Bulletin of Pharmaceutical Research 2015;5(2):51-8
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH PAPER

A SIMPLE AND SENSITIVE ASSAY METHOD FOR BORON ESTIMATION BY DIFFERENTIAL PULSE VOLTAMMETRY IN INDIAN TRADITIONAL HERBO-MINERAL FORMULATION, *MAHAMRUTYUNJAYA RASA*

Pallavi Rai[1]*, Jainendra Jain[1] and Sadhana J. Rajput[2]

[1]*Pharmacy Department, RamEesh Institute of Vocational and Technical Education, Greater Noida-201 310, Uttar Pradesh, India*
[2]*Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India*

**E-mails*: raipallav@gmail.com, sjrajput@rediffmail.com
Tel.: +91 9873848659, +91 265 2434187.

Received: Jul 30, 2015 / Revised: Aug 11, 2015 / Accepted: Aug 12, 2015

By optimizing the analytical conditions, a reliable, rapid, simple and accurate differential pulse voltammetric method was developed for the quantitative determination of Boron in an ayurvedic formulation, Mahamrutyunjaya rasa. The effects of several chemicals and instrumental variables were studied, and optimized operating conditions were identified. Boron was determined in the incinerated formulated by differential pulse voltammetry, according to the monitoring the anodic peak of the complex formed between boron and Alizarin Red S (ARS) at -521 mV in ammonium acetate- phosphate buffer (pH = 7). Based on the above method, a calibration curve was established by plotting the peak current of the boron-ARS complex to the boron concentration with a linear range of 1-10 μg/ml. The sample analysis was performed in the presence of 1 mM EDTA for the elimination of interference from metal ions. The results indicated that this method has a detection limit of 0.2 μg/ml, based on signal to ratio of 3, an average recovery of 98-101% and a relative standard deviation (RSD) of 2.0%. The results obtained from this method were compared with inductively coupled plasma optical emission spectrometry (ICP-AES) method, and no significant difference was found. This method can provide a scientific and technical platform to the product manufacturers for setting up a quality control standard as well as to the public for quality and safety assurance of the proprietary ayurvedic formulations.

Key words: Boron, *Mahamrutyunjaya rasa*, Differential pulse, Voltammetry, Alizarin red.

INTRODUCTION

Herbo-mineral formulations have reached extensive acceptability as therapeutic agents for several diseases. The development of authentic analytical methods which can reliably profile the organic and inorganic composition, including quantitative analysis of the active compound and other major constituents, is a major challenge to scientists. Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assured for the manufacturing of herbo-mineral drugs (Rai *et al* 2009). The WHO specifies guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization. *Mahamrutyunjaya rasa* (MHR) is an ayurvedic formulation used in the treatment of cardiac disorders. It contains roots of *Aconitum ferox*, *Solanum indicum*, fruits of *Piper longum, Piper nigrum,* Sulphur, Cinnabar and sodium metaborate. Borax or Sodium metaborate is an

alkaline salt known as Tankana in Sanskrit. It occurs as a natural deposit and has the chemical formula: $NaBO_2 \cdot 2H_2O$ [or $Na_2B_2O_4 \cdot 4H_2O$] and molecular weight 101.83 g (Ramakumar, 2005). In Ayurveda, Borax is given internally in acidity of the stomach, amenorrhoea, dysmenorrhoea, menorrhagia, puerperal convulsions and to promote uterine pains during labour. As a solvent, it is given in uric acid diathesis with good results. In small doses, it is given to children as a laxative. It is also used in the loss of appetite, painful dyspepsia, cough, asthma and diarrhoea. (Garcia Campana *et al* 1992).

