HPLC determination of caffeine in coffee beverage

HPLC determination of caffeine in coffee beverage

B E P Fajara, H Susanti

Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

E-mail: susantihari@gmail.com

Abstract. Coffee is the second largest beverage which is consumed by people in the world, besides the water. One of the compounds which contained in coffee is caffeine. Caffeine has the pharmacological effect such as stimulating the central nervous system. The purpose of this study is to determine the level of caffeine in coffee beverages with HPLC method. Three branded coffee beverages which include in 3 of Top Brand Index 2016 Phase 2 were used as samples. Qualitative analysis was performed by Parry method, Dragendorff reagent, and comparing the retention time between sample and caffeine standard. Quantitative analysis was done by HPLC method with methanol-water (95:5v/v) as mobile phase and ODS as stationary phasewith flow rate 1 mL/min and UV 272 nm as the detector. The level of caffeine data was statistically analyzed using Anova at 95% confidence level. The Qualitative analysis showed that the three samples contained caffeine. The average of caffeine level in coffee bottles of X, Y, and Z were 138.048 mg/bottle, 109.699 mg/bottle, and 147.669 mg/bottle, respectively. The caffeine content of the three coffee beverage samples are statistically different ($p<0.05$). The levels of caffeine contained in X, Y, and Z coffee beverage samples were not meet the requirements set by the Indonesian Standard Agency of 50 mg/serving.

Keywords: caffeine, beverage, coffee, HPLC

1. Introduction

Caffeine is an alkaloid compound of xanthine (Purine bases) which is naturally present in coffee. Caffeine has pharmacological effects, such as stimulating the central nervous system, eliminating drowsiness, and increasing concentration. However, excessive usage of caffeine can cause heartbeat, stomach upset, trembling hands, restlessness, diminished memory, and difficulty sleeping [1]. And if consumed above 500 mg (in one drink), will lead to poisoning [2]. And during the years 1995 to 2008, as many as 52.515 peoples in the world died from excessive coffee consumption [3].

To ensure the safety of coffee which is soled in the market, the National Standardization Agency (BSN) has set standards for caffeine levels in coffee ranging from 0.45-2% w/w (SNI 01-3542-2004) or 150 mg/day and 50 mg/serving (SNI 01-7152-2006) [4]. While based on the FDA (Food Drug Administration) [5], the dose of caffeine for adult is allowed <400 mg/day. The coffee which contained high levels of caffeine need to be decaffeinated, to suppress the activity of caffeine in the body [6].

Quantitative analysis of caffeine levels in some beverages and non-beverage products has been widely practiced by previous researchers with various methods, such as the determination of caffeine levels in cola soft drinks by HPLC, and the determination of caffeine levels in black coffee by the UV-Visible spectrophotometric method [7]. The good analytical method is required to provide

accuracy, accuracy, selectivity, and high speed. HPLC becomes the preferred method because it meets these specifications. Based on the description above, the objective of this research was to determine the caffeine content in the bottle drink branded X, Y, and Z by HPLC method.

2. Materials and Methods

2.1. Material

All solvents and reagent were analytical grade, chloroform, Dragendorff reagents, HCl, cobalt chloride, double distilled water, cobalt nitrate, methanol for liquid chromatography, ethanol.

2.2. Apparatus

The apparatus was used in this was LC-20AT HPLC instrument with SPD-20A Shimadzu detector. HPLC conditions referred to previous study [8] with slight modification in mobile phase composition ie : reversed phase, C18 ODS columns 250 x 4.6 mm, detector set at 278 nm wavelength, flow rate 1 mL/min at room temperature, 20.0 μL of sample volume, and methanol motion phase: double distilled water (95:5).

2.3. Experimental

2.3.1 Sample collection

Samples were obtained from several stores which selling coffee packaging with initials X, Y, and Z which are the "3 top brand index phase 2".

2.3.2. Qualitative analysis

2.3.2.1. Dragendorff test

A total of 1.0 mL of the sample was dissolved in 10.0 mL of chloroform and 4 drops of NH_4OH, then filtered and the filtrate was inserted into a closed reaction tube. The chloroform extract in the test tube was shaken with 6.0 mL H_2SO_4 2M and the acid layer was separated into another test tube. This acid layer is dripped onto the drop plate and added Dragendorff reagent which will cause the orange red sediment [9].

