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DETERMINATION OF BORON CONTENT IN CRACKERS USING CURCUMIN REAGENT IN A 2-ETHYL-1,3-HEXANEDIOL LIGAND THROUGH SPECTROPHOTOMETRIC UV-VIS ABSORPTION SPECTRA

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ABSTRACT

Curcumin reagent is a reagent that produces rosocyanin complex through Uv-Vis spectrophotometric absorption. The optimum state of boron curcumin in EHD ligand (2-ethyl-1,3-hexanediol) at a wavelength of 551 nm. The calibration curve is linear in the concentration range of 0.22-0.91 ppm in EHD with a correlation coefficient R2 of 0.9995 with a regression equation y = 0.9306x + 0.0005. This method produces a percent RSD of 0.303% with an average absorbance value of 0.226. The calculation results with the EHD ligand showed a detection limit value of 0.244 µg/L with a quantification limit value of 0.815 µg/L. The boron levels measured in the five cracker samples analyzed with EHD were 1.35 mg/kg, 1.80 mg/kg, 1.99 mg/kg, 1.44 mg/kg, 2.05 mg/kg.

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Keywords: Borax, Crackers, Curcumin, Spectrophotometry Uv-Vis.

INTRODUCTION

Boron is a beneficial element for plants and animals, but excessive boron is toxic to human health (Nielsen, 1993). Normally, boron is not found as a free element in nature but is commonly found in the form of boric acid, borax, and other complex forms. Borax is commonly used as an antiseptic and disinfectant, but its most common use is as a preservative in cosmetics, as well as an antifungal and preservative in wood, but not in food (Suseno, 2019). Regulation of the Minister of Health of the Republic of Indonesia No. 033 Year 2012 has regulated the prohibition of the use of borax in food. This is because borax is toxic to cells with a total lethal dose value of 3-6 grams for infants and 15-20 grams for adults (Swi See et al., 2010). Therefore, boron compounds such as boric acid and borax are prohibited from being used as food additives.

Today, borax is still widely used in the food industry, such as in the production of crackers. However, it is also possible to be found in other foods such as children's snacks. Borax is toxic and harmful to humans, so its use as a food ingredient is strictly prohibited. This will certainly harm consumers (Yuliarti, 2007). Borax belongs to a group of borate minerals formed from boron and oxygen. Boron is a beneficial element for plants and animals, but excessive boron is toxic to human health (Nielsen, 1993). The most commonly used method for boron analysis is the spectrophotometric method (Sah & Brown, 1997). Spectrophotometric analysis of boron requires special treatment of the sample matrix. The complex sample matrix needs to be separated through appropriate separation steps. Some of the separation methods that can be used are solvent extraction and distillation. Solvent extraction is done by extracting boron in the form of organic complexes such as boron-2-ethyl-1,3-hexanediol into solvents such as chloroform or benzene, followed by spectrophotometric determination.

Boron analysis with UV-VIS spectroscopy can utilize several types of

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complexes including curcumin, carminic acid, quinalizarine and azomethine-H (Sah & Brown, 1997). Curcumin is a commonly used complex in boron analysis. The reaction of boron and curcumin produces a stable rosocyanin complex characterized by the formation of a red solution (Sah & Brown, 1997). The rosocyanin complex is formed in strong acids and almost without water, making the rosocyanin complex very stable for boron analysis. The use of curcumin in the formation of boron-curcumin complexes can be achieved by separate extraction or distillation. Yamada and Hattori (1986) explained that boron analysis on soil samples using EHD in chloroform resulted in a percentage recovery of 98.3-98.9% with a relative standard deviation of 1.14%. The analysis showed that the relative standard deviation percentage value ranged from 1.5 to 3.3% (Donaldson, 1981). Egneus and Uppström (1973) explained that EHD has a distribution value in chloroform of 4.94 and a distribution constant of 5. Aznarez et al. (1985) conducted a study of boron analysis in plants by extraction using 2,2,4-trimethyl- 1,3-pentanddiol complex (TMPD) obtained at 549 nm.

Crackers are a type of food made from high-starchy ingredients and are well known to the public. Currently, some cracker producers add borax to their crackers for certain purposes, such as to increase crispness and flavor, so that the crackers they produce are of better quality and sales can increase (Sah & Brown, 1997). Crackers containing borax, if eaten continuously and for a long time, can cause adverse health effects in the form of chronic poisoning symptoms such as accumulation in the brain, bones and other body parts. If exposed to the body continuously, it will cause damage to the liver, cardiovascular system, central nervous system (CNS), peripheral nervous system, hematological system, urinary system (kidney, ureter, bladder) and endocrine (Pohanish, 2012).

