International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.12 No.04, pp 43-47, 2019

Determination of Caffeine Content of Indonesia Luwak Coffee (*Mongoose Coffee*)Using High Performance Liquid Chromatography (HPLC) Analysis

[1]Rani Rubiyanti

[1]Faculty of Pharmacy, PoliteknikKesehatanKementrianKesehatanTasikmalaya
Jl. Cilolohan No.35, Kahuripan, Tawang, Tasikmalaya, 46115
West Java, Indonesia

Abstract : Analysis of caffeine in Luwak coffee with Solid Phase Extraction using High Performance Liquid Chromatography. Coffee were analysedfor caffeine by HPLC with a UV detector at 274 nm and rate 1 ml/min. The column was a reversephase Enduro C-18G (250 x 4,6 mm) and the mobile phase consisted of water: methanol (70:30, v/v). A linear calibration curve was generated with caffeine concentration ranging from 1 to 200 ppm with correlation coefficient R2= 0,999. From sample of cultivated and luwak coffee beans, 20 µl of solution were injected to the HPLC. The result showed that the elution or the retention time for caffeine for luwak cofee bean cultivated coffee sample to be 4,287 min and 4,280 min. At this retention time, the concentration of caffeine was determined to be 36,189 mg/L; 36,780 mg/L and 35,448 mg/L or 1,134 ± 0.023% w/w in dry basis.
Keywords : Caffeine content; High Performance Liquid Chromatography, Luwak Coffee.

Introduction

Coffee is the most popular beverage in the world after water[2]. It is obtained from the processing of the fruits of coffee tree, whole plant of the genus Coffeea, Rubiaceae family[1].One mainstay of Indonesian commodity is coffee that result ranks third after the rubber and pepper[7]. Indonesia have several coffee type, such as arabica, robusta and luwak coffee. Civet (*Paradoxurus Hermaphrodirus*) or Luwak in Indonesian language is wild animal that actively at night and tends to behave cannibals when gathered with a smaller civet. Civet eat fruits such as palm fruit, papaya, and banana. Also, civet animal eat coffee berries because the sweet taste of outer skin of coffee berries. Coffee berries undergoes a fermentation process for 12 hours in a civet digestive system containing enzymes and bacterial isolates. Non-digestible coffee beans come out with feces in the excretion process. Civet feces are collected, cleaned and dried with sunlight[3].

DOI= http://dx.doi.org/10.20902/IJCTR.2019.120407

Luwak coffee has a distinctive taste and unique, so it has a high selling price in the international coffee market[5]. Benefits of coffee, among othersagainst type 2 diabetes mellitus,according to Van Dam (2005) withregular coffee consumption canreduce the risk of diabetestype 2 mellitus[9]. Also according to O'Keefe etal (2013) consume coffee regularlycan reduce the risk of deathcaused by cardiovascular disease[8].But coffee also contains caffeine. It can increase muscle tension, stimulates the heart, and increase gastric acid secretion due to caffeine is consumed in excess[4]

Robusta coffee has a caffeine content is almost twice as large, amounting to 2.4% compared with arabica coffee in the amount of 1.3% in dry conditions[10]. Tasikmalaya arabica coffee beans contain 0.967 mg/g(967 ppm), Garut 0.834 mg/g(834.025 ppm), and Pangalengan 0.482 mg/g(482.25 ppm)[6]. This leads to the need for the know percentage caffeine in luwakcoffea beans form Tasikmalaya.

Methods

Tools

Soxhlet, rotary evaporator (BuchiRotavapor R-3000), water bath, analytical balance, Simplicia grinder, glass cup, funnel,measuring cup, filter paper, vacuum, scales analytical sartorius, syringe, minipore 0.45 μm, Enduro C18G column, Dionex brand HPLC Ultimate 3000 with ultravioletsinar detector looks (Ultimate 3000 wavelenght detector), Visible Spectrophotometer(Analytikjenaspecord 200®).Chemicals used is 70% ethanol, distilled water, methanol, acetic acid.

