	AFS	-	10[b]	2	-	7	Chen *et al.* (2003)
	HG-AAS	-	0.02	0.004[a]	-	6	APHA *et al.* (2017)
	HG-ICP-AES	2-200$\mu g\ l^{-1}$ (0.4-40$\mu g\ kg^{-1}$)[e]	6.85[b]	1.37	2.3 (80$\mu g\ l^{-1}$)	3	Ilander and Väisänen (2011)
Mercury	CV-AFS	-	0.133	0.0266[a]	2.5	10	Shah *et al.* (2012)
	CV-AAS	0-5.0	1	0.2[a]	14	6	APHA *et al.* (2017)
	HG-ICP-	20-2000 (4-400$\mu g\ kg^{-1}$)[e]	10	2[a]	7.4 (20$\mu g\ l^{-1}$)	3	Fengxiang *et al.* (2006)

[a] Estimated from the MDL value considering a dry sample mass of 0.5g and 100ml of solution. [b] Estimated from the MDL value considering a dry sample mass of 0.5g and 100ml of solution. [c] Level determined at the concentration indicated in parentheses. [d] Estimated based on laboratory analysis of 20 samples under routine work conditions. [e] Estimated from the calibration curve reported by the authors, considering a dry sample mass of 0.5g and 100ml of solution. [f] Analysis time: routine work conditions.

plants or plants that grow in areas with high levels of chemical elements in their environment.

Linearity was evaluated by multi-element calibration standards (containing As, Se, Sb and Hg), in triplicate, with concentrations in the range of 5 to 300mg·kg^{-1} for As, Se and Hg, and in the range of 20 to 300mg·kg^{-1} for Sb. In each case the standards were prepared using cellulose as a matrix. They were then validated with three rounds of tests carried out on three different days. The intensities measured by EDXRF for each of the calibration standards were processed by plotting (Figure 3) the intensity ratio I_a/I_b *vs* standard concentration, where I_a is the intensity of the signal associated with the analyte and I_b is that of the background.

Accuracy and repeatability

Normalized methods based on HG-AAS and cold vapor-AAS techniques were used to compare the results obtained by means of the developed EDXRF methodology. With this purpose, AAS methodologies were previously verified. In this sense, when 'normalized methods' are used, a minor validation or verification should be carried out (ISP, 2010). This verification is used to check that the laboratory can adequately carry out the normalized method. In the case of a modified normalized method, this verification will only require checking that this verification does not affect the assays (ISP, 2010).

In this study, the determination of As, Sb and Hg was carried out by means of standardized methods (ISO, 1984, 2004, 2014), while a reported method was selected for Se (Baralkiewicz *et al.*, 2004). In all cases the methods were carried as described, without any modifications. Consequently, the only verification process used was for the Certipur® reference materials for AAS. Certipur® materials are ready-to-use solutions, certified and traceable to primary standard reference materials from the National Institute of Standards and Technology. The results showed good accuracy values in the range of 85-115% (SISS, 2007; ISP, 2010; APHA, 2017) for concentrations near the detection limits. Repeatability resulted in less than 20% relative standard deviation.

To evaluate the accuracy of the EDXRF method, the secondary reference materials COND1 and COND2 were analysed, and the concentrations obtained were compared with the values obtained by AAS-HG (Table II). For the evaluation of repeatability the data from Table III was considered, extracting the information associated with the highest and lowest concentrations determined for each of the chemical elements under study.

Once the AAS methodologies had been verified, these were used in the determination of As, Se, Sb and Hg content in the materials COND1 and COND2 (chilca). In this way, the samples COND1 and COND2 could then be used as secondary reference materials (MRS) during the development of the EDXRF methodology. This strategy was chosen because there is no available certified reference vegetable material with As, Se, Se, Sb and Hg concentrations above the detection limit.

