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Determination of Boron Content in Treated Wood Using an Azomethine-H Spectrophotometric

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Abstract

Boron compounds are leached quantitatively from the treated wood (wood treated by boric acid) using three different methods; heat extraction, refluxing and wet digestion methods. The extracted sample was analyzed using Azomethine-H spectrophotometric method. The absorbance at wavelength 411 nm was measured 30-40 minutes after the addition of the Azomethine-H reagent. A linear calibration graph for the sample extract using direct heat, reflux and wet digest method was obtained between 1-10 ppm with the R^2 = 0.9992. The selectivity, accuracy, within and between precision, limit of detection and quantification, and stability were examined as parts of the method validation. Recovery was determined after spiking with a typical range of analyte.

Keywords:

Heat-extraction; Reflux; Wet-digestion; UV-Visible Spectrophotometer; Colorimetric reagent

1. Introduction

Azomethine-H is a common and established reagent used for boron determination in grape [1], natural water and wastewater [2], health product likes lotions [3], wine [4] and plant [5] Azomethine-H is also used to detect microgram level of boron in glass and steel samples. The Azomethine-H (4-hydroxy-5 [salicylideneamino]-2,7-napthalene disulphonic acid) is the chelating agent, which is very sensitive to boron compound and forming a yellow compound in aqueous media. Krug *et al.* [5] reported that the Azomethine-H reagent dissociates according to boric oxide displaces this equilibrium towards the left, increasing the yellow color intensity proportionally to the boron concentration and the displacement is very slow depending on the pH value. The reaction of the Azomethine- H is carried out under slightly acidic condition. Matsuo *et al.* [6] reported that boric acid reacts with Azomethine-H to form a 1:1 bischelate complex. Azomehine-H is the shift base of salicylaldehyde and H-acid (1-amino-8-naphthol-3, 6-disulphonic acid) and the absorbance of the boron azomethine-H complex shows a broad absorbance peak in the range of 410-420 nm. Previous workers reported an absorption maximum at 412 nm [6], 415 nm [7] & 420 nm [8], which were used in their studies. However in this study, the absorbance at 411 nm was selected based on the screening test on the complexed of Azomehine-H and boric acid.

Several techniques have been reported in determining the value of boron- azomethine complex, such as differential pulse polarography and Flow Injection Spectrophotometric [5].

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Nevertheless all these methods are very expensive and difficult to handle. However, ultra-violet (UV) spectrophotometer methods are more suitable for the laboratory routine analysis because of the simple sample preparation, shorter time to get the result, easy handling, and high sensitivity and cheaper compared to other methods. Jakŝić [7] reported that the molar absorptivity of the boron-azomethine-H complex is 9 x 10^3 L mol^{-1} cm^{-1} and this represents an advantage over other colorimetric methods, which exhibit non-linear analytical curves at higher boron concentration.

In this study, Azomethine-H spectrophotometric method was used for determination of boron in spike samples (untreated rubber wood spiked with boric acid at different levels of concentration), which was extracted using three different extraction methods. Three different extraction methods are selected based on the most current practice in boron determination process form timber based products and solution. Heat extraction method is adopted from AWPA A2-06 standard [8], reflux extraction method is adopted Borax Limited Co. US and wet digestion is adopted from Japanese Agriculture Standard Method [9]. Standard solution and reagent were prepared according to AWPA A2-06 standard [8]. This method was validated with respect to linearity, precision (repeatability and reproducibility), accuracy, limit of detection (LoD), limit of quantification (LoQ) and recovery, as part of requirement in test method validation for the competence of testing lab [10].

2. Materials and Methods

2.1. Apparatus

UV-Visible Spectrophotometer, Perkin-Elmer model Lamda 35 provided with quartz sample cell and calibrated wavelength. The optimum absorbance value for determination of boron-azomethine complex at wavelength 411 nm and all glassware used should be calibrated.

2.2. Reagent

All chemicals used were of analytical grade. A stock solution, containing 100 ppm boron, was prepared by dissolving 0.5715 g of boric acid in one liter of water. (Note: boric acid contains 17.48% boron).

2.2.1. Preparation of blank solution

Blank solution for boron sample extract using direct heat or reflux method was prepared by gently heat to boil the amount of distilled water for 30 minutes and cool the solution to ambient temperature.

