SHORT COMMUNICATION

Caffeine content in dietary supplements: hidden risks and implications for public health

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Summary

The rising popularity of dietary supplements, especially those marketed for weight loss and athletic performance, has generated significant concerns due to their caffeine content. This study aimed to investigate the discrepancies between declared and actual caffeine content in 16 dietary supplements, which are widely available in stores and targeted to physically active population and professional athletes. Caffeine levels were measured using UV-Vis spectrophotometry at a maximum wavelength of 274 nm. The obtained values were then compared against the declared caffeine content on the selected product labels. The results were supported by a comparative review of relevant literature sources and discussed within the broader context of caffeine supplement use and its public health implications. Our study revealed notable inconsistencies in caffeine content, with several products containing by up to 149 % more caffeine than the amount declared. Our research suggested that while caffeine contents are often higher than declared, capsules of pure caffeine emerge as a more reliable option compared to powdered supplements and energy shots, which carry a higher risk of overdosing. We emphasize the need for stricter regulations and enhanced quality control in the manufacturing of dietary supplements, especially those marketed towards active individuals and athletes.

Keywords

caffeine; dietary supplement; public health

Accurate labeling in dietary supplements is crucial for consumer safety, yet discrepancies in declared and actual caffeine content remain prevalent. The dietary supplement industry has evolved into a large market, offering a vast range of products, including protein powders, creatine, branched-chain amino acids (BCAA), β-alanine, caffeine, ω-3 fatty acids (fish oil) and pre-workout formulas. Caffeine supplements, in particular, are notable for their ergogenic effects, which vary depending on the type of exercise, its intensity and the individual's response to caffeine [1]. Caffeine, generally regarded as safe, is known to enhance performance, reaction time, muscular endurance, isometric strength, anaerobic power, delay fatigue and to increase lean mass when consumed in doses of 3–6 $mg \cdot kg^{-1}$ before or during endurance exercises [2]. Anhydrous caffeine has been recognized as more effective compared to coffee, largely due to the presence of compounds like chlorogenic acid derivatives in coffee, which may reduce the efficacy of caffeine as an adenosine antagonist [3].

Despite its benefits, the regulation of dietary supplements is notably less stringent compared to pharmaceuticals. This regulatory gap often results in significant discrepancies between the declared and actual caffeine contents in dietary supplements. Some studies have revealed that pre-workout supplements may contain caffeine at levels averaging near the lower efficacy limit for a 70 kg individual, while others report that supplements may contain caffeine in multiple forms including herbal extracts with high caffeine content [4, 5]. Consequently, total daily dietary caffeine intake could increase by up to 384 % for average adults and by 157 % for high-level consumers, surpassing the recommended limit by approximately three to four times [6, 7]. These findings align with reports on adverse effects of caffeine or caffeine-containing products, which have increased eightfold from 1980 to 2013 [8].

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A study by Cappelletti et al. [9] confirmed that caffeine toxicity, especially at plasma levels above 15 $mg \cdot kg^{-1}$, has been linked to serious outcomes in athletes.

It is important to note that higher caffeine intake does not necessarily lead to better performance outcomes. For example, a study by Pasman et al. [10] investigating the effects of caffeine on endurance performance in aerobically trained cyclists found no significant difference in performance gains between moderate and high caffeine dosages. Furthermore, Pickering and Grgic [11] reported that caffeine might increase anxiety and disrupt sleep quality, thereby adversely affecting performance of athletes.

Highlighting the risks associated with specific supplements, Beauchamp et al. [12] drew attention to the dangers of powdered caffeine products, which in extreme cases can lead to fatal overdoses. Their study showed that a teaspoonful of powdered caffeine could contain as much caffeine as 25 cups of coffee. The health risks associated with excessive caffeine intake are well-documented and include cardiovascular complications like arrhythmia and seizure [13].

