KOVALEN: Jurnal Riset Kimia

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Validation of Boron Analytical Methods on Standard Reference Material Tomato Leaves from NIST No. 1573a with Isothermal Distillation by UV-Vis Spectrophotometry

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Abstract. Boron analysis by UV-Vis spectrophotometry has been developed through distilling triethoxy borane into the curcumin solution. In the distillation of ester borane, an esterification reaction occurs between borate and ethanol. Validation of isothermal distillation methods by UV-Vis spectrometry needs to be done to obtain specific, accurate, and reproducible results. The distillation for 24 hours at 25°C gave the optimum result. UV-Vis spectrophotometry wavelength was 535 nm. The standard curve was linear in the concentration range of 1-5 ppm (R^2 = 0.9995) with a sensitivity of 0.0902 ppm^{-1}, a limit of detection of 0.002±0.001 ppm, the limit of quantification of 0.006±0.001 ppm, and percent recovery of 88%.

Keywords: *Method validation, boron analysis, curcumin, distillation ester borane, spectrophotometry UV-Vis*

Abstrak. Analisis boron secara spektrofotometri UV-Vis telah dikembangkan melalui distilasi trietoksi borat ke dalam larutan kurkumin. Pada proses distilasi ester borat terjadi reaksi esterifikasi antara borat dan etanol. Validasi metode destilasi isotermal secara spektrometri UV-Vis perlu dilakukan untuk mendapatkan hasil yang spesifik, akurat dan reprodusibel. Hasil optimum didapatkan pada saat dilakukan distilasi selama 24 jam pada suhu 25°C. Analisis boron secara spektrofotometri UV-Vis dilakukan pada panjang gelombang 535 nm. Validasi metode yang digunakan yaitu kurva standar linear pada rentang konsentrasi 1-5 ppm dengan R^2=0,9995; sensitivitas sebesar 0,0902 ppm^{-1}; limit deteksi 0,002±0,001 ppm dan limit kuantifikasi 0,006±0,001 ppm; serta persen perolehan kembali 88%.

Kata kunci: *Validasi metode, analisis boron, kurkumin, destilasi ester borat, spektrofotometri UV-Vis*

Received: July 1st, 2022, Accepted: August 18th, 2022
Citation: Lailatusholihah, I., Roto., Kuncaka, A., and Susparini, NT. (2022). Validation of Boron Analytical Methods on Standard Reference Material Tomato Leaves from NIST No. 1573a with Isothermal Distillation by UV-Vis Spectrophotometry. *KOVALEN: Jurnal Riset Kimia*, 8(2): 178-183.

INTRODUCTION

Boron, the element which has the characteristics between those of metal and nonmetal, is the only metal in Group IIIA at the Periodic Table and the fifth element of the group. Boron has benefits for plants, animals, and the human body (Khaliq et al., 2018), however, if the accumulation of the boron which enters the body is excess, it can be a potential hazard (Duydu, 2017). It is necessary to analyze the existence of boron in the food.

Analytical methods that can be used to detect the existence of boron include ICP AES (Viso & Zachariadis, 2018), Gas

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https://doi.org/10.22487/kovalen.2022.v8.i2.15955

Chromatography-Mass Spectrometry, and spectrophotometry (Chiu & Kuo, 2020). Spectrophotometry technique that can be used is UV-Vis spectrophotometry. UV-Vis spectrophotometry is faster and requires smaller sample volumes than the other spectrophotometric techniques. Preparation methods that can be used for boron analysis include extraction (Ran et al., 2016), gas phase separation, ion chromatography (Yadav et al., 2017) and isothermal distillation (Adu et al., 2021). The conventional distillation in the acid is carried NO^{3-} and F^{-} which causes a serious interference in the analysis process.

