

A new methodology for the determination of silicon in plants by wavelength dispersive X-ray fluorescence

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Silicon is an important element for plants at their structure and physiology and plays an important role in bone mineralization and soft tissue development in human beings. Furthermore, its determination is being requested more frequently due to nutritional requirements. However, the methods found in the literature to determine silicon in this type of samples require a sample preparation step, which makes them time-consuming and provides high uncertainties. In this paper, a method for the determination of silicon in plants by wavelength dispersive X-ray fluorescence (WD-XRF) spectrometry has been developed. Horsetail (*Equisetum arvense* L.) and nettle leaf (*Urtica dioica*) have been used as a source of silicon due to its medical use. Sample preparation involved calcining the sample at 700°C and preparing fused beads from the calcined sample. Calibration standards for WD-XRF measurement were prepared by mixing certified reference materials and chemical products to reproduce the samples matrix. The linear range for silicon concentration ranges from 6 to 55 wt% SiO₂. The validation of the method was performed measuring a reference material (NCS DC73349 Bush branches and leaves) and comparing the results obtained by WD-XRF with those obtained by an independent method by atomic absorption spectrometry.

The developed methodology is rapid and accurate, provides low uncertainties, and is environmentally friendly, as it does require the use of less hazardous reagents.

1 | INTRODUCTION

Apart from oxygen, silicon is the most abundant element in the Earth's crust and plays an important role in biological processes. Silicon is found in human and animal tissue in three major forms: as water-soluble inorganic compounds, easily penetrating through the cell walls; as organic-soluble compounds, such as silicones and silicon complexes; and as insoluble polymeric compounds, such as polysilicic acids, silicates, amorphous silica, and quartz.^[1-4]

Regarding plants, silicon helps to raise the plant health by the creating of strongest and more resistant structures.^[5] For crops of rice or sugarcane, silicon

products are added to the yield such that Si is now considered as agronomically essential for sustained production. The quantification of silicon in plants is being requested more frequently, especially in the determination of nutritional requirements.^[6]

In regard to human beings, an important role for silicon in bone mineralization and soft tissue development has been demonstrated. Silicon is nowadays claimed to have beneficial effects on several human disorders, such as osteoporosis, ageing of skin, arteriosclerosis,^[7,8] or reducing the risk of developing Alzheimer's disease.^[9] The dietary standards of the United States and Canada specify the recommended daily dose of silicon at 19 mg

for women and 40 mg for men. Rich sources of silicon are rice, red beetroots, onions, horsetail, hemp nettle, and so forth.^[3]

A very limited number of methods for the determination of silicon in plants are described in the literature. All these methods require a sample preparation: mineralization by high-temperature fusion with NaOH followed by dissolution with H₂O and H₂SO₄, wet ashing with an acid solution of HNO₃:H₂SO₄:HClO₄, or dry ashing with ash dissolution in HF.^[3,10] Other sample preparation methods are based on microwave digestions using different mixtures of reagents, such as HNO₃-H₂O₂, HNO₃-HF, or HNO₃-H₂O₂-HF.^[5,7,11] These methods are all time-consuming and require highly dangerous acids at high temperatures.

Concerning the analytical methods for determining silicon, the gravimetric method is still widely used and is based on the precipitation of polymerized silicic acid followed up by drying, calcining, and weighing in the form of anhydrous silica.^[1] The photometric method is based on the conversion of silicon into a yellow or blue form of silicomolybdic acid, and visible light spectroscopy is used for determination of silicon.^[1,3,12] Although the determination by grazing-emission X-ray fluorescence, as a new opportunity to improve the results obtained by X-ray fluorescence, was developed in several publications, a sample pretreatment prior to analysis is needed, and it is not a quantitative technique; therefore, it can only be used as a complementary technique.^[11,13] The most widely used techniques nowadays are spectrophotometric techniques, graphite furnace atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry.^[3,5,7,8,11,14]