Evaluation of matrix effect

The so-called 'matrix effect' consists of a decrease or increase of the analyte instrumental response due to the presence of other components in the sample. In order to evaluate the necessity of instrument signal (fluorescence intensity)

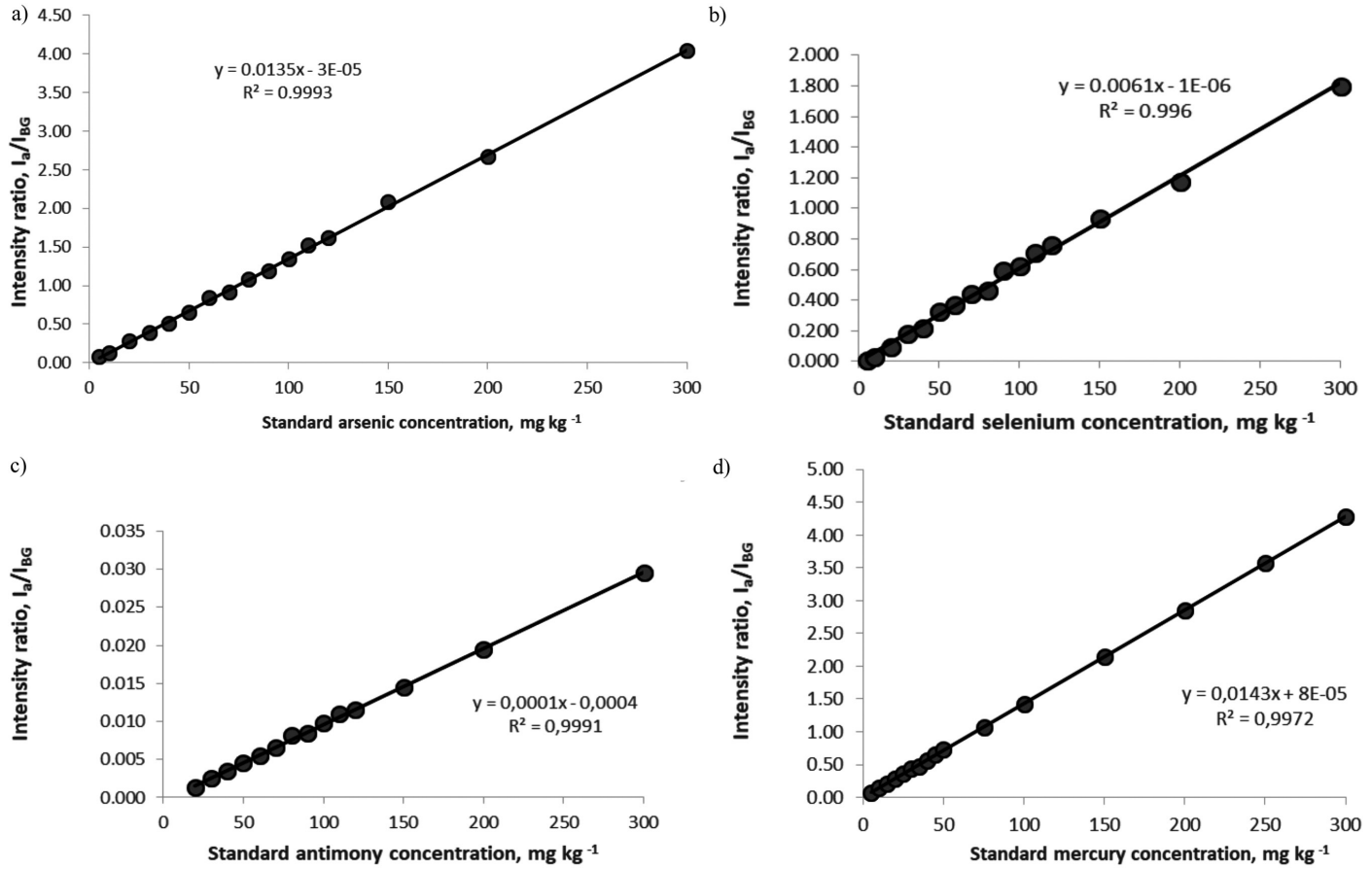

Figure 3. Standard curves for the determination of total arsenic (a), selenium (b), antimony (c) and mercury (d) by the optimised EDXRF methodology.

correction for the background in the proposed EDXRF method, two calibration options were considered: i) concentration of the standards as a function of the intensity signal I_a, and ii) concentration of the standards as a function of intensity signal corrected by background (I_a/I_{bg}). The efficiency of both signals was compared by means of MDL, linearity and sensitivity.

