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Analysis of Ethyl p-Methoxycinnamate from *Kaempferia galanga* L. Extract by High Performance Liquid Chromatography

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Abstract

Ethyl para-methoxycinamate (EPMS) is a major compound of *Kaempferia galanga* L that has anti-inflammatory effect. The purpose of this study was to determine of EPMS in *Kaempferiae galanga* L rhizome extract by High Performance Liquid Chromatography (HPLC) and evaluated the performance of the analysis. This study included determination of system suitability, accuracy, precision, linearity and range, limit of detection (LOD) and Limit of quantitation (LOQ) and selectivity. The results of system suitability test HPLC System for EPMS analysis were as follows isocratic elution system of a mobile phase mixture of methanol: water (70:30) containing 0.1% TFA, uv detector at a wavelength of 308 nm using column C18 (150×4,6 mm, 5µm) flow rate 1 ml / min. From the analysis, it was found that the average EPMS content was 78.74%. Then method had linear concentration range from 5-360 ppm, with R^2=0.9999. The LOD and LOQ were 7.0722 ppm and 21.4311 ppm respectively. The accuracy of this method that represented by % recovery was 98.02% - 101.26%. The precision of this method that expressed by Relative Standard Deviation (RSD) was 1.57%. The selectivity of this method that showed by resolution value was 2.6. Based on the results of the system suitability test and analysis performance evaluation,all parameters met the requirements.

Keywords: Ethyl para-methoxycinamate, high performance liquid chromatography, *Kaempferia galanga* L.

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1 Introduction

Kencur (*Kaempferiae galanga* L.) or known as kencur in Indonesia belongs to the Zingiberaceae family, which is one of the most common plants in Indonesia. Empirically kencur has activities to treat inflammation (anti-inflammatory) [1, 2]. In addition, the biological activity of kencur has been scientifically proven. The biological activity of

kencur is influenced by secondary metabolites and chemical compounds contained therein. The chemical compound that has anti-inflammatory properties from kencur rhizome (*Kaempferiae galanga* L.) is ethyl para-methoxycinamate (EPMS) [1, 2].

EPMS is a marker compound and the main component contained in kencur rhizome with a percentage of 80% [2]. This marker compound is needed as a comparison to confirm the presence of a plant extract in medicinal products and to determine the correctness of plant species in the analysis process. So far, the most widely used analytical process for EPMS analysis is Gas Chromatography Mass Spectrometry (GC-MS) [2]. This is because GC-MS is specific to volatile compounds. But besides that, GC-MS analysis requires a relatively high cost, and is quite complicated to only analyze the level of a sample,so that in order to reduce the cost of analysis, a cheaper method with high accuracy is needed.

At this time the most common method used for the analysis of a compound is High Performance Liquid Chromatography (HPLC). The advantage of this method lies in the accuracy of the analysis and high sensitivity and is suitable for separating nonvolatile compounds that are not resistant to heating. Research on EPMS analysis with HPLC has been conducted previously by [3] namely determining the levels of epms using HPLC at a temperature of 30 ℃. The above analysis conditions require special equipment such as a column heater. This is an obstacle for the analysis of EPMS using KCKT, because not all KCKT are equipped with column heater. Therefore it is necessary to develop a method for EPMS analysis on kencur rhizome extract at room temperature. Development of analytical methods in accordance with the United States Pharmacopeia by means of system suitability testing, assay of sample levels, and testing of validation parameters according to USP. From the research conducted, it is expected that the most optimum analytical conditions for EPMS analysis with HPLC at room temperature can be found [3,4].

2 Materials and Methods

2.1 Tools

The tools used in this research are analytical scales, blender, micropipette, filter membrane, sonicator, UV-Visible spectrophotmeter (Schimadzu UV 1800), High Kineja Liquid Chromatography instrument (Shimadzu LC 20 AT Prominance).

2.2 Materials

The materials used in this study were kencur rhizome (*Kaempferiae galanga* L.), standard ethyl p-methoxycinamate isolated in our laboratory, distilled water, pro injection water, 96% (Merck), methanol pro HPLC (Merck) ®, acetonitrile pro HPLC (Merck), Tri Fluoro acetic acid (TFA).

2.3 System Suitability Test

System Suitability Test was carried out by injecting six injections of the EPMS standard solution. Several parameters were calculated such as resolution (Rs), tailling factor, column capacity (K'), number of theoretical plates (N), relative standard deviation value (RSD) [5-10] .

2.4 Sample Analysis

100 g of *Kaempferiae galanga* L. Powder was extracted by maseration method with methanol solvent for 3 times solvent change for 3 days. The liquid extract was collected and evaporated at a temperature of not more than 50 ℃ to obtain a thick extract [4].

25 mg of the extract was then dissolved in 25ml methanol. Samples were sonicated for 5 minutes. Then filtered using a filter membrane and the solution was injected into HPLC [4].

