



Research article

Analytical method validation of cinnamaldehyde content in cinnamon (*Cinnamomum burmannii*) extract using high-performance liquid chromatography

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ABSTRACT

The use of herbal medicinal plants has been found widely in the community as traditional medicine. One example is the Cinnamon plant (*Cinnamomum burmannii*) which contains cinnamaldehyde as the main compound. Cinnamaldehyde is known to have a major role in lowering blood sugar levels. Determination of the content of bioactive compounds in extracts is beneficial in determining the safety, quality, and efficacy of the plants used. So it is necessary to do a quantitative analysis test on the cinnamaldehyde compound in cinnamon plants by determining its levels using the HPLC method. It is necessary to validate the method analysis based on parameters including selectivity, linearity, accuracy, precision, detection limit, and quantitation limit. The mobile phases in this method were acetonitrile mobile and 0.04% acetic acid solution (60:40), The stationary phase was octadecylsilane (C-18), The flow rate was adjusted at 1.0 mL/min with column temperature adjusted at 29°C, injection volume 20 µl, and using a Photodiode array detector at a wavelength of 280 nm. The results showed that the resolution value obtained was 3.401; the correlation coefficient value obtained is 0.9941; The percent recovery obtained between 98.74% - 101.95%; percent RSD obtained between 0.92% - 2.68%; and the LOD and LOQ values were 0.069 ppm and 0.23 ppm. The HPLC method met the validation requirement and can conclude to be valid.

Keywords: *Cinnamomum burmannii* extract, Cinnamaldehyde, HPLC, Method Validation.

INTRODUCTION

The use of herbal plants has been widely found in the community as traditional medicine. The potential for cost savings is an important reason for individuals to choose traditional medicine over conventional medicine. This is because traditional medicines are cheaper than conventional medicines [1]. Herbal medicine is one part of traditional medicine derived from plants or parts of plants that are used either directly, in the form of simplicia, in the form of extracts, or in the form of finished products that function to prevent or treat disease. Cinnamon is one of the plants in the Lauraceae family (in the form of woody plants) has many pharmacological activities such as anticancer, antidiabetic, antiinflammatory [2].

The Cinnamon genus, which consists of about 250 species, is mostly distributed in Asia and Australia. *Cinnamomum burmannii* Blume is the most commonly found cinnamon species in Indonesia [3].

Diabetes Still becomes a problem in low and middle-income countries [4]. Insulin therapy for diabetes is less efficient because pain at the injection site and lead to low patient compliance [5]. One of the opportunities for cinnamon to be used as a medicine is for the management of hyperglycemia; because cinnamon has hypoglycemic activity so it is very useful for controlling blood sugar levels [6]. The bioactive compounds found in cinnamon include cinnamaldehyde, cinnamic acid,

polyphenols, and flavonoids. From various existing studies on the pharmacological activity of cinnamon against diabetes, cinnamaldehyde is a marker compound with the largest composition in cinnamon which is known to have a major role in lowering blood sugar levels [7,8]. Cinnamaldehyde has an oral bioavailability of 20% and a half-life of 1.7 hours, with as much as 48% of its metabolites found in both urine and feces [9]. Excretion of cinnamaldehyde is mainly carried out by the liver and kidneys with most of the aldehyde ring of cinnamaldehyde being converted to cinnamic acid [10]. This component has an antihyperglycemic effect with its main way of working is to reduce the hormone ghrelin which can directly increase insulin sensitivity [11].

A herbal-based product to be manufactured or marketed must meet the applicable quality criteria and requirements. The quality of raw materials must be relatively constant and controlled so that product quality can be guaranteed. Determination of quality to ensure optimal safety of efficacy depends on the content of bioactive compounds contained in plants that have been shown to have certain physiological activities [12]. To meet these quality requirements, it is necessary to determine the content of the bioactive compound cinnamaldehyde contained in cinnamon extract (*Cinnamomum burmannii*).