Several spectrometric and electrometric analytical methods have been developed for the determination of boron in various samples. Among the spectrometric techniques, spectrophotometry (Zaijun *et al* 2001; de Azevedo *et al* 1998; Balogh *et al* 2009), spectrofluorimetry (Economou *et al* 2004), atomic absorption spectrometry (Burguera *et al* 2001), inductively coupled plasma-atomic emission spectrometry (Sun *et al* 1997; Probst *et al* 1997), inductively coupled plasma-mass spectrometry (Wilke *et al* 1997; Sun *et al* 2000; Al-Ammar *et al* 2000; Kozono *et al* 2002; Park and Song, 2003) and X-ray fluorescence spectrometry (Sanchez-Ramos *et al* 2000) have been used. A cathodic stripping polarographic method based on the peak current decrease of As (V) in the presence of mannitol, copper and selenium in sulphuric acid medium developed for the determination of boron in mg/l level (Sahin and Nakiboglu, 2006). A differential pulse polarographic method using tetraborate-copper complex formation has been reported for trace determination of tetraborate indirectly in waste and drinking water samples (Unal and Somer, 2009). On the other hand, Beryllon (III) (Jin *et al* 1993; Thunus, 1996; Tanaka *et al* 2006) has been used as complexing agent for voltammetric determination of boron at hanging mercury drop electrode (HMDE). The present study was focused on development of one of the standardization parameter of assay of Boron present in a herbo-mineral ayurvedic formulation, *Mahamrutyunjaya rasa.* A novel voltammetric method of Boron estimation was developed and validated. The results obtained were compared with the results of assay performed using inductively coupled plasma (ICP).

MATERIALS AND METHODS

Chemicals and reagents

Sodium metaborate was purchased from Qualigens. Alizarin red S was purchased from Fluka. The working solutions were prepared daily. The other chemicals used throughout the study were of analytical grade. All the solutions were prepared with triple distilled water.

Preparation of standard solution

Stock solution was prepared by weighing sodium borate equivalent to 10 mg of boron and diluting to 100 ml with triple distilled water. The solution was stored in a polyethylene bottle.

Preparation of reagents

Alizarin Red S solution (1 mM) was prepared by weighing exactly 0.171 g of the reagent and diluting to 500 ml with doubly distilled water. Buffer solution (pH 7) was prepared by mixing 1 M ammonium acetate and 1 M potassium dihydrogen phosphate.

Preparation of sample solution

About 1 g of powdered formulation was weighed accurately in a tarred silica crucible. The sample was incinerated in a muffle furnace at 500°C for 2 h and then left to cool inside the furnace. The ash was humidified using triple distilled water, and 1 ml of concentrated hydrochloric acid was added. The mixture was heated at 70°C on a heating mantle. The solution was filtered and washed with hot water. To above solution, 5 ml of 0.1 M EDTA solution was added. The solution was neutralized using sodium hydroxide solution and transferred into a 25 ml calibrated flask and diluted to the mark with doubly distilled water. Different aliquots of not more than 5 ml were taken for the determination of boron with Alizarin Red S.

General procedure

In a 10 ml of volumetric flask, 100 μl of 0.01 M Alizarin S Red, 1 ml of 1 M ammonium acetate-phosphate supporting electrolyte (pH 7) and the required amount of Boron was pippetted and volume was made up to 10 ml with distilled water. The solution was transferred to the quartz voltammetric cell and nitrogen was purged for 5 min. A new drop of mercury was extruded and the stripping was initiated immediately in the anodic direction starting from -700 mV by using differential pulse modulation without being stirred. The instrumental conditions were potential scan rate of 5 mV/s, pulse duration 0.02s and pulse amplitude 50 mV. All the measurements were

performed at room temperature. The sample analysis was performed in the presence of 1 mM EDTA for the elimination of interference from metal ions.

Effect of pH and supporting electrolyte

The peak current was measured by varying the pH in the range of 5.0-8.0. Additionally, different electrolytes at pH 7.0 were tested as supporting electrolyte (ammonium acetate, phosphate, ammonium actate-phosphate mixture, sodium nitrate, ammonium chloride, potassium perchlorate, sodium bromate). Among these, 0.1 M ammonium acetate and 0.1 M phosphate mixture (pH 7.0) has given the maximum peak current.

