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Spectrophotometric Methods for the Determination of Caffeine in Beverages Use Solvent Extraction Techniques and Adsorption of Activated Carbon

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Abstract

Caffeine has wide therapeutic uses, is widely used in the pharmaceutical field as an analgesic, and reduces fever. Caffeine is also widely added as a flavoring agent in soft drinks such as coca cola and energy drinks. Excess caffeine consumption or in the long term can have negative effects on health. The caffeine content in drinks varies by brand, from 10 to 50 mg per serving. This study aims to determine the amount of caffeine in carbonated drinks and energy drinks using chloroform solvent extraction techniques and activated carbon adsorption techniques. The amount of caffeine in the drink was analyzed by a spectrophotometric method using the maximum wavelength. The results showed that the amount of caffeine with the extraction technique in coca cola drinks was (31.39 ± 0.528 mg/serving), pepsi blue (27.93 ± 0.159 mg/serving), red bull (39.79 ± 0.233 mg/serving), and panther (43.37 ± 0.860 mg/serving). The adsorption technique obtained in coca cola drinks are (32.07 ± 0.164 mg/serving), pepsi blue (27.42 ± 0.174 mg/serving), red bull (31.35 ± 0.132 mg/serving), and panther (33.83 ± 0.205 mg/serving). In the coca cola drink, the best results are obtained close to the actual value as stated on the label. Meanwhile, for the three other types of drinks, the amount of caffeine obtained was lower than expected, and still below the maximum allowable limit. Of the two techniques investigated, the extraction technique still gave better results than the adsorption technique.

Keywords: Caffeine, Beverage, Extraction, adsorption, Spectrophotometry

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1 Introduction

Caffeine is an alkaloid compound derived from xanthine with the chemical name 1,3,7-trimethylxanthine, having the molecular formula $C_6H_{10}N_4O_2$ [1]. Pure caffeine is white hexagonal crystals, odorless, slightly bitter, has a melting point of 235-238 °C with a molecular weight of 194.19 g/mol. Caffeine is soluble in chloroform and partially soluble in water at room temperature (2 g/100 ml) [2-4]. Caffeine is the most widely consumed psychoactive substance in the world and acts as a stimulant for the central nervous system in humans [5–7]. Naturally, caffeine is found in coffee beans, cocoa beans, cola nuts, guarana fruit, tea leaves including yerba mate, and other plant species [8-9]. The amount of caffeine varies according to species, and plant origin [10].

Caffeine is widely used in pharmacological preparations as an analgesic, diet aid, and a fever-reducing drug. Clinically it was used for the treatment of neurasthenia and coma recovery [11], for the treatment of mild respiratory depression caused by narcotics and circulatory failure [12]. As one of the drugs, caffeine is most commonly consumed with more than 80 percent of the world's population consuming caffeine every day [13].

The physiological effects of caffeine have been studied extensively in various areas of human health and performance [14], including the central nervous system, cardiovascular, gastrointestinal tract, respiratory tract, and kidneys [15]. Caffeine is a central nervous system stimulant that has a mood-enhancing effect, reduces drowsiness, and restores alertness [7, 16]. Caffeine consumption may help reduce the risk of several chronic diseases including diabetes, liver disease, and cancer, as well as improve immune function [17]. Caffeine is also reported to play a role in the prevention of Parkinson's disease [18].

Consumption of excessive amounts of caffeine can have negative effects, including addiction [19], the risk of developing coronary artery disease, osteoporosis, gastritis, iron deficiency, anemia, and miscarriage [17]. Signs of caffeine poisoning include tachycardia, feeling nervous, restless, tremors, insomnia, hypertension, nausea, and seizures [19-20]. Caffeine can deplete important nutrients such as vitamin B6, interfere with the absorption of essential minerals including magnesium, iron, calcium, and vitamin B [21], and cause effects of DNA inhibitory mutations [22], long-term effects such as diuresis, stimulant disorders nervous system and gastric acid secretion [23]. The fatal dose of caffeine has been evaluated by more than 10 g (abaut 170 mg/kg of body weight). Caffeine was considered a species at risk for cardiovascular disease, kidney malfunction, asthma, and may also cause hyperactivity [24].

