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HPLC Method for Determination of Caffeine in Food Supplements for Weight Loss

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Introduction

Caffeine is 1,3,7-trimethylxantine [1], the main alkaloid of the coffee plant and an adenosine receptor antagonist. For the first time caffeine was described in 19th century. In 1820 the chemist F. Runge isolated caffeine from coffee beans [2].

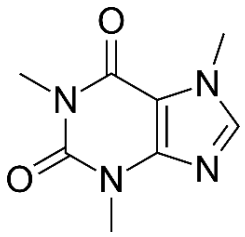

Figure 1. Structure of caffeine

The physiological effects of caffeine have been investigated for a long time and nowadays they are well know [1–10]. In humans, caffeine is rapidly and completely absorbed after oral intake, it freely crosses the blood-brain and placental barrier [6, 11, 12, 13]. Its main metabolites are paraxanthine (1,7-dimethylxanthine), theophylline and theobromine [6, 14]. The caffeine half-life is about 4 hours [6].

Caffeine intake influences the activity of neuronal control pathways in the central and peripheral nervous systems [3], cardiovascular system and diuresis.

Caffeine naturally occurs in plants like Coffee plants, Guarana, Thea sinensis, Theobroma cacao, Cola acuminata. Nowadays caffeine presents in a wide variety of beverages including coffee, tea, carbonated soft drinks, energy drinks, foods and food supplements (table 1). The average intake of caffeine depends on personal lifestyle and diet.

It is considered that the intake of moderate amounts of caffeine (20 – 200 mg day) is associated with "positive" effects on mood like alertness, efficiency, energy, concentration and motivation to work and has no harmful effects to healthy adults or occasional consumers [1, 4]. However single caffeine doses of 200 – 250 mg could acutely increase plasma renin activity, catecholamine concentrations, and blood pressure, and could induce arrhythmias in healthy adults [6, 15, 16].

The intake of of caffeine in amounts 200 – 500 mg/day may cause headache, tremor, nervousness and irritability [1, 2, 5].

The intake of higher amounts of caffeine (over 500 mg) is associated with some negative effects on health. People who suffer from hypertonia should minimise the consumption of caffeine containing foods, beverages and food supplements. All studies about the physiological effects of caffeine have been conducted on healthy adults. The adverse effects of caffeine intake in humans have not been investigated in studies which involve people suffering from neurological or psychiatric diseases, behavioural or sleep disorders, diabetes mellitus and other metabolic disorders, renal or hepatic insufficiency, open angle glaucoma etc [6]. The adverse effects of caffeine intake in hypertensive individuals has been investigated in limited studies [17]. People with medical conditions should avoid caffeine consumption.

Table 1. The caffeine content in different beverages.

Product	Caffeine content	Ref.
Espresso coffee	about 46 mg per serving/ 192 mg per 100 ml	[7]
Turkish coffee	about 60 mg per 50 ml/ 120 mg per 100 ml	[7]
Instant coffee	between 40 and 50 mg per 100 ml	[7]
Arabica – drip cofeee	80 – 120 mg per 150 ml	[7]
Decaffeinated coffee	about 3 mg per cup	
white, green, and black teas	between 14 to 90 mg per serving	[8, 9]
Instant tea	between 32 – 35 per serving	[10]
Energy drink	80 mg per 250 ml can	

Because of its stimulating and thermogenic effects caffeine is a common ingredient in many food supplements, especially in those used for weight reduction. Caffeine containing food supplements are promoted as metabolism boosters, which increase energy and suppress appetite.

Supplementation with caffeine products could have beneficial effect for weight reduction if it is combined with physical activity and low calorie diet but it hides some risks.

However the scientific data to support that caffeine is effective in promoting weight loss is limited.

The use of caffeine containing food supplements by obese people is a serious concern, because in many countries the manufacturing and labelling of supplements do not follow strict rules.

Obesity and overweight are considered as major factors in a number of diseases, including hypertonia, non-insulin-dependent diabetes, osteoarthritis etc [18, 19]. Most obese and overweight people suffer from hypertonia.

The caffeine content is often missing on the label of the food supplements used for "weigh reduction". The presence of caffeine in high doses may expose such patients on risk.

The aim of our study is to develop a fast and accurate HPLC method, which would permit us to quantify the caffeine in different food supplements in the weight loss category.

