# การประยุกต์ใช้วิธี HPLC ในการวิเคราะห์หาปริมาณคาเฟอีนในผลิตภัณฑ์กาแฟปรุงสำเร็จสำหรับลดน้ำหนักที่วางจำหน่ายในจังหวัดขอนแก่น

สุภาวดี ดาวดี[1*], เยาวเรศ ชูลิขิต[2], ดวงสุดา อภิวัฒน์ดำรงค์กิจ[3]

## บทคัดย่อ

**การประยุกต์ใช้วิธี HPLC ในการวิเคราะห์หาปริมาณคาเฟอีนในผลิตภัณฑ์กาแฟปรุงสำเร็จสำหรับลดน้ำหนักที่วางจำหน่ายในจังหวัดขอนแก่น**

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**บทนำ:**คาเฟอีนเป็นส่วนประกอบชนิดหนึ่งที่พบโดยทั่วไปในผลิตภัณฑ์กาแฟปรุงสำเร็จสำหรับลดน้ำหนักที่วางจำหน่ายในเขตอำเภอเมือง จังหวัดขอนแก่น งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อวิเคราะห์หาปริมาณคาเฟอีนในผลิตภัณฑ์ดังกล่าว **วัสดุและวิธีการทดลอง:** โครมาโทกราฟีแบบของเหลวสมรรถนะสูงได้ถูกประยุกต์ใช้ในการวิเคราะห์หาปริมาณคาเฟอีนในผลิตภัณฑ์เหล่านี้และใช้ตัวทำละลายในการสกัด การแยกสารจะใช้คอลัมน์ชนิด Hypersil ODS และตรวจวัดที่ความยาวคลื่น 254 นาโนเมตร ส่วนสถานะเคลื่อนที่ที่ใช้ในการแยกคาเฟอีนได้ชัดเจนคือ เมทธานอลกับน้ำในอัตราส่วน 65:35 วิธีการวิเคราะห์ได้ถูกตรวจสอบความถูกต้องและความน่าเชื่อถือโดยครอบคลุมถึง ความแม่น ความเที่ยง ความเป็นเส้นตรง ขีดจำกัดการตรวจพบและขีดจำกัดในการหาปริมาณ **ผลการศึกษา:**พบว่าคาเฟอีนที่พบในผลิตภัณฑ์เหล่านี้มีปริมาณแตกต่างกันไปตั้งแต่ 36.99-106.6 มิลลิกรัมต่อหน่วยบรรจุ ถ้าบริโภคมากเกินไปก็อาจจะก่อให้เกิดปัญหาสุขภาพได้ **สรุปผล:**ดังนั้นประชาชนที่จะบริโภคผลิตภัณฑ์เหล่านี้ในการควบคุมหรือลดน้ำหนัก ควรจะต้องตระหนักถึงผลข้างเคียงที่อาจจะเกิดขึ้นได้ในการได้รับคาเฟอีนในปริมาณที่สูงเกิน

**คำสำคัญ:** คาเฟอีน โครมาโทกราฟีแบบของเหลวสมรรถนะสูง ผลิตภัณฑ์กาแฟปรุงสำเร็จสำหรับควบคุมน้ำหนัก

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## Abstract

**Application of HPLC Method for Determination of Caffeine in Diet 3 in 1 Instant Coffee Products Commercially Sold in KhonKaen Province**

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**Introduction:** Caffeine is one of the constituent which generally found in Diet 3 in 1 instant coffee products. These products were commercially sold in Muang District, KhonKaenProvince so the aim of this study was to determine the amount of caffeine in these products. **Materials and Methods:** A simple HPLC method was applied to determine the amount of caffeine in these products. Liquid-liquid extraction was used for the extraction of caffeine from these samples. HPLC separation was carried out using Hypersil ODS C18 column and detected at wavelength 254 nm. This chromatographic condition using the mixture of methanol and water with constant ratio at 65:35 was found to complete resolution of caffeine. This HPLC method was validated in the topics of specificity, accuracy, precision, linearity, limit of detection and limit of quantitation and could give reliable results. **Results:** From the result of this study, the amount of caffeine in these products was found from 37 mg to 107 mg per pack which can cause health problem for over consumption of these products. **Conclusion:** Therefore, the awareness of consumption these products should be provided for the people who would like to use these products for the diet and weight control purpose to protect from the adverse side effect from caffeine overdose.