Several kinds of literature show the method of determining cinnamaldehyde levels using Gas Chromatography coupled with Mass Spectrometry (GC-MS)[13], Thin Layer Chromatography (TLC) [14], and High Performance Liquid Chromatography (HPLC) [15]. The selected method in this study is HPLC. The use of this method is because HPLC is sensitivity to analyze a compound with a very small concentration. Beside that, HPLC is selectivity because it can detect and separate compounds based on polarity differences [16]. The cinnamon extract has many compounds, so a simple and selective analytical method is needed to separate these multicomponent compounds for the determination of cinnamaldehyde in raw materials and products [17].

It is necessary to develop methods for assay and validation before the method is routinely used for determination of marker compound in raw materials and products [18]. Method validation is an process of evaluating certain parameters based

on laboratory experiments to prove that the parameters of the analytical method meet the requirements for their use. Testing the validity of the method using HPLC for the analysis of cinnamaldehyde compounds in the cinnamon extract was carried out with several parameters including selectivity, linearity, detection limit, quantitation limit, accuracy, and precision [19].

The aim this study to validate HPLC method for determining the levels of cinnamaldehyde in cinnamon extract (*Cinnamomum burmannii*). The result of this study show the development method can be used for analysis routine for analysis cinnamaldehyde in *Cinnamomum burmannii* extract.

MATERIALS AND METHODS

The materials used in this study were cinnamon extract powder (local herbal industry), trans-cinnamaldehyde standard (Sigma Aldrich), methanol pro-HPLC (Merck), acetonitrile pro-HPLC (Merck), glacial acetic acid pro analysis (Merck). The instrument used in this study were HPLC instruments (Shimadzu® i-series LC-2030 LT), Shim-pack GIST C-18 column (150 mm x 4.6 mm, 5µm), analytical balance (Ohaus), ultrasonicator (Sonica), digital pH meter (Schott), 0.20 µm pore size Nylon membrane filter (Millex®-GN).

Preparation Of Standard Solution and Sample Solution

The Cinnamaldehyde standard was prepared at a concentration of 1000 ppm by dissolving 10 mg of cinnamaldehyde standard into 10 mL of methanol. Then 1 mL was pipetted and diluted into 10 mL of methanol to obtain a standard solution of 100 ppm. The standard solution of 100 ppm was diluted through graded dilutions into several concentrations, namely 20; 30; 50; 65; and 75 ppm. The solution was then filtered using a 0.20 µm pore size filter.

Cinnamon extract samples were weighed 200 mg. Then the sample was dissolved in 5 mL of methanol and sonicated for 30 minutes at room temperature. Then methanol was added to the final volume of the volumetric flask. The solution was filtered using a 0.20 µm pore size filter.

HPLC Conditions

The isocratic mode was used for HPLC system. Mobile phase A consists of acetonitrile pro-HPLC and mobile phase B consists of 0.04% glacial acetic acid in water. The flow rate of the mobile phase is 1 mL/min with the setting of the ratio of giving the mobile phase as shown in (Table 1), the injection

volume was 20 µl, the column temperature was 29°C, and the wavelength was 280 nm. Then the System Suitability Test (SST) was carried out by injecting the standard solution 6 times.

Table 1: Mobile Phase Conditions HPLC

Mobile phase composition	Mobile phase ratio (v/v)
Acetonitrile : 0.04% acetic acid	40 : 60
Acetonitrile : 0.04% acetic acid	50 : 50
Acetonitrile : 0.04% acetic acid	60 : 40

Optimization Method

A standard solution of 100 ppm and a sample solution was injected into the HPLC instrument with an injection volume of 20 µl using the mobile phase composition (Table 1). The peak shape, retention time, resolution, tailing factor, and several theoretical plates were observed from the chromatogram data obtained. The mobile phase which provides a faster separation of the compounds will be used in the analysis.

Method Validation

Selectivity

The standard solution and the sample solution were injected into the HPLC instrument with an injection volume of 20 µl. Then observed the retention time produced by the standard solution and the sample solution. Compare the peak of the analyzed compound in the sample and the peak produced by the standard. Then calculate the Resolution (Rs).