Some reagents that can be used in boron analysis are carminic acid (Floquet et al., 2016), azomathine-H (Ali et al., 2015), alizarin red (Zhu, C. Et al., 2018), quinalizarin and curcumin (Kim et al., 2018). Among these reagents there are two reagents that give optimal results when used, quinalizarin and curcumin. The quinalizarin method is less subject to interference by other elements. It is more specific for boron. However, the curcumin method is more sensitive. The use of curcumin reagent is gives maximum results (Kim et al., 2018)

Validation method is an act of evaluating certain parameters, based on laboratory experiments, to prove that these parameters meet the requirements for their use. Validation of isothermal distillation methods by UV-Vis spectrophotometry needs to be done to obtain specific, accurate, and reproducible results. Validation method to determination of borax levels in meatballs have been done by sudjarwo (2018). The results showed linear regression y = 1.3127x – 0.0994, r = 0.9690 > r table (n = 5) is 0.878 and p = 0.007 (p< 0.01) and Vxo is was 15.53%. The detection limit and quantitation limit were $9.7x10^{-4}$ ppm and $2.94x\ 10^{-3}$ ppm respectively. The recovery and coefficient variation were 47.56±3.92%.

Based on the results of the analysis, the researchers investigated the development of boron analysis method using isothermal distillation and curcumin as a boron compound which was analyzed by spectrophotometry UV-Vis.

MATERIAL AND METHODS

Materials

The material was used 4 different brands of sausage sample, curcumin from Chemix. Absolute ethanol, H_2SO_4, CH_3COOH, methanol, HCl, oxalic acid dehydrated by Merck, and Acetone from Mallinckrodt.

Instrumentation

This research used Shimadzu UV-Vis spectrophotometer, Socorex micropipette 0.5 µL – 10 µL, Socorex micropipette 10 µL - 100 µL, Socorex micropipette 100 µL - 1000 µL, magnetic stirrer, analytical balance (Kern), water bath, mortal and agat, distillation bottle made from Teflon consists of inner teflon and outer teflon, and some glassware.

Procedure

The research procedure started from sample destruction, distillation, optimization, validation and sample analysis. Validation procedure used Standard Reference Material Tomato Leaves from NIST no. 1573a with 33.3 mg/kg boron content.

Distillation system

10 mg boric acid dissolved in 0.5 mL ethanol then put into the inner teflon and added with 2.5 µL H_2SO_4. 10 mL curcumin solution was put into the outer teflon. The teflon bottle was distilled at room temperature for 24 hours.

Filled the outer Teflon bottle that a boron curcumin complex form then put into plastic beaker glass (Adu et al., 2021).

Linearity

Analysis of boron standard solution 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 ppm was put into a double-walled Teflon distillation device and distilled the same as the previous procedure. It was analyzed in triplicate (Staufer, 2018).

Accuracy

Analysis of boron standard solution with concentration 1.5, 2.5, and 3.5 ppm was put into a double-walled Teflon distillation device and distilled the same as the previous procedure. It was analyzed in triplicate. The treatment was repeated 3 times on different days and a day to calculate RSD. LOD and LOQ were calculated using the calibration curve (Revisankar, 2015).

$$RSD=\frac{100 \text{ x Standard Deviation}}{x} \quad \ldots\ldots(1)$$

$$LOD=\frac{3 \text{ x Standard Deviation}}{m} \quad \ldots\ldots(2)$$

$$LOQ=\frac{10 \text{ x Standard Deviation}}{m} \quad \ldots\ldots(3)$$

Boron analysis for standard reference material tomato leaves

50.0 mg Standard Reference Material Tomato Leaves were put into a double-walled Teflon distillation device and distilled same with previous procedure. Filled the outer Teflon containing boron-curcumin complex then poured into a plastic beaker glass and diluted with a flask until the mark emerges. The complex solution of boron-curcumin was heated using water bath at 75° C until dry. The dried boron-curcumin complex was removed and diluted with acetone. It was analyzed in triplicate (Adu et al., 2021).

$$\%\ \text{Recovery}=\frac{\text{Consentration}}{\text{Consentration SRM}} \text{ x } 100 \quad \ldots(4)$$

RESULT AND DISCUSSION

The use of spectrophotometry requires a colorimetric method. One of the capable reagent of complexing boron is curcumin. The reaction of boron with curcumin oxalate will be formed become rubocurcumin which has a red complex is show in Figure 1. Meanwhile, the reaction of boron with sulfuric acid curcumin will be formed rosocyanin is show in Figure 2.

