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ORIGINAL RESEARCH PAPER

CHROMATOGRAPHIC DETERMINATION OF CAFFEINE CONTENTS IN SOFT AND ENERGY DRINKS AVAILABLE ON THE ROMANIAN MARKET

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Abstract: Caffeine is a stimulant that is commonly found in many foods and drinks that we consume. Concerns exist about the potential adverse health effects of high consumption of dietary caffeine, especially in children and pregnant women. Recommended caffeine intakes corresponding to no adverse health effects have been suggested recently for healthy adults (400 – 450 mg/day), for women contemplating pregnancy (300 mg/day), and for young children age 4 – 6 years (45 mg/day). Different brands of soft and energy carbonated beverages available on the Romanian market were analysed for caffeine by HPLC with a diode array UV-VIS detector at 217 nm. The column was a reverse phase C18 and the mobile phase consisted of potassium dihydrogen orthophosphate buffer (0.02 mol/L, pH 4.3) and acetonitrile (88:12, v/v). The caffeine contents in energy drink samples ranged from 16.82 mg/100 mL to 39.48 mg/100 mL while the carbonated soft drink group showed caffeine content in the range of 9.79 – 14.38 mg/100 mL. In addition, the concentrations of caffeine have been converted into the daily intake doses based on beverages consumption. The mean values of caffeine daily intakes were 124 mg and 49 mg through the ingestion of energy drinks and soft drinks, respectively.

Keywords: *caffeine, HPLC, soft drinks, energy drinks, daily intakes*

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is an alkaloid that occurs naturally in the leaves, seeds and fruit of tea, coffee, cocoa, kola trees and more than 60 other plants [1].
The widespread occurrence of caffeine in a variety of plants played a major role in the long-standing popularity of caffeine-containing products. The most important sources of caffeine are coffee (*Coffea* spp.), tea (*Camellia sinensis*), guarana (*Paullinia cupana*), maté (*Ilex paraguariensis*), cola nuts (*Cola vera*), and cocoa (*Theobroma cacao*). The amount of caffeine found in these products varies – the highest amounts are found in guarana (4 – 7%), followed by tea leaves (3.5%), maté tea leaves (0.89 – 1.73%), coffee beans (1.1 – 2.2%), cola nuts (1.5%), and cocoa beans (0.03%) [2].
Recreationally, caffeine is used to provide a boost of energy or a feeling of heightened alertness. It is often used to stay awake longer. The pleasant stimulant feeling, which often occurs at low doses, may be replaced by psychological symptoms, which resemble anxiety and depressive neuroses at high doses. Those with more severe psychological problems may have their symptoms exaggerated with excessive caffeine usage, or such symptoms can actually be caused by excess. Diagnosis of such conditions must take caffeine usage into account. Caffeine abuse is more prevalent than we may imagine [3].
Caffeine is added to soft drinks as a flavoring agent, and from dietary sources is the most frequently and widely consumed central-nervous-system stimulant today. Nevertheless, it was classified as a drug of abuse by the International Olympic Committee (IOC) when present in urine at concentration levels of more than 12 μg/mL. The high levels of caffeine consumption have been implicated in various disorders including the increase of gastric-acid secretion, kidney malfunction, heart disease (cardiac arrhythmia), and disturbances of the central nervous system such as seizures and delirium. However, caffeine is utilized as a co adjuvant in many pharmaceutical formulations [4].
The reported caffeine content in the main dietary sources varies significantly: 93.0 – 163.5 mg per cup in ground coffee, 46.7 – 67.6 mg per cup in instant coffee, 30.2 – 67.4 mg per cup in bag tea and 0.32 – 0.54 mg/g in dark sweet chocolate. The differences have been attributed to the variety of coffee bean or tea leaf, method of preparation (e.g. the brewing of coffee and tea), volume of a cup and analytical methods utilized for caffeine determination. Although the level of caffeine in chocolate is less variable, it still depends on the origin of the beans. In the case of carbonated beverages the variability occurs among brands, since most of the caffeine content in these products is added from other natural sources, i.e. less than 5% of the total present caffeine is from cola nuts [5].
Cola like drinks account for 80 to 90% of the caffeine added to foods today. These products with caffeine artificially stimulate the body and increase the heart rate. While this artificial stimulation temporarily arouses the intellect and fatigue seams to disappear, it is short lived. The excess stimulation depletes the body of vital energy as it struggles to deal with this poison that has entered its system. In the amounts presently being consumed, it can cause insomnia, nervousness, irritability, anxiety and disturbances in the heart rate and rhythm. There are other effects from the consumption of caffeine, including increased incidence of bladder and stomach cancer, raised blood pressure and it aggravates diabetes and damages the lining of the stomach [3].