Linearity

A standard solution of cinnamaldehyde with a concentration of 20; 30; 50; 65; and 75 ppm was injected into the HPLC instrument with an injection volume of 20 µl. The area of the peak plotted against the concentration of the standard solution of cinnamaldehyde. After that, the value of the linear regression equation $y = bx + a$; correlation coefficient (r) and coefficient of variation of the function (Vxo) was determined.

Detection Limit and Quantitation Limit

The detection limit and quantitation limit were calculated using the residual standard deviation method from the standard curve so that the LOD and LOQ values of cinnamaldehyde were obtained. The LOD and LOQ formulas are as follows:

$$\text{LOD} = 3 \times \frac{S_y}{\text{slope}} \text{ dan } \text{LOQ} = 10 \times \frac{S_y}{\text{slope}}$$

Accuracy and Precision

A standard solution of cinnamaldehyde with 3 different concentration levels, namely 80%, 100%, and 120% were injected into the HPLC instrument with an injection volume of 20 µl (3 times injection for each standard solution of cinnamaldehyde). The area of the peak was calculated to the

equation of the linear regression line. Then the percent recovery and the %RSD were calculated.

Determination of Cinnamaldehyde Levels in Cinnamon Extract

The sample solution was injected into the HPLC instrument with an injection volume of 20 µl (injecting the sample solution 3 times). The area of the peak was calculated to the equation of the linear regression line. Then the cinnamaldehyde content in the sample was calculated.

RESULTS AND DISCUSSION

Method validation is a process carried out with certain parameters to ensure that the selected analytical method is reliable, consistent, and of good quality according to its intended purpose, as well as to inform that the selected method will be suitable for use in the further analysis [19]. These parameters include selectivity, linearity, accuracy, precision, LOD, and LOQ. Validation of the method in this study aims to inform that the HPLC method chosen has a guarantee that it is feasible to use, quality, consistent, and meets the requirements for the analysis of cinnamaldehyde determination in the cinnamon extract.

Optimization of the mobile phase was carried out which aims to determine the composition of the mobile phases that is good for use in separating the components of the analyzed target compound. The optimization results showed that the first method elicited the target compound at a retention time of 8.4 minutes, the second method produced the target compound at a retention time of 5.0 minutes, while the third method produced the target compound at a retention time of 3.6 minutes. The three methods meet the requirements for the resolution value set by the AOAC guidelines, but in this study, the third method was chosen because it produces the target compound faster as shown in (Figure 1).

Selectivity is one of the parameters to determine the extent to which the analytical method to be used can later measure the target compound in the presence of other compounds contained in the sample as well as the presence of a matrix or other material that has the potential to interfere with the analysis of the target compound [19]. It can be seen in (Figures 1 and 2), that the retention time and spectrum are the same for both the standard and the sample. In addition, the peak resolution value of the cinnamaldehyde compound obtained

was 3.4. These results meet the specified requirements, namely, a good resolution is indicated by a value of $R_s \geq 1.5$ [19].

Figure 1. (a) Chromatogram of 100 ppm standard solution, (b) Chromatogram of cinnamon extract sample solution

