ISSN : 0974-7419 Volume 10 Issue 12

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(12), 2011 [776-780]

Simple and sensitive solid phase spectrophotometric batch procedure for boron determination in botanical samples

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Received: 28th June, 2011 ; Accepted: 28th July, 2011

ABSTRACT

A solid phase spectrophotometric batch procedure for boron in botanical samples has been developed. Boron (from borate ion) was complex with quinalizarin reactive that form coloured specie. The absorbance of the complex fixed on silica gel resin, was measured directly at 560 and 800 nm after packing the gel beads in a 1-mm cell. Using a sample volume of 50 ml, the calibration graph was linear over the range 10.0–100.0 μg l^{-1} with a R.S.D. of 2.75 % (*n*=8). The method was applied to the determination of boron in botanical samples and was corroborated by standard addition.

KEYWORDS

Micro-nutrients;
Plants;
Boron determination;
Solid-phase Spectrometry;
Quinalizarine.

INTRODUCTION

Boron (cas no.7440-42-8) is a naturally occurring element, does not occur free in the environment, it is always bond to oxygen[1].

In the nature, boron (B) occurs as an anion or neutral molecule in most soils. Whether B is actively transported into plants is a subject of considerable interest in current literature[2]. B is an essential element for plants. B deficiency in plant may result in reduced growth yield loss, and even death, depending on the severity of deficiency. Excess B is toxic to plants[3, 4]. Its bioavailability is therefore of fundamental importance. Likewise, humans could be exposed to B through fruit and vegetables, water, air and consumer products, too. Essential for healthy bones, brain function, alertness, and the metabolism of bulk minerals such as calcium, phosphorus, and magnesium[5, 6].

Quantification of B is an important task in a wide range of disciplines, such as agriculture, biomonitoring, human and other biological analysis. At present, there are several spectrophotometric methods based on the use of specific reagents for the determination of B[7]. The main reagents used for this purpose are curcumin[8], carminic acid[9], alizarin red S[10], quinalizarine (QZN)[11, 12] and azomethine-H[13]. Among these, QZN has extractive properties and has frequently been used as a reagent for solid phase extraction and spectrophotometric determination of cations[14]. Although atomic spectrometries, such as electrothermal atomic absorption spectrometry (ETAAS)[15], inductively coupled plasma–atomic emission spectrometry (ICP-AES)[16] and inductively coupled plasma–mass spectrometry (ICP-MS) are sensitive and selective for B determina-

tion, moreover the total amounts of B can be determined despite the fairly expensive equipment.

In this context, solid-phase spectrophotometry (SPS)[17,18], appears as advantageous technique, because its low-cost and simplicity, with similar analytical quality results. This technique is based on the direct spectrophotometric measurement of a coloured specie on solid phase, that has sorbed a sample component. This method made it possible to determine trace components in natural samples without preconcentration, because a sensitivity enhancement was easily accomplished by increasing the sample volume.

The aim of this study was to develop and evaluate a simple and sensitive solid-phase spectrophotometric batch method using a commercially available, UV-Vis spectrophotometer, which would be applicable to the determination of micro amounts of B in natural samples. The different experimental parameters such as acidity, type and amount of resin, surfactant concentration, ligand concentration, and type of buffer have been thoroughly investigated. The results showed that QNZ can selectively react with B in the presence of common coexisting interfering ions. This new SPS method has been used for determination of B from different botanical samples. The most interesting features of the proposed method are simplicity in operation, high sensitivity and selectivity for the spectrophotometric determination of B.

EXPERIMENTAL

Reagents

High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA), was used exclusively. A B standard solution (1000 μg ml^{-1}) was obtained from Fluka and diluted as necessary to obtain calibration solutions. Reagent grade 1,2,5,8-tetrahydroxy anthraquinone (QZN) (from Merck) was dissolved in reagent grade ethanol. All acids used were of the highest purity available from Merck and used as received. Analytical-grade Octylphenol poly(ethyleneglycol) ether (Triton X-100, Merck®) non ionic surfactant solution was of the highest purity available and was used without any further purification. Active silica gel was prepared by weighting 200 g of silica gel and mixing with 500 ml of 4.0 mol l^{-1} hydrochloric acid. This mixture was allowed to reflux with stirring for 4 h, filtered, washed with doubly distilled water until acid free silica gel phase. The active silica gel was then dried in an oven at 150–160 °C for 6 h and left in a vacuum desiccator for future use.