Since the consumption of soft drinks and beverages is a widespread habit all around the world, hence, such foodstuff products are considered of great economically and socially importance. As it is the case with any food, the composition of soft drinks is regulated by legislations [6]. According to Directive 2000/13/EC, quinine and/or caffeine used as a flavoring in the production or preparation of a foodstuff must be mentioned by name in the list of ingredients immediately after the term "flavoring". In addition, according to Directive 2002/67/EC of 18 July 2002, drinks containing caffeine in excess of 150 mg/L must also provide a warning message on the label followed by an indication of the caffeine content such that: "High caffeine content (X mg/100 mL)".
Therefore, the determination of caffeine compounds in soft and energy drinks for assurance of food safety and quality control is mandatory. Several analytical methods have been developed for the determination of caffeine and the quality control of products containing caffeine including titrimetry, voltammetry [7], NIR-spectroscopy, derivative spectrophotometry [8], polarography, GC [9], and HPLC [5, 10 – 12].
Considering the remarks mentioned above, this present research study has been aimed to assess the compliance of the concentration levels of caffeine in various commercial drink brands available from the local market in Romania. The obtained analytical data combined with the estimated food consumption were used to calculate approximately the average daily intake doses of caffeine through the consumption of beverages.

MATERIALS AND METHODS

Reagents

All the reagents, acetonitrile (Baker, 8257), potassium dihydrogen orthophosphate 98+% (Alfa Aesar, A 12142), phosphoric acid 85% (Merck, 1805), caffeine 99.0% (Alfa Aesar, A 10431) were of analytical purity or for chromatographic use. The water used was ultra-pure, Basic TWF. The stock solutions and the corresponding dilutions were made in ultra-pure water and were stored in dark places at +4 °C between experiments.

Sample preparation

Different kinds of beverages brands, including regular and diet cola, carbonated refreshment drinks and energy drinks were purchased from different local supermarkets and 15 samples were analyzed using the indicated HPLC method. All measurements were performed in triplicate. Once sample bottles were open, the drinks were degassed, homogenized and filtered. Then each sample was filtered through a 0.45 µm syringe filter with a 5 mL syringe. Filtered drink samples of 2 mL were 10 times diluted in water.

HPLC analysis of caffeine

Filtered samples were injected for HPLC analysis according to the method developed and validated in our previous study [13]. Equipment used consisted of a Surveyor Thermo Electron system including vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP auto sampler, "diode array" detector with 5 cm flow cell and Chrom

Quest 4.2 software. The determinations were made under isocratic conditions, at 20 °C, by using a mobile phase made of 88% phosphate solution (KH_2PO_4 0.02 mol/L), adjusted to pH 4.3 with 5% phosphoric acid, filtered through a polyamide membrane (0.2 µm) and 12% acetonitrile. The volume injected was 5 µL and the flow rate of the mobile phase was 1 mL/min.
Caffeine in the degassed and filtered sample is separated by reversed phase chromatography on a 250 × 4.6 mm i.d., 5 µm particle DS HYPERSIL C18 column, detected by absorbance at the wavelength of 217 nm and quantified with a calibration graph. Caffeine was identified by comparing the retention times and spectral data with those of authentic standards. All analyses were repeated three times. The relative peak areas were determined for three replicates of each dilute sample.

RESULTS AND DISCUSSION

The HPLC method with diode array detection developed in our laboratory for the quantitative determination of caffeine in carbonated beverages provides stable retention times and a detection limit of 0.01 mg/L for a signal-to-noise ratio of 3. The method was validated in terms of sensitivity, linearity range, reproducibility, repeatability, analytical recovery and robustness. The mean recovery of caffeine was 99.25% [13].
Figure 1 presents the chromatogram obtained for one of the injections of an energy drink sample.