Caffeine is widely used as an ingredient in non-alcoholic beverages such as coca cola and soft drinks [25]. The caffeine content in soft drinks varies by brand, ranging from 10 to 50 mg of caffeine per serving [26]. The maximum limit of caffeine allowed in beverages has been set by the government based on regulations according to the Decree of the Director-General of POM No. PO.04.02.3.01510 and SNI No. 01- 6684-2002, which is 50 mg per serving and not more than 150 mg per day [27]. The purpose of setting these standards is to protect consumers from the negative effects of excessive caffeine use.

Caffeinated drinks, both types of carbonated drinks and energy drinks are often consumed by the public. However, daily caffeine intake varies depending on the amount and type of beverage product consumed. The dose of 100-150 mg of caffeine is a safe limit, so the recommended dose for the consumption of energy drink manufacturers is 2-3 times or the equivalent of 100-150 mg of caffeine per day. Consumption of energy drinks in the maximum amount is also a potential risk if accompanied by consumption of coffee, tea, or other ingredients that contain caffeine. Consumption of energy drinks is not recommended for children, pregnant women, and nursing mothers.

Quantitative analysis of caffeine in both beverages and pharmaceutical preparations can be carried out by various methods. Some of these methods such as HPLC [28-29], Gas Chromatography [30-31], solid-phase extraction (SPE) [32], electrochemistry [22], spectrophotometry and titration [33-34]. Caffeine separation techniques are usually performed using organic solvents [35]. The use of chloroform for caffeine extraction has been reported as a good solvent [36-37].

The results of recent studies have reported the separation of caffeine in energy drinks using an adsorption technique using adsorbent from activated carbon [38]. Adsorption is an effective method commonly used to remove dyes and dissolved organic pollutants including organic dyes, toxic chemicals, phenols, pesticides, and cyanides [39-40]. The most widely used adsorbent in decolorizing is activated carbon [41]. The use of adsorbents is not only easy to obtain but also cheaper. This study aims to determine the amount of caffeine in carbonated drinks and energy drinks using solvent extraction and adsorption techniques which were analyzed using the Spectrophotometric method.

2 Materials and Methods

2.1 Material

All reagents and chemicals used in this study have an analytical grade. Pure caffeine is obtained from Sigma-Aldrich (Merck), Chloroform ($CHCl_3$), activated carbon, sodium carbonate (Na_2CO_3), and double-distilled water. Soft drinks coca cola and energy drinks pepsi blue, red bull, and panther are taken directly from supermarkets in the city of Gresik.

2.2 Instrument

This study used a UV-1600 PC spectrophotometer instrument, with 10 mm quartz cells used to measure the absorbance of standard solutions and sample solutions. Other equipment is analytical balance, magnetic stirrer, separating funnel, Erlenmeyer flask, beaker, stirrer device, and Whatman filter paper (No. 42).

2.3 Preparation of Solutions

Sodium carbonate (20%) was prepared by weighing 10 g of Na_2CO_3 powder and diluted in 50 ml of double-distilled water.

2.4 Preparation of Caffeine Standard Solution

Caffeine standard stock solution (1000 µg/ml) was prepared by dissolving 0.1 g of caffeine each separately in 100 ml of the solvent used (chloroform and double-distilled water). Caffeine standard working solution (100 µg/ml) was prepared by pipetting 10 ml of the aliquot of caffeine standard stock solution and put into a 100 ml volumetric flask, then diluted with a solvent to the mark.

2.5 Preparation of Standard Curves with Organic Solvents

Standard solutions of caffeine was prepared by taking a working standard solution of caffeine (100 µg/ml), respectively 0.5 ml; 1.0 ml; 1.5 ml; 2.0 ml and 2.5 ml, were transferred into a series of 25 ml volumetric flasks then diluted with chloroform to the mark. The absorbance of each standard solution was measured at maximum wavelength against the chloroform blank using 10 mm quartz cuvettes. The standard curve was obtained by plotting the concentration of the caffeine standard solution with the absorbance.