Apparatus

A single beam microprocessor controlled Metrolab® 1700 UV/Vis spectrophotometer controlled by personal computer with a 1.0 mm quartz cell was used for all spectral measurements. An Altronix model TPX pH meter was used for checking the pH of solutions. To decrease the risk of contamination, glassware was avoided, and plastic (polypropylene) vessels were used to prepare and store the solutions or suspensions. All plastic ware was nitric acid-washed and rinsed with ultrapure water.

Absorbance measurements

The absorbance of complex retained on the solid phase was measured in a 1.0 mm quartz cell at 466 and 800 nm against a 1.0 mm cell packed with resin previously equilibrated with blank solution. The net absorbance for boron-QZN complex A_{net} in solid phase was obtained from: $A_{net} = A_{complrx} - A_{resin}$ Where $A_{complrx} = A_{466} - A_{800}$ for the sample and $A_{resin} = A_{466} - A_{800}$ for the blank solution[19]. When the absorbance was measured at two different wavelengths, one corresponding to absorption maximum of the complex (466 nm) and the other in a region where the complex absorbs very low (800 nm), the absorbance difference, could be assumed to be constant under the similar packing conditions.

General Procedure

For 50 mL final volume, an appropriate volume of sample containing 0.1 to 20.0 μg of B was transferred into a 50 ml plastic tube with stopper and then 0.5 ml of 5% v/v Triton X-100 solution, 0.5 ml of 2.5 mol l^{-1} H_2SO_4 and 1.0 ml QZN $1x10^{-3}$ mol l^{-1} solution were placed leveling off to the mark with water. After standing for 5 min in the dark, 70 mg of silica gel resin was added to the solution. The mixture was stirred mechanically during 20 min then the colored resin was collected and transferred to 1.0 mm quartz cell with the aid of a dropping pipette. A blank solution containing all reagents, with the exception of B, was prepared and treated in the same way as the sample. The absorbance differ-

ence between sample and blank was measured after 5.0 min as described in Section 2.3.

Reference method (ICP-OES method)

The measurements were carried out with an ICP atomic emission spectrometer Varian ICP-OES model ICP-OES Vista Pro, with a Czerny-Turner monocromator, holographic diffraction grid and a VistaChip charge coupled device (CCD) array detector. The used emission lines of B were B I 249.773, B I 249.678, and B I 208.959 nm. The line of internal standard indium (In I 325.609 nm) was used for matrix effect correction.

Botanical samples treatment

Prior to the determination, the vegetable samples were pretreated in the following way. An edible portion of vegetable was firstly washed clean with tap water and then rewashed with deionized water. After removing deionized water on the surface of vegetable, the sample was cut into small pieces and dried at 65 °C in oven. Then the sample was ground and passed through a 60-mesh sieve. The powdered sample (1.0 g) was accurately weighted into a porcelain crucible and calcined at 550 ºC for 2 hours. The contents of the crucible were cooled to room temperature and moistened with 10 drops of water. Upon addition to the crucible and 3.0 ml of (1+1) HNO_3, then the nitric phase is evaporated at 100 ºC on a hot plate. The crucible was returned to the furnace for an additional hour of calcinations at 550 ºC. The contents of the crucible were allowed to cool room temperature, dissolved in concentrated H_2SO_4, quantitatively transferred to a 50 ml volumetric flask, and brought to volume with distilled water. The usual general precautions were taken to avoid contamination.

RESULTS AND DISCUSSION

The fixation of complex

It was found that the ionic pair is not fixed on ion-exchangers as Dowex 1X8 or DEAE Sephadex anion exchangers, as well as in the cation exchangers (SP) or lipophilic Sephadex. On the contrary, the ionic pair was strongly fixed in hydrophilic adsorbents like active silica gel and Amberlite XAD-7, giving a red color with an absorption maximum at 466 nm, compared with 460 nm observed in solution. Subsequently, silica gel was selected by its high stability on strong acid media, environmental friendly and low economical cost.