2.3.2.2. Parry method

Parry method analysis was performed by means of a number of substances dissolved in alcohol, then added Parry reagents and dilute ammonia. The dark blue/green solution states that show caffeine exists (MOH, 1995). Parry reagents were made by reacting Cobalt Nitrate [$Co(NO_3)_2$] with methanol (CH_3OH)[10]

2.3.3. Quantitative analysis

The system suitability test was performed by injecting a standard solution of caffeine 60 μg/mL as 6 times in a predetermined HPLC system. The RSD value is specified for the values of t_R, AUC, and tailing factors value.

The linearity test was performed by injecting the caffeine standard soluble in ethanol with concentrations of 40, 60, 80, 100, 120, 140, 160, 180, and 200 μg/mL, respectively, into the HPLC system. The standard curve was created between the concentration of caffeine standard and AUC. [8].

In order to determine the caffeine content in coffee beverages, 5.0 mL coffee sample was inserted in a separating funnel, then extracted 4 times, each with 25.0 ml of chloroform addition. The bottom layer is taken, then the extract (chloroform layer) is evaporated over the water bath until the chloroform evaporates entirely. The solvent-free caffeine extract is dissolved with ethanol up to 5.0 mL [10].

The standard solution and sample were injected into HPLC system, with HPLC conditions as follows: reversed phase, C18 / ODS columns 250 x 4.6 mm, detector set at 278 nm wavelength, flow rate 1 mL/min at room temperature, sample volume 20, 0 μL, and mobile phase methanol: double distilled water (95:5). The caffeine content is calculated with the linear regression equation $y = bx + a$ from the standard calibration curve [11].

3. Results and Discussion

3.1. Qualitative analysis

3.1.1. Dragendorff test

The result of the qualitative test with Dragendorff test was presented in Table 1.

Tabel 1. The result of Dragendorff test on coffee beverages

Sample	Before added reagent	After added reagent	Result
Caffeine Standard	Clear	Brown precipitation	+
Sample "X"	Clear	Brown precipitation	+
Sample "Y"	Clear	Brown precipitation	+
Sample "Z"	Clear	Brown precipitation	+

Note: + = contained alkaloid

Table 1 showed that three samples reacted with Dragendorff reagent and formed a red brick/brown precipitation. The caffeine standard also showed a red brick precipitate with Dragendorff. This result showed that in the three samples contained alkaloid compounds [12]. Caffeine was a purin alkaloid compound in a plant.

3.1.2. Parry Test

The positive result of caffeine compounds in the sample is shown by the formation of a dark blue/green solution. In this test, the Cobalt (Co) ions in the reagent will form a green complex. The cobalt ion has positively charged and allow to bind to the nitrogen group present in the caffeine compound [11]. The result of the qualitative test with Parry method can be seen in Table 2.

Tabel 2. The result of Parry method test of coffee beverages

Sample	Before added reagent	After added reagent	Result
Caffeine Standard	Clear	Green	+
Sample X	Clear	Green	+
Sample Y	Clear	Green	+
Sample Z	Clear	Green	+

Table 2 showed that 3 samples which tested using Parry reagents produced a green color. The same result was shown by caffeine standard. This result indicates the presence of caffeine in the sample. Maramis was also found the same result in the study of [6] samples which contain caffeine compounds.

3.1.3. Retention Time (t_R)

Qualitative analysis of caffeine content with HPLC was done by comparing the retention time of caffeine standard (pure compound) with the sample. From the experiment, the average standard caffeine tR was 2.518 minutes and the mean t_R of X, Y, and Z samples were 2.508 minutes, 2.491 minutes, and 2.495 minutes, respectively. From the data, it is clear that t_R of standard caffeine and the three samples are relatively the same, so it can be concluded that three samples had caffeine content. The solvent, standard caffeine and samples chromatogram profiles X, Y, and Z are presented in Figure 1.