Figure 1. The result solution of complex boron-curcumin

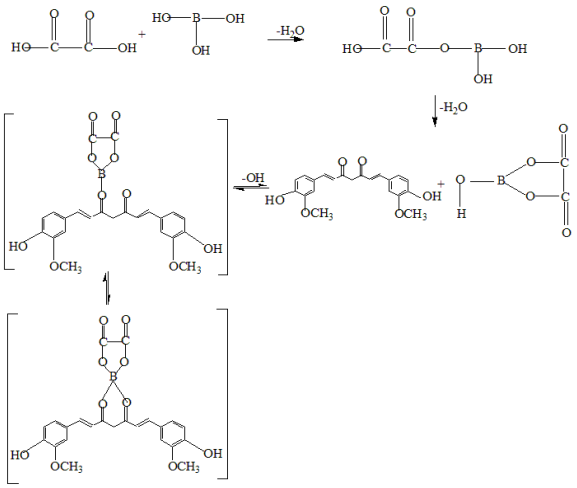

Figure 2. Mechanism of complex boron-curcumin

Validation Result

The maximum wavelength obtained was 535 nm at 3 ppm concentrations, 4 ppm and 5 ppm. The maximum wavelength of boron-curcumin oxalate was not much different from 540 nm (Kim et al., 2018). While pure curcumin

complexes have maximum absorbance at 420 nm wavelength. The spectra complex boron-curcumin are shown in Figure 3, while the spectra pure curcumin is shown in Figure 4.

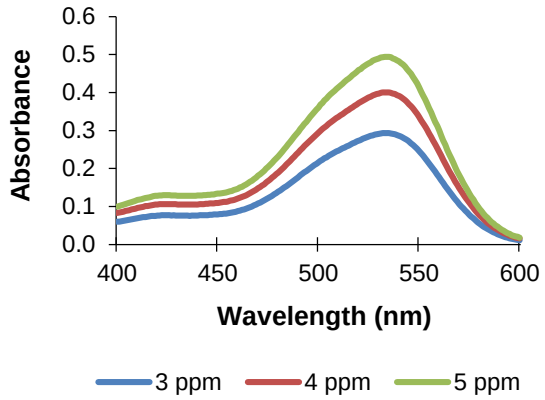

Figure 3. The spectra complex boron-curcumin of UV-Vis Spectrophotometry wavelength

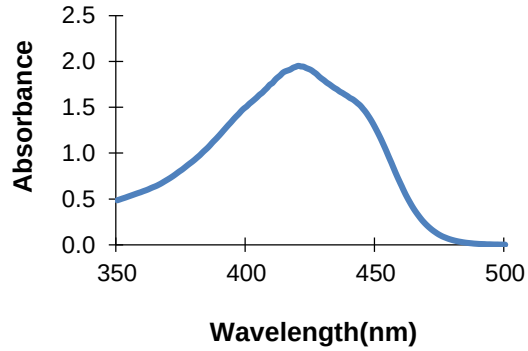

Figure 4. The spectra pure curcumin of UV-Vis spectrophotometry wavelength

Linearity

Based on the measurement, it obtained linear regression with R^2=0.9995 with regression equation y = 0.0902 x – 0.0064. Following the equation, then the so slope formed was 0.0902. An acceptable regression equation when having $R^2 \geq 0.995$ or $R^2 \geq 0.999$ for the better (Belouafa et al., 2017). In this regression equation showed value R^2=0.9995, so according to the theory then this calibration curve can be used as a calculation. The calibration curve are shown in Figure 5.