Some studies tried to develop a direct solid analysis of the samples in order to avoid exhaustive sample preparation procedures and possible contamination. A method using electrothermal vaporization as a method of sample introduction in ICP-OES for small size plant samples was developed. However, the precision of the method is sometimes limited by the inhomogeneity of the distribution of elements in powdered samples because a very low amount of sample (1–3 mg) is used.^[15] Another direct solid analysis method by graphite furnace atomic absorption spectrometry was employed, but limit of detection and limit of quantification were higher than those provided by the methods in which higher amounts of sample are used.^[9]

A recent study developed a method by capillary electrophoresis. Sample preparation included dry ashing, followed by oxidative degradation using HClO₄ and dissolution of formed silica in a mixture solution of KOH and NaEDTA; thus, the method takes considerable time and provides a high limit of detection,^[1] so this is not a suitable method for the determination of silicon in plants.

Consequently, there is an urgent need for further improvement of analytical methods for the determination of silicon in plants. Silicon determination in a simple matrix can be properly carried out by spectrometric techniques, but problems arise when more complex organic matrices are analysed by direct calibration or when silicate-containing materials require sample destruction prior to analysis. Losses of silicon can occur during the pretreatment process, and the overall analysis cannot be assumed to be accurate. Furthermore, the analytical techniques that allow the direct measurement of silicon in this type of materials cannot be used as quantitative techniques and have some limitations, such as the amount of sample used or the high detection limits provided. Therefore, there is a lack of a rapid, accurate, and reliable method for the determination of silicon in plants.

In this paper, a method for the determination of silicon in plants by wavelength dispersive X-ray fluorescence (WD-XRF) spectrometry has been developed. The development of the method has been conducted using two different plants: horsetail aerial parts (*Equisetum arvense* L.) and nettle leaf (*Urtica dioica*), which were selected because it has found extensive application in medicine as a source of silicon and they can amount to 25% of the dry weight of the plant.^[5]

The development of the new methodology included the sample preparation and the determination of silicon by WD-XRF. The sample preparation consisted of calcining the sample at a temperature of 700°C and preparing fused beads from the calcined sample. This sample preparation method is not as tedious as digestion methods required in other analytical techniques, and matrix effects are avoided. The determination of silicon by WD-XRF was carried out preparing a calibration curve by mixing different certified reference materials. The validation was performed by measuring a reference material (NCS DC73349 Bush branches and leaves) and comparing the results obtained with an independent method by atomic absorption spectrometry (AAS).

The developed methodology is faster, more accurate, and more environmentally friendly than other methodologies previously mentioned, as it does not require the digestion of the sample, and thus, the use of irritating and corrosive reagents, and minimizes wastes generation.

2 | MATERIALS AND EQUIPMENT

2.1 | Materials and reagents

Three different brands of horsetail and two of nettle leaf were selected to carry out the study. The samples were identified by the following references: HORSETAIL 1,

HORSETAIL 2, HORSETAIL 3, NETTLE LEAF 1, and NETTLE LEAF 2.

In order to simulate the matrix of the samples to be analysed, that is, the plants, the following certified reference materials, and chemical products were used for the preparation of the calibration standards for the measurement by WD-XRF: GBW 03123 Wollastonite supplied by the National Research for Certified Reference Materials – GBW (China), SRM 120c Florida phosphate rock supplied by the National Institute of Standards and Technology – NIST (USA), Euronorm-ZRM 777/1 Silikastein supplied by the Federal Institute for Materials Research and Testing – BAM (Germany), CERAM AN37 Magnesite supplied by CERAM Research Ltd – Lucideon (England), BCS-CRM 201a Nepheline supplied by Bureau of Analysed Samples Limited – BAS (England), Euronorm-CRM No. 782-1 Dolomite supplied by Bureau of Analysed Samples Limited (BAS) (England), K_2SO_4 supplied by Sigma-Aldrich, Na_2SO_4 supplied by Sigma-Aldrich, $CaSO_4$ supplied by Sigma-Aldrich, Na_2CO_3 supplied by Merck, and K_2CO_3 supplied by Aldrich.