Musgrave et al. [14] underscored the dose-dependent adverse effects of caffeine and its varied metabolism, particularly in slow metabolizers, highlighting the risks for specific groups using high-caffeine supplements. This finding is critical for the athletic community, emphasizing the need for cautious and informed consumption of these products. The importance of this issue is further emphasized by forensic studies and case reports detailing caffeine toxicity, including fatalities, which demonstrate the severe health risks associated with high caffeine intake. While fatalities from caffeine overdose are infrequent, the potential for such events is notably higher in individuals with underlying health issues or when caffeine is used in conjunction with other substances.

In this study, we investigated the consistency between declared and actual caffeine content in sports supplements available on the Serbian market, aimed at the active population and professional athletes. For this purpose, we used a UV-Vis method with an adapted standard sample preparation, with some findings confirmed by HPLC analysis.

MATERIALS AND METHODS

In this study, 16 dietary supplements in the form of gels, shots or capsules, offered for sale in the markets in Serbia between November and December 2023, were analysed. The supplements came from various manufacturers. For the two caffeine capsule preparations, five capsules from each were randomly sampled and analysed (Tab. 1).

All reagents used were of analytical-reagent grade (Merck, Darmstadt, Germany). All solutions were prepared using distilled and deionized water. For the spectrophotometric method, the following reagents were prepared: 1 $mol \cdot l^{-1}$ $Zn(CH_3COO)_2$ solution, 0.25 $mol \cdot l^{-1}$ $K_4[Fe(CN)_6]$ solution and 0.05 $mol \cdot l^{-1}$ H_2SO_4. Sample preparation for non-capsule supplements involved measuring 2 ml for shot preparations and 0.5 g for powder supplements. These samples were prepared according to an optimized UV-Vis spectrophotometric method for caffeine analysis, as detailed in the work of Dobrinas et al. [15]. For 5 out of the 16 samples, results obtained by this method were confirmed using HPLC analysis. Analyses were carried out on Agilent 1260 RR HPLC instrument (Agilent Technologies, Santa Clara, California, USA) equipped with diode-array detector working in the range of 190–550 nm. The samples were separated using reverse phase Zorbax SB-C18 (Agilent Technologies) analytical column (150 mm × 4.6 mm; 5 μm particle size). Isocratic elution was performed using water-acetonitrile (65 : 35, v/v) as a mobile phase at a flow rate of 1 $ml \cdot min^{-1}$. The detection wavelength was set at 274 nm. The injection volume was 10 μl and the column temperature was maintained at 25 °C. Identification of the compounds was achieved by comparing their UV spectra and retention time with those from authentic substances. The amount of caffeine was calculated using a calibration curve.

For the calibration curve, caffeine solutions were prepared using caffeine powder (Reagent Plus, Sigma Aldrich, St. Louis, Missouri, USA). An initial solution of 0.1 $mg \cdot ml^{-1}$ was prepared by dissolving 0.0100 g of caffeine in a 100 ml volumetric flask with distilled water, followed by sonication in an ultrasonic bath at 65 °C. Working standard solutions (3, 6, 9, 12 and 15 $mg \cdot l^{-1}$) were prepared and used to generate a standard calibration curve, where absorbance values were plotted against concentrations. The absorption spectrum showed a maximum at 274 nm, and both the standard solutions and samples were analysed at this wavelength at room temperature. Deionized water was used as the blank.

The spectrophotometric analysis was conducted using a UV-Vis spectrophotometer LLG-uniSPEC 2 (Lab Logistics Group, Meckenheim, Germany) with a quartz cuvette of a 10 mm

Tab. 1. Declared and determined caffeine content in dietary supplements.

