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Determining Caffeine in Fat-Burning Supplements Using Thin Layer Chromatography-Densitometry and UV-Vis Spectrophotometer

Muhammad Luthfi Maulana*, Muhammad Theza Ghozali
School of Pharmacy, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Jl Brawijaya, Tamantirto, Kasihan, Bantul, Yogyakarta 55183.

Abstract

There are so many ways to lose weight, such as consuming fat burner supplements to burn fat faster. A fat burner supplement consists of various ingredients, such as caffeine. Fat burner supplements are usually not registered in BPOM RI. This study aims to evaluate caffeine in fat burner supplements qualitatively and quantitatively. These supplements were analyzed in the pharmaceutical laboratory in Universitas Muhammadiyah Yogyakarta using Thin Layer Chromatography (TLC)–Densitometry and UV–Vis Spectrophotometer. Sample preparation was conducted by separating caffeine from supplements by a separatory funnel with chloroform as an organic solvent. Qualitative analysis was carried out by TLC and analyzing the standard spectrum, and the sampling technique was carried out with UV–Vis Spectrophotometer. The first quantitative analysis used densitometry by measuring the spot on the TLC plate. Meanwhile, the second quantitative analysis used UV–Vis Spectrophotometer by observing absorbency value on samples with λ 273.5 nm. The result of the qualitative test using TLC was analyzed by comparing the Rf value of the standard and the sample. The Rf value of caffeine standard was 0.63, and every sample showed similar value with caffeine standard, indicating that all samples contain caffeine. The result of the quantitative test with TLC - Densitometry method revealed 1st sample 5.68 mg/ml, 2nd sample 5.74 mg/ml, 3rd sample 3.43 mg/ml, 4th sample 8.90 mg/ml, and 5th sample 1.88 mg/ml. The qualitative test result was analyzed using the UV–Vis Spectrophotometer method, and all of the caffeine standard spectra can be read at wavelength 273.5 nm, which means all samples contain caffeine. The second quantitative test result analyzed by using the UV–Vis Spectrophotometer method showed 1st sample 3.22 mg/g, 2nd sample 4.56 mg/g, 3rd sample 2.23 mg/g, 4th sample 11.22 mg/g, and 5th sample 0.26 mg/g. It can be concluded that all samples (fat burners) contained caffeine.

Keywords: Caffeine; Densitometry; Fat burner; TLC; UV-Vis Spectrophotometer

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* *Corresponding author, e-mail: luthfiiimaulanaa21@gmail.com*

INTRODUCTION

Today's modern life currently focuses on healthy lifestyles, whether based on healthy food or exercise. Lifestyle is a pattern of life expressed by someone through activity, interest, and opinion.[1] It is carried out by paying attention to lifestyle related to fitness or individual bodyweight. A person will usually pay attention to his body weight in daily life. People do exercises to regulate their body weight, such as fitness or taking fat-burned supplements. However, supplements are not necessary when someone wants a healthy fit body. A person can simply adopt a healthy lifestyle and adjust their diet to get their body fit. Therefore, before deciding to take supplements, being informative about the products is a crucial thing to do.

Supplements are products intended to complete the nutritional needs of food, containing one or more ingredients in the form of vitamins, minerals, amino acids, and other elements having nutritional values and physiological effects in the concentrated amounts.[2] An example of supplements used daily is a fat burner. Fat-burner supplements are usually used to lose bodyweight in accelerating fat burning. In this supplement, there are a number of various kinds of ingredients, such as caffeine. This substance can mostly be identified in the content of coffee beans, tea leaves, chocolate.[3] Caffeine in fat-burners usually functions to accelerate fat burning. It is also used to improve performance to be active, suppress hunger, and increase the body's metabolism.[4]

It is one of the factors that someone uses fat-burning supplements to lose weight instantly.[5] However, excessive consumption of caffeine has a number of adverse side effects for the body, such as nervousness, anxiety, insomnia, tremors, nausea, and vomiting. In Indonesia, caffeine is one of the chemical substances categorized as psychoactive that has limitations on daily consumption, which is 150 milligrams a day.[2] This study aims to identify the levels of caffeine in fat-burning supplement products on Indonesian markets by using Thin Layer Chromatography – Densitometry and UV-vis spectrophotometry. The benefit of using this method is that the method is simple, fast, and inexpensive in its application.