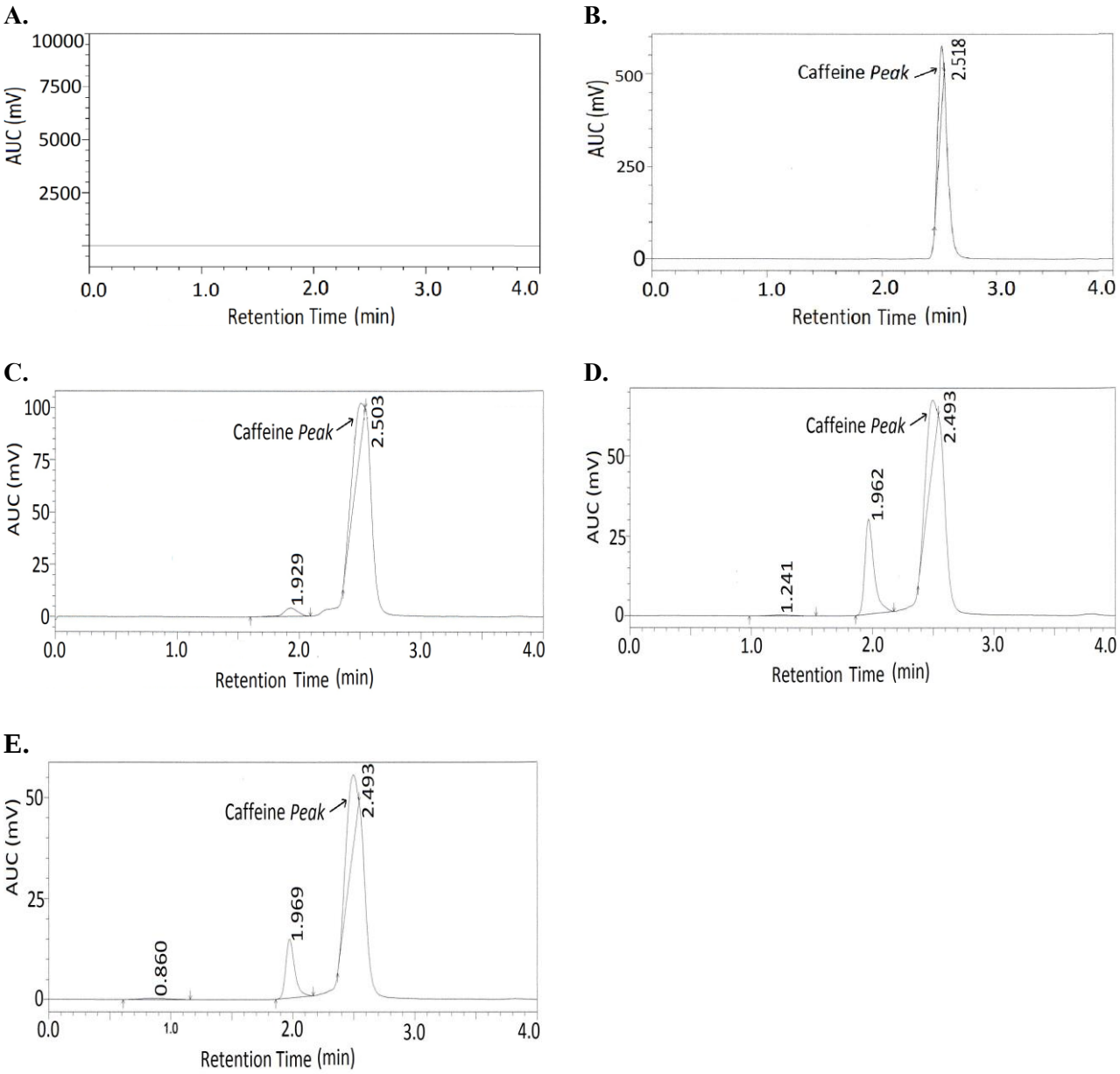

Figure 1. Chromatograms of (A) solvent, (B) caffeine standard solution, (C) sample X, (D) sample Y, and (E) sample Z

3.2. Quantitative Test

The result of system suitability test obtained RSD of tR equal to 0.199%, RSD of AUC equal to 1.476% and tailing factor equal to 0.67. These data showed that chromatographic system meets the requirements of RSD t_R and AUC < 2% and 0.5 < TF < 2[13]. The results showed a linear relationship between the concentration of standard caffeine solution with the concentration range of 40-200 µg/ml. the result was obtained linear regression equation y = 15044x + 231298.87 with the R value = 0.992 ($p < 0.05$) where X is the standard concentration of caffeine (µg/mL) and Y as AUC (mV).

Determination of caffeine content in coffee beverage samples
The caffeine content of the three coffee beverage samples as listed in Table 3 below.