2.6 Preparation of Standard Curves with Double-Distilled Water Solvent

Caffeine standard solution was prepared by taking a working standard solution of caffeine (100 µg/ml), respectively 0.4 ml; 0.5 ml; 0.8 ml; 1.0 ml; 1.5 ml and 2.0 ml were transferred into a series of 25 ml volumetric flasks and then diluted with double- distilled water to the mark. The concentration of the caffeine standard solution obtained was 1.6 µg/ml; 2.0 µg/ml; 3,2 µg/ml; 4.0 µg/ml; 6.0 µg/ml and 8.0 µg/ml. The absorbance of each standard solution was measured at maximum wavelength against a double-distilled water blank using 10 mm quartz cuvettes.

2.7 Sample Preparation

Before analysis, the beverage sample was de-carbonated. Each bottle was opened and poured into an Erlenmeyer flask, stirred briefly, and then left for 72 hours to remove carbon dioxide gas in the drink.

2.8 Determination of caffeine by extraction technique

Caffeine extraction was determined by the technique used [35] with slight modifications. The drink sample (5 ml) was pipetted and put into a 125 ml separating funnel, then 10 ml of double-distilled water, 1.0 ml of 20% Na_2CO_3 solution, and 25 ml of chloroform were added, respectively. The solution was extracted by reverse the separating funnel several times. Each reversal of the separating funnel at least three times the lid was opened to release gas. Furthermore, the mixture is left to stand for ±10 minutes to form two layers. The non-aqueous layer of chloroform was collected in a 50 ml volumetric flask, while the aqueous layer was added another 25 ml of chloroform. The extraction procedure was repeated twice and the resulting chloroform layers were combined and then diluted with chloroform to the mark. The absorbance of the solution was measured using a spectrophotometer at a wavelength of maximum use solvent blank.

2.9 Determination of caffeine by adsorption techniques

Separation of caffeine by adsorption technique is according to the method proposed by [38]. The drink sample (15 ml) was pipetted, put into a 250 ml Erlenmeyer flask and 0.150 g of activated carbon was added. The mixture was stirred briefly, let stand for 5 minutes then filtered using Whatman filter paper (No. 42). The filtrate obtained was diluted with double-distilled water in a 100 ml volumetric flask to the mark. The absorbance of the solution was measured using a spectrophotometer at a maximum wavelength against a double-distilled water blank. The amount of caffeine in the drink was calculated from the regression equation obtained from the standard curve of caffeine solution.

3 Results and Discussion

3.1 Determination of Calibration Curves

Before the drink samples were analyzed, the maximum wavelength (λ_{maks}) was measured from a standard solution of caffeine with a UV-1600PC spectrophotometer at a wavelength of 200-400 nm against a solvent blank. The measurement results obtained the maximum absorbance with chloroform solvent and double-distilled water, respectively 275 nm and 272 nm. These wavelengths are then used to measure the absorbance of standard solutions of caffeine and carbonated and energy drink samples.

Based on the standard curve of both the chloroform solvent and the double-distilled water solvent, good linearity was obtained. Each complies with the Lambert-Beer law with a concentration range of 1.6–8.0 μg/ml (Figure 1a) and 2.0–10 μg/ml (Figure 1b). Based on this curve, a linear regression equation was obtained $y = 0.0433x + 0.0192$ for organic solvents with a correlation coefficient of $r = 0.997$. Meanwhile, with double-distilled water solvent, the regression equation is $y = 0.0511x + 0.0074$ with a correlation coefficient of $r = 0.999$. Both equations produce r values close to 1.0, indicating good linearity or there is a correlation between caffeine concentration and absorbance.

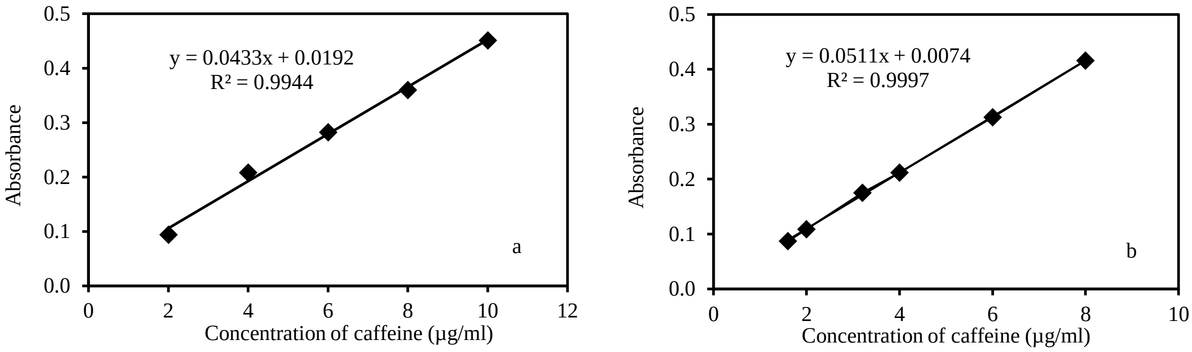

Figure 1. Calibration curve of caffeine standard solution using solvent (a). Chloroform, (b). Double distilled water.

