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Validation of Reversed-phase High-performance Liquid Chromatography Method for Determination of Alpha Tocopherol in Corn Oil

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Abstract

Determination of alpha tocopherol in corn oil can be analyzed by using Reversed-Phase High Performance Liquid Chromatography (RP-HPLC). This research aimed to validate RP-HPLC method for determination of alpha tocopherol in corn oil. The method was based on linearity, precision, accuracy, limit detection (LOD) and limit quantization (LOQ). The Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method includes the determination of the wavelength, the mobile phase composition and the flow rate of mobile phase, then validation of each of the above methods. The maximum wavelength was found in 290 nm, composition of mobile phase was 100% methanol, flow rate of mobile phase was 1,5 mL/min and retention time was 9,67 min. The researched result indicated that the method of RP-HPLC had a linearity with the correlation coefficient of 0.999, a relative standard deviation (%RSD) for intra and inter-day precision was < 5%, accuracy/recovery was 95.69-100.99 %, detection limit was 0,43 mg/L, quantization limit was 0,55 mg/L and alpha tocopherol content in corn oil was 40.06 ± 0.24 mg/L.

Keywords: Corn oil, alpha tocopherol, RP-HPLC, and validation method

1. INTRODUCTION

In the last decade, the attention of the oil consumed by humans is increasing. Oil for human consumption can be derived from fried foods or consumed directly as an addition to the dressing, salad and mayonnaise, which is better known as vegetable oil (vegetable oils). Vegetable oils contain high polyunsaturated fatty acids and tocopherol which is useful for body. Olive oil and corn oil are vegetable oil consumed frequently. Corn oil is the oil obtained from the processing of corn seeds (Zea Mays) and had undergone a process of purification with or without the addition of other additives [1].

Corn oil is used to decrease cholesterol levels in the blood because it contains polyunsaturated fatty acids. The content of phytosterol in corn oil also inhibits the absorption of cholesterol in the intestine. In addition, the corn oil is a source of tocopherols which act as antioxidants in the body [2].

Tocopherol is a group of fat-soluble vitamins contained in four vitamin different forms, namely alpha (α), beta (β), gamma (γ) and delta (δ) tocopherol. α-Tocopherol is a component of vitamin E, which has highest antioxidant activity, which serves to fight free radicals cause arthritis, liver diseases, the aging process, various types of cancer,

cataracts, Alzheimer's disease and atherosclerosis [3].

α- Tocopherol could not be synthesized by the body and is found in small quantities in a variety of foodstuffs. Fat-soluble antioxidants can generally be stored in the liver for long time, however α- tocopherol is actually stored in body fat and used very fast [3].

Analysis of the compounds contained in corn oil can provide nutrition information that is naturally present in it such as fatty acids, phenol compounds, sterols and tocopherols as well as synthetic antioxidants when added to the production process. The matrix in corn oil is quite complex so that different analytical methods are used for identification and quantitative analysis, which is not likely to give different results [4].

Validation method is a process used to confirm that the analytical procedures used for a particular test has the appropriate designation. The results of the validation method can be used to assess the quality, reliability and consistency of analysis results[5]. The validity of a specific method of analysis that must be demonstrated in a laboratory experiment using the same standard or sample to the test sample to be analyzed regularly [6]. The analytical method should be validated is a non-standard method, a method designed, developed by the laboratory, the standard method used outside the scope prescribed and standard methods were modified [7]. The parameters should be tested in the validation of analysis methods including specificity, linearity, limit of detection, limit of quantization, range, precision, accuracy and perseverance [8].

Tocopherol can be analyzed by High Performance Liquid Chromatography (HPLC) (normal phase or reversed phase) with isocratic or gradient elution system, using uv-vis, fluorescence or electrochemical detector [9]. In normal phase HPLC, the mobile phase is n-hexane which is relatively easy to separate all forms of tocopherol while the mobile phase of the reversed phase HPLC is methanol or acetonitrile which is rather difficult to separate the β and γ-tocopherol [10].

HPLC is now the most widely method to determine the concentration of α-tocopherol. The advantages of the HPLC compared to spectrophotometry, fluorometry or gas chromatography. Parameters usually use for the study are speed, relatively free from interference of impurities, the condition of non- destructive ends and a simple method to be applied in a very small sample. Analysis of α-tocopherol using HPLC has been done [3, 11 – 14]. The studies showed that HPLC had good linearity, precision, accuracy and sensitivity.

Based on those researches, the aim of this research is to validate methods of analysis of α-tocopherol in corn oil by reversed-phase HPLC with the parameters of linearity, precision, accuracy, detection limit and the limit of quantization.