Optimization of the reaction medium

Previous studies have shown that the QZN-boron complex occurs in sulfuric acid media. From sulfuric acid, phosphoric acid, hydrochloric acid and acetic acid, the first gave the highest absorbance and stability. The experimental data indicated the optimum pH for the formation and fixation of the complex species was in the range 1.65–3.15 using different concentration of sulfuric solutions (Figure 1). Therefore, diluted sulfuric acid solution was used to maintain the optimum one which gives highest absorbance value in addition to the stability of the color fixed species, so, all subsequent studies were performed at pH 2.00. Moreover, 1.0 ml of 0.25 mol l^{-1} H_2SO_4 solution was selected for 50 ml sample volume.

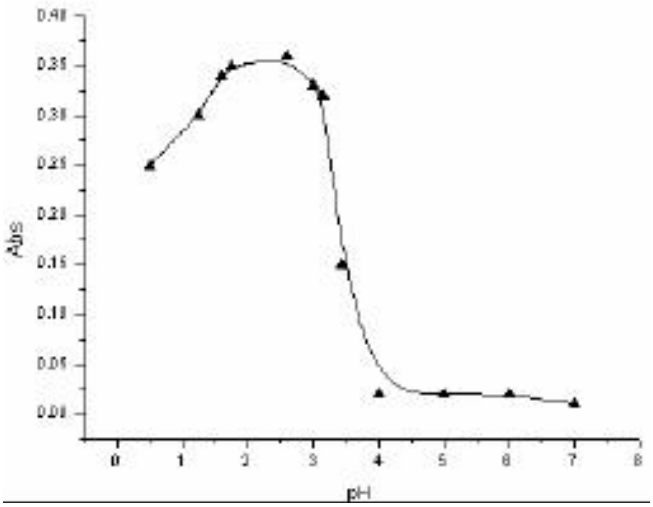

Figure 1 : Effect of pH. [B] = 25 µg l^{-1}; [QZN] = 1x10^{-3} mol l^{-1}; resin = 70 mg.

Then, a tensioactive agent was necessary to help the dissolution of the reagent and its complexes in water or ethanol. Triton X-100, was tested at different concentrations, and 0.05% v/v was selected (Figure 2).

Effect of reagent concentration

With the increase of the concentration of QZN, the absorbance of the fixed complex on resin increases rapidly and reaches a plateau. After that, it dropped slowly. In 50 ml of solution, the optimum volume of QZN 1x10^{-3} mol l^{-1} solution (in ethanol) was between 0.4 and 0.8 ml. Thus, 0.6 ml of the reagent was employed for this work.

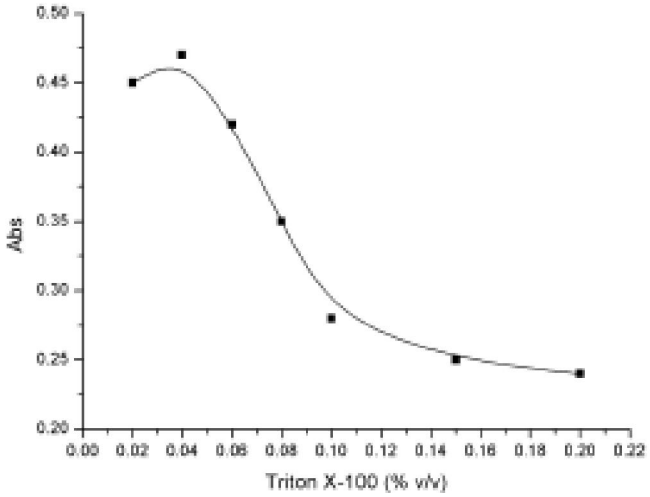

Figure 2 : Effect of amount of surfactant (Triton X-100). [B] = 25 µg l^{-1}; [QZN] = 1x10^{-3} mol l^{-1}; pH = 2.00; resin = 70 mg.

Effect of time

The effect of stirring time on color intensity was investigated. The rate of equilibration was not influenced by the rate of formation of QZN-boron complex. The color development was complete within 15.0 min. A stirring time of 20.0 min was chosen to keep the analysis time. The fixed complex is stable for at least 8.0 h after equilibration.