Blank solution for boron sample extract using wet digest method was prepared heating the mixer of 7.5 mL of hydrogen peroxide, 30% w/w, 1.0 mL of sulphuric acid concentrated (ρ_{20}=1.84 g mL^{-1}), 1.0 mL ortho-phosphoric acid concentrated (ρ_{20}=1.0046 g mL^{-1}) and 5 mL distilled water until the white fume is released. The mixture was then cooled and diluted to 100 mL with distilled water and mixed well. Repeat the same process to prepare more blank solution.

The blank solution shall be used to dilute the sample solution according to analysis method before subjected to UV-Visible analysis.

2.2.2. Preparation of azomethine-H reagent

Buffer solution for azomethine-H analysis was prepared by dissolve 250 g ammonium acetate in 400 mL of distilled water. Add with 15 g of disodium EDTA and 125 mL of glacial acetic acid into the mixture and mix well.

Azomethine-H solution was prepared by adding 1.0 g of ascorbic acid and 0.45 g of azomethine-H to 100 mL volumetric flask and bringing to volume with distilled water. Fresh reagent was prepared weekly and stored in the refrigerator since azomethine-H reagent degrades over time, calibration standards was analyzed on daily basis.

2.3. Raw material

Local rubberwood (*Hevea brasiliensis*) was used in this study. Approximately 1000 g of wood was chipped and ground to fine sawdust at size of 1 mm for about 1000 g.

2.4. Spike sample

Two different levels of concentration (i.e. 0.5 and 1 % w/w) in the form of boric oxide (B_2O_3) were selected for this study. It was based on the normal concentration range found in treated wood to ensure the timber is fully protected from wood borer and termite. 1 mL of different levels of concentration of boric acid was pipette onto 1 g of untreated rubber wood sawdust in six replicates. Mix well the chemical and sample. The spike samples were allowed to stand for 48 hours before extraction to allow the spike solution to penetrate the test material. These samples are ready for extraction process.

2.5. Extraction method

2.5.1. Direct heating process

Weigh properly 1 g of sample to the nearest 0.01 g and put the sample into 250 mL Erlenmeyer flask. Add 50 mL of distilled water to the flask and heat to boil for 30 minutes. Allow the content to cool at room temperature and filter using filter paper size 4. Rinse the flask 3 times with 10 mL of blank solution, pouring the washings through the filter paper and add the blank solution to mark and mix well.

2.5.2. Reflux process

Weigh properly 1 g of sample to the nearest 0.01 g and put the sample into 250 mL round bottom flask. Add 50 mL of distilled water to the flask and connect to the condenser. Turn on the cooling water to condense the vapor inside the condenser column. Cover the top of the condenser with a plastic cap. Turn on the heater to heat the contents of the flask. Bring the temperature to boil and maintain the boiling temperature for 30 minutes. Turn off the heater and allow the contents in the flask to cool to room temperature and filtered using filter paper size 4. Rinse the flask 3 times with 10 mL of blank solution, pouring the washings through the filter paper and add the blank solution to mark and mix well.

2.5.3. Wet digestion process

Weigh properly 1 g of sample to the nearest 0.01 g and put the sample to a 100 mL kjeldahl flask. Add slowly 7.5 mL of 30% hydrogen peroxide. Add 1 mL of concentrated of ortho-phosphoric acid and 1 mL of concentrated sulfuric acid. Warm gently the solution in the digestion stand. Heat the solution until charring occurs. Add 5 mL of 30% hydrogen peroxide and continue heating until white fumes evolved and the solution decolorized. Filter the samples in 100 mL volumetric flask after cooling in room temperature and dilute with blank solution.

2.6. Sample analysis

1 mL of unknown solution was pipetted into 1 cm cuvettes. 1 mL of the buffer solution was added to cuvette and the solutions were mixed. 1 mL of azomethine-H reagent was added and the solutions were mixed again. The absorbance at 411 nm was measured 30 to 40 minutes after the addition of the azomethine-H. The reagent blank was used to zero the instrument. The ppm boron of the unknown(s) could be determined from the graph by reading

the concentration on the x-axis. If the absorbance of the unknown exceeds the 10 ppm standard, dilute the unknown (s) solution with blank solution until the absorbance falls within the calibrated range.

2.7. Validation

Method validation was performed following the EURACHEM, NATA and IUPAC recommendations. The linearity was verified by analysis in duplicate of five points in the range of 2-10 ppm boron. The solutions of the standards were prepared from known purity boron with certificate of the content. For samples accuracy, the appropriate quantity of untreated sawdust was spike with different levels of concentration of B_2O_3; 0.5 and 1.0 % w/w. Recovery was evaluated with the same method. Results for recovery are reported as % RSD (CV). Method precisions (repeatability) were determined by analyzing two different operators and the reproducibility was determined by two different laboratories. Results are reported as % RSD (CV).