The certified reference material NCS DC 73349 Bush branches and leaves ($0.60 \pm 0.07\%$ Si), supplied by the National Research for Certified Reference Materials – GBW (China), was used for validation measurements.

Beads preparation for WD-XRF measurements were performed using a 50:50 mixture of lithium metaborate and lithium tetraborate (EQF-TML-5050) supplied by EQUILAB as a flux and LiI supplied by Merck as a non-stick agent. Pellets were prepared using a solution of n-butyl methacrylate in acetone as a binder.

The calibration standards for AAS measurement were prepared from a standard solution containing $1,000 \text{ mg}\cdot\text{L}^{-1}$ of Si supplied by Sigma-Aldrich. The validation was carried out with a standard solution containing $1,000 \text{ mg}\cdot\text{L}^{-1}$ of Si supplied by Merck. The sample preparation was carried out by fusion with the same flux used in beads preparation and subsequent dissolution of the melt with HCl 50:50 supplied by Merck.

2.2 | Equipment

WD-XRF analysis was performed with a PANalytical model AXIOS X-ray fluorescence wavelength-dispersive spectrometer, with 4 kW power and Rh tube, fitted with flow, scintillation, and sealed detectors, and eight analysing crystals: LiF200, LiF220, Ge 111, TLAP, InSb 111-C, PET 002, PX1, and PX7.

The measurements by AAS were carried out using a PerkinElmer model AAnalyst 400 atomic absorption spectrometer.

Sample drying process was carried out in a laboratory oven supplied by Salvis Thermocenter. Samples were

milled in a HERZOG tungsten carbide mill. The calcination of the samples and the fusion for AAS measurement were carried out in a NANETTI muffle furnace model M-1.

Beads for the measurement by WD-XRF were prepared in an EQUILAB fusion bead preparation machine model F2 and using Pt-Au crucibles and 30-mm diameter Pt-Au casting dish to conform the bead. Pellets were formed at a pressure of 100 kN in a CASMON hydraulic press using a 37-mm diameter die.

The loss on ignition (LOI) was performed in a LECO thermogravimetric analyser model TGA-701.

3 | EXPERIMENTAL PART

3.1 | Sample preparation

The first step of the new method development was the sample preparation. First, the samples were dried in a laboratory oven at 105°C . Next, the samples were milled in a tungsten carbide mill to obtain a sample with 95% of the particles below $100 \mu\text{m}$ to get a homogeneous sample with an appropriate particle size to subsequent preparations. And then, the samples were calcined at a maximum temperature of 700°C for 30 min.

As samples were calcined, the LOI at 700°C must be considered to calculate the real concentration in the sample. The correction factor (f) from the ignited to pre-ignited basis for the sample is^[16]:

$$f = (100 - L)/100,$$

where L is the loss on ignition at 700°C .

3.2 | Semi-quantitative analysis

The following step to establish the measurement procedure by WD-XRF was the semi-quantitative analysis of the calcined samples in order to know the matrix of the sample, which allows for reproducing it faithfully and establishing the appropriate composition of the calibration standards.

The samples were prepared as pressed pellets for the semi-quantitative analysis. The pressed pellets were measured using a semi-quantitative analysis program named “Uniquant” supplied by PANalytical and based on fundamental parameters, which provides information about the concentration of the major and minor elements present in the sample. All the elements were analysed using the following measurement conditions: voltage 40 kV and intensity 60 mA. The measurement of Si, Al, Fe, Mg, and Na was conducted using the TLAP crystal, and the measurement of Ca, K, P, and S was carried out using the Ge111.