Effect of alizarin red S concentration

The effect of the concentration of Alizarin red S on peak current of the Boron-ARS complex was tested. Various concentrations of the Alizarin red S ranged from 0.1-10 μM in the presence of 5 μg/ml boron and supporting electrolyte at pH 7.0 were studied. The results have shown that the peak height of boron-Alizarin red S complex increases with the ligand concentration.

Method validation

The analytical method validation was done by ICH Guidelines (ICH, Q2B, 1996).

Linearity and range

The linearity of the Boron was evaluated by analyzing a series of different concentrations of Boron. In this study, five different concentrations of Boron-Alizarin red S complex were chosen within the linearity range, and each was repeated three times. A linear relationship was found between the current and the concentration of the complex in the range 1-10 μg/ml. The linearity range and regression equation (slope, intercept, correlation co-efficient and standard error of estimation) were determined.

Detection and quantitation limits

The LOD and LOQ values for Boron were calculated from the calibration curves as kSD/b where k = 3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve.

Precision

For evaluation of the precision, within the day (intra-day) and between-day (inter-day) precision variability was performed at three concentration levels 1, 5 and 10 μg/ml for Boron. The experiments were repeated six times a day for intra-day precision and on three different days for inter-day precision.

Accuracy

To confirm the accuracy of proposed method, recovery study was performed by the standard addition technique. Three different levels (80, 100 and 120%) of standards were added to pre-analyzed tablet samples, and each level was repeated three times.

Robustness

The robustness of the method was checked by examining the reflection of the slight changes to the results in methodological parameters. The brand of the reagents used in the analysis was purposely altered in order to determine the robustness of the method.

Estimation of boron using inductively coupled plasma

Preparation of standard solution

Standard stock solution of Boron in the concentration range of 100 μg/ml was prepared in de-ionized water.

Preparation of sample solution

Twenty tablets were triturated to fine powder and about 1 g of each was incinerated in muffle furnace at 500°C until a white color was obtained. Solutions of the ash were dissolved in 0.1 N HCl by sonication and the volume was made up in the concentration range of 5 ppm.

General procedure

Boron was estimated in the formulations using inductively coupled plasma-AES (Perkin Elmer, Optical Emission Spectrometer, Optima 2100 DV attached to Winlab 32 software). A calibration plot was prepared using standard Boron solution in the range (1-6 μg/ml). The samples were analyzed at 249.43 nm. The estimation was repeated three times and the %RSDs were calculated.

RESULTS AND DISCUSSION

Voltammetric peak characteristics of the boron-ARS complex

The differential pulse voltammograms of Alizarin red S and boron-Alizarin red S complex are shown in **Figure 1, 2**. A peak was observed at -590 mV in a solution of Alizarin red S at pH

7.0 when the potential was scanned towards anodic direction. After the addition of Boron to this solution a new peak at -520 mV appeared. This new peak proportionally increased with increasing concentration of Boron while the peak at -590 mV significantly decreased. The peak at -590 mV practically disappeared when the excess boron was added to the solution higher than 0.5 μg/ml. This indicated that Alizarin red S reacts with boron to form a complex, borosulfoalizarin and a new anodic peak related to this complex appeared. No peak was observed corresponding to boron-Alizarin red S complex when the potential scanning was done towards more negative values.