**Keywords:** caffeine, HPLC, diet instant coffee products

## Introduction

Caffeine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione is a bitter and white crystalline in the group of xanthine alkaloidsand is a psychoactive stimulant drug (Maryadele, 2006). Its chemical structure is shown in Figure 1. It is found in varying quantities in the beans, leaves and fruit of some plants. It is most commonly consumed by humans in infusions extracted from the cherries of the coffee plant, the leaves of the tea bush and from various foods and drinks containing products derived from the kola nut. In humans, caffeine is a central nervous system (CNS) stimulant, having the effect of temporarily warding off drowsiness and restoring alertness. Nowadays, beverages containing caffeine such as coffee, tea, soft drinks and energy drinks are very popular. Soft drinks typically contain about 10 to 50 milligrams of caffeine per serving. By contrast, energy drinks can start at 80 milligrams of caffeine per serving. Caffeine is absorbed by the stomach and small intestine within 45 minutes of ingestion and then distributed throughout all tissues of the body. Consumption of caffeine does not eliminate the need for sleep but only temporary reduces the sensation of being tired throughout the day. In general, 25 to 50 milligrams of caffeine is sufficient for most people to increase alertness. Caffeine citrate has been used in treating of breathing disorders of apnea of prematurity and bronchopulmonary dysplasia in premature infants for short and long term. Only short term treatment has risk associated with a temporary reduction of weight gain during the therapy. In large amount of ingestion and especially over extended periods of time, caffeine can lead to a condition known as caffeinism. This condition usually combines caffeine dependency with a wide range of unpleasant conditions including nervousness, irritability, anxiety, tremulous-

ness, muscle twitching, insomnia, headaches, and respiratory alkalosis and heart palpitations. Moreover, it can increase the acid production in stomach which can lead to peptic ulcers, erosive esophagitis and gastroesophageal reflux disease (Sweetman, 2011).

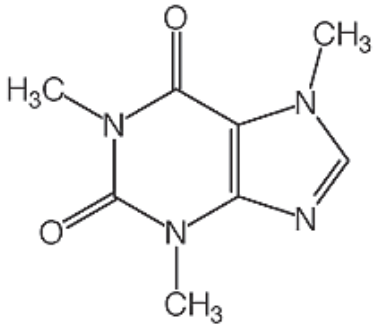


**Figure 1** Chemical structure of caffeine

In recent years, various manufacturers have begun producing Diet 3 in 1 instant coffee product which claims to be used for weight control purpose. Caffeine and other ingredients such as L-carnitine, sucralose, cactus extract and white kidney bean extract are added in these products. However, the effectiveness of such products has not been proven and they are likely to consume large amount of caffeine from these products. Therefore, it is interesting to know exactly how much caffeine is in certain products. Moreover, the possibility for the over consumption of caffeine is very high and will cause the condition of caffeinism. The simple way to analyze the amount of caffeine in these products is by using HPLC method. (Xu and Stewart 2000; Ferguson 1998; Branislava *et al.* 2008; Franeta *et al.* 2002; Altun 2002; Potard *et al.* 1999; Chutrtong *et al.* 2008).This study aims to determine the amount of caffeine in the Diet 3 in 1 instant coffee products by HPLC method.

## Materials and methods

Caffeine was purchased from Fluka® (Switzerland). All solvents were of AR and HPLC grade and were purchased from Merck® (Darmstadt, Germany). All thirteen Diet 3 in 1 instant coffee productswere collected and purchased in the area ofMuang district, KhonKaen province. A Hewlett Packard LC-1100 liquid chromatography instrument, equipped with UV detector was used in the determination of this compound under the following operating conditions; methanol-water (65:35, %v/v) as mobile phase, flow rate 1.0 ml/min. The analysis was performed on a Hypersil® ODS column (250x4.0 mm, 5µm) and this compound was detected at 254 nm. All the calculations concerning the analysis were performed with external standardization by the measurement of peak areas.

***Preparation of stock standard solution***

Stock standard solution of caffeine (1120 µg/mL) was prepared by accurately weighing (11.2 mg) in a 10 ml volumetric flask, dissolving with methanol and adjusting to volume with methanol.

***Preparation of Standard working solution***

Standard working solutions were prepared from aliquotsof the stock standard caffeine solution and diluted to volume with mobile phase used in the HPLC system to yield a solution with final concentrations of 11.2, 22.4, 44.8, 67.2, 89.6 and112 µg/mL.