METHODS

The supplements were analyzed in the pharmaceutical laboratory in the Universitas Muhammadiyah Yogyakarta. The analytical methods used in this study were UV–Vis Spectrophotometer, and Densitometry or TLC Scanner (Camag TLC Scanner 4®), GF 254 silica gel (Merck KGaA®), separatory funnel (Pyrex®), analytical scales (Mettler Tolledo®), UV box, stirrer (Well Spencer®), glass beaker (Pyrex®), measuring flask (Pyrex®), glass funnel (Pyrex®), volume pipette (Pyrex®), Erlenmeyer (Pyrex®), cup, capillary tube (Vitrex®), and a stove. Meanwhile, materials used included Caffeine anhydrous (PT Brataco Yogyakarta), distilled water (PT Brataco Yogyakarta), sodium carbonate/Na_2CO_3 (PT Brataco Yogyakarta), ethanol pa (PT Brataco), pro-analysis chloroform / $CHCl_3$ (PT Brataco Yogyakarta), and commercial fat burners with brands (HHE, CSHD, UR, HHNG, FBL-C). They were then labeled 1 – 5.

Sample Preparation

A total of 2 grams of a fat burner sample was put into a glass beaker and then dissolved with 100 ml of boiling distilled

water and then filtered. The filtration was added and placed in a separatory funnel and extracted with 25 ml of chloroform in a row four times. The filtration obtained was then collected in a container. The chloroform sample was taken to be injected on a Thin Layer Chromatography (TLC) plate.

Thin Layer Chromatography Analysis

A GF254 silica plate was cut to a size of 10 cm x 3 cm and then bordered 1 cm on the top edge and bottom, marked with a little pencil stroke. The mobile phase used in this study consisted of a mixture of pro-analysis chloroform and pro-analysis ethanol by comparison 9: 1.[6] Preparation of a comparative solution to identify chemical substances using Thin Layer Chromatography was conducted by dissolving 2 mg of caffeine powder in 10 ml of chloroform. The standard relative and sample solution were injected on each TLC plate as much as one using a capillary tube at 1 cm of the bottom. An analysis was then conducted by observing the elution leaking on the TLC plate. The stain on the TLC plate was identified with a 254-nm UV light, and then the stain was marked. The Rf value was calculated and then compared to the Rf value of the sample and standard for comparison.

Analysis with Densitometry

On the TLC plate that has been analyzed containing caffeine, the levels were calculated using a densitometer. The plate was then inserted into the densitometer to be detected with a-254 nm UV light. Furthermore, an analysis using linear regression was carried out to obtain the value of the standard curve equation as shown in Figure 1 and 2.

Analysis by UV -Vis Spectrophotometry

In this study, a stock solution was prepared by weighing 100 mg of standardized caffeine and then put into a 1000 ml measuring flask and dissolved with distilled water to obtain a solution with a concentration of 100 ppm. The next step was to determine the maximum wavelength, conducted by taking 5 ml of the stock solution into a 100 ml measuring flask using a pipette, and then dissolved with distilled water to obtain a 5-ppm standard solution. The step was continued by measuring the absorption at wavelengths between 270-300 nm using a UV-Vis spectrophotometer. In this study, a calibration curve was created using a series of concentration solutions, with the following concentrations 0; 2.5; 5; 7.5; 10; and 12.5 ppm. The next step was to measure the absorption using a maximum-absorbance UV-Vis spectrophotometer, and the distilled water was used as a blank. In terms of samples analyzed by using UV-Vis spectrophotometry, chloroform solvent in Erlenmeyer was evaporated by utilizing a sublimation method in the cup; thus, the caffeine extract was obtained. The resulting caffeine extract was then put into a 100 ml volumetric flask and dissolved with distilled water to the mark, and then absorbance was measured.