Research on borax in crackers has been conducted in several places in Indonesia. Andyningtias (2013) conducted a study on several types of puli crackers in the traditional market of Malang City in 2013 showing 6/20 samples tested positive for borax. Another study conducted by Kurnia in several markets in Surabaya city in 2017 found 12 biscuit samples that tested positive for borax (Kurnia Hartati, 2017). Therefore, a study of boron content in crackers by UV-Vis spectrophotometry using curcumin reagent in EHD ligand was conducted. Sampling was conducted in five locations for each sample in the Special Region of Yogyakarta.

METHODS

Materials and Equipment

$Na_2B_4O_{7}.{}_{10}H_2O$, concentrated HCl (12 M), H_2SO_4 (Pro analysis, Merck), chloroform (Pro analysis, Merck), 2-ethyl-1,3-hexanediol (EHD) (Pro analysis, Aldrich), curcumin and glacial acetic acid (Pro analysis, Merck), 96% ethanol (Pro analysis, Merck), aquabides and distilled water.

The equipment used in this study included: analytical balance (Kern ABT 220-4M), glassware, porcelain crucible, separatory funnel, shaker, blender, oven (Memmert UN 55), furnace (Muffle furnace FM 13), and UV-Vis spectrophotometry (Thermo Scientific Evolution 201).

Method

1 Preparation of solution

This study started with the preparation of 1000 ppm curcumin-acetic acid solution. In addition, optimization of the wavelength of the boron-curcumin complex was achieved. Each borax standard solution of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm was placed in a 25 ml volumetric flask. Add 2 ml of 6 M HCl to each flask and adjust with aquabid to the mark. The extraction step was carried out so that each solution from the volumetric flask was transferred to a 100 mL conical flask then 4 mL of EHD: chloroform (2:4) v/v mixture was added. The solution was stirred for 30 minutes, then transferred to a separatory funnel. The solution was allowed to stand for 30 minutes until two layers were formed. The organic phase (below) was taken to 0.5 ml. Then the complexation was carried out until 0.5 ml of organic phase was added to 2 ml.

Curcumin-acetic acid: H_2SO_4 (4:1) v/v solution. The solution was allowed to stand for 45 minutes in the dark, then diluted with ethanol until the volume was 25 ml. The absorbance of the solution was measured using UV-Vis spectrophotometer from 480-620 nm against the blank. Furthermore, optimization of the stabilization time for boron-curcumin complex formation was achieved by allowing the solution to stand continuously for 5 minutes to 65 minutes in the dark.

2 Wavelength optimization of boron-curcumin complexes.

Standard solutions of borax at 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm were each put into a 25 mL measuring flask. In each tube, 2 mL of 6 M HCl was added and aquabides was added until the limit mark. The extraction stage was carried out in a way that each solution in the measuring flask was put into a 100 mL Erlenmeyer then added 4 mL of a mixture of EHD: chloroform (2: 4) v/v. The solution was shaken for 30 minutes, after which it was put into a separating funnel. The solution was allowed to stand for 30 minutes until two layers were formed perfectly. The organic phase (below) was taken as much as 0.5 mL. Then the complex formation process is carried out, namely as much as 0.5 mL of organic phase is added to 2 mL. Solution of curcumin-acetic acid: H_2SO_4 (4:1) v/v. The solution was allowed to stand for 45 minutes in a dark place, then diluted with ethanol until the volume was 25 mL. The absorbance of the solution was measured using UV-Vis spectrophotometer from 480-620 nm wavelength against the blank.

The same thing was also done in a different solvent, namely the TMPD solution. Determination of the wavelength of curcumin in ethanol was done by dissolving 1 mg of curcumin solids into 96% ethanol until the volume was 100 mL using a 100 mL volumetric flask. The solution was measured for absorbance using a UV-Vis spectrophotometer from a wavelength of 300-600 nm against the blank. The highest absorbance indicates the maximum wavelength.