***Validation of HPLC method***

The optimized HPLC method was validated for specificity, accuracy, precision, linearity, limit of detection and limit of quantitation. Accuracy of this HPLC method could be defined in terms of percent recovery from the spiked concentrationof five concentrations. Within-day and between-day precision were done to assure the precision of the method. Linearity was tested by using linear regression analysis and the coefficient of determination was then calculated. For detection limit and limit of quantitation, standard solutions were diluted until signal/noise ratio approximately close to 3 and 10 respectively.

***Preparation of sample solution and extraction method***

A sample from each pack of products was weighed and boiled in 150 mL of water. Then, 2 mL of sample solution was extracted with 2 mL of chloroform 2 times and evaporated until dry with nitrogen gas. The extract was reconstituted with 1 mL of mobile phase and 20 µL of the solution was injected into the HPLC system to determine the amount of caffeine.

## Results and discussions

From this method, caffeine was eluted at 3.68 min. The chromatogram of caffeine standard solution using this HPLC system showed complete resolution (Figure 2). In addition, this condition was found to allow complete resolution of caffeine from the other components in the sample solution (Figure 3) which showed the specificity of this method.

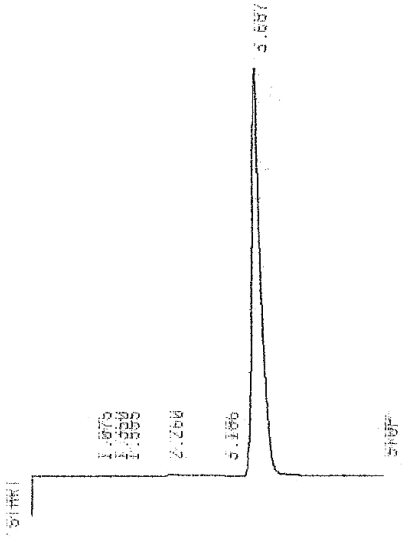

**Figure 2** HPLC chromatogram of caffeine standard solution using methanol and water (65:35) as mobile phase at a flow rate of 1 mL/min (concentration 112 µg/mL, retention time = 3.68 min)

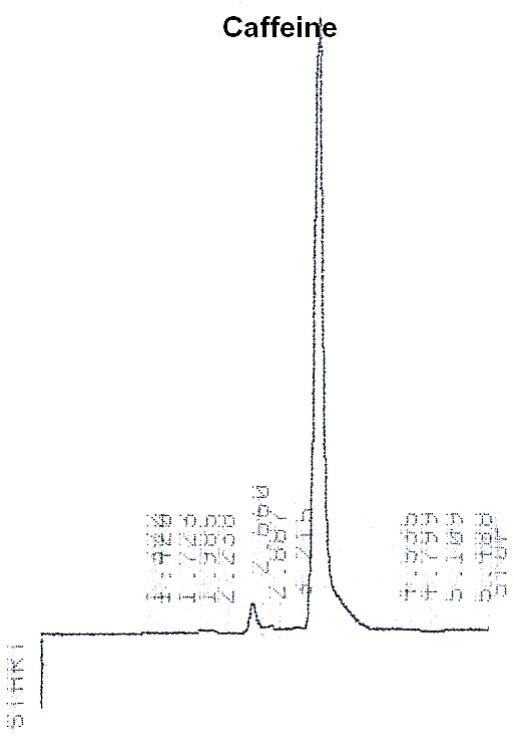


**Figure 3** HPLC chromatogram of caffeine in the extracted sample solution (Sample No. 2) using methanol and water (65:35) as mobile phase at a flow rate of 1 mL/min.

This method was validated including the criteria of accuracy, precision, linearity, limit of detection and limit of quantitation. The validation results were also shown in Table 1. The accuracy, defined in terms of percent recovery from the spiked concentrationwas closed to 100 percent (99.19 - 100.7%). The precision including within-day and between-day precision was established and expressed as the percent relative standard deviation (%RSD) was less than 1.32% for within-day and 3.19% for between-day. The precision of extraction was also calculated as percent relative standard deviation which was 2.68% for ten replicate extractions. Excellent linearity was obtained for standard solutions between the peak areas and concentrations of 11.2-112 µg/mL (n=5) with $r^2$ equal 0.999. Limit of

detection (LOD) and limit of quantitation (LOQ) were verified by five injections of caffeine solution and were calculated to be 0.011 and 0.028 µg/mL, respectively.

