

Determination of Vitamin B₃ Levels on Red Dragon Fruit (*Hylocereus Costaricensis* (F.A.C Weber) Britton & Rose) Using HPLC

Ridho Asra¹, Zulharmita², Putri Regina Prayoga³

^{1,2,3} School of Pharmaceutical Science (STIFARM) Padang, Indonesia

ABSTRACT

Dragon fruit (*Hylocereus costaricensis* (F.A.C Weber) Britton & Rose) contains bioactive substances that are beneficial to the body, including antioxidants. This study aims to determine the levels of vitamin B₃ in red dragon fruit (*Hylocereus costaricensis* (F.A.C Weber) Britton & Rose) using High Performance Liquid Chromatography. Red dragon fruit is taken from Kamang Magek District, Agam Regency, West Sumatra. Red dragon fruit samples (*Hylocereus costaricensis* (F.A.C Weber) Britton & Rose) were extracted by maceration. Then the sample extract was added with 5M NaOH and HCl and analyzed by High Performance Liquid Chromatography using methanol and phosphate buffers (10:90) as the mobile phase against UV light at a wavelength of 261 nm. The results showed that the mean level of Vitamin B₃ from (*Hylocereus costaricensis* (F.A.C Weber) Britton & Rose) was 0.0173465 mg with a detection limit of 0.515 μ g/mL and a limit of quantization of 1.7190 μ g/mL.

KEYWORDS: Vitamin B₃; HPLC; Dragon fruit.

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INTRODUCTION

Dragon fruit is one of the fruits of several types of cacti from the clans *Hylocereus* and *Selenicereus*¹. *Hylocereus polyrhizus* or red dragon fruit has a higher level of sweetness than white dragon fruit (*Hylocereus undatus*), which reaches 13-150 Brix. This red dragon fruit has the same level of sweetness as super red dragon fruit (*Hylocereus costaricensis*) but has its advantages because the flowers of this red dragon fruit plant always appear every season and are always abundant². Dragon fruit is one that contains bioactive substances that are beneficial to the body including antioxidants (ascorbic acid, beta-carotene, and anthocyanins) and contains dietary fiber in the form of pectin. Apart from that, dragon fruit contains several minerals such as calcium, iron, and others. Vitamins contained in dragon fruit include vitamin B₁, vitamin B₂, vitamin B₃, and vitamin C³.

Vitamins are organic molecules that are needed by the body for normal metabolism and growth. Vitamins cannot be made by the human body in sufficient quantities, therefore they must be obtained from the food consumed. Vitamins also have specific roles in the body and can also provide health benefits. If the levels of this compound are insufficient, the

body can experience a disease. The body only needs vitamins in small amounts, but if this need is ignored, the metabolism in the body will be disrupted because its function cannot be replaced by other compounds⁴. Vitamin B₃ (Nicotinic Acid) is an organic compound that is needed by the body. Vitamin B₃ plays a role in converting food into energy. Vitamin B₃ is one of the five vitamins, if the body is deficient in Vitamin B₃, the body will experience the effects of pellagra disease and can cause symptoms that affect the skin, digestive system, and nervous system⁵.

From the results of previous research, testing of vitamin B₃ in formula milk using High-Performance Liquid Chromatography with a simple Modified sample preparation technique from previous method^{6,7}, where the correlation coefficient value for linearity is 0.999 in the range. concentration of 0.108 to 8.64 mg / L and detection limit of 0.083 mg / L and concentration of quantitation limit of 0.28 mg / kg⁸. Based on the above, the research was interested in testing the levels of vitamin B₃ in red dragon fruit using the HPLC method, this study aimed to determine the levels of vitamin B₃ contained in red dragon fruit. The vitamins that will be tested for levels are vitamin B₃ in red dragon fruit

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(*Hylocereus costaricensis* (F. A. C Weber) Britton & Rose) by HPLC.