3. Result and Discussion

The successful analyzed boron content in treated wood using Azomethine-H spectrophotometric method was determined. Three types of extraction method were used to extract boron content from wood; direct heat, reflux and wet digest. This study relies on the optimization of the operators and different of UV-Visible used. Each of the steps was carefully optimized for developing sensitive, accurate, repeatability and reproducibility assay methods for determination of boron compound in treated wood. Calibration curves obtained for the samples extract using direct heat; reflux or wet digest was found to be linear over range 1-10 ppm boron. The linear regression scheme (y = mx + c) was used to perform standard calibration. All the data are given in Table 1. Fig.1a, 1b and 1c showed the calibration curve for each extraction method.

Table 1: Calibration curve equations for boron when extracted using direct heat, reflux and wet digest method.

Extraction method used	Equation	R	R^2	Regression Equation
Direct heat	y = 0.1523 x + 0.2211	0.9996	0.9992	'x' = (y-0.2211)/0.1523
Reflux	y = 0.1535 x + 0.2031	0.9996	0.9992	'x' = (y-0.2031)/0.1535
Wet digest	y = 0.1535 x + 0.2061	0.9996	0.9992	'x' = (y-0.2061)/0.1535

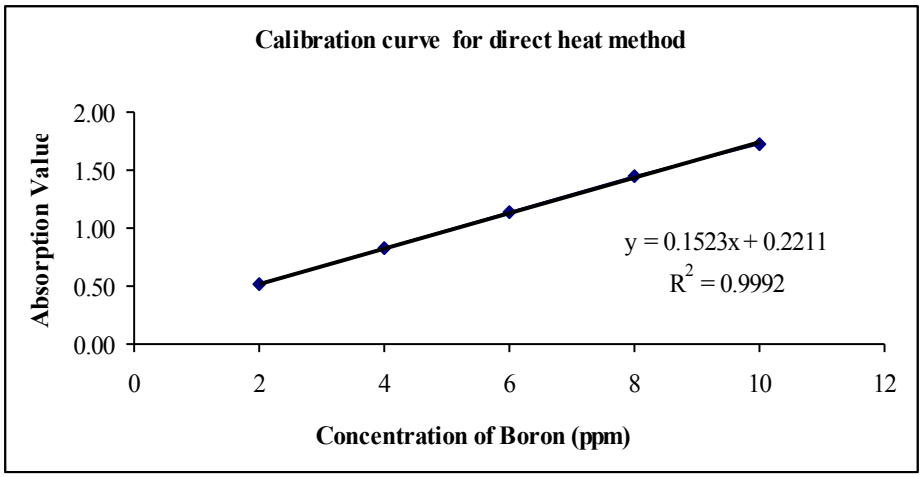

Fig. 1a Calibration curve for direct heat method

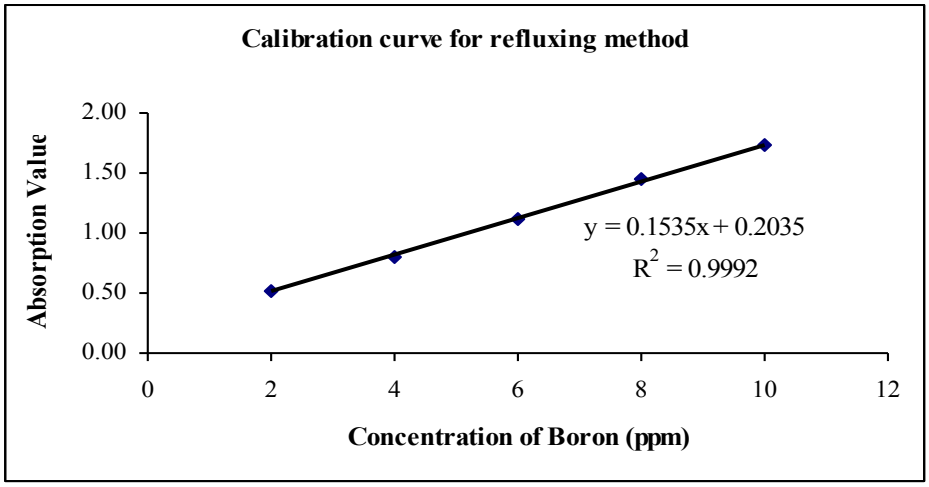

Fig. 1b: Calibration curve for refluxing method

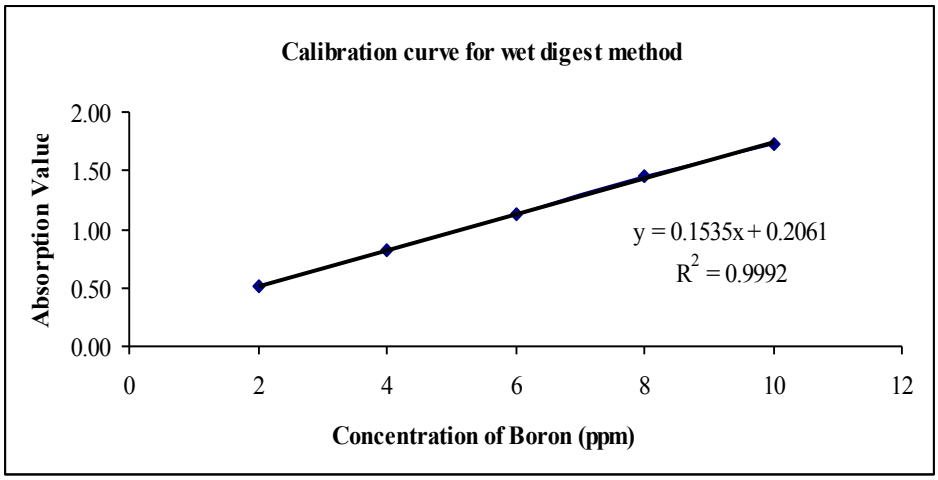

Fig. 1c. Calibration curve for wet digest method

The linear index can be calculated using the following equation;

'x'/ x = linear index

Which,

'x' = Regression value

x = Concentration of boron

In this study, the calibration curve for the three extraction methods are considered linear in the range 2-10 μg mL^{-1} because R value is very close to one. The linear index calculated using the above equation is also very close to one indicating the linearity of the calibration is linear (Table 2).

Limit of detection (LoD) and limit of quantification (LoQ) values for this method are determined by spiking the sample with the lowest concentration of B_2O_3 (0.005 % w/w). LoD is the lowest concentration of analyte in the sample that can be detected and LoQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy under the state conditions of the test (NATA). The LoD and LoQ values were calculated by using

