ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Study On Sample Preparation Method In Analysis Of Borax In Dates Palm Using Visible Spectrophotometry

Wiwiek Indriyati, Maria Paramita, Aliya Nur Hasanah.

Faculty of Pharmacy Universitas Padjadjaran, Jl Raya Jatinangor KM 21,5 Jatinangor-Sumedang, postal code : 45363

ABSTRACT

Dates palm is one of the fruits which are consumed by the people of Indonesia during the fasting month. However, there are some rogue traders who add borax into palm to increase the shelf-life. The use of borax in food has been banned by the government based on Indonesian Minister of Health Regulation No. 003 of 2012 on Food Additives (BTP). Sample preparation was the most critical step in the analysis and spend more than 80% of analysis time. Until now, no research has been done in comparing sample preparation method to quantify borax in dates palm. Therefore, this study was conducted to determine the sample preparation method that meets with validation criteria. Analysis of borax in dates palm was done with three different sample preparation method which are centrifugation, incineration, and reflux. Samples then being reacted with curcumin and the reaction result was analyzed by using a visible spectrophotometer. Sample preparation method that gives the highest recovery and meets validation criteria then was determined. From the study, centrifugation was the best preparation sample method in preparing borax analysis from dates palm with recovery 96.21%. Analytical method validation result using centrifugation as sample preparation was meet validation requirement with accuracy in the range of 80-110%, relative standard deviation <2% with linearity (r) 0.995. LOD and LOQ were 0.0108 mg/L and 0.0359 mg/L respectively. The results found that analysis of borax in dates palm could be done by visible spectrophotometry using centrifugation as sample preparation.

Keywords: Borax, Spectrophotometry UV-Vis, Dates Palm, Curcumin Reagent, samples preparation

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Dates palm fruit is often found in Indonesia, especially during Ramadan. Fruit that has a sweet taste is believed can restore power after a day of fasting because it has a very high sugar content, reaching 50-57% so that it can be used as an energy source. Dates storage processes affect the quality of the fruit. In industry, the storage usually conducted at a -3°C temperature for a year. Palm fruit also has a shelf life of up to 2 years at room temperature (25°C) [1].

The palm fruit was only sold out during certain moments such as during Ramadan, while this fruit can only survive for 2 years at room temperature. This is why the traders was using borax as a preservative to preserve the palm fruit. Based on SNI 01-0222-1995, borax is a substance that is not allowed to use in food [2]. The use of borax in dates are usually accompanied by the use of coconut oil for recycling the dates which were almost rotten. Coconut oil is used to separate the palm fruit mutually attached to each other and to shine the colors of the dates are almost rotten and given borax to preserve these dates. After the expire date and the traders used coconut oil and borax to make it shine, they were sold palm fruit at a cheap price [3].

Concerning the condition that happened around, analysis of borax content on dates palm fruit was urgently required. Analysis of borax from samples could be done by three different method preparation which are centrifuge, ashing, and reflux. Borax which has been extracted from the sample was then analyzed using visible spectrophotometry after treated with curcumine. Until now, no research has been done in comparing sample preparation method to quantify borax in dates palm. Therefore, this study was conducted to determine the sample preparation method that meets with validation criteria.

MATERIALS AND METHODS

The tools used in this study were mixer, blender (MIYAKO), bottle vial, funnel glass, erlenmeyer lids, beakers, measuring cups, paper filter, flask rounded, flask, micropipette, oven (HARAEUS), a water bath , pipette, reflux (GOPAL), centrifuges (YENACO), spatula, spectrophotometer (SPECORD 200 - 222U203), test tubes, furnaces (THERMO SCIENTIFIC), and scales electrically (OHAUS) used in the Laboratory of Pharmaceutical Chemistry.

The materials used in this study were distilled water, dates palm fruit, borax (MERCK), curcumin p(MERCK), ethanol absolute (MERCK), hydrochloric acid (MERCK), glacial acetic acid (MERCK), sodium hydroxide (MERCK), acid glacial sulphate (MERCK), filter paper, 35% peroxide (MERCK) all in pro analysis grade.