Table 1. The amount of caffeine in beverages with extraction techniques

Type of drink (Amount of caffeine)	Amount of caffeine (mg/100 ml)	Amount of caffeine (mg/serving)	Result %	E_{rel} %	Mean ± SD (mg/serving)
Coca cola	8.15	31.78	102.50	1.25	31.39 ± 0.528
(31 mg/390 ml)	8.10	31.60	101.92		
	7.89	30.79	99.31		
Pepsi blue	8.45	27.88	79.65	-20.27	27.93 ± 0.159
(35 mg/330 ml)	8.42	27.80	79.44		
	8.52	28.11	80.31		
Red bull	15.82	39.54	79.08	-20.42	39.79 ± 0.233
(50 mg/250 ml)	15.93	39.83	79.65		
	16.00	40.00	80.00		
Panther	17.57	43.93	87.85	-13.26	43.37 ± 0.860
(50 mg/250 ml)	16.95	42.37	84.73		
	17.52	43.81	87.62		

3.2 Determination of Caffeine by Extraction Techniques

Determination of caffeine in carbonated drinks and energy drinks by extraction techniques using chloroform solvents, obtained the results as listed in Table 1. Various types of organic solvents for caffeine separation have been widely used. Studies on the use of solvents for separation of caffeine have been reported such as dichloromethane, water, chloroform, and ethyl acetate [42], water, ethyl acetate, chloroform, and methanol [37]. The extraction in this study used chloroform because it is a good solvent to separate and purify caffeine from the solution compared to organic solvents such as benzene, diethyl ether, ethyl acetate, and hexane [36 [. The same thing is according to the results of research by Maidon et al., (2012), chloroform is the best selective solvent because of its polarity and has a good ability to dissolve the caffeine.

Based on Table 1, the results of the average amount of caffeine in coca cola drinks are (31.39±0.528 mg/serving), blue pepsi (27.93±0.159 mg/serving), red bull (39.79±0.233 mg/serving) and panther (43.37±0.860 mg/serving). Meanwhile, the average percentage of the amount of caffeine in coca cola drinks was 101.25%, pepsi blue was 79.80%, red bull 79.58%, and panther 86.74%. The amount of caffeine obtained in coca cola drinks is close to the true value with the smallest relative error of 1.25%. This result is slightly higher, it is suspected that some additives are also extracted, or carried away when moving the solution from the separating funnel so that it absorbs light at the wavelength used when analyzed. For the other three drinks, the results were slightly lower than expected with the relative errors of pepsi blue, red bull, and panther drinks -20.27%, -20.42%, and -13.26%, respectively.

The accuracy can be increased by a repetition of the extraction and increasing the amount of solvent so that the extraction is impeccable. The shuffling factor can also determine the solubility level of caffeine in a chloroform solvent. The composition of many ingredients, such as red bull and Pepsi blue drink, is thought to inhibit caffeine transfer during the extraction process. Alternative extraction procedures need to be investigated for additives that could interfere with the absorbance readings when analyzed.

3.3 Determination of Caffeine by Adsorption Techniques

The results of the average amount of caffeine in beverages with adsorption techniques using activated carbon are listed in Table 2. The use of activated carbon proposed in this study is to absorb the dyes contained in beverages. The purpose of removing dyes in beverages is so as not to interfere with the absorption of caffeine when analyzed using a spectrophotometer.