Figure 4 shows the comparison between both options for total As determination. Significant differences are seen in the standard curves of both options. The corrected one was the best analytical option. With respect to the detection limits, when the intensity signals were directly used, the MDL_{Ia} obtained were 28.4 (As), 44.8 (Se), 205 (Sb) and 22.4 (Hg) mg·kg^{-1}, more than 10 times higher than the values obtained with the corrected signal (Table II).

Additionally, when using the intensity signal without correction by background, there was a decrease in the linearity (R^2_{As}= 0.926, R^2_{Se}= 0.926, R^2_{Sb}= 0.926 and R^2_{Hg}= 0.9261), below the acceptance criterion of R^2=0.995 (SISS, 2007; APHA, 2017).

Finally, the sensitivity evaluation resulted in values of 0.8×10^{-3} (As), 0.6×10^{-3} (Se), 0.02×10^{-3} (Sb) and 0.5×10^{-3} (Hg), which are 17, 10, 5 and 29 times lower, respectively, than the values obtained using the corrected intensity.

The general improvement in the analytical performance parameters when using the corrected instrumental signals (Ia/Ibg) during the calibration process can be understood as an effect of compensation and reduction of the random variability associated with the stages of preparation of the standards and samples (small changes in the composition of standards and micro-heterogeneity of pellets) and during the measurement process (fluctuations in electrical voltage, instrumental signal or changes in the environmental conditions of the laboratory). The strategy of using the ratio (Ia/Ibg) makes it possible to better compensate for these effects (in comparison with the direct use of the fluorescence intensity signals), improving the accuracy of each individual measurement when testing standards and samples.

In this sense, the option to consider the I_a/I_b ratio as a variable during calibration allows to efficiently compensate the small differences between the matrix of each sample and the standards. The above implies that for the same analyte concentration, the analysis of a real sample or a standard solution of the pure analyte provides approximately the same instrumental response, in addition to the absence of systematic errors.

Application of the method

The optimized EDXRF methodology was applied to a total of 18 plant samples, in order to determine their total content in terms of the elements As, Se, Sb and Hg. These samples represent three different species of wild plants found in the Arica and Parinacota region, northern Chile (Atacama Desert), in areas with high levels of environmental arsenic. The obtained results of As, Se, Sb and Hg determination, via EDXRF, are summarized in Table III.