2. MATERIALS AND METHODS

A. Equipment

This study used Ultra Fast Liquid Chromatography instrument with detector Shimadzu SPD 20A Prominence UV VIS Detector, LC 20AD pump system, Online Degasser DGU-20A3/DGU-20A5 Prominence, and System Controller CBM - 20A/20Alite Prominence to record and process the data, column used was Shimpack VPODS (250x4,6 mm ID), Branson sonicator, Vortex VM 300 and a set of glassware.

B. Materials

Materials used were methanol, 2-propanol (Merck), syringe Whatman PTFE filter Puradisc 13, α- tocopherol purely from Sigma Aldrich, aquabidest and commercial corn oil.

C. Methods

Standard of pure α-tocopherol (M_r = 430.71 g/mol) was prepared with a concentration of 1000 mg/L in methanol. Standard solution was then diluted to 100 mg/L and created a standard series with a concentration of 0.5; 1.0; 2.0; 4.0; 8.0; and 16.0 mg/L. Each standard solution was then filtered using a filter syringe 0:45 µm.

0.25 mL of corn oil was dissolved with 2 mL to 10 mL of propanol, was shaked for five minutes and then centrifuged at 3000 rpm for five minutes. Before injected into the HPLC instrument, corn oil sample was filtered using syringe filter of 0.45 µm.

Optimization wavelength was done by the injection of the standard solution at wavelengths of 240, 290 and 360 nm, with a flow rate of 1.5 ml/min and the mobile phase composition of 100% methanol. After the maximum wavelength was obtained, the selection of a mobile phase composition was done consecutively with 100% methanol, methanol:water a ratio of methanol to water of 100:1, 98:2 and 99: 1.

After the maximum wavelength and optimal mobile phase composition being obtained, the flow rate election was conducted under conditions of 1mL/min, 1.5mL/min and 2mL/min with a mobile phase of 100% methanol.

The standard curve was obtained by injecting a standard at concentrations of 0.5, 1, 2, 4, 8, and 16mg/L, and then plotting the peak area against concentration. Linearity was determined from the correlation coefficient (r) of the standard curve.

Precision was determined by evaluating the area and the retention time. Standards were analyzed at concentrations of 0.5, 1, 2, 4, 8, and 16mg/L in intra-day and inter-day. For intra-day measurement, five repetitions were conducted at the same day, while for inter measurements, five times experiments were performed a day for five consecutive days. From the results obtained %RSD was determined.

Accuracy is defined as the percent recovery of α-tocopherol standard (spike) added to a sample of corn oil. Recovery was done against 1, 8 and 16 mg/L α-tocopherol standard. Concentration of spike samples (A),

the concentration of the sample (B) and the spike concentration (C).

The recovery percentage was calculated as:

$$[(A- B)] / C \times 100\%$$

Limit of detection and limit of quantization were calculated based on the standard deviation of the reading of the blank or standard that is closest to the blank.

$$LOD = X + 3\ SD$$
$$LOQ = X + 10\ SD$$

0.25 mL corn oil sample was pipetted, put into a 10 mL volumetric flask and diluted with 2-propanol to 10 mL, shaked for 5 minutes, and filtered with a PTFE filter syringe of 0.45µm.

Samples were injected as much as 5 replicates into the HPLC apparatus with the mobile phase, the flow rate and the wavelength corresponding to the optimum conditions obtained. The concentration of α-tocopherol in the sample was calculated based on the regression equation of the calibration curve obtained.

3. RESULTS AND DISCUSSION

The discussion of results includes the determination of the maximum wavelength, the mobile phase composition and the flow rate, the calibration curve as well as the determination of linearity, precision, accuracy, the detection limit and the quantization limit, along with the determination of α- tocopherol in corn oil.

The results showed that the maximum wavelength was 290 nm which was the best for the determination of α- tocopherol. The best mobile phase obtained from experiments at various compositions of the mobile phase was 100% methanol. The best flow rate of the mobile phase was 1.5mL/min.

Based on the calculation, the linear regression correlation coefficient obtained for α-tocopherol was 0.999 with the equation of y = 5469x + 558.7. This showed that the method for determining α- tocopherol using HPLC had good linearity. The calibration curve is given in Figure 1.

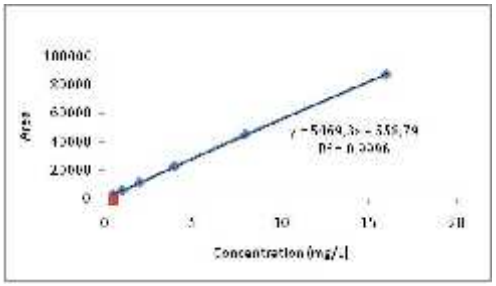

Figure 1. Calibration Curve of α-tocopherol

In this study, α-tocopherol was detected in 9.67 minutes on the condition as follows: a wavelength = 290 nm, mobile phase composition = 100% methanol and a flow rate = 1.5 mL/min. The linearity of the standard curve in the range from 0.5 to 16 mg/L resulted in a correlation coefficient of 0.999, which means that the linearity and sensitivity of this method is good enough to be applied to the analysis of α- tocopherol.