3 Linearity and Sensitivity

Standard solutions of borax with concentrations of 1 ppm, 5 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm, 140 ppm, 160 ppm, 180 ppm, and 200 ppm of 1 mL each were put into a 25 mL volumetric flask. A total of 2 mL of 6 M HCl was added and sufficed with aquabides until the limit mark. The extraction and complex formation process was the same as the previous procedure. The solution was measured for absorbance using UV-Vis spectrophotometer at λ maximum and optimum time of complex formation against the blank. The treatment was repeated for 3 consecutive days, then a calibration curve was made and the R^2 value was calculated. Sensitivity calculation was performed using the following formula (Chan et al., 2004):

$$S = \frac{(Act-Acr)}{(Ct-Cr)} \qquad (1)$$

Description:

Acr : Absorbance of the lowest concentration

Act : Absorbance of the highest concentration

Cr : Lowest concentration

Ct : Highest concentration

4 Accuracy

Standard borax solution with concentrations of 20 ppm, 60 ppm, and 100 ppm each as much as 1 mL was put into a 25 mL measuring flask, then added 2 mL of 6 M HCl and sufficed with aquabides until the limit mark. The extraction and complex formation process was the same as the previous procedure. The solution was measured for absorbance using UV-Vis specrophotometer at the maximum wavelength and optimum time of complex formation against the blank. The treatment was repeated for 3 consecutive days (interday) and repeated 3 times in the same day (intraday), then calculated % RSD. Percent RSD was measured by calculating % recovery using the formula (Chan et al., 2004):

$$\% RSD = \frac{SD}{X} \times 100\% \qquad (2)$$

Description:

SD : Standard Deviation of a series of data

X : Average Data

5 Limit of detection (LoD) and limit of quantification (LoQ)

After obtaining the calibration curve equation and R2 value, the LoD and LoQ values can be calculated. The limit of detection can also be calculated based on the standard deviation (SD) of the response and the slope of the standard curve at levels close to the LoD according to the formula (Chan et al., 2004):

$$LoD = \frac{3{,}3\ Sb}{m} \qquad (3)$$

Description:

LoD : Limit of detection

Sb : Standard deviation of absorbance value from the lowest concentration

M : Slope

The limit of quantification (LoQ) is defined as the lowest concentration of analyte in the sample that is acceptable under the operational conditions of the method used (Shrivastava & Gupta, 2011). The limit of detection (LoD) is also based on the standard deviation (SD) of the response and the slope of the standard curve at levels close to the LoQ according to the formula (Chan et al., 2004):

$$LoQ = \frac{10\ Sb}{m} \qquad (4)$$

Description:

LoQ : Limit of quantitation

Sb : Standard deviation of absorbance value from the lowest concentration

M : slope

6 Analysis in food samples

The food samples used in this study were crackers. The five cracker samples were each weighed as much as 20.00 grams, mashed until homogeneous then each was dried using an oven at 80 °C for 24 hours. The dried samples were ashred using a furnace at 550 °C for 24 hours. Each ash sample was put into a 100 mL measuring flask by adding 2 mL of 6 M HCl and sufficed with aquabides until the limit mark. Each sample was taken 1 mL by putting it into a 25 mL volumetric flask. The solution was measured for absorbance using a UV-Vis spectrophotometer from 480-620 nm wavelength against the blank. Determination of the wavelength of curcumin in ethanol was carried out by dissolving 1 mg of curcumin solids into 96% ethanol until the volume was 100 mL using a 100 mL volumetric flask. The solution was measured for absorbance using UV-Vis spectrophotometer at the maximum wavelength and optimum time of complex formation against the blank.

RESULTS AND DISCUSSION

Wavelength of Boron-Curcumin Complex

The boron-curcumin complex with EHD solvent formed has a maximum λ of 551 nm. The complex formed is characterized by a red solution illustrating the formation of Rosocyanin Complex. The maximum wavelength obtained in the boron-curcumin complex using EHD is in accordance with various studies in boron analysis using UV-Vis spectrophotometric method with curcumin complexing in acetic acid which is 550 nm (Donaldson, 1981; Amada and Hattori, 1986; Betty and Day, 1986; Mair and Day, 1972). The absorbance measurement results of solutions with EHD solvent in various concentrations are presented in **Figure 1**.