Effect of resin

The use of a large amount of resin lowered the absorbance as usual. The solid support level in the cell is a very important variable. Only the amount required filling the cell and to facilitate handling (i.e. 70 mg) was used for all studies. This level has to be sufficiently high to let the light beam pass through the colored resin.

TABLE 1 : Analytical parameters for SPS boron determination

Parameter	
Intercept	0.14
Slope (µg^{-1} l)	0.016
Linear range (µg l^{-1})	10 – 100
Correlation coefficient	0.97
% R. S. D. (n = 10)[a]	2.75
Detection limit (µg l^{-1})[b]	1.50
Determination limit (µg l^{-1})[c]	5.0

[a]R. S. D. was established at 10 µg l^{-1} B; [b] 3 σ criterion.; [c] 10 σ criterion

Analytical figures of merit

TABLE 1 summarizes the analytical figures of merit of the optimized method, including regression equation, linear range, reproducibility, and limit of detection. The limit of detection, defined as $C_L = 3S_B/m$ (where C_L, S_B, and m are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively), was 1.5 µg l^{-1}. Reproducibility was measured for a series of ten independent determinations containing 25.0 µg l^{-1} of B in 50 ml sample using 70 mg of the silica gel resin.

Foreign ion effects

A study of potential interferences in the determination of B was carried out for ions, in amounts ranging up to 10.0 mg l^{-1}, with 20 µg of B. A relative error of less than 5 % was considered to be within the range of experimental error, so ions were considered as not interfering if they produced a relative error less than ±5%. The tolerance limits for ions studied are shown in TABLE 2.

TABLE 2 : Tolerated limit of various foreign ions. Boron concentration: 20 µg l^{-1}

	Tolerated limit (mg l^{-1})
Na(I), K(I), Cl(I), NO_3(I)	10.0
Ca(II), PO_4(III)	5.0
Al(III), Ba(II), Mg(II)	1.0
As(III), Cr(III)	0.9
Cu(II), Co(III), Zn(II)	0.7
Cd(II), Ge(IV), Mo(VI)	0.5
Fe(III)	0.2

TABLE 3 : Analytical applications

Sample	Concentration of boron (µg g^{-1})			
	Added	Found by proposed method[a]	Recovery (%)	Found by ICP-OES[a]
Mandarina leaves	--	52.6 ± 0.4		51.5 ± 0.2
	5.0	58.4 ± 1.5	101.3	55.8 ± 0.3
	20.0	70.2 ± 1.0	96.6	73.2 ± 0.5
Lemmon juice	--	10.5 ± 0.4		10.6 ± 0.1
	5.0	15.8 ± 0.8	101.8	15.6 ± 0.3
	20.0	28.1 ± 0.9	92.1	30.1 ± 0.2
Seed soybean	--	8.4 ± 0.3		8.2 ± 0.1
	5.0	15.2 ± 0.5	113.5	14.6 ± 0.2
	20.0	29.8 ± 0.8	104.9	28.9 ± 0.2

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Application to botanical samples and validation

In order to test the reliability of the proposed method, the method was applied to the determination of B in botanical samples. The recovery of B in the samples was checked by the addition of standard B to 50 mL samples. The results are shown in TABLE 3. The recoveries were ranging from 93.5 to 107.6% (n = 5), while indicates that no interfering substances encountered with the determination of B. The analytical data for various samples given in TABLE 3 indicate a high degree of correlation between the results of ICP-OES and proposed procedure.

CONCLUSIONS

The proposed procedure gives a simple, very sensitive and low-cost spectrophotometric procedure for determination of B that can be applied to botanical samples. Solid-phase spectrophotometry combines the pre concentration of the species of interest on a solid matrix and subsequent measurements of the absorbance of the complex in the previous solid phase. This provides an increase in selectivity and sensitivity with respect to conventional spectrophotometric method without requiring expensive and sophisticates instrumentation such as ICP-OES. The proposed method could be successfully applied to the determination of B in plant materials with reliable results.

ACKNOWLEDGEMENTS

The author Roberto Gerardo Pellerano wants to thank for grants received from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina that provided financial support.

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