3.3 | Preparation of the calibration curve

The calibration curves were constructed according to the concentrations found in the semi-quantitative analysis of the samples. Certified reference materials detailed in Section 2.1 were appropriately mixed to obtain the calibration standards with adequate concentration to yield a proper range of measurement. Table 1 shows the percentage in weight of each reference material used to prepare each calibration standard, as well as their composition.

The calibration standards were prepared as fused beads. They were weighed into a platinum crucible, and the flux was added in a 1:15 sample : flux ratio. A volume of 0.25 ml of 250 g L⁻¹ LiI solution was then added. The mixture was heated in a fusion bead preparation machine at a maximum temperature of 1050°C for 6 min.

The fused beads obtained were measured using the conditions detailed in Table 2.^[17]

The experimental data of the calibration curves were fitted minimizing the root mean square (RMS) value, obtained from the following equation:

$$\text{RMS} = \sqrt{\frac{\sum (C^* - C)^2}{n - p}},$$

where C^* is the theoretical concentration, C is the calculated concentration, n is the number of calibration standards, and p is the number of parameters calculated from the regression (slope, ordinate at the origin, and inter-element coefficients).

3.4 | Validation

The developed methodology by WD-XRF was validated with the certified reference material NCS DC 73349 Bush branches and leaves.

Additionally, silicon was measured by an independent method: fusion and dissolution of the melt in acid media and subsequent measurement by AAS. The measurement conditions were as follows: $\lambda = 251.6$ nm, nitrous oxide/acetylene flame, slit width 0.2, and 40-mA lamp current.

The sample preparation was carried out by fusion at 1000°C with the flux and subsequent dissolution of the melt in acid media with HCl 50:50.

Three measurements of each material were conducted to estimate the uncertainty of the measurements, which was calculated using the expression^[18]:

$$U = k \cdot u_{method},$$

where u_{method} is the combined uncertainty and k is the coverage factor, which is determined from the Student's

t distribution corresponding to the appropriate degrees of freedom and 95% confidence. The u_{method} was calculated from the expression:

$$u_{method}^2 = u_{V_R}^2 + u_{V_L}^2 + u_{REPRO}^2,$$

where u_{V_R} is the uncertainty of the certified value of the reference material, u_{V_L} is the uncertainty of the measurement of the reference material, and u_{REPRO} is the uncertainty of the measurement of the sample. The coverage factor k is determined from the Student's t distribution corresponding to the appropriate degrees of freedom and 95% confidence.

4 | RESULTS

4.1 | Sample preparation

The results of the LOI at 700°C for the reference material was 87.2 wt%, whereas HORSETAIL samples 1, 2, and 3 provided the following values: 85.9, 75.4, and 83.4 wt%, respectively, and NETTLE LEAF samples 1 and 2 provided 82.0 and 86.1 wt%, respectively.

The LOI obtained for all the materials ranges between 75% and 90%, which are usual values in this type of materials, due to their high cellulose and organic compounds content.

4.2 | Semi-quantitative analysis

The analysis provided by the semi-quantitative program "Uniquant" for the samples are detailed in Table 3.

The results obtained show a variable concentration of some elements such as Si, Ca, K, P, and S, which has been considered in the calibration standards preparation.

4.3 | Preparation of the calibration curves

The silicon concentration range obtained in the construction of the calibration curves ranges from 6.02 to 54.57 wt% SiO₂ and the fitting of the curve provided a root mean square value of 0.21, which is a satisfactory value because it is lower than 5% of the silicon concentration throughout the working range.