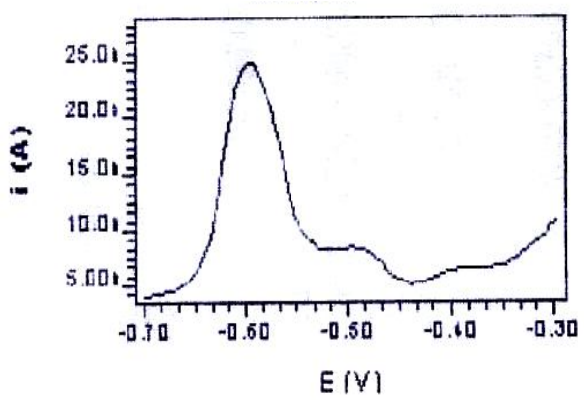

Fig. 1. Differential voltammogram of Alizarin red S

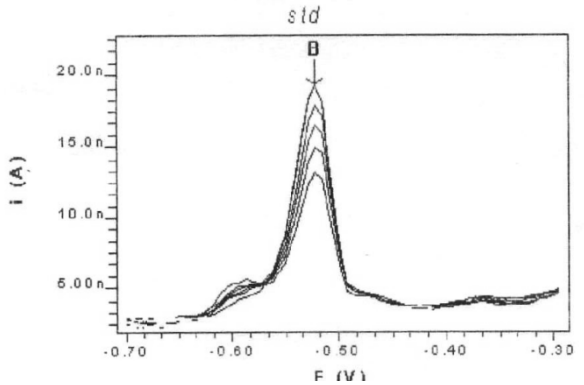

Fig. 2. Differential voltammograms of Boron-Alizarin red S complex

As the anodic peak height of boron-Alizarin red S complex was plotted versus potential scan rate, the peak current linearity changed with potential scan rate indicating that it is an adsorption process. In this case pre-treatment conditions such as accumulation time and potential were investigated.

The variation of peak current as a function of accumulation time at -0.7 V is shown in **Figure 3**. As per figure, the current increases with accumulation time and reached a maximum at 5s and then, decreased. For accumulation times longer than 5s, massive adsorption of the free ligand on the electrode surface took place and hindered the anodic peak of the complex. The precision of the method was poor for accumulation time of 5s, therefore, the measurements were performed without applying any accumulation time.

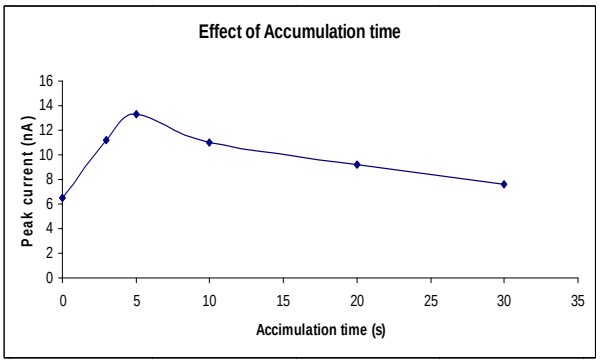

Fig. 3. Effect of accumulation time on peak current

On the other hand, as the scan rate is 5 mV/s and the starting potential is - 0.7V, 36 s was virtually passed during the scan to potential -0.521V where the anodic peak was observed. Accumulation at a certain degree is maintained during this period. In fact, the scan rate was quite critical for the peak height as it determines the time passed during the scan and so adsorption /desorption ratio of the complex and free ligand. The competitive adsorption of the free ligand and the complex could be clearly observed in the scan where the scan rate was 5 mV/s. At higher scan rates, the anodic peak of free ligand increased and the complex peak appeared as a shoulder on the former peak in particular for low boron concentrations. This resulted in bad resolution threatening the selectivity of the method.

Besides, the effect of temperature on the peak current was investigated. The reaction mixture including boron, alizarin red S at pH 7.0 was heated for 15 min at 40, 60 and 80°C and then allowed to stand for 15 min, 4h and 10h. No significant difference was observed between the results of these experiments and of the ones performed at room temperature.

The effect of accumulation potential on the peak current was investigated for a very short accumulation time (3s) at different potential values but the scan was initiated from -0.7 V in each experiment. **Figure 4** shows dependence of

the peak current on the accumulation potential. The peak current gives a maximum at -700 mV and thereafter, this potential was chosen.