2.5 Analysis Performance Evaluation

2.5.1 Linearity and Range

The linearity between the peak area and the concentration was analyzed using a calibration curve obtained from seven standard solutions with a concentration of 5 ppm, 10 ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm and 360ppm. Judging by the linear regression value [5-10].

2.5.2 Limits of quantification and limits of detection

The determination of the limit of quantification (LOQ) and the limit of detection (LOD) is based on the standard deviation of the response and the slope (slope) obtained from the calibration curve [5-10].

LOD and LOQ can be calculated by the formula 1 and 2.

$$\text{LOD} = \frac{3.3 \times \text{SD}}{\text{Slope}} \qquad \text{(equation 1)}$$

$$\text{LOQ} = \frac{10 \times \text{SD}}{\text{Slope}} \qquad \text{(equation 2)}$$

2.5.3 Accuracy

The accuracy of a method is carried out by means of recovery using the standard method at several concentrations. Then the percent of recovery is calculated [5-10].

2.5.4 Precision

Measurement of the standard solution was carried out for six replications with six preparations, then calculate the % RSD.

2.5.5 Selectivity

The Selectivity of this method was determined by its resolution. Resolution describe the degree of separation of analyte peak with another compound peak [5-10].

3 Results and Discussion

Methanol was chosen as the solvent for extraction because it can dissolve EPMS. Maceration method was used to extract the compound because it suitable for thermolabile substance like EPMS. The yield of EPMS extract carried out by this method was 8.4%.

Optimization of the analytical conditions was carried out by doing system suitability test. To assess the suitability of the system several parameters such as tailing factor, capacity factor, theoretical plate, resolution, RSD of retention factor, RSD of Area Under Curve (AUC) in various mobile phase was determined [5-10]. From several mobile phase that had been used, the most suitable mobile phase was methanol: water (70:30) contained 0.1% TFA. This mobile phase with isocratic elution in C18 column, a diameter of 150 × 4.6mm, 5μm, the flow rate was 1 ml/min, detected by UV detector at 308 nm, gave better resolution compared to another mobile phase. The result of system suitability test was tabulated in table 1.

Table 1. System Suitability Test Result

No.	Parameter	Result	Requirement	Conclusion
1.	Tailing factor	1.068	< 1,5	Qualify
2.	Capacity factor (K')	5.540	2-10	Qualify
3.	Theoretical plate (*N plate*)	4445.767	>2000	Qualify
4.	Resolusion	2.069	>1.5	Qualify
5.	RSD of Retention time	0.270	>1.5	Qualify
6.	RSD of AUC	0.1	>1.5	Qualify

Prior injection to HPLC system, the sample was prepared by sonicated the thick extract in methanol. From this study it was found that EPMS had retention time at 10.046 and it was faster than method that was carried out by [2] which had retention time of 12 minutes. So, the new HPLC system can reduce analytical time. To found EPMS content in *Kaempferia galanga* L extract the AUC of EPMS in the sample was extrapolated to the calibration curve. From the calculation it was found that EPMS content in the extract was 78.76 %. Chromatogram of *Kaempferia galanga* extract and standard EPMS was depicted in figure 1.

Linearity and range were determined based on the R^2 value of the calibration curve. From calibration curve it was found that linear range concentration was between 5-360 ppm. Linearity of this curve that express by R^2 was 0.9999 and it met the requirement [5-10]. Calibration curve of EPMS showed in figure 2.

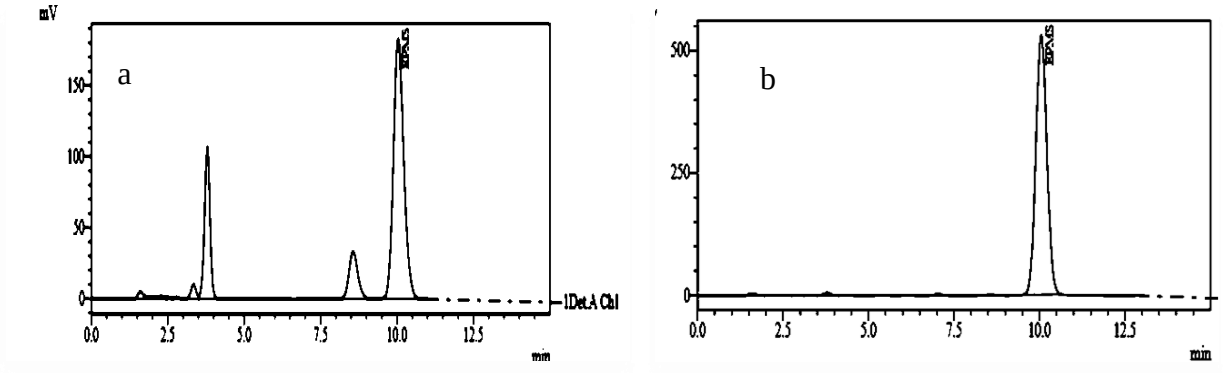

Figure 1. Chromatogram of EPMS in sample (a) and chromatogram of standard EPMS (b)