Method

Samples Simulation of Dates Which Contain Borax

A total of 20 grams of dates contains no borax was weighed and then smeared with a little coconut oil and borax were added as much as 8 mg and then stirring until blended. 5 grams of dates were used for each extraction method.

Sample Preparation for Extraction of Borax from Samples Simulation

a. Centrifugation

5 grams of sample simulation was added with 100 mL of distilled water and put in a blender until it was smooth. The solution is put in a centrifuge tube and centrifuged for 2 minutes at a speed of 3000 rpm. Part supernatants were collected and then filtered to obtain a borax concentration of 20 ppm.

b. Ashing

5 gram sample of dates was ignited in a furnace at a temperature of 600°C for approximately 5 hours. Samples were ignited until it become white ashes. The ash then was dissolved using 100mL distilled water, filtered and the filtrate was taken to obtain a borax concentration of 20 ppm.

c. Reflux

5 grams of sample was introduced into a 250 ml round bottom flask, then was added 20 ml of concentrated H_2SO_4. Refluks was done for 5 minutes, and continue by adding 20 ml of 30% H_2O_2. After 15 seconds, put the liquid from reflux process in a 100 ml flask and diluted with distilled water at a room temperature to obtain borax concentration of 20 ppm.

Qualitative Analysis

a. Flame Test

First, extract from the three types of extraction method is added 1 ml of concentrated sulfuric acid and 1 ml of ethanol. After that, burnt it with fire. Postive results come from green flame caused by reaction between ethanol and borax in the samples.

b. Turmeric Paper Test

Turmeric paper test is identification of borax by using a piece of paper that is dipped into the supernatant turmeric that have been acidified with 5N HCl 1 ml. When a sample contains borax, there will be color changes in a paper from yellow to dark blue green after the addition of aqueous ammonia.

Evaluation Method of Preparation of Sample and Analysis of Borax Dates

1 mL of samples from each extraction methos was taken and added with 1 ml solution of 10% NaOH, heated until dry. Then the heating was continued in the oven at a temperature of 100° ± 5 ° for 5 minutes, then cooled. 3 ml of 0.125% curcumin solution was added to the solution, then heated with stirring for 5 minutes and then cooled again. After that, 3 ml solution of sulfuric acid: acetic acid (1: 1) was added and then heated with stirring until no yellow color (either on a plate or on a rod stirrer). Ethanol then was added until mark to get sample concentration in a range 0.4 ; 0.02 ; 0.04 ; 0.06; 0.08 and 0.1 ppm respectively. Each concentration then was determined using spectrophotometer UV Visible in a maximum wavelength that already measured.

All data then was analyzed by using ANOVA and Newman Keuls advanced test.

RESULTS AND DISCUSSION

Result of Samples Simulation of Dates Which Contain Borax

The sample used in this research was palm fruit sold at fruit store in the city of Cirebon. Samples selected for the sample did not contain borax therefore it was being added with borax.

Result of Sample Preparation for Extraction of Borax from Samples Simulation

Preparation with centrifuge method was done by weighing 5 grams of dates then blended with 100 mL of distilled water for approximately 2 minutes until smooth. After that the samples were centrifuged at 3000 rpm for 10 minutes to separate the supernatant and sediment. Part of the supernatant was taken for further analysis.

Preparation ashing method was done by weighing 5 grams of dates then inserted into the porcelain cup and then ignited with a temperature of 600°C for 5 hours. Temperatures are used under 742°C because at these temperatures in the form of anhydrous borax melt [4]. Ash that was obtained then was diluted with distilled water in a 100 mL volumetric flask.

Preparation with reflux method performed by weighing 5 grams of dates then put in a 250-ml round bottom flask and concentrated H_2SO_4 was added to accelerates the oxidation because sulfuric acid is a strong oxidizing agent. 30% H_2O_2 then was added to serves as an oxidant which is useful for reducing organic

compounds into CO_2 and H_2O, so the solution will become clear [5]. After that reflux until a clear solution. The filtrate obtained was then diluted with distilled water in a 100 mL volumetric flask.