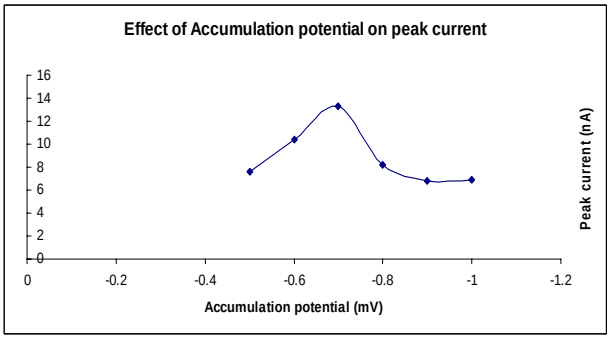

Fig. 4. Effect of accumulation potential on the peak current (nA)

Effect of pH and supporting electrolyte

Figure 5 shows the effect of pH on the peak current. The peak current slightly increases from pH 5.0 to 7.0 and then, decreases with increasing in pH. A positive shift in the peak potential of the complex was observed with decrease in pH. No signal related to the boron complex was obtained below pH 5.0. The optimum pH was selected as 7.0 to carry out subsequent experiments.

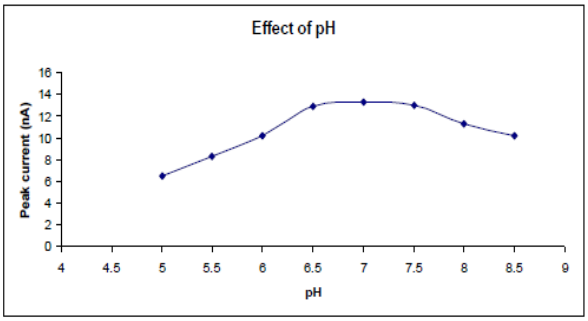

Fig. 5. Effect of pH on peak current

Different electrolytes at pH 7.0 were tested as a supporting electrolyte (ammonium acetate, phosphate, ammonium acetate-phosphate mixture, sodium acetate, ammonium nitrate, ammonium chloride, potassium perchlorate, sodium bromate). Among these, 0.1 M ammonium acetate - 0.1 M phosphate mixture (pH 7.0) has given the maximum peak current and the best resolution.

Effect of Alizarin red S concentrations

The effect of the ligand concentration on peak current of the boron alizarin red S complex was tested. Various concentrations of ligand ranged from 0.1 to 10 μM in the presence of 100 μg/l Boron and supporting electrolyte at pH 7.0 were studied. The results have shown that peak height of boron-Alizarin red S complex increases with the ligand concentration. However, the free ARS peak overlaped with the peak of boron-Alizarin S complex for low boron concentrations. Thus, 1 μM of Alizarin red S was selected for subsequent experiments to obtain good resolution between the alizarin red S peak and boron alizarin red S peak.

Method validation

Linearity and range

Under the recommended conditions in the **Table 1**, a calibration curve for boron was constructed. **Figure 6** shows the voltammograms obtained for the calibration curve (**Table 2**). The curve is linear in the concentration range of 1-10 μg/ml in boron and the regression equation of the curve is y = 0.8015x + 8.3787, where y is the peak height (in nA) and x is the concentration of Boron (in μg/ml). The correlation coefficient was 0.9975 for n = 6.

Detection and quantitation limits

The limits of detection (LOD) and quantitation (LOQ) calculated at a signal-to-noise (S/N) ratio of 3 and 10 were 0.2 and 0.66 μg/ml, respectively.

Table1. Voltammetric parameters for determination of boron

Parameter	Description
Accumulation potential (mV)	-700
Accumulation time (s)	0
Supporting Electrolyte, pH	Ammonium acetate-phosphate buffer, 7.0
Concentration of ARS (mM)	0.001
Scan rate (mV/s)	5
Pulse amplitude (mV)	50
Pulse duration (ms)	20