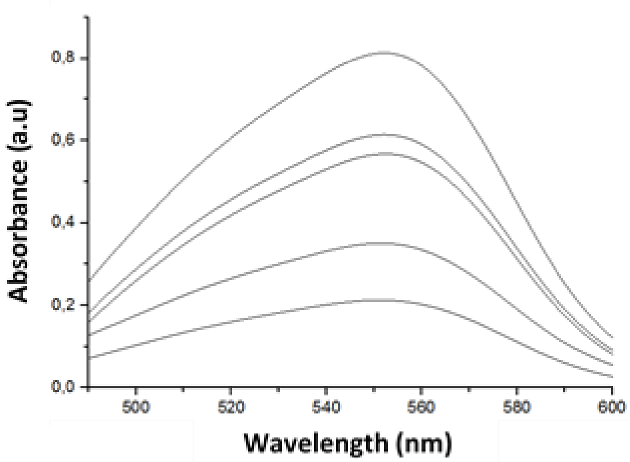

Figure 1 Visible Spectra of Boron-Curcumin Complex with EHD solvent

The possible reactions between boron and EHD are as follows **Figure 2**.

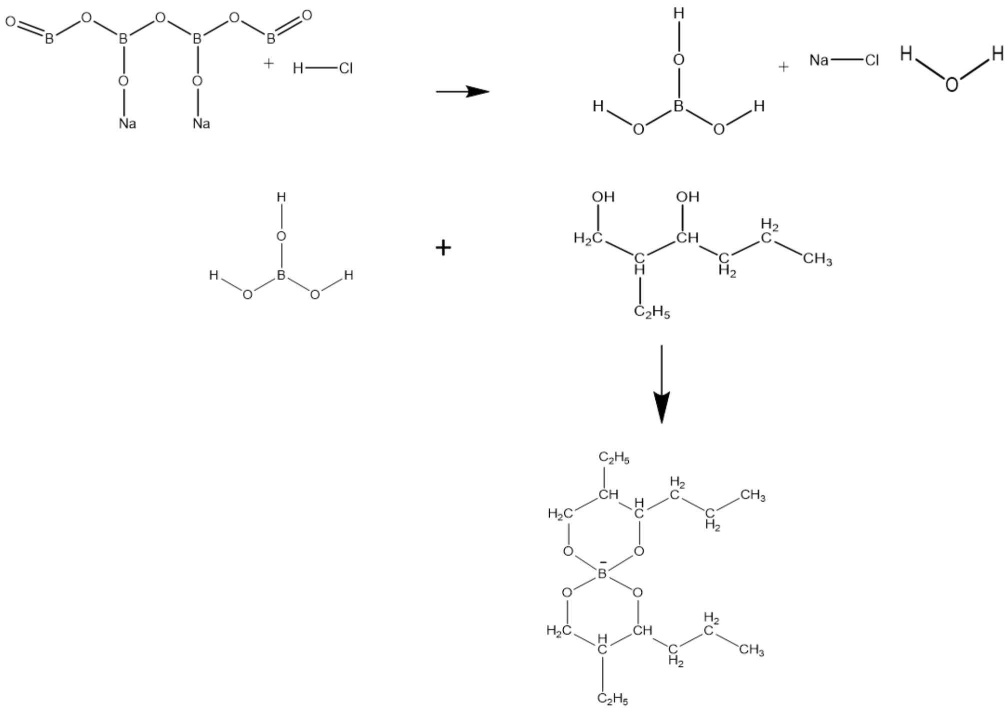

Figure 2 Boron-EHD Complex (organic phase)

Validation of Boron Analysis Method

The results of UV-Vis spectrophotometry to determine linearity in boron analysis by making calibration curves at various boron concentrations with EHD solvent can be seen in **Figure 3**.