4.4 | Validation

As mentioned, the developed methodology was validated by measuring a certified reference material: NCS DC 73349 Bush branches and leaves by WD-XRF and AAS. Three replicates were measured in order to estimate the

TABLE 1 Calibration standards prepared for WD-XRF measurement

Calibration standard	Reference materials	Mass fraction (wt%)	Chemical composition (wt%)	
Standard 1	GBW 03123 Wollastonite	13.1	SiO₂	54.57
	SRM 120c Florida phosphate rock	17.9	Al ₂ O ₃	0.67
	K ₂ SO ₄	19.6	CaO	15.30
	Euronorm-ZRM 777/1 Silikastein	49.4	MgO	0.22
			Na ₂ O	0.11
			K ₂ O	10.71
			P ₂ O ₅	5.97
		S	3.66	
Standard 2	Euronorm-ZRM 777/1 Silikastein	6.0	SiO₂	29.87
	K ₂ SO ₄	9.0	Al ₂ O ₃	0.38
	SRM 120c Florida phosphate rock	11.9	CaO	24.70
	Na ₂ CO ₃	26.5	MgO	0.48
	GBW 03123 Wollastonite	46.6	Na ₂ O	15.58
			K ₂ O	4.98
			P ₂ O ₅	3.99
		S	1.70	
Standard 3	GBW 03123 Wollastonite	6.3	SiO₂	6.18
	Na ₂ SO ₄	11.5	Al ₂ O ₃	0.73
	K ₂ CO ₃	27.6	CaO	28.77
	SRM 120c Florida phosphate rock	54.6	MgO	0.23
			Na ₂ O	5.30
			K ₂ O	18.91
			P ₂ O ₅	18.21
		S	2.79	
Standard 4	CERAM AN37 Magnesite	5.2	SiO₂	10.64
	SRM 120c Florida phosphate rock	4.7	Al ₂ O ₃	4.35
	K ₂ CO ₃	9.1	CaO	16.21
	BCS-CRM 201a Nepheline	18.0	MgO	4.9
	Na ₂ CO ₃	29.8	Na ₂ O	18.83
	CaSO ₄	33.2	K ₂ O	7.80
			P ₂ O ₅	1.58
		S	7.82	
Standard 5	SRM 120c Florida phosphate rock	7.1	SiO₂	23.29
	GBW 03123 Wollastonite	14.8	Al ₂ O ₃	0.30
	Euronorm-ZRM 777/1 Silikastein	16.2	CaO	15.89
	Euronorm-CRM no. 782-1 Dolomite	19.8	MgO	4.40
	K ₂ SO ₄	42.1	Na ₂ O	0.05
			K ₂ O	22.80
			P ₂ O ₅	2.38
		S	7.75	
Standard 6	GBW 03123 Wollastonite	6.0	SiO₂	6.02
	Na ₂ SO ₄	11.5	Al ₂ O ₃	0.73
	K ₂ CO ₃	27.9	CaO	28.64
	SRM 120c Florida phosphate rock	54.6	MgO	0.23
			Na ₂ O	5.28
			K ₂ O	19.14
			P ₂ O ₅	18.22
		S	2.782	

Note. WD-XRF: wavelength dispersive X-ray fluorescence.

uncertainty as explained in Section 3.4. The results obtained by WD-XRF and AAS expressed as the average of the three replicates together with its uncertainty was

(1.29 ± 0.12) wt% SiO₂ and (1.27 ± 0.19) wt% SiO₂, respectively; thus, no significant differences were observed between both values.

TABLE 2 Measurement conditions for chemical analysis by WD-XRF

Element	Line	Crystal	Detector	Voltage (kV)	Intensity (mA)	Angle (2 θ)	Time (s)
Si	K α	InSb 111-C	Flow ^a	30	90	144.80	40
Al	K α	PET 002	Flow	30	90	144.96	40
Fe	K α	LiF 200	Duplex ^b	60	50	57.52	10
Ca	K α	LiF 200	Flow	30	90	113.15	16
Mg	K α	PX1	Flow	30	90	22.70	30
Na	K α	PX1	Flow	30	90	27.48	40
K	K α	LiF 200	Flow	30	90	136.74	12
P	K α	Ge 111	Flow	30	90	141.02	16
S	K α	Ge 111	Flow	30	90	110.77	16

Note. WD-XRF: wavelength dispersive X-ray fluorescence.

^aFlow: Ar 90%/Methane 10%.