Qualitative Test Results

Following the results of qualitative tests were obtained:

Table 1. Qualitative Test Results

No.	Samples	Turmeric paper	Flame test
1	Standard solutin of borax	+	-
2	Filtrate from Reflux method	+	-
3	Filtrate from Ashing method	+	-
4	Filtrate from centrifugation method	+	-
5	Filtrate from free borax dates	-	-

According to the table above, it was concluded from the turmeric paper test, the standard solution and the filtrate of the three methods contain borax. Meanwhile, from flame test, all samples have a negative results. This is due to borax levels that are below the detection limit of the method.

Evaluation Method of Preparation of Sample and Analysis of Borax Dates

a. **Determining Maximum Wavelength**

The maximum wavelength measurement is done using a standard solution concentration of 0.08 ppm and obtained maximum wavelength at 554 nm.

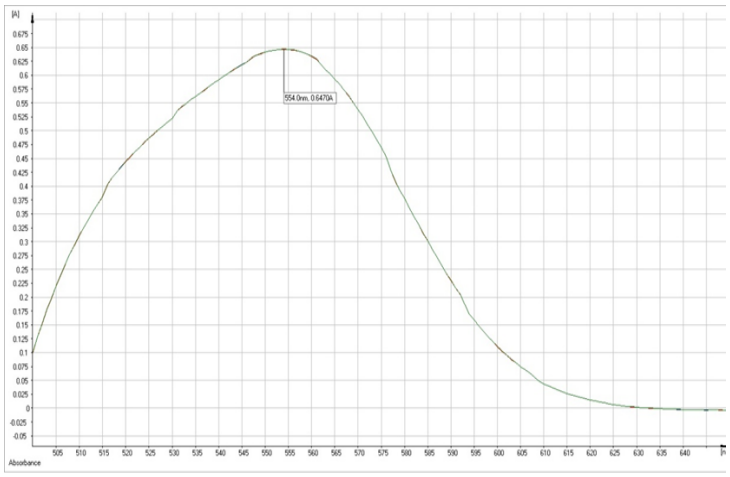

Figure 1. Wavelength Maximum Results The reaction of borax and Curcumin.

b. Calibration Curve

Calibration curve was made using five variations of concentration is 0.02 ppm; 0.04 ppm; 0.06 ppm; 0.08 ppm; and 0.1 ppm. line equation y = 8,998x - 0,038 with R = 0.995 were obtained from this test. The equation was then used to determine the concentration of the sample. The correlation coefficient close to 1 or R2 ≈ 1 states that a linear relationship between absorbance with concentration so that the linear regression equation can be used for sample measurements [6].

ISSN: 0975-8585

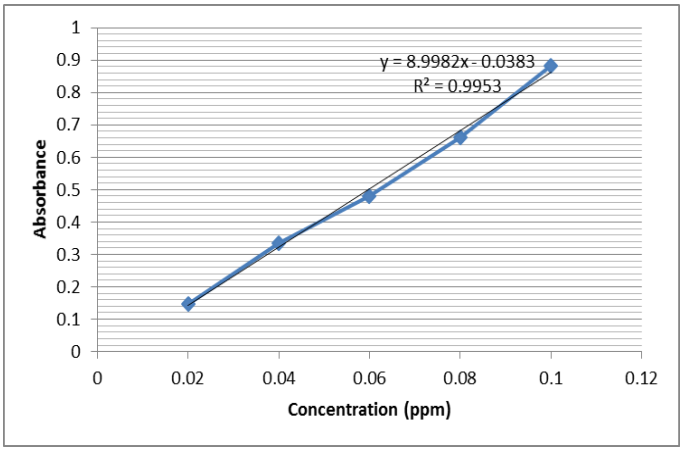

Figure 2. Calibration Curve of Borax Solution

c. Accuracy test

Accuracy test was done by making three variations of the standard solution of borax with concentration 0.04 ppm ; 0.06 ppm; and 0.08 ppm respectively. The results of accuracy test was ddepicted bellow (Table 2):

Table 2. Result of Accuracy Test

Concentration	Absorbance	Measured concentration	%recovery
0.04	0.3353	0.0415	103.7175
	0.3358	0.0415	103.8564
	0.3359	0.0416	103.8842
0.06	0.4802	0.0576	95.9843
	0.4805	0.0576	96.0398
	0.4802	0.0576	95.9843
0.08	0.6621	0.0778	97.2577
	0.6621	0.0778	97.2577
	0.6621	0.0778	97.2577
		Mean	99.0266

Based on the above table is obtained from borax test the accuracy of the standard solution of 99.029%. It meets the requirements of [7] in the amount of 80-110%.