Materials and methods

Materials and reagents:

- Caffeine reference standard, Sigma-Aldrich;
- Acetonitril, HPLC grade, Sigma-Aldrich;
- Ultra pure water;
- Test samples – caffeine containing food supplements.

HPLC equipment and conditions:

Equipment: Varian Pro Star HPLC system, UV detector, chromatographic column – Microsorb – MV100-5 C18 150 × 4.6 mm, thermostat, ultrasonic bath, Hamilton syringes designed for use with manual HPLC injection ports (25 mcl).

Chromatographic conditions: mobile phase water 65%/ acetonitrile 35%, UV detection – 274 nm, flow rate 1 ml/min. Column – C18, thermostat – temperature 25°C.

Preparation of standard solutions:

Dilution with acetonitrile: water and ultrasonication in ultrasonic bath for 15 minutes.

Preparation of test samples:

Sample preparation included extraction with acetonitrile/water, ultrasonication in ultrasonic bath for 15 minutes. All test solutions were filtrated twice. The first step included prefiltration to remove large particles from the solutions. The finer filtration was performed with syringe filters (pore size 0.45 μm). All samples were analysed in triplicate.

Results and discussion

For the screening of caffeine we have used Varian Pro Star HPLC system: chromatographic column – Microsorb-MV 100 – 5 C18 150 × 4.6 mm and UV detector. The detector was set at 274 nm. We have used isocratic elution and the mobile phase composition was: acetonitril and water in a ratio 35:65 (v/v). Flow rate 1 ml/min. Retention time for caffeine – 1,86 min (fig. 2, fig. 3).

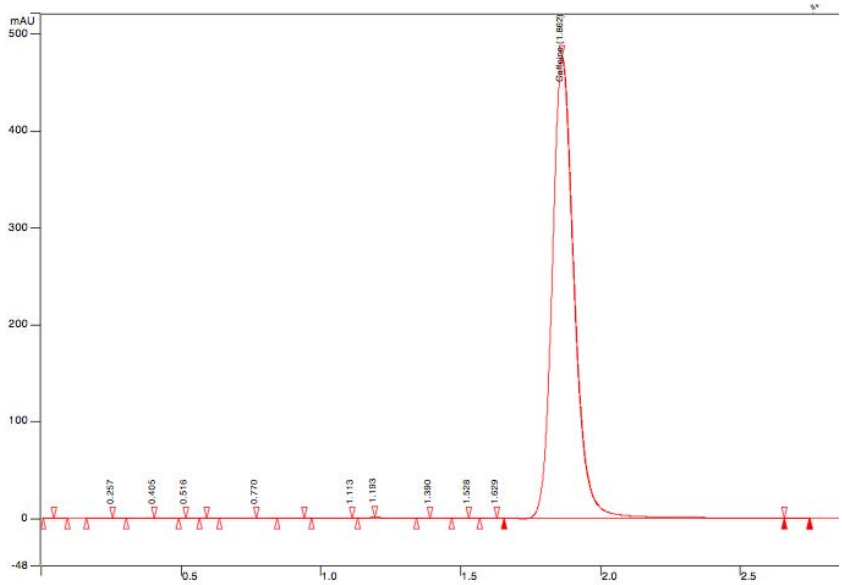

Figure 2. Chromatogram of standard solution of caffeine in concentration 60 mcg/ml