equation; LoD = mean + 3SD and LoQ = mean + 10 SD and the results are presented in Table 3.

Table 2 The linear index value

Extraction method used	x (boron cont.) ppm	y (Abs. value)	'x'	Linear Index
Direct Heat	2	0.5158	1.9354	0.9677
	4	0.8329	4.0180	1.0045
	6	1.1419	6.0478	1.0080
	8	1.4557	8.1088	1.0136
	10	1.7269	9.8900	0.9890
Reflux	2	0.5212	2.0696	1.0348
	4	0.8001	3.8865	0.9716
	6	1.1209	5.9763	0.9960
	8	1.4484	8.1098	1.0137
	10	1.7321	9.9579	0.9958
Wet digest	2	0.5074	1.9625	0.9812
	4	0.8174	3.9818	0.9954
	6	1.1329	6.0369	1.0061
	8	1.4544	8.1311	1.0164
	10	1.7241	9.8878	0.9888

Table 3. The LoD and LoQ values for boron determination in treated wood

Extraction method used	LoD	LoQ
Direct heat	0.2040 ppm	0.3672 ppm
Reflux	0.2040 ppm	0.3672 ppm
Wet digest	0.2196 ppm	0.3623 ppm

The data shows that this method can be used to detect boron extracted from treated wood by using direct heat, reflux and wet digest larger than 0.2040, 0.2040, 0.2019 μg mL^{-1} respectively. This method can only quantify boron larger than 0.3672, 0.3672, 0.3623 μg mL^{-1} after extracted from wood using direct heat, reflux and wet digest methods respectively. All the boron concentration detected and quantified in the samples are of higher value.

Selectivity of the assay method was defined as non-interference in the regions of the compound of interest with endogenous substance. Ca, Mg and Fe [1], Al, Cu and Zn [5] present in great amounts in timber may interfere when boron is determined directly in wood samples. Previous study found that the addition of the disodium EDTA in the reaction systems can very effective in masking reagent to overcome interferences caused by these ions and greatly improved the selectivity of this method.

The value of accuracy which is referring to precision and recovery for this study are listed in Table 4. The results obtained show that the Azomethine-H spectrophotometric

method was precise and accurate to detect boron compound in treated wood by using all the type of extraction method used. The repeatability describes the precision when the samples were analyzed by the same operator, equipment, laboratory and method being used. The reproducibility describes the precision when the same samples were analyzed by different laboratory.