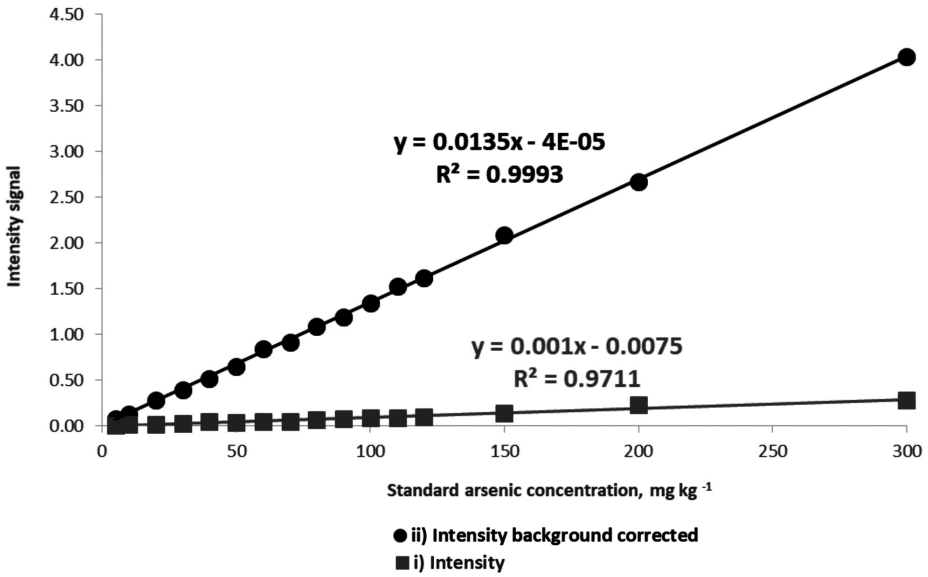

Figure 4. Comparison of two calibration options for total arsenic determination by EDXRF: i) concentration of the standards as a function of intensity signal (I_a) and ii) concentration of the standards as a function of intensity signal corrected by background (I_a/I_{bg}).

It should be emphasized that the optimized EDXRF methodology delivers results on a dry basis, a methodological strategy that allows even lower analyte concentrations to be used than when calculating the results on a wet basis (wet fresh plant tissue).

Regarding the applicability of the method for the analysis of real samples, all of the samples analyzed had concentrations of As and Se over the MDL. In the case of Sb and Hg, only 3 samples had concentrations below the respective MDL values (2 for Sb and 1 for Hg), all of them corresponding to samples from the Azapa valley sector, an area characterized by low levels of heavy metals in the environment and used as the control site.

From the information presented in Table IV it can be seen that different MDL values are reported in the literature for the determination of As, Sb, Se and Hg. These differences are related to the analytical procedure, where AFS and ICP-MS present the lowest detection limits. Although the detection limits of the EDXRF methodology are higher, there are other advantages, such as: large lineal range (mg/kg to %), low interference with other chemical elements, short analysis times (<5min), low-cost (no reagents due to the absence of digestion stage), good accuracy and precision, no waste and simple sample preparation.

In this study EDXRF methodology was proposed to analyze vegetable samples from three areas in the Arica and Parinacota region, North of Chile. Concentrations of As, Sb, Se and Hg above the detection limit were determined. The highest As concentrations were found in the Illapata sector, which is an area with high levels of As in water and soil (Cornejo and Acarapi, 2011). In a previous study (Cornejo and Acarapi, 2011), the total As content in the soil was determined for different regions of Arica and Parinacota. The results were in the range of 69.5 to 245mg·kg^{-1} (dw). Considering this information and relating it to the EDXRF methodology developed in the present work, the concentration factors of *Tessaria absinthioides*, *Typha angustifolia* y *Cyperaceae Scirpus* were determined (Figure 5). Linearity between total As content in the plant and total As content in its corresponding soil was used in the calculation. In this way, the As accumulation factors (mg·kg$^{-1}_{plant}$ /mg·kg$^{-1}_{soil}$) were: 2.30 for *T. absinthioides* (R^2= 0.81, p=0.038), 1.02 for *T. angustifolia* (R^2= 0.90, p=0.015) and 0.81 for *C. Scirpus* (R^2= 0.88, p=0.017). Similar determinations could not be performed for Sb, Se and Hg as no information is available regarding their presence in the soil in the study areas.