Number of product	Country of production	Type of product	Caffeine content per package		
			Declared total [mg]	Determined [mg]	Percentage of declared [%]
1	United Kingdom	Gel	75	86	+14
2	United Kingdom	Powder	350	376	+7
3	Belgium	Shot	400	433	+8
4	Czechia	Powder	200	498	+149
5	United Kingdom	Shot	250	368	+47
6	Czechia	Shot	200	329	+64
7	Serbia	Shot	200	204	+2
8	Czechia	Shot	310	543	+75
9	EU	Shot	400	384	–4
10	Belgium	Shot	400	526	+32
11	United Kingdom	Powder	400	174	–56
12	Poland	Shot	480	446	–7
13	Poland	Shot	400	445	+11
14	EU	Shot	150	301	+101
15	Hungary	Capsule	100	96	–4
			100	98	–2
			100	93	–7
			100	94	–6
			100	92	–8
16	USA	Capsule	200	191	–4
			200	182	–9
			200	181	–9
			200	188	–6
			200	196	–2

path length. A linear relationship was observed between the absorbance and caffeine concentration in the standard solutions, expressed by the equation (Eq. 1)

$$y = 53.685x \quad (1)$$

where y is absorbance (A) and x is concentration (expressed in milligrams per litre), and a correlation coefficient (Eq. 2)

$$R^2 = 0.9961 \quad (2)$$

indicating high linearity (Fig. 1).

Measurements of absorbance were taken in triplicate. Quality control measures included the use of blank solutions, replicates, and regular calibration checks to ensure the reliability and accuracy of the spectrophotometric data.

RESULTS AND DISCUSSION

The results of our analysis of caffeine content in dietary supplements are presented in Tab. 1. Some samples had caffeine content that matched the declared amounts, while in six out of 16 products (37.5 %), the content was significantly higher, up to 149 % of the declared value. The HPLC analysis of five supplements revealed that the caffeine contents detected by UV-Vis spectroscopy differed by up to 5.4 % from those measured by HPLC.

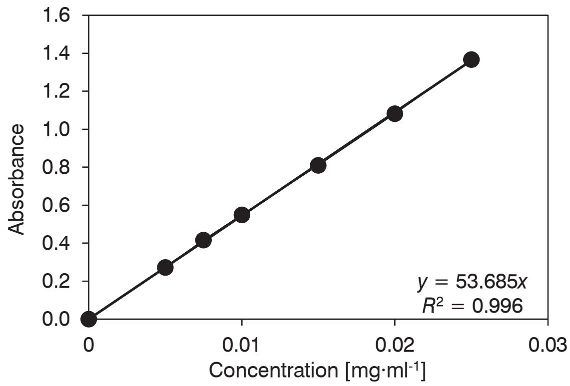

Fig. 1. Linear relationship of absorbance to caffeine concentration in standard solutions.

Our study demonstrated that, compared to liquid and powder supplements, capsules exhibited remarkable labelling accuracy, with actual caffeine content closely matching the declared amounts and standard deviations of less than 10 %. Similarly, Bailey et al. [16] found that caffeine content in liquid supplement forms deviated more significantly from the declared values compared to non-liquid supplements. At the time of their study, Dietary Supplement Label Database, developed by the Office of Dietary Supplements (National Institutes of Health, Bethesda, Maryland, USA), included over 30 000 products, of which 157 were specifically labeled as "energy" supplements. Of these, 126 were non-liquid forms, primarily pills and 31 were liquid forms with serving sizes greater than 29.57 ml. Among the non-liquid products, 52 (41 %) declared caffeine on their labels and of those, 43 (83 %) provided the exact amount of caffeine. In contrast, nearly all liquid forms (30 out of 31) declared caffeine, but only 9 (30 %) of those listed the precise caffeine content.

In our study, there was only one sample with low caffeine content; most samples either matched or exceeded the declared amounts. However, Andrews et al. [17], which included a larger sample size, identified products with both significantly high and negligible caffeine content. They analysed the caffeine content of various dietary supplements, including tablets, caplets, and capsules containing caffeine-rich ingredients such as guarana, yerba mate, kola nut, and green tea extract. Using high-pressure liquid chromatography (HPLC), they tested 53 products and found caffeine intake ranging from 1 mg to 829 mg per day. For products with labeled caffeine amounts (n = 28), 89 % (n = 25) had caffeine levels per day within ±16 % of the labeled values. Approximately 72 % of the products showed lot-to-lot variability of less than 10 %, which aligns with our findings for capsule supplements. In the remaining samples from the Andrews et al. study [17], 17 % of the products had relative standard deviations between 10 % and 25 %, and 11 % exhibited deviations between 25 % and 75 %, with 5 products containing levels less than 50 mg of caffeine per day.