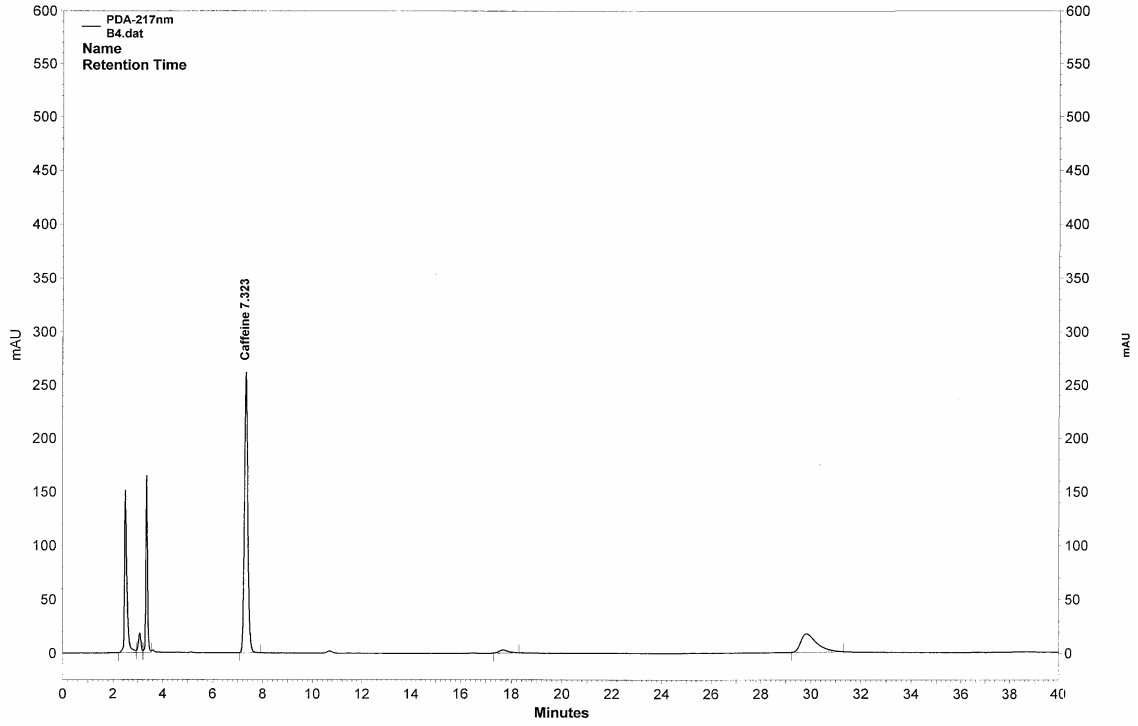

Figure 1. *HPLC at λ = 217 nm of an energy drink sample*

The concentrations of caffeine food additive (flavor enhancer) in energy drinks collected from local supermarkets are noticeably higher than their counterpart concentration levels in the refreshment soft drinks. The caffeine content levels in energy drinks and carbonated soft drinks respectively are presented in Tables 1 and 2.

Table 1. *Analytical results of caffeine contents in the energy drink samples*

Energy drinks	Caffeine content (mg/100 mL)	Daily intakes* (mg)
Rienergy Refresher	39.48 ± 0.08	157.92
Effect	32.97 ± 0.05	131.88
Energia Power Drink	24.83 ± 0.1	99.32
Maximum Speed	29.73 ± 0.14	118.92
Red Bull Energy Drink	33.45 ± 0.07	133.80
Burn Energy Drink	33.41 ± 0.11	133.64
Tiger Energy Drink	30.81 ± 0.09	123.24
B-52 Energy Drink	33.72 ± 0.05	134.88
Mem	16.82 ± 0.12	67.28
Imola	34.38 ± 0.08	137.52

* - based on a 400 mL/day beverage consumption by a 70 kg adult

Table 2. *Analytical results of caffeine contents in the carbonated soft drinks*

Soft drinks	Caffeine content (mg/100 mL)	Daily intakes* (mg)
Coca Cola	9.79 ± 0.02	39.16
Coca Cola light	14.38 ± 0.06	57.52
Pepsi Cola	11.27 ± 0.05	45.08
Pepsi Cola light	12.19 ± 0.10	48.76
Action Cola	14.03 ± 0.20	56.12

* - based on a 400 mL/day beverage consumption by a 70 kg adult

The caffeine contents in energy drink samples ranged from 16.82 mg/100 mL to 39.48 mg/100 mL. The minimum caffeine content level was observed in *Mem* energy drink sample, while *Rienergy Refresher* sample showed the highest caffeine content. The mean of caffeine quantity in the analyzed energy drinks was found to be in the level of 30.96 mg/100 mL. However, the analyzed carbonated soft drink samples presented much lower caffeine contents since its mean concentration level was of 12.33 mg/100 mL. The analyzed samples from the carbonated soft drink group showed caffeine content in the range of 9.79 – 14.38 mg/100 mL.