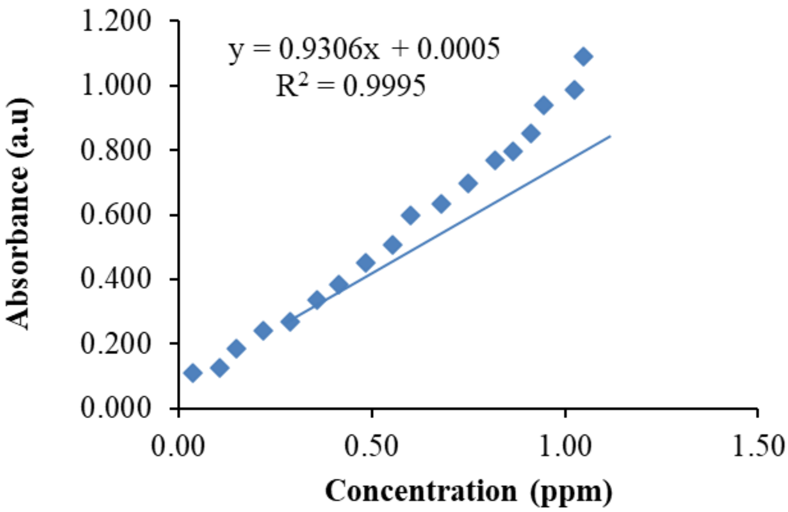

Figure 3 Calibration curve of boron-curcumin complex in EHD solvent

Figure 3 shows the calibration curve in the boron concentration range of 0.3-1.05 ppm with a linear curve in the concentration range of 0.22-0.91 ppm. The R^2 value in that concentration range is 0.9995 and the equation y = 0.9306x + 0.0005 is obtained. Linearity is determined based on the correlation coefficient (R^2) value of the linear regression equation obtained from the calculation results. The values of slope, intercept, and correlation coefficient (R^2) were 0.9306; 0.0005; and 0.9995, respectively. The UV-Vis spectrophotometric boron analysis method used in this study has met the linearity parameter with intercept values close to zero and R^2 values above 0.9995.

Table 1 Results of sensitivity determination of boron analysis with EHD solvent

Variable	1	2	Difference	Sensitivity (Lmg^{-1})
X	0.290	0.91	0.62	0.9387
Y	0.269	0.851	0.582	

Table 1 shows the results of determining the sensitivity of boron analysis in EHD solvent. The equation obtained from the calibration curve is y = 0.9306x + 0.0005.

Accuracy in boron analysis.

The criteria for precision are very flexible and depend on the concentration of the analyte being examined, the number of samples, and the laboratory conditions. Precision is described by a precision value that is determined by measuring the absorbance of standard solutions with different concentrations. In measuring precision, the calculation of the average (mean), standard deviation and percent standard deviation (% RSD) is carried out. Measurements were made three times on the same day (intraday) and three times on different days (interday). The absorbance measurement results of the interday standard solution with EHD solvent can be seen in **Figure 4**.

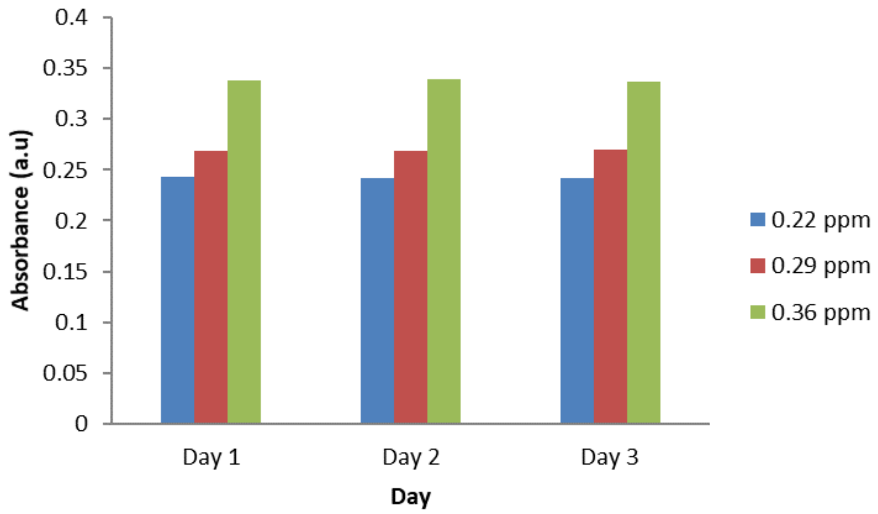

Figure 4 Absorbance of boron-curcumin complex solution at concentrations of 0.22 ppm, 0.29 ppm and 0.36 ppm on different days with three repetitions

Figure 4 shows that the repetition results obtained on the first, second, and third days do not provide significant differences. At a concentration of 0.22 ppm produces a percent RSD of 0.303% with an average absorbance value of 0.226. At a concentration of 0.29 ppm produces a percent RSD of 0.405% with an average absorbance value of 0.289, and at a concentration of 0.36 ppm produces a percent RSD of 0.583% with an average absorbance value of 0.367.

The limit of detection and limit of quantification were calculated based on the calibration curve. The standard deviation of the lowest concentration used and the slope of the standard curve were calculated to determine the LoD and LoQ. The limit of detection was performed to determine the smallest concentration of borax in the sample that can still be detected by this method. The calculation result with EHD solvent showed that the detection limit value was 0.244 µg/L, while the calculation result of the limit of quantification value was 0.815 µg/L. This shows that the smallest amount of analyte that can be detected and then give a significant response compared to the blank solution is 0.244 µg/L and the smallest quantity of analyte that can meet the accuracy criteria is 0.815 µg/L.