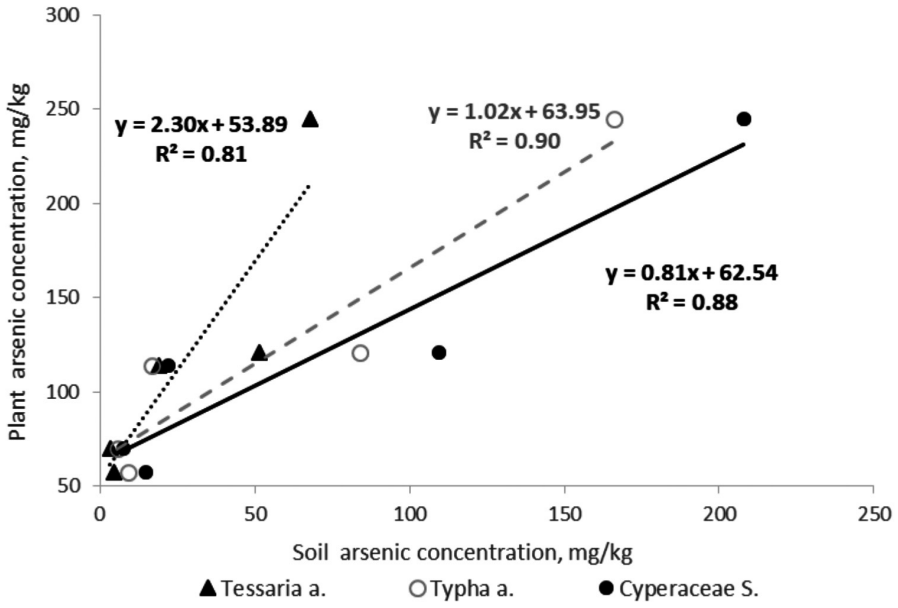

Figure 5. Correlation between the As total content in the vegetable and the As total content in soils for the species *Tessaria absinthioides*, *Typha angustifolia* and *Cyperaceae Scirpus*.

Conclusion

The present work considered the development and optimization of an analytical methodology based on the EDXRF technique for the determination of the content of the chemical elements As, Se, Sb and Hg in plant tissue samples. This is a non-destructive sample analysis method with good analytical performance, reduced analysis time, fast sample and standard preparation processes, and low or no residual emissions to the environment.

It is hoped that in the future new applications for the EDXRF technique will be explored that can be presented as an analytical alternative to conventional test methods and provide a useful tool in studies oriented to the environmental assessment or risk evaluation of exposure to hazardous and/or potentially hazardous chemical elements.

ACKNOWLEDGEMENTS

The authors wish to thank the support of FONDECYT Project Nº 1120881, Ayllu Solar Project and the Solar Energy Research Center, SERC-Chile (FONDAP/15110019).

REFERENCES

APHA (2005) *Standard Methods for the Examination of Water and Wastewater*. 21st ed. American Public Health Association. Washington, DC, EEUU.

Baralkiewicz D, El-Sayed U, Filipiak M, Gramowska H, Mleczek M (2004) Determination of selenium in infant foods using electrothermal atomic absorption spectrometry with direct slurry sample introduction. *Cent. Eur. J. Chem. 2*: 334-346.

Bundschuh J, Nathd B, Bhattacharya P, Liu CW, Armienta MA, Moreno MV, Lopez DL, Jean JS, Cornejo L, Lauer LF, Filho AT (2012) Arsenic in the human food chain: the Latin American perspective. *Sci. Total Environ. 429*: 92-106.

Carvalho M L, Magalhães T, Becker M, Von Bohlen A (2007) Trace elements in human cancerous and healthy tissues: A comparative study by EDXRF, TXRF, synchrotron radiation and PIXE Review Article. *Spectrochim. Acta B 62*: 1004-1011.

Chen B, Krachler M, Shotyk M (2003) Determination of antimony in plant and peat samples by hydride generation-atomic fluorescence spectrometry (HG-AFS). J. *Anal. At. Spectrom. 18*: 1256-1262.

Clarkson TW (1998) Human toxicology of mercury. *J. Trace Elem. Exp. Med. 11*: 303-317.

Cornejo L, Acarapi J (2011) Fractionation and bioavailability of arsenic in agricultural soils: solvent extraction tests and their relevance in risk assessment. *J. Environ. Sci. Health A Tox. Haz. Subst. Environ. Eng. 46*: 1247-1258.

D'Amato M, Forte G, Caroli S (2004) Identification and quantification of major species of arsenic in rice. *J. AOAC Int. 87*: 238-243.