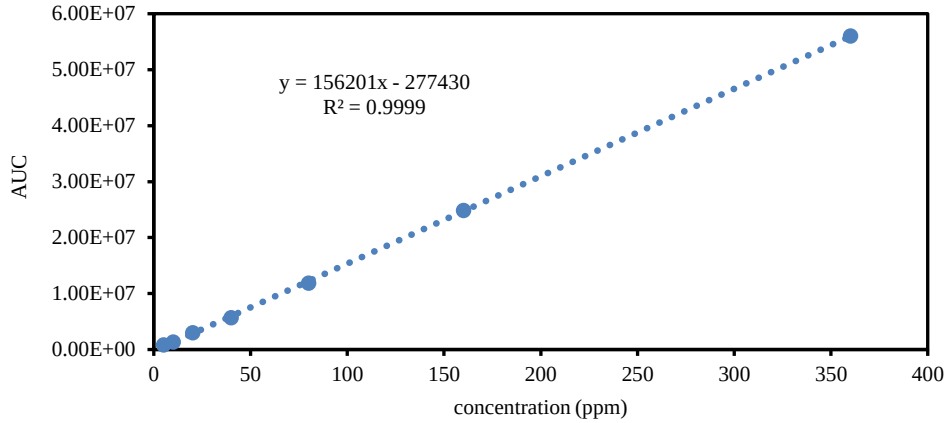

Figure 2. Calibration curve of EPMS

LOD and LOQ of the system were determined from calibration curve. The detection limit was the lowest detectable concentration of analyte. Whereas the Quantification Limit was the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy [5-10]. The detection limit and quantification limit values can be obtained from the linear regression line from the calibration curve with the linear regression equation y = 156201x - 277430. Based on the results of the calculation, the LOD was 7.0722 ppm and the LOQ was 21.4311 ppm

Precision evaluation aimed to assess the closeness of the repeatability measurement results at the same concentration and the same day. The results of the precision test were expressed in% Relative Standard Deviation (RSD) or % Coefficient of Variation (KV) [5-10]. Precision determination was carried out by injection of EPMS standard for six times at a concentration of 40 ppm, from the results of the precision test experiment on the EPMS standard, the% KV value was 1.57%. Based on the literature, the% KV value is 2% or <2% [5-10]. From the results of the analysis which is obtained on the EPMS standard has a good% KV value that is <2%.

Accuracy was done to find out the closeness of the test results to the actual level. The accuracy test was carried out against the standard with three different concentrations, namely 20 ppm, 40 ppm, and 80 ppm. each concentration is injected 3 times each. From the analysis results obtained the average value % recovery of 101.26% at a concentration of 20 ppm, 98.11% at a concentration 40 ppm, and 98.02% at a concentration of 80%. The results of the recovery calculations show that these results meet the requirements. According to the literature the average % of recovery is 98-102 [5-10]. Then it can be concluded that this method has good accuracy

Table 2. Result of Accuracy Evaluation

Concentration	Area	Experimental concentration	% Recovery	Average % Recovery
20 ppm	2806456	19.74306242	98.71531211	101.26%
	2917046	20.45106048	102.2553024	
	2934546	20.56309562	102.8154781	
40 ppm	5974465	40.02468114	100.0617029	98.11%
	5722811	38.41359024	96.0339756	
	5860504	39.29510192	98.23775479	
80 ppm	11345609	77.72472937	97.15591172	98.02%
	11863250	79.53337984	99.4167248	
	12145763	77.99988127	97.49985159	
Requirement				98-102

Selectivity is the ability of an analytical method to separate analyte from another compound in the sample [5-10]. Selectivity determination was carried out on samples containing EPMS, then analysed the resolution of the analyte with the matrix on the left and right of the analyte. From the results of the selectivity test, it shows that the analyte and matrix have a good separation with a resolution value of 2.609. According to the literature, the specificity test requirements for the resolution value of the analyte are addressed with a matrix of ≥ 2 [5-10].

4 Conclusions

From the results of the study it can be concluded that the HPLC system suitable for EPMS analysis of kencur extract was a mixture of the mobile phase methanol: water (70:30). containing 0.1% TFA with a UV detector at a wavelength of 308 nm, an isocratic elution system using a C18 column (150×4.6 mm, 5 μm) flow rate of 1 mL/min. From the analysis, it was found that the average level of EPMS in 25 mg of kencur rhizome extract contained 19.71 mg of EPMS with a percentage level of 78.74%.

The results of the system suitability test and analysis performance evaluation showed that all the parameters met the requirements therefore this method had good performance and can be used to analysis EPMS in other matrix.

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