Table 2. The amount of caffeine in beverages with adsorption techniques

Type of drink (Amount of caffeine)	Amount of caffeine (mg/100 ml)	Amount of caffeine (mg/serving)	Result %	E_{rel} %	Mean ± SD (mg/serving)
Coca cola	8.24	32.14	103.67	3.45	32.07 ± 0.164
(31 mg/390 ml)	8.25	32.19	103.83		
	8.17	31.88	102.84		
Pepsi blue	8.36	27.58	78.80	-21.65	27.42± 0.174
(35 mg/330 ml)	8.25	27.24	77.81		
	8.32	27.45	78.43		
Red bull	12.60	31.49	62.98	-37.30	31.35 ± 0.132
(50 mg/250 ml)	12.49	31.23	62.46		
	12.53	31.33	62.66		
Panther	13.62	34.05	68.10	-32.33	33.83 ± 0.205
(50 mg/250 ml)	13.52	33.81	67.61		
	13.46	33.64	67.29		

Activated carbon is the most widely used adsorbent for removing color, and other pollutants in industrial waste treatment [39, 41], for purification in the chemical and pharmaceutical industries, and in wastewater treatment [43]. The surface of activated carbon is nonpolar, easier to absorb color, adsorbs certain gases and chemical compounds which are selective, depending on the size or volume of the pores and their surface area [44]. Activated carbon also known as solid sponge [45], has a specific surface area, a high adsorption capacity, a higher level of surface reactivity, and a micro-pore structure sufficient to remove various types of dyes [46-47]. Activated carbon has a very large absorption power, namely 25-100 % by weight of activated carbon [44]. When compared with organic solvents, the use of adsorbents is cheaper, easier to obtain, and more efficient. Adsorbent material can be obtained from industrial waste, and agricultural byproducts such as bark, rice grain, soybean skin, straw, and others. These by-products are generally rich in cellulose and hemicellulose so that they can function as absorbents [48-49].

Based on Table 2, the results of the average amount of caffeine in coca-cola drinks was found (32.07±0.164 mg/serving), pepsi blue (27.42±0.174 mg/serving), red bull (31.35±0.132 mg/serving), and panther (33.83±0.205 mg/serving). The average percentage of the amount of caffeine in coca cola, pepsi blue, red bull, and panther drinks respectively was 103.45%; 78.35%; 62.70%, and 62.67%. The amount of caffeine in coca cola drinks is close to the true value with the smallest relative error of 3.45%. These results are almost the same as the values obtained in the extraction technique using chloroform. The amount of caffeine in pepsi blue, red bull, and panther drinks were lower than expected. Each of them was found a relative error of -20.67%; -37.30%; and -32.33%. The comparison of the amount of caffeine in the extraction technique and the adsorption technique is shown in Figure 2.

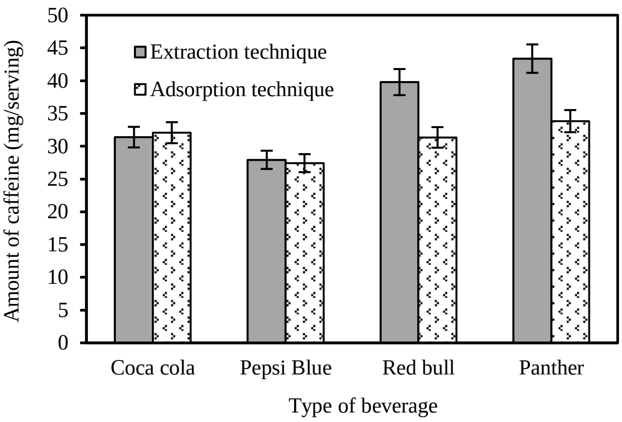

Figure 2. The amount of caffeine in beverage with extraction and adsorption techniques

From Figure 2, it can be seen that the amount of caffeine in the two methods investigated was obtained results that were not much different. In the coca cola drink the best results were obtained close to the amount of caffeine claimed on the label. Meanwhile, in the three other types of drinks, the average amount of caffeine was lower than expected. The average amount of caffeine from the three types of drinks in the resulting adsorption technique is still below the extraction technique. Except for the coca cola drink, the yield was found to be slightly higher than the extraction technique.

4 Conclusions

Determination of caffeine in beverages by extraction and adsorption techniques obtained the best results in coca cola drinks. The three other drinks, pepsi blue, red bull, and panther, were slightly lower than expected, respectively. All three are still below the maximum allowable limits. Of the two techniques studied, the extraction technique still gave better results than the adsorption technique.

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