De Jesus R, Silva L, Castro J, de Azevedo T, Ferreira S (2013) Determination of mercury in phosphate fertilizers by cold vapor atomic absorption spectrometry. *Talanta 106*: 293-297.

De La Calle I, Costas M, Cabaleiro N, Lavilla I, Bendicho C (2013) Fast method for multielemental analysis of plants and discrimination according to the anatomical part by total reflection X-ray fluorescence spectrometry. *Food Chem. 138*: 234-241.

Dumont E, Vanhaecke F, Cornelis R (2006) Selenium speciation from food source to metabolites: a critical review. *Anal Bioanal Chem. 385*: 1304-1323.

Fengxiang H, Dean W, Yunju X, Maruthi B, Yi S (2006) Rapid determination of mercury in plant and soil samples using inductively coupled plasma atomic emission spectroscopy, a comparative study. *Water Air Soil Pollut. 170*: 161-171.

Feng X, Li P, Qiu G, Wang S, Li G, Shang L, Mehg B, Jiang H, Bai W, Li Z, Fu X (2008) Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou province, China. *Environ. Sci. Technol. 42*: 326-332.

Fortes AD, Wood IG, Dobson DP, Fewster PF (2009) An icy mineralogy package (IMP) for in-situ studies of Titan's surface Original Research Article. *Adv. Space Res. 44*: 124-137.

Frank J, Krachler M, Shotyk W (2006) Determination of arsenic in peat samples using HG-AFS and L-cysteine as pre-reductant *J. Anal. Atom. Spectrom. 21*: 204-207.

Galvão C, Almeida M, Galvão E, Pinto A, Lago I, Santos J (2016) A review of multivariate designs applied to the optimization of methods based on inductively coupled plasma optical emission spectrometry (ICP OES). *Microchem. J. 128*: 331-346.

Habte G, Min I, Jae Sung K, Joon H, Young S, Ji Y, Eun Y, Jamila N, Khan N, Kyong S (2016) Elemental profiling and geographical differentiation of Ethiopian coffee samples through inductively coupled plasma-optical emission spectroscopy (ICP-OES), ICP-mass spectrometry (ICP-MS) and direct mercury analyzer (DMA). *Food Chem. 212*: 512-520.

Hu Y, Wang D, Wei L, Zhang X, Song B (2014) Bioaccumulation of heavy metals in plant leaves from Yan′an city of the Loess Plateau, China. *Ecotoxicol. Environ. Saf. 110*: 82-88.

Hughes M, Beck B, Chen Y, Lewis A, Thomas D (2011) Arsenic Exposure and Toxicology: A Historical Perspective. *Toxicol. Sci. 123*: 305-332.

Ilander A, Väisänen A (2011) The determination of antimony and arsenic concentrations in fly ash by hydride generation inductively coupled plasma optical emission spectrometry. *Anal. Chim. Acta. 689*: 178-183.

ISP (2010) *Validación de Métodos y Determinación de la Incertidumbre de la Medición: Aspectos Generales sobre la Validación de Métodos*. Instituto de Salud Pública de Chile.

INIA (2007) *Métodos de Análisis de Tejidos Vegetales*. Centro Regional La Platina. Instituto de Investigaciones Agropecuarias. Santiago de Chile. 139 pp.

ISO (1984) *ISO 6637:1984 Fruits, Vegetables and Derived Products - Determination of Mercury Content - Flameless Atomic Absorption Method*. International Organization for Standardization. Geneva, Switzerland. 5 pp.

ISO (2004) *ISO 17239:2004 Fruits, Vegetables and Derived Products - Determination of Arsenic Content - Method Using Hydride Generation Atomic Absorption Spectrometry*. International Organization for Standardization. Geneva, Switzerland. 10 pp.