^bDuplex: Xe sealed in tandem with flow detector.

TABLE 3 Semi-quantitative analysis of the calcined sample at 700°C obtained by WD-XRF

Oxide	Mass fraction (wt%)				
	HORSETAIL 1	HORSETAIL 2	HORSETAIL 3	NETTLE LEAF 1	NETTLE LEAF 2
SiO ₂	27.5	47.0	47.0	11.7	12.4
Al ₂ O ₃	0.4	2.6	1.5	0.2	0.5
CaO	19.7	11.5	11.5	13.5	12.6
MgO	3.6	3.2	3.1	6.8	5.3
Na ₂ O	0.1	0.4	0.5	1.7	2.5
K ₂ O	23.9	15.5	15.6	45.0	45.0
P ₂ O ₅	4.3	2.1	2.5	6.1	4.4
SO ₃	13.7	12.5	12.4	7.4	9.6

Note. WD-XRF: wavelength dispersive X-ray fluorescence.

To verify the goodness of the method, the results obtained were compared with the certified values as specified in the literature.^[18,19] For that purpose, the absolute value of the difference between the measured and the known value (Δ_m) was compared with the expanded uncertainty (U_{Δ_m}), such that if $\Delta_m \leq U_{\Delta_m}$, there was no significant difference between the measured value and the known value.

The results obtained in this comparison showed good agreement between the certified and the experimental values for both the measurement with the developed methodology by WD-XRF, because Δ_m (0.01) is smaller than U_{Δ_m} (0.38); thus, the developed method has been validated.

Table 4 shows the results obtained for the measurement of the samples where no significant differences had been found between the results obtained by WD-XRF and those obtained by AAS. The WD-XRF method

TABLE 4 Determination of silicon in the reference material NCS DC 73349 Bush branches and leaves and in the samples studied

Sample	SiO ₂ measured by WD-XRF (wt%)	SiO ₂ measured by AAS (wt%)
HORSETAIL 1	5.5 ± 0.2	5.6 ± 0.4
HORSETAIL 2	9.4 ± 0.2	9.2 ± 0.4
HORSETAIL 3	8.0 ± 0.2	8.2 ± 0.4
NETTLE LEAF 1	1.90 ± 0.12	2.0 ± 0.2
NETTLE LEAF 2	1.56 ± 0.11	1.5 ± 0.2

Note. AAS: atomic absorption spectrometry; WD-XRF: wavelength dispersive X-ray fluorescence.

provides low uncertainties than the AAS method, probably because the sample preparation is less complicated in WD-XRF method and the error in the measurement procedure is lower.

In conclusion, the fine-tuned WD-XRF methodology to determine Si in plants has been validated using reference materials and an independent method by AAS.

5 | CONCLUSIONS

In this study, a methodology for the determination of silicon in plants by WD-XRF has been developed, preparing the sample in the form of fused beads. The methodology was established optimizing the sample preparation and the measurement conditions. The following conclusions can be drawn from the study:

- 1) The proposed methodology is rapid and accurate, and the low uncertainties of the results obtained enable this method to be used as a control method.
- 2) Sample preparation for WD-XRF measurement preparing fused beads of the calcined sample is rapid, reproducible, and allows for the use of higher amounts of sample than the methods described in the literature, which use grazing-emission X-ray fluorescence, AAS, ICP-OES, or inductively coupled plasma mass spectrometry, leading to higher representativeness and lower limits of quantification. In addition, this sample preparation method allows for reanalysing the samples as many times as desired due to the long useful lifetime of the fused beads.
- 3) The developed methodology provided significantly low uncertainties and shorter operative times than other methods found in the literature. The calibration curves can remain for a long time; therefore, a daily calibration is not necessary. Thus, the determination of silicon using the developed methodology can be carried out in less than 1 hr.
- 4) The established methodology is more environmentally friendly than other methods, which require the use of different reagents, such as acids or oxidizing agents, which are corrosive and irritating.

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