Plant Materials

Plant material used is crude drugluwakcoffee beans.

Preparation of standard solutions

Caffeine stock solution of 2000 ppm was prepared by dissolving 50 mg of standard caffeine powder with 25 ml of pure water. The caffeine working standard solutions of different concentrations 1, 5, 10, 25, 50, 100, and 200 ppm.

HPLC analyses of caffeine

The HPLC conditions followed during the experiment were: column size: Enduro C18G, 4.6 x 250 mm. Theflow rate was set at 1.0 ml/min, detector UV at 274 nm, and the sample injection volume at 20 μL. The mobile phase used in the experiment was solution of water: methanol (70:30, v/v). A calibration curve of peak areas versus concentration of the standardswas plotted using standard solutions of different concentrations (1, 5, 10, 25, 50, 100, and 200 ppm).

Results and Discussion

Method validation

To determine the caffeine content of the luwak coffee samples, the method was validated using the standard solution prepared for the experiment. The standard solutions used in experiment were 1, 5, 10, 25, 50, 100, and 200 ppm. The solutions were then injected into the HPLC machine following the chosen chromatographic conditions.

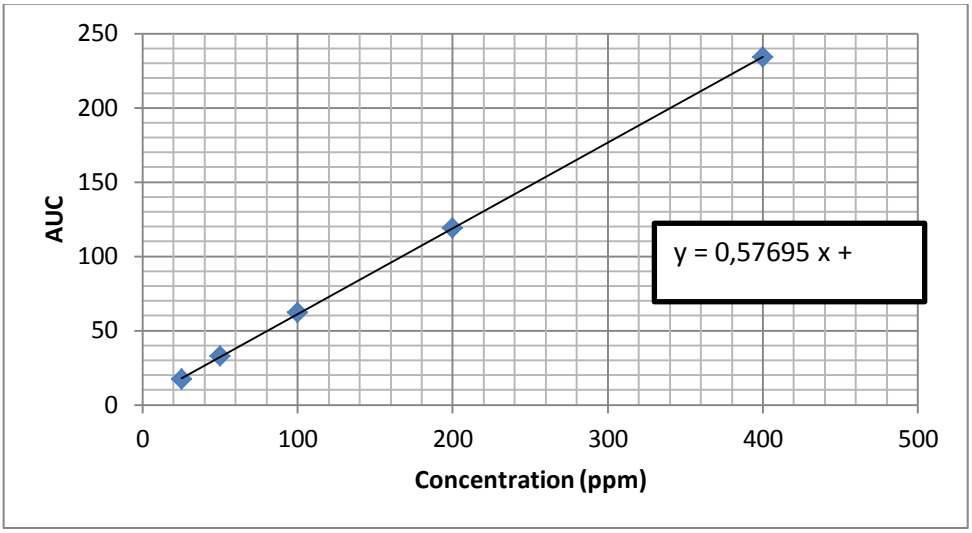

Figure 1. Linieritas Caffeine Graphic

Tabel 1 Concentration standard solution Caffeine

Concentration (ppm)	Retention time (menit)	AUC	AUC Mean
400 ppm	4,480	239.44	234,07
	4.367	232.55	
	4.327	230.23	
200 ppm	4.293	117.92	118,64
	4.287	117.54	
	4.393	120.44	
100 ppm	4.400	62.16	61,83
	4.387	61.59	
	4.360	61.74	
50 ppm	4.360	32.67	32,72
	4.353	32.78	
	4.353	32.72	
25 ppm	4.353	16.96	16,87
	4.347	16.68	
	4.347	16.98	

The solution with 0-10 ppm (blank solution) did not give any peak. A calibration curve for peak area against concentration of working caffeine standards was constructed to validate the HPLC quantification of caffeine in terms of linearity, sensitivity, precision and for calibration purpose. The curve showed good linear relationship between the peak area and concentrations of the standard solutions. Its equation was derived as Y =0,57695 x + 3,403 and calibration curve of standard (R2 = 0,999) where Y is peak area, X is concentration of caffeine (mg/L) and R is the linear correlation factor. Hence, the chosen method was taken as suitable and reproducible for the quantitative determination of caffeine extracted in coffee samples

Solid Phase Extraction

Conditioning the SPE sorbent: Conditioning step - activate an SPE cartridge stationary phase, the sorbent to by passing 6mL 50%methanol through the sorbent slowly; use a vacuum manifold. Make sure to keep the sorbent wet. Turn off the vacuum as methanol level approaches the top of packing.