ISO (2014) *ISO 17378-2:2014 Water quality - Determination of Arsenic and Antimony - Part 2: Method Using Hydride Generation Atomic Absorption Spectrometry (HG-AAS)*. International Organization for Standardization. Geneva, Switzerland. 13 pp.

INN (2005) *Norma Chilena NCh409/1:2005 Drinking water - Part 1: Requirements*. Instituto Nacional de Normalización. Santiago. Chile. 9 pp.

Khana S, Rehmana S, Khana A, Khana M, Shah M (2010) Soil and vegetables enrichment

with heavy metals from geological sources in Gilgit, northern Pakistan. *Ecotoxicol. Environ. Saf. 73*: 1820-1827.

López D, Bundschuh J, Birkle P, Armienta M, Cumbal L, Sracek O, Cornejo L, Ormachea M (2012) Arsenic in volcanic geothermal fluids of Latin America. *Sci. Total Environ. 429*: 57-75.

Marguí E, Hidalgo M, Queralt I (2005) Multielemental fast analysis of vegetation samples by wavelength dispersive X-ray fluorescence spectrometry: Possibilities and drawbacks. *Spectrochim. Acta B 60*: 1363-1372.

Marguí E, Queralt I, Hidalgo M (2009) Application of X-ray fluorescence spectrometry to determination and quantitation of metals in vegetal material. *Trends Anal. Chem*. 28:362-372.

Matos-Reyes M, Cervera M, Campos R, De la Guardia M (2007) Determination of arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid in cereals by hydride generation atomic fluorescence spectrometry. *Spectrochim. Acta B 62*: 1078-1082.

Miller J, Miller J (2005) *Statistics and Chemometrics for Analytical Chemistry.* 5th ed. Pearson. Harlow, RU. 288 pp.

Niedzielski P, Siepak M (2003) Analytical methods for determining arsenic, antimony and selenium in environmental samples. *Pol. J. Environ. Stud. 2*: 653-667.

Petrick K, Krivan V (1987) Interferences of hydride forming elements and of mercury in the determination of antimony, arsenic, selenium and tin by hydride-generation AAS. *Fresen. Zeitsch. Anal. Chem. 327*(3-4): 338-342.

Ranesh A, Riyazuddin P (2005) Mechanism of volatile hydride formation and their atomization in hydride generation atomic absorption spectrometry. *Anal. Sci. 21*: 1401-1410.

Reidnger S, Ramsey M, Hartley S (2012) Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. *New Phytol. 195*: 699-706.

Sanz E, Muños-Oliva R, Camara C (2005) A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Anal. Chim. Acta*. *535*: 227-235.

Shah A, Kazi T, Baig J, Afridi H, Arain M (2012) Simultaneously determination of methyl and inorganic mercury in fish species by cold vapour generation atomic absorption spectrometry. *Food Chem. 134*: 2345-2349.

SISS (2007) *Manual de Métodos de Ensayo para Agua Potable*. Superintendencia de Servicios Sanitarios. Chile. 268 pp.

Takahashi G (2015) Technical articles: Sample Preparation for X-ray fluorescence analysis. Pressed and loose powder methods. *Rigaku J. 31*: 26-30.

USEPA (1992) *Method 7062: Antimony and Arsenic (Atomic Absorption, Borohydride Reduction)*. United States Environmental Protection Agency. 8 pp.

USEPA (1994) *Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry*. United States Environmental Protection Agency. 18 pp.

Vassileva E, Hoenig M (2001) Determination of arsenic in plant samples by inductively coupled plasma atomic emission spectrometry with ultrasonic nebulization: a complex problem. *Spectrochim. Acta B. Atom. Spectrosc*. *56*: 223-232.

Vessman J, Stefan R I, Van Staden J F, Danzer K, Lindner W, Thorburn D, Fajgelj A, Müller H (2001) Selectivity in analytical chemistry. *Pure Appl. Chem. 73*: 1381-1386.

Welna M, Szymczycha-Madeja A (2014) Effect of sample preparation procedure for the determination of As, Sb and Se in fruit juices by HG-ICP-OES. *Food Chem. 159*: 414-419.