All over the world, the caffeine contents in soft drinks varies according to the type of the brand, yet its average content in soft drinks is approximately 18 mg per six ounces (i.e. 10 mg/100 mL) [14]. In fact, the US Food and Drug Administration (FDA) limits the maximum caffeine amount in carbonated beverages to 6 mg/oz (20 mg/100 mL). Clearly, the caffeine mean content level in the analyzed beverage samples marketed in Romania is well below the above food industry guidelines. Also, the caffeine content of energy drinks can vary greatly. A 250 mL energy drink (about 8.5 ounces) can have anywhere from 50 – 160 mg of caffeine (i.e. 20 – 66 mg/100 mL). Comparatively, an average 8-ounce cup of coffee has about 100 mg caffeine [15].

Rationally, the daily intakes of caffeine through beverages consumption rely mainly on both the content levels of caffeine in the drinks and the amounts consumed of these drinks. Due to the fact that the amounts of the consumed foodstuffs depends on the food habits in particular population group, and in order to estimate the daily intake of caffeine, the following assumptions have been put forward: on average, two to three bottles of the drink are consumed daily by every adult person. However, the size of the drink bottle varied considerably (125-330 mL), hence, daily consumption rate of 400 mL of the drink and also an average adult body weight of 70 kg were also assumed. Contributions from other foodstuffs such as tea, coffee and as alternative source for caffeine are not considered [6].

Tables 1 and 2 illustrate the estimated values of caffeine daily intakes through the consumption of the indicated foodstuff products. As expected, the data presented in these tables indicates an elevated caffeine intake doses through the consumption of energy drinks in comparison to the consumption of the carbonated fizzy drinks. The mean values of caffeine daily intakes were 124 mg and 49 mg through the ingestion of energy drinks and soft drinks, respectively.

According to the International Food Information Council, moderate caffeine consumption for most individuals, including sensitive populations such as pregnant women and children, is about 300 mg per day. Therefore, on average, one energy drink would fall within moderate consumption levels [15].

Brown *et al*. (2001), in a study conducted in Ontario, Canada on 481 men and women aged 30-75 years find that in all strata, the four main sources of caffeine were regular coffee, instant coffee, regular tea, and cola soft drinks. These sources accounted for 90-98% of total daily caffeine intake. Males had a higher caffeine intake than females, and respondents aged 45-59 years had a higher caffeine intake than the other two age groups. Regular coffee was the main source of caffeine (42-63%) for all age-sex strata. Tea and cola soft drinks ranked second or third as a caffeine source, depending on the age-sex stratum. Cola soft drinks tended to be the preferred drink for the youngest age group and tea for the oldest [16].

Intake from major dietary sources (coffee, tea and carbonated soft drinks) were measured in 10,712 caffeinated beverage consumers in the 1999 US Share of Intake Panel, a targeted beverage survey. The data showed that while mean caffeine intakes are within recommended safe levels, heavy consumers of certain subpopulations, including young children and women contemplating pregnancy, might benefit from dietary advice. Also, the study showed that children and young adults consumed most of their caffeine in carbonated soft drinks [17]. Beverage consumption patterns and caffeine intakes were evaluated also from another beverage marketing survey: the 2001 Canadian Facts study. Throughout childhood there is a shift from predominantly milk-based beverage consumption to other types of beverages, including those containing caffeine. Considerably fewer Canadian children than American children consume caffeinated beverages (36% versus 56%). Canadian children consume approximately half the amount of caffeine (7 versus 14 mg/day in American children). Differences were largely because of higher intakes of carbonated soft drinks in the US [18].

CONCLUSIONS

Although the number of drink samples analyzed is still small, the data presented in this study gave a preliminarily outline about the content levels in soft and energy beverages frequently consumed in Romania. Based on these analytical data, it seems that the content levels of caffeine are within or even significantly lower than the maximum authorized levels.

Like all caffeinated foods and beverages, energy drinks can be consumed safely in moderation. The collective evidence from both scientific reviews and clinical studies concludes that moderate consumption of 300 mg caffeine per day is safe, even for more sensitive members of the population, such as children and pregnant women.

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