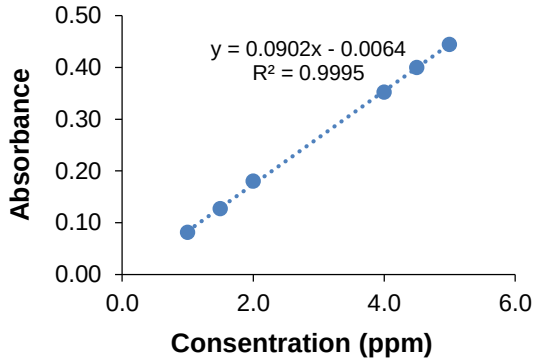

Figure 5. The UV-Vis spectrophotometry calibration curve at wavelength 535 nm.

Limit of detection and limit of quantification

Based on calculations, it obtained the amount of LOD and LOQ at 1 ppm concentration in sequence was 0.002 ± 0.001 ppm and 0.006 ± 0.001 ppm.

Accuracy

Determination of accuracy using UV-Vis spectrophotometry with boron concentration on Standard Reference Material Tomato Leaves of 33.3 ppm gave a concentration 29.305 ± 0.002 ppm. This means the determination of accuracy using Standard Reference Material Tomato Leaves from NIST no. 1573a gives a 88% recovery percentage is show in Table 1. This recovery percentage was categorized as accurate according to Lopes (2019), since it falls within the range of 82% - 120%.

Table 1. Recovery percentage

Absorbance	Consentration (ppm)	Recovery Percentage (%)
0.265	29.305±0.002	88

Precision

In the same day (intraday) produced RSD sequentially 1.274, 1.147, and 0.497 are show in Figure 6. While in different days (interday) the RSD produced sequentially 0.433, 1.466, and 1.388 are show in Figure 7. Based on the study

data, it indicates that the accuracy of the boron determination method was included in the range % RSD <2% (Belouafa et al., 2017). According to Belouafa (2017) range % RSD <2% included in the category was very good.

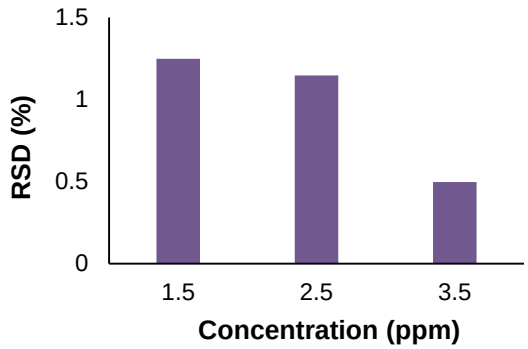

Figure 6. Precision on the same day (Intraday)

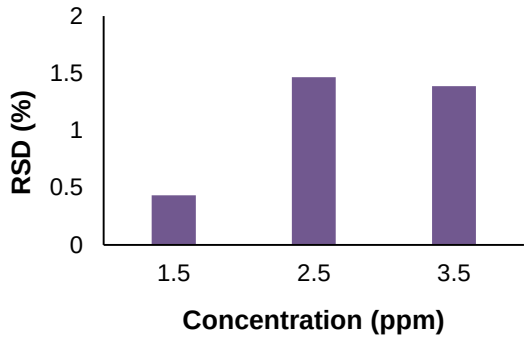

Figure 7. Precision on different day (Interday)

The result of method validation showed that the complex method of boron curcumin oxalate can be measured using UV-Vis spectrophotometry.

CONCLUSION

Boron analysis using distillation by esterification process can be used to separate boron in food samples. This validation method had sensitivity method of UV-Vis spectrophotometry in the sequence was 0.0902 ppm^{-1} and the use of UV-Vis spectrophotometry can be used further in the analysis of boron in food because it has % recovery rate by 88%. This recovery percentage was categorized as accurate according to Lopez (2019), since it falls within the range of 80% - 120%.

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