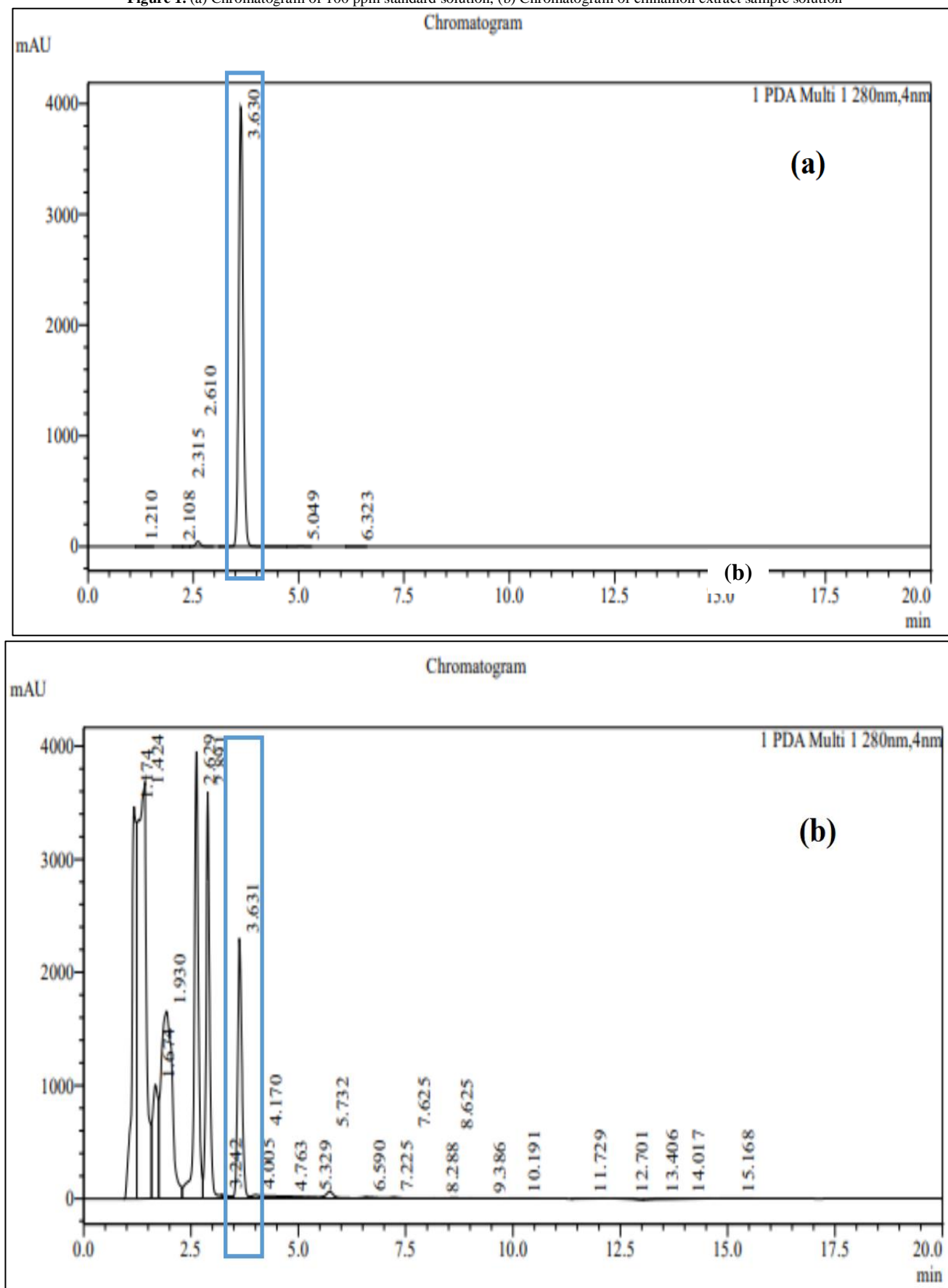
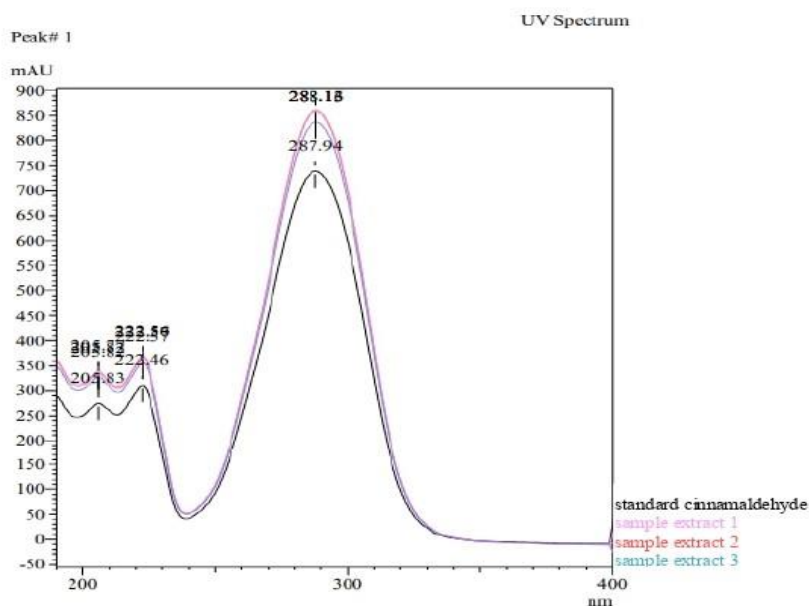