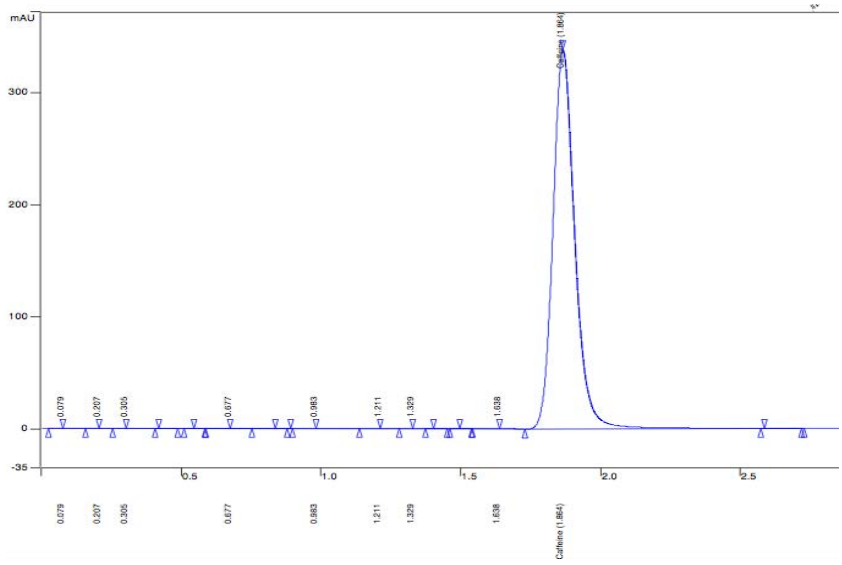

Figure 3. Chromatogram of standard solution of caffeine in concentration 40 mcg/ml

Validation of the method

The method was validated according ICH guidelines.

Linearity: a calibration curve of caffeine peak area within a concentration range of 250 μg/ml – 2 μg/ml was obtained with the following linear regression line: y = 486182x + 178544 (R2 = 0.974).

Accuracy: The accuracy was evaluated by replicating the analysis of the samples from three different concentrations 50, 75, and 100%. For each concentration level, we have performed three tests. Mean recovery: 98.76 ± 0.10% respectively. The results show that the method is accurate.

Precision: We have evaluated the intra-day precision (repeatability) and inter-day precision (intermediate precision). The RSD values, in all cases, were less than 0,9%.

Detection limit (DL): for determining the detection limit we have used the approach based on signal to noise ratio. According ICH a signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. We have estimated DL of the method to be 0,13 μg/ml (S/N = 3:1).

Quantitation limit (QL): for determining the quantitation limit we have used the approach based on signal to noise ratio – S/N = 10:1. We have estimated QL of the method to be 0,45 μg/ml.

Robustness: it was evaluated by changing the temperature of the thermostat +/- 2 degrees. The small changes in the temperature did not have significant effect on chromatographic resolution for the method.

With the proposed method we have evaluated the caffeine content in different "natural" food supplements containing green tea, coffee extracts, guarana extracts and combinations.

Before the HPLC screening we have found some disturbing results about six of the samples (capsules). The weight of the capsule content was quite different from the declared: 10 – 20% difference. In these samples we have also found great variations in the weight between different capsules in the same batch. For example: food supplement X – 500 mg (declared weight), we have estimated that the weight in five different capsules is 596, 578, 552, 543 and 598 mg. Another example: food supplement Y – 300 mg (declared weight), we have estimated that the weight in five different capsules is 420, 435, 433, 410 and 460 mg.

All capsules and tablets were weighed, powdered and dissolved in 50 ml of mobile phase and filtrated. At the end the extract was diluted in the mobile phase (100/200-fold, depends of the product) before injection into the chromatographic system. All test solutions were introduced in the chromatographic system through manual injector port. We used Hamilton Syringes for HPLC (25 mcl). Injection – 20 mcl of each sample.

We have calculated the daily intake of caffeine for every of the analysed products. The results are presented in table 2. Figure 4 represents the chromatograms of some of the analysed samples.

Weight loss strategy for overweight people should be carefully done. The supplementation programme should be recommended by a physician or another health specialist. Because most of obese/overweight people suffer from hypertonia and other medical conditions, food supplements containing caffeine should be avoided, because of the risk of intake of high single dose of caffeine. Caffeine containing supplements should be included in the diets only of healthy adults.