METHODS

Tools

The tool used is a set of High Performance Liquid Chromatography or HPLC (Shimadzu LC-20AD) which is obtained from the Natural Material Chemistry Laboratory (UNAND) and other glass tools that support the implementation of blender research (Philips), analytical scales, measuring pipettes (Pyrex Iwaki®), beaker (Pyrex Iwaki®), volumetric flask (Pyrex Iwaki®), volume pipette (Pyrex Iwaki®), funnel (Pyrex Iwaki®), KCKT vial, spatel, dropper pipette, stir bar, suction ball, tissue, and Whatman No.1 filter paper and Whatman filter paper No.42, Vortex Mixer (Gemmy VM-300), pH Meter (Hanna Instrument 2211).

Materials

The materials used are red dragon fruit (*Hylocereus castaricensis*), Hydrochloric Acid (HCl), MeOH: phosphate buffer (10:90), Methanol (CH₃OH), Sodium Hydroxide (NaOH), Aquabides (Water For Injection) and Vitamin B₃ (PT. Indo Pharma).

Sampling Method

The sample used was *Hylocereus Castaricensis* (F. A.C Weber) Britton & Rose. A total of 1 kg obtained from the plantation area of Kamang Magek, Agam Regency, West Sumatra, Indonesia.

Plant Identification

Identification was carried out at the Herbarium of Andalas University, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang Indonesia.

Sample Preparation

Ripe red dragon fruit is cleaned then peeled, then the flesh is cut into small pieces then blended until smooth then weighed 2 grams. Put it in a glass beaker, dissolve it with 30 mL of aqua bides, let stand for 1 hour. Then in the Vortex mixer and filter it with filter paper.

Extraction by Maceration

Red dragon fruit extraction was carried out by the maceration method. Maceration of the sample by soaking 2 grams of red dragon fruit flesh with 30 mL of Aqua bides solvent for 30 minutes⁹.

High-Performance Liquid Chromatography (HPLC) Study

The content of Vitamin B₃ from red dragon fruit can be determined by HPLC with a UV-Vis detector based on the difference in affinity for the stationary phase of the reverse phase column C18 using the mobile phase of the methanol mixture: phosphate buffer (10:90) with an isocratic program against UV light at a wavelength of 261nm¹⁰.

Preparation of a standard solution of Vitamin B₃

Weigh 50 mg of vitamin B₃ put in a 100 mL volumetric flask, add 0.1 N HCl to the limit mark (500 ppm). Then 20 ml pipettes are put into 100 ml volumetric flasks, sufficient with 0.1 N HCl until the boundary mark (100 ppm), then another 1 ml pipette into 10 ml volumetric flasks added with 0.1 N HCl to the limit mark, then measured by a chromatogram with HPLC according to the optimum analysis conditions, the area of the standard solution was recorded¹⁰.

Preparation of Vitamin B₃ calibration curve

A concentration series of vitamin B₃ main solution with a concentration of 100 ppm was created. The concentrations made were 2,4,6,8,10 ppm by pipetting the mother liquor as much as 0.2; 0.4; 0.6; 0.8; and 1 mL into a 10 mL volumetric flask, then add 0.1 N HCl, put it in the cuvette, then the area is determined by HPLC with the mobile phase of methanol: phosphate buffer 10: 90¹¹.

Determination of Vitamin B₃ Levels in Red Dragon Fruit with HPLC

10 mL pipette red dragon fruit extract is put in a test tube. Then 5 mL of 5M NaOH was added, then heated in a water bath for 1 hour, then cooled to room temperature. Then the solution was changed to pH from 2.5 to 3.0 by adding HCl. Furthermore, the solution was filtered with Whatman filter no.42 and then measured by a chromatogram HPLC according to the optimum analysis conditions, the area of the test solution was recorded, Measurements were carried out in three repetitions¹⁰.

RESULT AND DISCUSSION

In the study, the sample used was red dragon fruit (*Hylocereus Castaricensis* (FAC Weber) Britton & Rose). 1 kg was obtained from the plantation area of Kamang Magek, Agam Regency, West Sumatra. The aim of the study was to determine the levels of Vitamin B₃ found in red dragon fruit.