Chromatogram of α- tocopherol for precision determination can be seen in Figure 2.

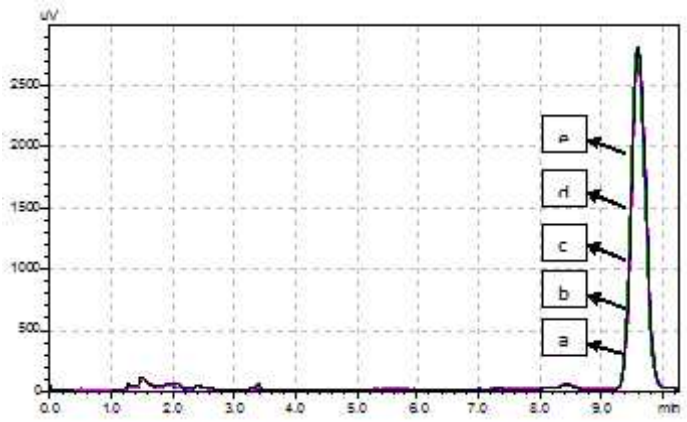

Figure 2. Chromatogram of α-tocopherol for precision determination, a) repetition 1, b) repetition 2, c) repetition 3, d) repetition 4, and e) repetition 5

It is clear that the method has good precision because the peaks obtained are similar for the 5 repititions of measurements. The accuracy of the method was determined by calculating the recovery of the fortified corn oil samples (sample spike method). The experiments were performed at a concentration of 1, 4 and 8 mg/L with the result of 95.69, 99.53 and 100.99%,, respectively. This indicated that the accuracy was in agreement with the specified requirements (95-100%). The results of the examination accuracy/recovery can be seen in Table 1.

Table 1. Results of recovery measurements

Repitition	Sample concentration +Spike	% Recovery
1	2.0468	93.59
2	2.1938	106.76
3	1.9795	87.5
4	2.1123	99.46
5	2.0194	91.14
	Total	478.45
	Average	95.69

The limit of detection of α-tocopherol using HPLC was 0:43 mg/L, whereas the limit of quantization was 0:55 mg/L with a range of 0.5-16.0 mg/L.

The determination of α-tocopherol in corn oil can be seen in chromatogram of Figure 3.

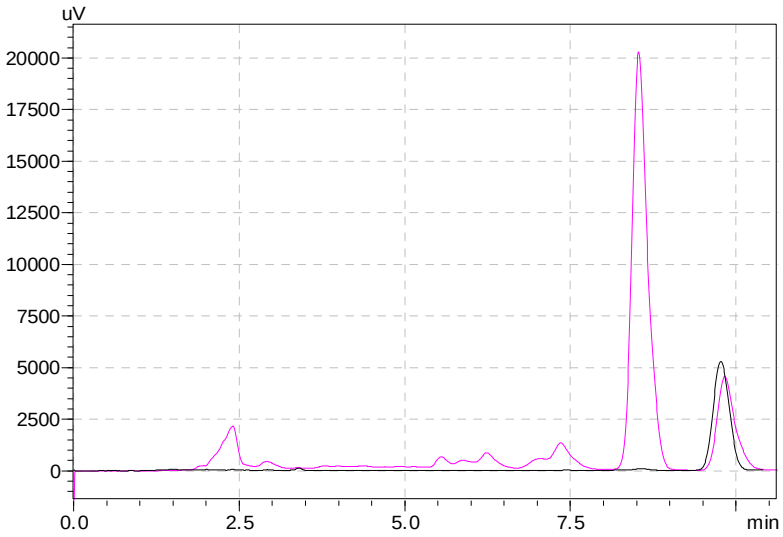

Figure 3. Chromatograms of (a) corn oil (a) and (b) α-tocopherol standard

The concentration of α-tocopherol corn oil obtained in this study was 40.60±0.24mg/L. In the analysis, α- tocopherol corn oil had the same retention time with the standard retention time of α- tocopherol. It is also seen that the α-tocopherol in corn samples have a peak and a base line that is quite good. It shows that this method is quite specific and selective for the determination of α-tocopherol in corn oil.

4. CONCLUSIONS

HPLC conditions for determination of α-tocopherol corn oil in this study was at the wavelength of 290 nm, the mobile phase composition of 100% methanol and the flow rate of 1.5 mL/min. Parameters validation of analytical methods include linearity, precision and accuracy in the determination of α-tocopherol corn oil gave results that satisfyied the validation requirements for the analysis method based on the ICH 2005. The lemit of detection and the limit of quantization was 0.43 mg/L and 0.55 mg/L, respectively. This method is good enough to be applied as a method of routine analysis in the laboratory. A subsequent study is suggested to continue the research until calculation of estimated measurement uncertainty.

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