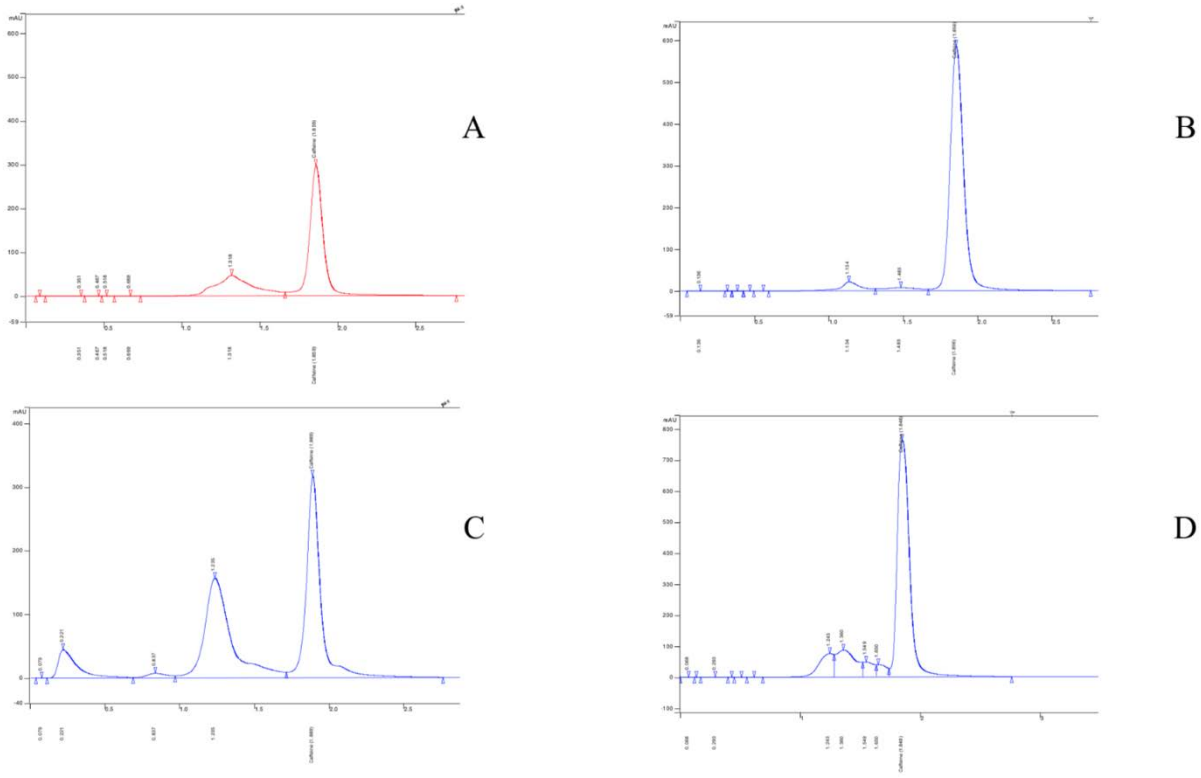

Figure 4. Chromatograms of some of the analysed samples

Table 2. Caffeine content of the analysed samples

Type of product	Caffeine in mg per one dose	Content of caffeine declared on the label per one dose	Recommended dose (on the label of the product)	Total (daily) intake of caffeine with the FS in mg
Cola extract	7,94	-	1 dose 3 times per day	23,82
Guarana extract	14,76	-	1 dose 3 times per day	44,28
Guarana extract	14	10	2 dose 4 times per day (2x4)	112
Combination – plant extracts	17,54	-	1 dose 3 times per day	52,62
Green cofee	10		2 doses per day (2x1)	20
Caffeine, vitamins, amino acids	78,12	79	1 per day	78,12
Combination – plant extracts	15,54	-	1 dose 3 times per day	46,62
Green – tea extract	18,8	13,5	2 doses per day (2x1)	37,6
Combination – plant extracts	6,6	-	2 doses per day (2x1)	13,2
Combination – plant extracts	48	60	2 doses 2 times per day (2x2)	192
Combination – plant extracts	6,9	-	2 doses per day (2x1)	13,8
Green tea extract	6,7	-	1 dose 3 times per day	20,1
Green tea extract	39	-	1 dose per day	39
Green tea extract	109,51	-	2 doses per day (2x1)	219,02
Green coffee	36	-	2 doses per day (2x1)	72
Combination – plant extracts	8,2	-	2 doses per day (2x1)	16,4
Combination – plant extracts	11,2	-	2 doses per day (2x1)	22,4
Caffeine, plant extracts	198,9	200	1 or 2 doses per day	198,9/ 397,8
Caffeine, plant extracts	197,5	200	2 doses per day (2x1)	395
Combination – plant extracts	48,9	50	2 doses per day (2x1)	146,7

Conclusion

The validated HPLC-UV method was applied for the analysis of 20 food supplements in the category "weight reduction". The content of caffeine was in moderate concentrations for almost all of the analysed samples. The highest single dose of caffeine was found to be 198.9 mg. Single dose of caffeine in this content could significantly increase the blood pressure even for healthy adults. In our view food supplements containing caffeine should be avoided by obese people, because obesity is usually accompanied by comorbid disorders, such as cardiovascular disease and others. In our view the content of caffeine should be declared on the label of every caffeine containing food supplement.

Acknowledgment

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