Table 2. Linearity data of boron-alizarin complex

Sample	Potential (V)	Peak Current (nA)	i.mean	i.delta
1-1	-0.521	8.96	9.02	1.02
1-2	-0.521	9.08		
2-1	-0.521	10.11	10.07	1.71
2-2	-0.521	10.03		
3-1	-0.521	11.59	10.57	1.73
3-2	-0.521	11.55		
4-1	-0.521	13.72	13.34	1.56
4-2	-0.521	12.96		
5-1	-0.521	14.5	14.9	1.12
5-2	-0.521	15.3		
6-1	-0.521	16.58	16.22	1.32
6-2	-0.521	16.22		

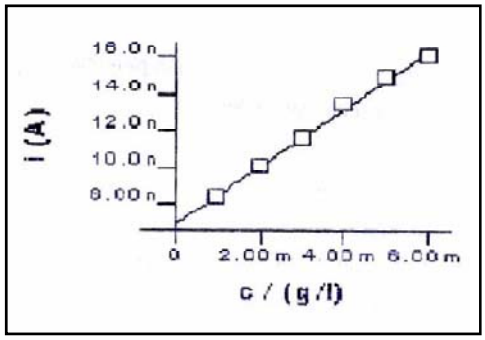

Fig. 6. Calibration curve of boron-alizarin red S using voltammetry

Precision

The precision of the method was tested with regard to both the intra-day and inter-day precision of the assay. The intra-day variability of the assay was determined by repeated analysis of three concentrations (n = 6). Similarly, the inter-day variability of the assay was determined through replicate analysis of three concentrations (n = 3), and the results are listed in **Table 3**. Both intra-day and inter-day precision of the presented methods were fairly good.

Table 3. Intra-day and inter-day precision of spectrofluorimetric method (n=6)

Amount (μg/ml)	Intra-day precision		
	Peak current (nA)	S.D.	% RSD
1	9.01	0.56	1.2
5	12.63	0.34	1.32
10	16.18	0.72	0.96
Amount (μg/ml)	Inter-day precision		
	Peak current (nA)	S.D.	% RSD
1	9.05	1.1	0.93
5	12.52	0.42	0.72
10	16.31	0.31	0.91

Recovery

The proposed method when used for estimation of boron from the herbal dosage form after spiking with 80, 100 and 120% of additional drug afforded recovery of 98–101% as listed in **Table 4**.

Table 4. Data of recovery studies

Excess drug added to the analyte (%)	Theoretical content (μg/ml)	Recovery (%)	% RSD
0	4.25	98.53	1.29
80	7.65	99.21	1.54
100	8.5	98.10	1.71
120	9.35	98.23	1.83

Robustness

No significant difference could be observed in the results found out. The recovery obtained individually and the mean was between 98% and 101% boron. Therefore, it can be concluded that method is consistent for the reagent brand.

Implementation of the developed method in formulations

The proposed method was applied to the determination of boron in formulations of three different batches. The label claim of Boron in the formulation is about 4.3 mg per tablet. The recoveries obtained from the spiked samples are shown in **Table 4** and the assayed boron concentration in the samples by voltammetry and ICP are shown in **Table 5**. The recoveries were in the range 96-104%

Table 5. Results of analysis of MHR by voltammatry and ICP-AES methods

S. No.	Formulation	Boron, mg per tablet by voltammetric method	Boron, mg per tablet by inductively coupled plasma method
1	MHR-1	4.25±0.19	4.25±0.23
2	MHR-2	4.15±0.15	4.14±0.11
3	MHR-3	4.19±0.24	4.2±0.16

Estimation of boron in *mahamrutyunjaya rasa* by ICP-AES

In this study, analytical methods have been applied for determination of boron in three different batches of *Mahamrutyunjaya rasa*. The results obtained by ICP-AES at 249.43 nm. The operating conditions are shown in **Table 6**. A calibration results gave good linearity as shown in **Table 7**. The content found was comparable with content estimated by voltammetry method.