This is not much different from the TMPD solvent which shows that the detection limit value is 0.227 µg/L, while the calculation of the limit of quantification value is 0.756 µg/L. This shows that the smallest amount of analyte that can be detected and then give a significant response compared to the blank solution is 0.227 µg/L and the smallest quantity of analyte that can meet the accuracy criteria is 0.756 µg/L.

Results of Boron Analysis in Crackers.

The boron concentration measured in the five samples analyzed by EHD. In the sample of cracker A, the boron concentration was 1.35 mg/kg in the organic phase and 0.14 mg/kg in the water phase. In the sample of cracker B, the boron concentration was 1.80 mg/kg in the organic phase and 0.32 mg/kg in the water phase. In the sample of cracker C, the boron concentration was 1.99 mg/kg in the organic phase and 0.71 mg/kg in the aqueous phase. In the sample of cracker D, the boron concentration was 1.44 mg/kg in the organic phase and 0.27 mg/kg in the aqueous phase. In cracker sample E, the boron concentration was 2.05 mg/kg in the organic phase and 0.79 mg/kg in the aqueous phase.

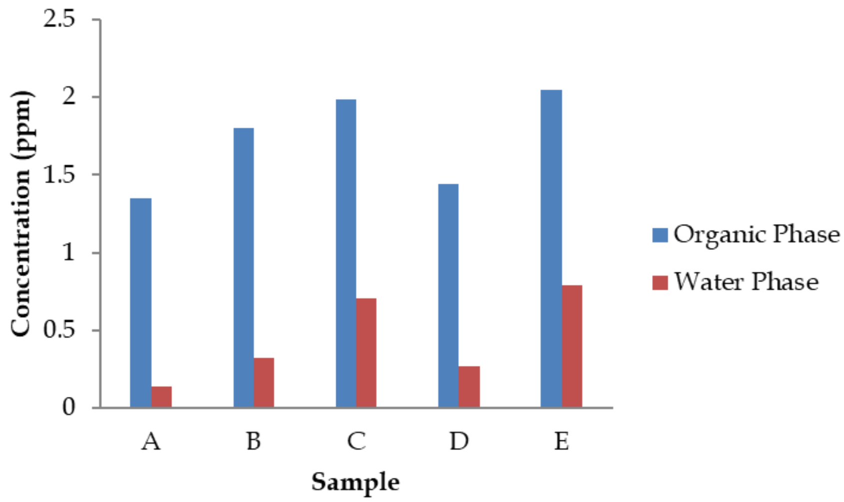

Figure 5 Boron concentration in Crackers samples with EHD solvent

Boron levels detected in the five cracker samples in Yogyakarta Special Region indicate that borax is still used as a food preservative. The amount of borax consumed has a threshold of 1-2 mg/day for adults (Belitz et al., 2009). The threshold for the use of borax is in toddlers and infants with a dose in the body of 5 grams can cause death, while in adults it occurs at a dose of 10-20 grams. Boron levels derived from borax as a food additive are consumed excessively and continuously, so borax will be absorbed in the body and accumulate in the liver, kidneys, and brain (Sugiyatmi, 2006). The boron concentration in the five samples of crackers in Yogyakarta Special Region is still below the threshold level, making them safe for consumption.

CONCLUSION

UV-Vis spectrophotometric determination of boron in cracker samples through extraction with EHD ligand (2-ethyl-1,3-hexanediol) meets the validation parameters. The linear regression equation obtained is y = 0.9306x + 0.0005 in the boron concentration range of 0.3-1.05 ppm with a linear curve in the concentration range of 0.22-0.91 ppm. The limit of detection and limit of quantification with EHD solvent were 0.244 µg/L and 0.815 µg/L. The UV-Vis spectrophotometric method resulted in a percent standard deviation (%RSD) of 0.303% with an average absorbance value of 0.226. boron levels measured in the five cracker samples analyzed with EHD were 1.35 mg/kg, 1.80 mg/kg, 1.99 mg/kg, 1.44 mg/kg, 2.05 mg/kg.

Author's declaration

Authors' contributions and responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation and discussion of results. The authors read and approved the final manuscript.

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Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interest.

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