Desbrow et al. [18] found greater variability in caffeine content both within and between batches of the same product. They reported that only one of 15 pre-workout supplements showed consistent caffeine content within batches, while another had an average caffeine content ranging from 93 % to 176 % of the manufacturer‘s claimed amount across different batches. Had we analysed more samples of each liquid and powder supplement, we might have observed greater lot-to-lot variability as well. Additionally, this study showed results most similar to ours in terms of discrepancies between measured and declared caffeine amounts. They reported that the caffeine content in supplements ranged significantly from 91 mg to 387 mg per serving, with values varying widely from what was reported on the labels, ranging from 59 % to 176 % of the labeled amount. Given that this study is more recent than those previously cited, it is possible that its findings, along with ours, indicate that as the supplement market expands and the number of products increases, there are greater risks that caffeine content will not match the declared values.

The study by Okuroglu et al. [7], which also reflects the current state of supplements on the market, showed extreme discrepancies in the caffeine content in supplements available in the Turkish market and caffeine content on product labels. They reported that caffeine concentration in 17 products ranged from 4.52 $mg\cdot ml^{-1}$ to 471 $mg\cdot ml^{-1}$. Although the reported values are consistent with our findings, Okuroglu et al. [7] reported that eight of the 17 products had caffeine amounts between 151 % and 948 % higher than stated on the labels, indicating that the declared values were unusually low. The study also found that 29.4 % of the caffeine-containing products exceeded the recommended daily intake limit of 400 mg per day, with five products surpassing this threshold.

We investigated supplements from the European market, but the problems with caffeine level in supplements from the market in USA seems to be even more extreme, also showing significant discrepancies from the declared values. In a study [19] examining the caffeine content in dietary supplements, 213 samples from 52 different products were analysed. The findings indicated significant inconsistencies in labeling and the presence of undeclared active ingredients. The supplements included 11 tablets, 97 solid capsules, and 105 liquid capsules, primarily aimed at weight loss, bodybuilding, and diuretic purposes. Most products were manufactured in or for the United States, with one weight loss supplement of Brazilian origin. The caffeine content in the supplements varied significantly. Some samples contained less than 80 % of the declared amount, and some exceeded 120 % of the declared amount, with some reaching up to 382.2 %. The authors mentioned that Brazilian legislation mandates that caffeine supplements for athletes should contain 210–420 mg caffeine per serving, added only as anhydrous caffeine.

However, the estimated daily caffeine intake from these supplements often exceeded the safe limit of 400 mg for adults, with some products reaching up to 1 181.4 mg per day. The study also screened for undeclared active ingredients, finding that about 13 % of the samples contained undeclared substances such as phenylethylamines, synephrine, yohimbine, and 1,3-dimethylamylamine.

Additionally, VIANA et al. [20] investigated the presence of caffeine in 94 different products purchased from 30 Brazilian websites, focusing on supplements claimed to aid in weight loss, appetite reduction, fat burning, and metabolism acceleration. Focusing on caffeine, the study revealed that it was present in doses from 25.0 mg to 1 476.7 mg per day.

CONCLUSIONS

This study identified significant discrepancies between declared and actual caffeine content in dietary supplements available in the Serbian market. Specifically, 37.5 % of the products analysed had caffeine content up to 149 % higher than stated. Capsules demonstrated the highest labeling accuracy, with deviations of less than 10 %, in contrast to liquid and powder supplements which showed greater variability. Given the expansion of the supplement market and the increasing number of products, our findings, along with those from other recent studies, suggest a growing risk of discrepancies in caffeine content. This underscores the need for stricter regulatory measures, accurate labeling, and consistent quality control to ensure consumer safety.

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Received 20 February 2024; 1st revised 12 June 2024; accepted 15 July 2024; published online 22 August 2024.