Equilibration - pass 3mL of water through the packing; keep the sorbent wet and turn off the vacuum as

water level approaches the top of packing.

Loading - Introduce 1 mL of the sample made above to thepreconditionedSPE cartridge/tube/column and apply a vacuum, make sure to keep the sorbent wet. Do not discard the collected filtrateMove the SPE tube to a different location witha test tube set up to collect the filtrate. Reintroduce the above filtrate into the same SPE tube. Apply a vacuum, this time draw all liquid through. Draw air through the SPE tube for 3-5 minutes until the sorbent is dry.

Elution – Move the SPE tube with the adsorbed analyte (caffeine) from the SPE column to a new location in the manifold setup with clean dry test tube. Introduce 10 mL methanol into the SPE tube, apply a vacuum slowly, and collect the eluate in the fresh clean dry test tube. Dry the solution thoroughly using a rotor-evaporator; re-dissolve the residue in 10.00 mL aquadest.

Quantitative determination of caffeine using HPLC method The validated method, was used to determine the concentration of caffeine in the coffee samples were determined quantiatively by injecting the prepared solutions of crude caffeine of the coffee. samples used in the study. From sample of cultivated and luwak coffee beans, 20 µl of solution were injected to the HPLC. The result showed that the elution or the retention time for caffeine for luwak cofee bean cultivated coffee sample to be 4,287 min and 4,280 min (Figure 2,3). At this retention time, the concentration of caffeine was determined to be 36,189 mg/L; 36,780 mg/L and 35,448 mg/L or 1,134 ± 0.023% w/w in dry basis (Table 2)