For the thirteen samples of Diet 3 in 1 instant coffee products, the amount of caffeine in each product is shown in Table 2 and varied from 37to 107 mg per pack. The weight of each product was varied in the range of 10.84 to 17.32 g. Therefore the amount of caffeine in these products was in the range of 0.34 to 0.72 %w/w when calculated in percent weight per pack. From the Notification of the Ministry of Public Health (No.197) B.E. 2543 (2000) Re: Coffee, dried ready-to-drink coffee, when dissolved according to label, shall have caffeine content not more than 100 mg per 100 mL, the results of this study showed that only one sample (sample No.2) contained more than 100 mg of caffeine which did not followed the Notification of the Ministry of Public Health (No.197).

## Conclusions

In this study, the simple HPLC method was applied and used in the determination of caffeine in Diet 3 in 1 instant coffee products. The results revealed varying caffeine content in these products which can cause health problemsif over consumed. Moreover, none of the products specified the exact amount of caffeine. They mentioned only the percent instant coffee or coffee in the range of 12.0 to 25.8 percent. The information about the amount of caffeine in these products was not revealed to the consumer. Therefore, the government sector who has responsibility should be aware of these products including Diet 3 in 1 instant coffee products which contain caffeine and require list of contents to the consumer. The consumer can then decide to consume these products or not themselves. Therefore, the awareness of consumption these products should be provided for people who would like to use these products for diet and weight control purposes to protect the adverse side effect from overdose caffeine.

**Table 1** Validation results for the analysis of standard caffeine solution

| Validation criterias | results |
|---|---|
| **Accuracy** | |
| % recovery from the spiked | |
| concentration (%RSD), n=5 | 99.19 (2.70) |
| Conc. 11.2 µg/mL | 100.4 (2.03) |
| 22.4 µg/mL | 99.38 (1.14) |
| 44.8 µg/mL | 100.7 (1.63) |
| 67.2 µg/mL | 100.1 (1.00) |
| 89.6 µg/mL | 99.84 (0.38) |
| 112 µg/mL | |
| **Precision** | |
| Within-day (n=5) | %RSD |
| Conc. 11.2 µg/mL | 0.96 |
| 22.4 µg/mL | 1.32 |
| 44.8 µg/mL | 0.86 |
| 67.2 µg/mL | 1.10 |
| 89.6 µg/mL | 0.70 |
| 112 µg/mL | 0.13 |
| **Between-day (n=5)** | %RSD |
| Conc. 11.2 µg/mL | 2.40 |
| 22.4 µg/mL | 2.03 |
| 44.8 µg/mL | 2.79 |
| 67.2 µg/mL | 3.19 |
| 89.6 µg/mL | 3.01 |
| 112 µg/mL | 3.19 |
| Precision of extraction at conc. | |
| 89.6 µg/mL (n=10) %RSD | 2.68 |
| **Linearity (n=5)** | |
| Regression equation | Y= 168757X+203443 |
| | $r^2$= 0.999 |
| Limit of detection (n=5) | 0.011 µg/mL |
| Limit of quantitation (n=5) | 0.028 µg/mL |

## Acknowledgements

We thank Faculty of Pharmaceutical Sciences, KhonKaen University for supporting all facilities.

**Table 2** The amount of caffeine found in thirteen samples of Diet 3 in 1 instant coffee products.

| Sample Number | Weight per pack (g) | Milligrams of caffeine per pack (mg) | Percent of caffeine content (%w/w±SD) |
|---|---|---|---|
| 1 | 16.12 (16.00*) | 91.2 | 0.57±0.03 |
| 2 | 17.32 (17.00*) | 106.6 | 0.61±0.05 |
| 3 | 15.40 (15.00*) | 75.0 | 0.49±0.06 |
| 4 | 15.07 (15.00*) | 72.9 | 0.48±0.03 |
| 5 | 15.10 (15.00*) | 57.8 | 0.38±0.02 |
| 6 | 13.72 (11.00*) | 87.2 | 0.64±0.04 |
| 7 | 16.03 (15.00*) | 100.1 | 0.62±0.05 |
| 8 | 10.84 (11.00*) | 37.0 | 0.34±0.01 |
| 9 | 13.15 (13.00*) | 95.2 | 0.72±0.04 |
| 10 | 14.36 (14.00*) | 52.3 | 0.36±0.03 |
| 11 | 12.70 (13.00*) | 74.8 | 0.59±0.01 |
| 12 | 11.35 (11.00*) | 65.6 | 0.58±0.04 |
| 13 | 13.79 (14.00*) | 90.0 | 0.65±0.06 |

* weight per pack from the label of each product.

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