The stability of the Azomethine-H species was studied by Angeles [11]. The finding from study is the complex of azomethine-H can stable not more than 10 minutes in the absence of oxygen and light, however under phosphate buffered conditions its remained stable for longer periods. In this study, buffer solution (2.2 a) was used to buffer the Azomethine-boron complex and the complex was found stable more than 8 hours. The stability of the complex indicates that the analysis time can be prolong within 8 hours.

Analysis of data on different type of extraction method using analysis of variance (ANOVA) confirmed that the types of extraction method used have no significant effect on total of boron compound leached from wood results (Table 5). The result indicates that boron compounds can be leached quantitatively from the treated wood by either direct heating process or reflux with water or wet digest method. However wet digestion method which is using ortho-phosphoric acid is highly recommended to digest boron from wood gluing based product such as plywood and particle board as required by JAS standard.

Applicability of the assay to boron determination in treated wood

This method has been successfully applied to the analysis of samples from a locally treated timber. The samples were analyzed at two different sampling sections, which is crosscut section and core section. Table 6 represents the concentration of the boron when the samples were extracted using direct heat method and analyzed using Azomethine-H Spectrophotometric method.

Table 4. Accuracy, precision and recovery values for boron determination in treated wood when boron compound was extracted using direct heat, reflux and wet digest method and analyzed using Azomethine-H Spectrophotometric

Extraction Method	**Element**	**Expressed as oxide compound**	**Conc. level (% w/w)**	**Accuracy***	**Limiting percentage**		**Recovery (%)**		
					Repeatability	Reproducibility	Lab A	Lab B	Lab C
Direct Heat	Boron	B_2O_3	0.5	0.5214	0.0216	0.0250	104.3	103.6	107.6
	Boron	B_2O_3	1.0	1.0375	0.0229	0.1125	99.3	105.2	103.8
Reflux	Boron	B_2O_3	0.5	0.5459	0.0232	0.0272	109.1	103.7	100.1
	Boron	B_2O_3	1.0	0.9900	0.0380	0.0537	99.0	105.7	105.1
Wet Digest	Boron	B_2O_3	0.5	0.5247	0.0426	0.0453	104.9	100.1	103.2
	Boron	B_2O_3	1.0	1.0154	0.0410	0.0926	101.5	105.1	108.4

Note: * Actual value is 0.5 and 1.0 % w/w B_2O_3, so within 15 % of the actual values are:-

a) For 0.5 % w/w B_2O_3 = 0.5 ± 0.075
b) For 1.0 % w/w B_2O_3 = 1.0 ± 0.15

Table 5. Analysis of variance on the effect of extraction method used to extract boron compound from treated wood

Conc. of B_2O_3 (% w/w)	Replicate	F critical value	F value	Conclusion
0.5	6	3.4028	2.7545	Not significant different
1.0	6	3.4028	0.0941	Not significant different

Note: Not significant at $p<0.05$

Table 6: The amount of boron in different types of treated timber analyzed using Azomethine-H Spectrophotometric.

Type of wood	Density (Kg/m^3)	Boron content (ppm)	
		Crosscut	Core
Tualang, *Koompassia excelsa* (leguminosae)	800-865	8.967	7.374
Kembang Semangkok, *Scaphium spp.(*Sterculiaceae)	515-755	22.040	15.910
Kasai, *Pometia spp*.(Sapindaceae)	735-915	2.8730	0.9410
Keruing, *Dipterocarpus spp*.(Dipterocarpaceae)	690-945	10.4890	8.2790
Kapur, *Dryobalanops spp*.(Dipterocarpaceae)	575-815	2.3740	1.4880
Red Balau, Genus *Shorea* (Dipterocarpaceae)	800-880	1.6630	1.4620
Medang, Family Lauraceae	350-880	0.4980	0.1980
Mengkulang, *Heritiera spp*. (Sterculiaceae)	625-895	7.4422	7.0730
Light Red Meranti	385-755	8.1810	1.0989
Kempas *Koompassia malaccensis* (leguminosae)	770-1120	16.1620	15.8794

4. Conclusion

The study revealed Azomethine-H Spectrophotometric method is a practical method for determination of boron in wood samples. This method is the most common technique for boron determination and easily available in most laboratories. ANOVA analysis showed that no significant difference between the extraction methods used to extract boron from treated wood. According to the validation data, all three types of extraction method are suitable to extract boron from wood.

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