WHO (2011) *Guidelines for Drinking-Water Quality*. 4th ed. World Health Organization. Geneva, Switzerland. 541 pp.

Zhang H, Feng X, Larssen T, Qiu G, Vogt R (2010) In inland China, rice, rather than fish, is the major pathway for methylmercury exposure. *Environ. Health Persp. 118*: 1183-1188.

DESARROLLO Y VALIDACIÓN DE UN MÉTODO PARA LA DETERMINACIÓN SIMULTÁNEA DE ARSÉNICO, ANTIMONIO, SELENIO Y MERCURIO EN PLANTAS MEDIANTE ESPECTROMETRÍA DE FLUORESCENCIA DE RAYOS X DE ENERGÍA DISPERSIVA

Lorena Cornejo-Ponce, Jorge Acarapi-Cartes y María Arenas-Herrera

RESUMEN

El objetivo del presente trabajo fue desarrollar, implementar y validar una metodología analítica, basada en la técnica de espectrometría de fluorescencia de rayos X de energía dispersiva (EDXRF) para la determinación directa, rápida y simultánea de arsénico (As), antimonio (Sb), selenio (Se) y mercurio (Hg) en muestras de plantas silvestres presentes en la región de Arica y Parinacota, área ubicada en el centro del desierto de Atacama. El método fue optimizado y validado para alcanzar los límites de detección más bajos. El plan de validación de la metodología propuesta consideró los parámetros de linealidad, sensibilidad, límites de detección, precisión y efecto matriz. Se determinaron límites de detección de 2,3; 3,6; 19,8 y 1,6mg kg^{-1} (base seca) para As, Se, Sb y Hg, respectivamente y se midieron las concentraciones de esos elementos en diferentes especies de plantas que crecen en la zona. Se concluye que la metodología propuesta es una alternativa analítica adecuada a los métodos convencionales usados para la determinación de estos elementos en tejidos vegetales, no requiriéndose etapas tales como disolución de la muestra o transformación del analito en vapores de hidruro/metales volátiles para su separación y/o extracción de la matriz, como paso previo a su análisis instrumental.

DESENVOLVIMENTO E VALIDAÇÃO DE UM MÉTODO PARA A DETERMINAÇÃO SIMULTÂNEA DE ARSÊNIO, ANTIMONIA, SELÊNIO E MERCÚRIO EM PLANTAS POR ESPECTROMETRIA DE FLUORESCÊNCIA DE RAIOS X DE ENERGIA DISPERSIVA

Lorena Cornejo-Ponce, Jorge Acarapi-Cartes e María Arenas-Herrera

RESUMO

O objetivo do presente trabalho foi desenvolver, implementar e validar uma metodologia analítica, baseada na técnica de espectrometria de fluorescência de raios X por energia dispersiva (EDXRF) para a determinação direta, rápida e simultânea de arsênio (As), antimônio (Sb), Selênio (S) e mercúrio (Hg) em amostras de plantas silvestres presentes na região de Arica e Parinacota, uma área localizada no meio do deserto de Atacama. O método foi otimizado e validado para atingir os limites de detecção mais baixo. O plano de validação da metodologia proposta considerou os parâmetros linearidade, sensibilidade, limites de detecção, precisão e efeito de matriz. Determinaram-se os limites de detecção de 2,3, 3,6, 19,8 e 1,6mg kg^{-1} (base seca) para As, Se, Sb e Hg, respectivamente, e suas concentrações em diversas espécies vegetais de a região. Concluiu-se que a metodologia proposta é apresentada como uma alternativa analítica adequada aos métodos convencionais utilizados para a determinação destes elementos em tecidos vegetais, não requerendo passos tais como dissolução de amostras ou transformação de analito em vapores de metal hidreto / voláteis para a sua separação e / ou extração a partir da matriz, antes da etapa de análise instrumental.