LOD and LOQ

LOD and LOQ value is calculated through linear regression equation of the standard curve [6]. Based on linear regression equation, LOD value was 0.0108 mg / L and LOQ value of 0.0359 mg / L.

Analysis Borax In Dates

Quantitative analysis of borax starting with sample preparation. After the samples were prepared, obtained borax filtrate. Curcumin concentration that used for analysis was 0.125% based on previous research, that the range of 0.100% -0.150% curcumin can be dissolved in acetic acid without filtering process [8].

Stability of the complex color can only be maintained for 2 hours after the color complex formed in acidic conditions.

Borax solution colorless become an obstacle in absorbance inspection using UV-Vis spectrophotometry which must have a chromophore group marked with a color. Curcumin is reacted with a sample to form complexes that produce color rosocyanin color rosa (red brick).

Below (Table 4) is a result of quantitative analysis of samples using visible spectrophotometry:

Table 4. Results of quantitative analysis of samples for each method

No	Method	Mean Recovery (%)
1	Centrifugation	96.21 %
2	Ashing	96.13 %
3	Reflux	87.37%

Results of statistical analysis showed that all three of these methods have differences, which according to the purpose of this study is to compare the three methods of sample preparation borax in the palm fruit. Meanwhile, based on validation tests that include tests of accuracy and precision, three of these methods have met the requirements. Dry destruction which is ashing was a good method for destruction of stable metals [9], boron that was a metal in borax was not a stable metal. Boron is an element with metaloid properties. Based on statistics, reflux and centrifugation have the same impact on borax analysis in dates. Centrifugation has a better result compare to others because borax is dissolve in 20 parts of water that was used in dissolution step of centrifugation method, hence more borax being extracted from dates.

CONCLUSIONS

Centrifugation was the best method for preparation of borax analysis from dates palm fruit using visible spectrophotometry with highest % recovery.

REFERENCES

[1] FSSAI. 2012. *Manual of Methods of Analysis of Food- Food Additives*. New Delhi: Food Safety and Standards Authority of India Ministry of Health and Family Welfare Government of India.

[2] FAO. 1991. *Manuals of Food Quality Control 1980, 14/ 2 page 27/ Pearsons Composition and Analysis of Food 9th ed. Page 82*

[3] Rock, W. 2009. *Effects of Date (Phoenix dactylifera L., Medjool or Hallawi Variety) Consumption by Healthy Subjects on Serum Glucose and Lipid Levels and on Serum Oxidative Status: A Pilot Study*. Journal of Agricultural and Food Chemistry 57(17): p. 8010-8017.

[4] Departemen Kesehatan RI. 2012. Peraturan Menteri Kesehatan Republik Indonesia No. 003/MENKES/PER/X/2012. Tentang Bahan Tambahan Makanan. Jakarta: Departemen Kesehatan Republik Indonesia.

[5] Biglari, Foroogh. 2009. Assessment of Antioxidant Potential of Date (Phoenix Dactylifera) Fruits From Iran, Effect of Cold Storage And Addition To Minced Chicken Meat. Tesis (Malaysia: Universiti Sains Malaysia, 2009)

[6] Flanaga, R.J.,Braithwaite, R.A. Brown, S.S., Widdop, B., de Wolff,F.A. 1995. *Basic Analytical Toxicology*. Geneva: World Health Organization.

[7] Haddad, L.M., Winschester, J.F. 1990. *Borats on Clinical Management of Poisoning and Drug Overdose*. Philadelphia: Saunders Co; 1447-1449.

[8] Underwood, R., M. 1998. *Spectrometric Identification of Organic Compound.* New York: John Willey and Sons Inc.

[9] Azas, Q.S. 2013. Analisis Kadar Boraks pada Kurma yang Beredar di Pasar Tanah Abang dengan Menggunakan Spektrofotometer UV-Vis. Jakarta: UIN Syarif Hidayatullah.