Figure 2: Overlay of 25 ppm standard spectrum and cinnamon extract sample

System Suitability Test (SST) is a test carried out to ensure the quality of the performance of the chromatographic system and to ensure that the validity of analytical procedures can be maintained every time it is used [20]. The test results show the average resolution value obtained is 4.4; the average value of the tailings factor was obtained 1.1, the percent RSD at retention time and area was 0.042% and 0.615%, respectively. These results meet the specified requirements, namely a good resolution or separation of two chromatogram peaks indicated by a value of $R_s \geq 1.5$; tailing factor on the chromatogram was indicated by the value of $TF \leq 2$; the percentage limit of RSD allowed for 6 repetitions is less than 1% [21].

Linearity is one of the parameters to measure whether the standard series of cinnamaldehyde at a certain concentration is directly proportional to the response of the instrument. In linearity testing, it is recommended to use a minimum of 5 concentration points [19]. The results of the linearity test obtained the linear equation $y = 271424x - 1945211$ with a value of $r = 0.9941$ and $V_{xo} = 0.05\%$. Linearity can be determined to be linear if the value of the correlation coefficient $r \geq 0.99$ [19].

LOD or detection limit is the lowest amount of analyte in a sample that can still be detected by an instrument but cannot be quantified precisely, while LOQ or quantitation limit is the lowest amount of analyte in a sample that can be quantified or precisely determined by an instrument [19]. In this study, the

LOD value was 0.0686 ppm and the LOQ value was 0.2287 ppm.

Table 2: Accuracy and Precision Test Results

Conc.	Replication	Area	Conc (ppm)	Recovery (%)	Average %recovery	% RSD
80%	1	1092 7798	52.89	97.94	98.99	0.92
	2	1110 5772	53.75	99.54		
	3	1109 7695	53.72	99.48		
100%	1	1230 0708	59.54	99.23	98.74	2.68
	2	1188 6782	57.53	95.88		
	3	1253 5272	60.67	101.12		
120%	1	1357 0548	65.68	99.51	101.95	2.55
	2	1385 9281	67.08	101.64		
	3	1427 5740	69.10	104.69		

Table 3: Analysis of Cinnamaldehyde Levels in Cinnamon Extract

Solution	Replication	Area	Conc (ppm)	% W/W	Average % W/W ($\pm\%$ rsd)
Sample cinnamon extract	1	6050486	31.33	0.195	0.193 \pm 0.594
	2	6023630	31.20	0.195	
	3	5876877	30.45	0.190	

Accuracy indicates that a method used can provide closeness of test results to the actual value, while precision indicates that a method used can provide conformity of test results if the treatment is repeated on several homogeneous samples. Tests for accuracy and precision were carried out using 3 different concentrations with 3 replications at each concentration so that nine data were obtained to be processed [19,20]. From the calculation results, the average value

of %recovery is in the range of 98.74 - 101.95%, and the average value of %RSD is in the range of 0.92 - 2.68%. The calculation results of the accuracy and precision obtained can be seen in (Table 2). In this study, the concentration of the standard solution was used in the range of 0.1 ppm, so the allowable percent recovery value was 90-108% and the maximum allowable RSD value was 3%^[19].

The results of the determination of cinnamaldehyde levels in cinnamon extract powder are listed in (Table 3). Based on the results of the determination of the cinnamaldehyde levels in the cinnamon extract, the average cinnamaldehyde content in the cinnamon extract was 0.193%.

CONCLUSION

The HPLC method met the validation requirement and can conclude to be valid. This method can be used for routine analysis for determination cinnamaldehyde in *Cinnamomum burmannii* extract.

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