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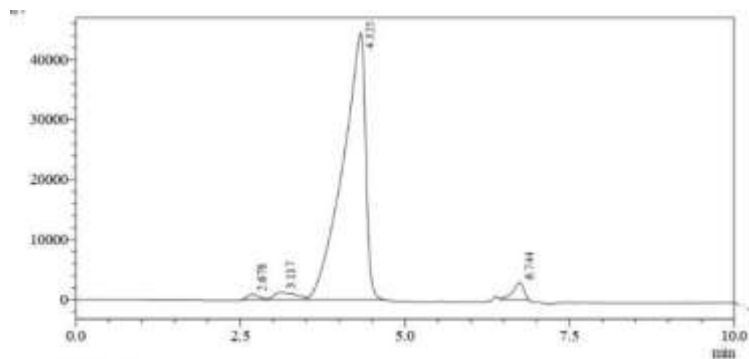


Figure 1. Vitamin B₃ Standard Chromatogram

At first, a standard preliminary test of vitamin B₃ was carried out using a mobile phase, namely methanol and phosphate buffer (10:90) so that the peak area of vitamin B₃ was 1071925 with a wavelength of 261 nm.

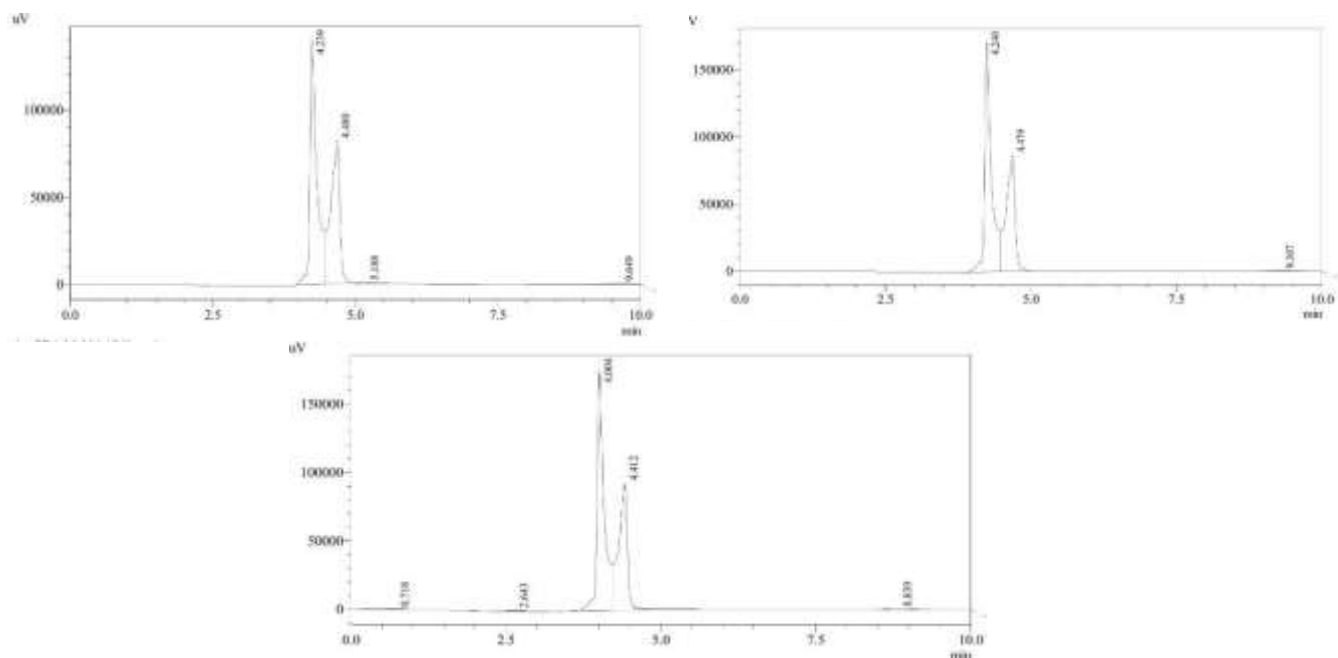


Figure 2. Red dragon fruit sample chromatogram (Repetition 1-3)