Table 6. Operating conditions of ICP-AES

S. No.	Parameter	Description
1	Plasma view	15 mm radial
2	Nebulizer type	Concentric
3	Washing time	30 s
4	Plasma power	1300 W
5	Argon flow rate	
	Plasma	15 l/min
	Auxillary	0.5 l/min
	Nebulizer	0.8 l/min
6	Replicates	5
7	Read time/replicate	1s
8	Emission wavelength	249.43 nm
9	R_f power	1.20 kW
10	Argon gas purity	99.99%

Table 7. Data for calibration curve of boron

Boron conc. (μg/ml)	G. correlative intensity	% RSD
1	22326	0.54
2	47563	0.32
3	76190	0.18
4	113234	0.43
5	148932	0.34
6	186342	0.56

Validation of the method

Linearity

The linearity of the present ICP-AES method for boron determination was good (r^2 = 0.99) for a boron concentration range of 1-6 μg/ml. The results revealed a good linearity.

LOD and LOQ

The limits of detection and quantification were estimated from the SD of the boron signal obtained by repeated measurements (n = 6) of a zero calibrator containing the internal standard. The limit of detection was 0.1 ppm, and the limit of quantification was 0.33 ppm (corresponding to the boron signals equivalent to 3 and 10 times the SD of the boron signal in the zero calibrator, respectively).

Recovery study

Analytical recovery in the ICP-AES procedure was further studied by supplementing the test samples with standard boron. The observed recoveries were in the range of 96-103%.

CONCLUSION

The proposed method provides a simple, precise, accurate and sensitive voltammetric technique which can be applied for boron determination in the herbo-mineral formulations. The results were very close to the results obtained by inductively coupled plasma method. Thus, the method provides comparable results with atomic spectrometric methods in terms of accuracy and sensitivity and is much simple and cost effective. Since ayurvedic herbo-mineral medicines containing boron has been extensively

used in ayurveda and other traditional systems of medicine, method for standardization of those medicines are in demand. The contents of such substances can significantly vary in proprietary medicines. So, it is highly recommended that determination of boron in the proprietary ayurvedic medicines must be done as a routine measurement, so as to provide a safe application to patients in clinics, and good manufacture practices.

REFERENCES

Al-Ammar AS, Gupta RK, Barnes RM. Elimination of boron memory effect in inductively coupled plasma-mass spectrometry by ammonia gas injection into the spray chamber during analysis. *Spectrochim. Acta Part B Atom. Spect.* 2000;55(6):629-35.

Balogh IS, Andruch V, Kadar M, Billes F, Posta J, Szabova E. A simple method of boron determination in mineral waters using Victoria blue 4R. *Int. J. Environ. Anal. Chem.* 2009; 89(6):449-59. [DOI: 10.1080/03067310802710621]

Burguera M, Burguera JL, Rondon C, Carrero P. Determination of boron in blood, urine and bone by electrothermal atomic absorption spectrometry using zirconium and citric acid as modifiers. *Spectrochim. Acta Part B: Atom. Spec.* 2001;56(10):1845-57. [DOI: 10.1016/ S0584-8547(01)00340-8]

de Azevedo C, de Luca GC, Fernandes RN, Reis BF, Krug FJ. Multicommutation in flow analysis exploiting a multizone trapping approach: spectrophotometric determination of boron in plants. *Anal. Chim. Acta* 1998;374(1):53-9. [DOI: 10.1016/S0003-2670(98)00395-X]

Economou A, Themelis DG, Bikou H, Tzanavaras PD, Rigas PG. Determination of boron in water and pharmaceuticals by sequential-injection analysis and fluorimetric detection. *Anal. Chim. Acta* 2004;510(2):219-24. [DOI: 10.1016/ j.aca.2004.01.002]

ICH, Q2B. Validation of analytical procedures: Methodology. International conference on harmonization, Geneva, Switzerland, 1996.

Jin W, Jiao K, Metzner H. On the adsorption voltammetry of the boron-Beryllon III system: Part I. Determination of boron in plants and soils. *Electroanalysis* 1993;5(5-6):437-43. [DOI: 10.1002/elan.1140050511]

Kozono S, Takahashi S, Haraquchi H. Determination of boron in high-purity tantalum materials by on-line matrix separation/inductively coupled plasma mass spectro metry. *Analyst* 2002:127(7):930-4.