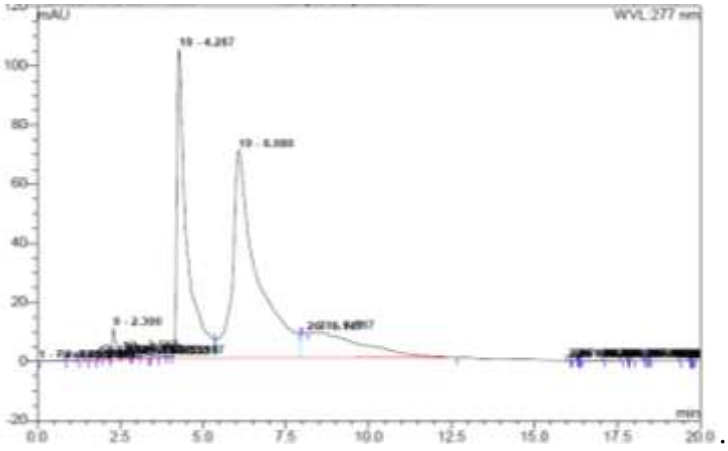

Figure 2. The HPLC chromatogram of luwak coffee beans sample 1

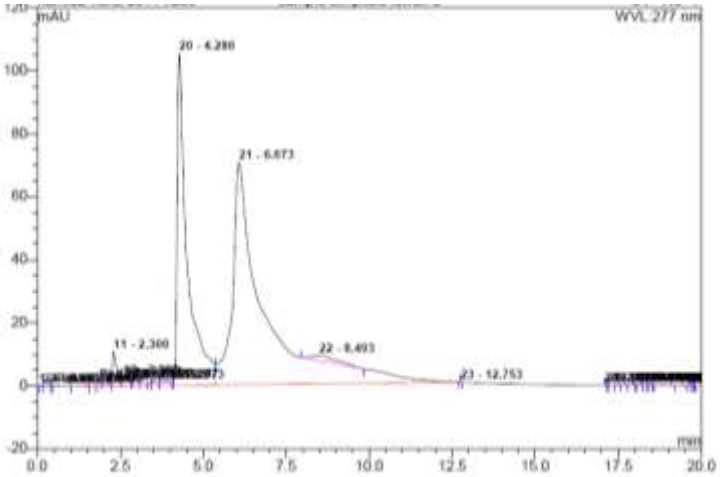

Figure 3. The HPLC chromatogram of luwak coffee beans sample 2

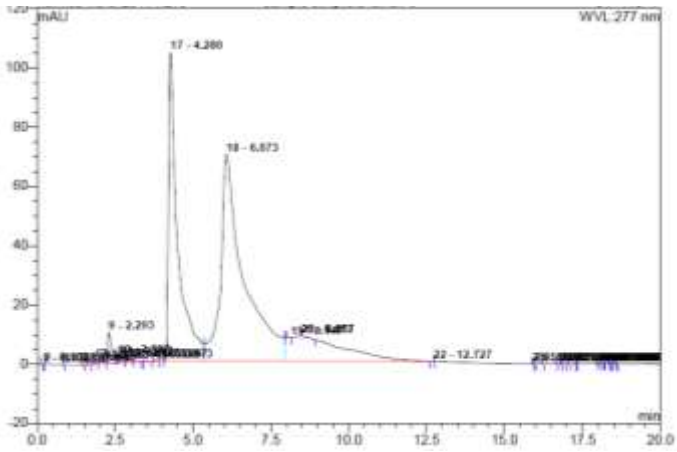

Figure 4. The HPLC chromatogram of luwak coffee beans sample 3

Tabel 2 Concentration Caffeine Luwak Coffee

Sampel	**Retention time (menit)**	**AUC**	**Concentration Caffeine (%b/v)**	**Concentration CaffeineMean (%b/v)**	**R**
Kopi Luwak	4,290	36,189	1,136	1,134	0,023
	4.280	36,780	1,157		
	4.280	35,448	1,111		

Conclusions

Quantification of caffeine contents of luwak coffee beans using HPLC analyses showed that there is difference in the caffeine contents of the arabica coffee beans in Tasikmalaya. The HPLC analysis was obtained the following results: the sample had a caffeine content of 1.134 ± 0.023%.

References

1. Davies, A., R. Govaerts, D. Bridson and P.Stoffelen. 2006. An annotated taxonomic conspectus of the genus Coffeea (Rubiaceae).Bot. J. Linn. Soc. 152:465-512.
2. George, S., K. Ramalakshmi and R. Mohan. 2008. A perception on health benefits of coffee. Crit. Rev. Food Sci. Nutr. 48:464-486.
3. Hadipernata and Nugraha. 2017. Process technology of luwak coffee through bioreactor utilization. IOP Conf. Ser.: Earth Environ. Sci. **102** 012092
4. Higdon, J. and B. Frei. 2006. Coffee and health: A review of recent Humen Research. Crit. Rev. Food Sci. Nutr. 46:101-23.
5. Kompas 2010 Luwak Coffee, from Culture Stelseel Era to Oprah Winfrey Show 18 December2010 p 1
6. Muchtaridi, Rubiyanti Rani. 2017. Determination Of Parameters Standardization Crude Drug And Extract Arabica Coffee Beans (Coffea Arabica L.).International Journal Of Scientific & Technology Research Volume 6, Issue 02, February 2017
7. Mulato, S. 2001. Pelarutan Kafein Biji Robusta Dengan Kolom Tetap Menggunakan Pelarut Air. Jakarta: Pelita Perkebunan.
8. O'Keefe et al 2013. Effects of habitual coffeeconsumption on cardiometabolic disease,cardiovascular health, and all-cause mortality
9. Van Dam and Hu FB. 2005. Coffee Consumption and Risk of Type 2 Diabetes: a Systematic review.
10. Weinberg, Bennett alan. 2001. The Word Of Caffeine. The Scientist and Culture of The Word's Most Popular Drug. Routledge: New York.

*****