Testing was carried out on samples of red dragon fruit, seen from the peak of vitamin B₃ in the sample, it can be better separated, namely for repeated samples 1 obtained a peak area of 914524, 2 repetition samples obtained a peak area of 931534, and for 3 repetition samples obtained a peak area of 930428. The area of the predicted peak for vitamin B₃ needs to be confirmed by reading the spectrum from the sample and compared with the standard spectrum of vitamin B₃. The spectrum for each compound is unique so two compounds can't have the same spectrum. In the standard spectrum of vitamin B₃, it can be seen that the maximum wavelength is 261 nm. In optimum conditions, the peak of vitamin B₃ will appear at a retention time of 4.23 - 4.48 minutes, which is for the qualitative HPLC test in the form of observations of the retention time of raw compounds and unknown compounds compared by sequential chromatography under stable

instrument conditions with the smallest possible difference in operating time and for quantitative HPLC can be done by measuring peak height or by the area of the peak. Peak height is measured as the distance from the baseline to the maximum peak. While the peak area is measured as a product of the peak height and the width at half the height¹²

Based on the evaluation data for the standard series of vitamin B₃ for the standard series between 2–10 µg / L as in table 1, a regression curve is obtained as shown in Figure 4, with the regression equation $y = 453745x + 663760$ with a correlation coefficient value of 0.9978. The theoretical concentration of the analyte is on the x-axis and the analyte's response (area) of the instrument used is on the y-axis. These results meet the acceptance requirements, namely $r \geq 0.99$ ^{13,14, 15}.

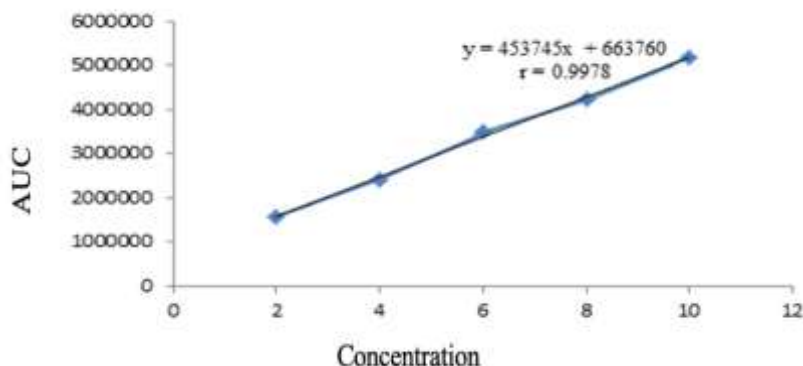


Figure 3. Vitamin B3 Calibration Curve

Table 1. Table of results of determination of Vitamin B3 levels in Dragon Fruit with HPLC.

Sample	AUC	Retention Time (minutes)	Niacin Levels (mg/mL)	Mg sample	% Content
Sample 1	914524	4,480	0,0005526	0,0165796	0,000828
Sample 2	931534	4,479	0,0005900	0,0177000	0,000885
Sample 3	930428	4,412	0,0005920	0,0177600	0,000888
Average Content			0,0005782	0,0173465	0,000867

After obtaining the sample peak area and the standard peak area for vitamin B3, the levels of vitamin B3 in red dragon fruit were calculated. For red dragon fruit sample 1 repetition with a peak area of 914524 and a retention time of 4.480, the vitamin B3 levels were 0.0165796 mg. For samples of red dragon fruit 2 repetitions with a peak area of 931534 and a retention time of 4.479, the vitamin B3 levels were 0.01770 mg and for red dragon fruit samples 3 repetitions with a peak area of 930428 and a retention time of 4.412, the vitamin B3 levels were 0.01776 mg. From the research, it was found that the average level of vitamin B3 from 2 grams of red dragon fruit was 0.0173465 mg.

CONCLUTIONS

From the data obtained in this study, it can be concluded that: The content of vitamin B3 in red dragon fruit (*Hylocereus costaricensis*) was tested with using HPLC using a comparison of vitamin B3 as evidenced by the value of red dragon fruit sample retention time 1 repetition with a retention time of 4,480 minutes, for red dragon fruit sample 2 repetitions with a retention time of 4,479 minutes and for red dragon fruit samples, 3 repetitions obtained a retention time of 4,412 minutes. The average level of vitamin B3 for red dragon fruit samples is 0.0173465 mg. So in 2 grams of red dragon fruit, there is a vitamin B3 content of 0.0173465 mg.

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