Garcia Campana AM, Ales Barrero F, Roman Ceba M. Spectrofluorimetric determination of boron in soils, plants and natural waters with alizarin red S. *Analyst* 1992;117(7):1189-91. [DOI: 10.1039/AN9921701189]

Park CJ, Song S. Determination of boron in high-purity sulfuric acid by ester generation and isotope dilution inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* 2003;18(10):1248-51. [DOI: 10.1039/B306897H]

Probst TU, Berryman NG, Lemmen P, Weissfloch L, Auberger T, Gabel D, Carlsson J, Larsson B. Comparison of inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry with quantitative neutron capture radiography for the determination of boron in biological samples from cancer therapy. *J. Anal. At. Spectrom.* 1997;12(10):1115-22. [DOI: 10.1039/A700445A]

Ramakumar KL. Analytical challenges in characterization of high purity materials. *Bull. Mater. Sci.* 2005;28(4):331-7. [DOI: 10.1007/BF02704245]

Rai P, Pathak A, Rajput SJ. Stability-indicating reversed-phase liquid chromatographic methods for the determination of aconitine and piperine in a polyherbal formulation. *J. AOAC Int.* 2009;92(4):1044-54.

Sahin I, Nakiboglu N. Indirect determination of Boron in water by cathodic stripping voltammetry. *Fresen. Environ. Bull.* 2006;15(5):457-61.

Sanchez-Ramos S, Bosch-Reig F, Gimeno-Adelantado JV, Yusa-Marco DJ, Domenech-Carbo A, Berza-Perez JA. Validation of a method for the determination of boron in ceramic materials by X-ray fluorescence spectrometry. *Spectrochim. Acta Part B Atom. Spect.* 2000;55(11):1669-77. [DOI: 10.1016/S0584-8547(00)00267-6]

Sun D-H, Ma R-L, McLeod CW, Wang X-R, Cox AG. Determination of boron in serum, plasma and urine by inductively coupled plasma mass spectrometry (ICP-MS). Use of mannitol-ammonia as diluent and for eliminating memory effect. *J. Anal. At. Spectrom.* 2000;15(3):257-61. [DOI: 10.1039/A908250F]

Sun D-H, Waters JK, Mawhinney TP. Microwave digestion and ultrasonic nebulization for determination of boron in animal tissues by inductively coupled plasma atomic emission spectrometry with internal standardization and addition of mannitol. *J. Anal. At. Spectrom.* 1997; 12(6):675-9. [DOI: 10.1039/A606283K]

Tanaka T, Nishu K, Nabekawa H, Hayashi H. Determination of trace boron in iron and steel by adsorptive stripping voltammetry using Beryllon III. *ISIJ Int.* 2006;46(9):1318-23. [DOI: 10.2355/isijinternational.46.1318]

Thunus L. Voltammetric determination of boron in plasma using Beryllon(III) as a ligand. *Anal. Chim. Acta* 1996;318(3):303-8. [DOI: 10.1016/0003-2670(95)00462-9]

Unal U, Somer G. Indirect determination of trace tetraborate by differential pulse polarography using its copper complex and application to waste and drinking water. *Electroanalysis* 2009;21(20):2234-40. [DOI: 10.1002/elan.200904674]

Wilke T, Wildner H, Wunsch G. Ester generation for the determination of ultratrace amounts of boron in volatile high-purity process chemicals by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* 1997; 12(9):1083-6. [DOI: 10.1039/A701654I]

Zaijun L, Yuling Y, Jiaomai P, Jan T. 1-(2-hydroxy-3-methoxybenzylideneamino)- 8-hydroxynaphthalene-3,6-disulfonic acid as a reagent for the spectrophotometric determination of boron in ceramic materials *Analyst* 2001;126(7):1160-3. [DOI: 10.1039/B102863B]

*****