Table 3. Caffeine levels in samples X, Y, dan Z

No	Caffeine Const. (mg/botle		
	X	Y	Z
1	133.159	106.953	148.797
2	130.683	109.057	146.292
3	132.969	109.583	145.541
4	132.969	112.038	148.547
5	131.445	110.810	150.049
6	131.064	109.758	146.793
mean	**132.048***	**109.699***	**147.669***
SD	**1.107**	**1.713**	**1.726**
%CV	**0.838%**	**1.562%**	**1.169%**

Note: * $p<0.05$

The caffeine content of the three coffee beverage samples are statistically different ($p<0.05$). According to Indonesian Standard Agency , the allowed caffeine levels are 150 mg/day (SNI 01-3542-2004) and 50 mg/serving (SNI 01-7152-2006) [4]. While based on the FDA (Food Drug Administration) [5] the dose of caffeine is allowed for adult <400 mg/day. However, the present study showed that caffeine content does not comply with the SNI 01-7152-2006, where in a serving should only contain a maximum of 50 mg of caffeine while in samples X, Y, and Z levels obtained are 132.048 mg/bottle; 109.699 mg/bottle, and 147.669 mg/bottle (Table 3). This level exceeds the limit set by Indonesian Standard Agency for one serving of coffee. However, based on SNI 01-3542-2004 the limit of caffeine allowed to be consumed is 150 mg/day, while the FDA regulation allowed the level of caffein <400 mg/day.

4. Conclusions

The caffeine content of the X, Y, and Z coffee beverage samples respectively were 138.048 mg/ bottle; 109.699 mg / bottle, and 147.669 mg / bottle.

The levels of caffeine contained in the X, Y, and Z coffee beverage are not comply with Indonesian Standard Agency regulation.

References

[1] Tjay H and Rahardja K *Essential Drug, It's Benefits, uses and Side effect* (Jakarta: Elexmedia Komputindo).
[2] Danasrayaningsih V 2007 Penetapan kadar kafein dalam minuman berenergi merk 'X' dengan metode spektrofotometri derivatif aplikasi peak to peak, Universitas Sanata Dharma.
[3] Freedman N D 2012 Association of coffee drinking with total and all cause mortality *Nejm*, 1–14.
[4] Arwangga A F, Asih I A and Sudiarta I W 2016 Analisis kandungan kafein pada kopi di desa sesaot narmada menggunakan spektrofotometri Uv-Vis *J. Kim. Univ. Udayana* **10** (1) 110–114.
[5] FDA FDA to Investigate Added Caffeine 2013 [Online]. Available: https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm350570.htm.
[6] Fatoni A 2015 Analisis secara kualitatif dan kuantitatif kadar kafein dalam kopi bubuk lokal yang beredar di kota Palembang menggunakan spektrofotometer UV-Vis. Palembang.

[7] Aptika N, Tunas I and Sutema I 2015 Analisis kadar kafein pada kpi hitam di Lebah Bukian Gianyar menggunakan spektofotometri UV-Vis *J. Chem. Lab.* **2 (**1) 30-37.
[8] Wanyika H N, Gatebe E G, Gitu L M, Ngumba E K, and Maritim C W 2010 Determination of caffeine content of tea and instant coffee brands found in the Kenyan market **4** 353-358.
[9] Marliana S D and Suryanti V 2005 Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah labu siam (*Sechium edule J*acq. Swartz.) dalam ekstrak etanol *Biofarmasi* **3** (1) 26–31.
[10] Maramis R K, Citraningtyas G and Wehantouw F 2013 Analisis kafein dalam kopi bubuk di kota manado menggunakan spektrofotometri Uv-Vis *J. Ilm. Farm.***2** (4) 122-128.
[11] Artanti A N and Nikmah W R Setiawan H and Prihapsara F 2016 Perbedaan kadar kafein daun teh (*Camellia sinensis* (L.) Kuntze) berdasarkan status ketinggian tempat tanam dengan metode HPLC *J. Pharm. Sci. Clin. Res.* **1** (1) 37–44.
[12] Kusrahman A 2012 Isolasi, karakterisasi senyawa aktif dan uji farmaka ekstrak biji kebiul pada mencit Universitas Bengkulu.
[13] Snyder L R, Kirkland J J and Dolan J W *Introduction to Modern Liquid Chromatography, Third Edition*, Third. **22** (1) (USA: John Willey and Son's).