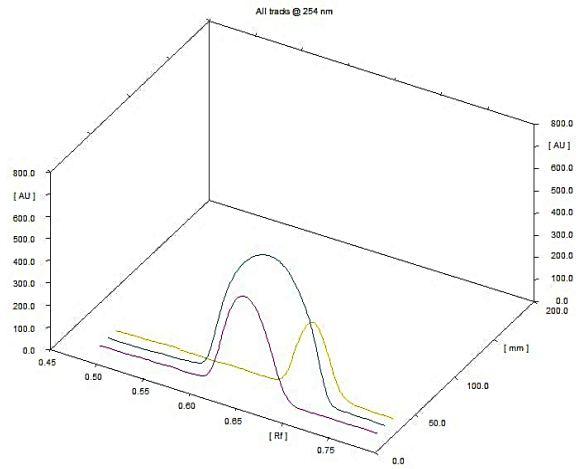

Figure 1. Densitometry value of standard, 1st sample, and 2nd sample

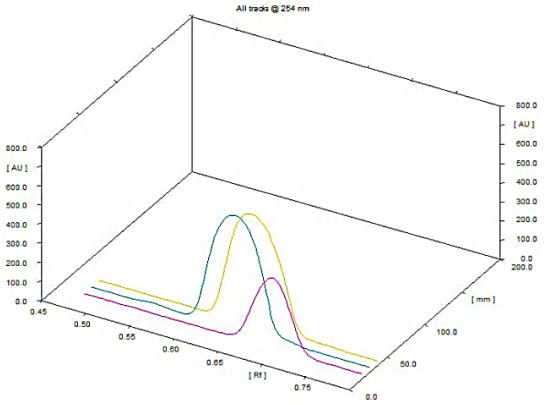

Figure 2. Densitometry value of 3rd sample, 4th sample, and 5th sample

RESULTS AND DISCUSSION

The results of this study showed that many commercial fat-burners contained caffeine. It was obtained from caffeine identification by utilizing Thin Layer Chromatography. The mobile phase in this method was a mixture of chloroform and ethanol with a ratio of 9 : 1.[6] This study used these solvents for the mobile phase as caffeine was very soluble in chloroform solvents.[7] The identification of TLC was carried out by comparing the Rf value of pure caffeine and the samples. Furthermore, caffeine was analyzed by using densitometry; thus, the Area Under Curve (AUC) and chromatogram could be identified.

Table 1. Rf value of sample

No	Sample Code	Rf Value
1	A (pure caffeine)	0.63
2	HHE (1st sample)	0.58
3	CSHD (2nd sample)	0.59
4	UR (3rd sample)	0.58
5	HHNG (4th sample)	0.55
6	FBL-C (5th sample)	0.65

In determining the levels of each fat-burner, a quantitative analysis was performed using densitometry. This study used a TLC - densitometry as this analysis method was cheaper and easier to work on.[7]

Table 2. The level of caffeine with TLC-desitometry method

Sample Code	AUC*	Concentration (ppm)
HHE	25921.4	1137.56
CHSD	26158.6	1148.21
UR	15906.5	687.87
HHNG	40258.3	1781.30
FBL-C	8961.2	376.02

*AUC = Area Under Curve

The area obtained was put into the standard curve equation, y = 22.271x + 586.75, and then the concentration of each sample could be identified. Each concentration that has been obtained was later converted, and the result of the 1st sample was 5.68 mg/ml; the 2nd was 5.74 mg/ml; the 3rd sample was 3.43 mg/ml; the 4th sample was 8, 90 mg/ml; and the 5th sample was 1.88 mg/ml.[8] This study also used UV-Vis spectrophotometry in determining caffeine in fat-burners that have been tested. In terms of calculating caffeine, samples were analyzed using UV-Vis spectrophotometry, and then absorbance of each sample was obtained. This phase was replicated three times to reduce errors the moment the absorbance was read. The absorbance obtained was entered into the equation y = 0.0457x + 0.0034. Furthermore, the concentration of each sample was found and then calculated.

Table 3. The level of caffeine with UV-Vis spectrophotometry method

No	Sample Code	Average Concentration
1	A (pure caffeine)	6.443
2	HHE (1st sample)	9.139
3	CSHD (2nd sample)	4.472
4	UR (3rd sample)	4.491
5	HHNG (4th sample)	5.386

Each concentration was later converted, and the results showed as follows: the 1st sample was 3.22 mg/g; the 2nd sample was 4.56 mg/g; the 3rd sample was 2.23 mg/g; the 4th sample was 11, 22 mg/g; and the 5th sample was 0.26 mg/g.

CONCLUSION

Based on the analysis, all samples (fat burners) contained caffeine with the result showed by TLC – densitometry as follows: the 1st sample was 5.68 mg/ml; the 2nd sample was 5.74 mg/ml; the 3rd sample was 3.43 mg/ml; the 4th sample was 8.90 mg/ml, and the 5th sample was 1.88 mg/ml. Meanwhile, UV-Vis spectrophotometry showed the results as follows: the 1st sample was 3.22 mg/g; the 2nd sample was 4.56 mg /g, the 3rd sample was 2.23 mg/g; the 4th sample was 11.22 mg/g, and the 5th sample was 0.26 mg/g.

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