Original

A fast and efficient method for the study of caffeine levels in energy drinks using micellar electrokinetic chromatography (MEKC)

Um método eficiente e rápido para determinação de cafeína em bebidas energéticas usando a técnica de eletroforese capilar (MEKC)

Cristiano Augusto BALLUS[1], Adriana Dillenburg MEINHART[1], Carolina Schaper BIZZOTTO[1], José TEIXEIRA FILHO[2], Helena Teixeira GODOY[1]*

Abstract

Energy drinks are becoming popular in Brazil and in the world due to their stimulant properties. Caffeine is present in energy drinks with the aim of stimulating the central nervous system and intensifying brain activity. On the other hand, the ingestion of high doses of caffeine can cause undesirable symptoms such as anxiety and tachycardia. Therefore, it is necessary to monitor the caffeine content added to energy drinks to guarantee that the levels in the final product are in accordance with the labeling and within the legislation limits. The goal of this work was to validate a fast, efficient, and low-cost method for the determination of caffeine in energy drinks by micellar electrokinetic chromatography (MEKC). A total of seven brands were analyzed, each in three lots. The electrolyte was prepared with 50 mmol.L^{-1} of sodium dodecyl sulfate (SDS) and 10 mmol.L^{-1} of sodium carbonate (pH 11.0). The mean concentration of caffeine ranged from 122.8 to 318.6 mg.L^{-1}. None of the brands had caffeine levels above the maximum limit. Considering the interval of confidence (95%), 72% of the samples had less caffeine than the amount informed on the product label.

Keywords: *caffeine; energy drinks; micellar electrokinetic chromatography.*

Resumo

As bebidas energéticas tornaram-se populares no Brasil e no mundo devido às suas propriedades estimulantes. A cafeína está presente na bebida energética com a finalidade de estimular o sistema nervoso central e intensificar a atividade cerebral. Por outro lado, a ingestão de altas doses pode causar sintomas indesejáveis como ansiedade e taquicardia. Por isso, é necessário monitorar a quantidade de cafeína adicionada às bebidas energéticas a fim de comprovar se os níveis no produto final estão de acordo com a rotulagem e dentro dos limites legais. O objetivo deste trabalho foi validar um método rápido, eficiente e econômico para determinação de cafeína nessas bebidas por cromatografia eletrocinética micelar (MEKC). Foram analisadas sete marcas, cada uma em três lotes. O eletrólito foi preparado com 50 mmol.L^{-1} de dodecil sulfato de sódio (SDS) e 10 mmol.L^{-1} de carbonato de sódio (pH 11,0). A concentração média de cafeína variou entre 122,8 e 318,6 mg.L^{-1}. Nenhuma das marcas apresentou níveis de cafeína acima do limite máximo permitido. Considerando-se os intervalos de confiança (95%), verificou-se que 72% das amostras possuíam teor de cafeína menor que o informado aos consumidores através dos rótulos.

Palavras-chave: *cafeína; bebidas energéticas; cromatografia eletrocinética micelar.*

1 Introduction

Since drinks containing stimulants were launched in 1987, the best known brands have expanded their shares of the market in Brazil and in the world (FINNEGAN, 2003; CARVALHO et al., 2006; REISSIG; STRAIN; GRIFFITHS, 2009). These drinks are typically constituted of caffeine, taurine, and vitamins. They may contain a source of energy (carbohydrates) and other substances. They are sold with the purpose of either real or perceived psychological or physical improvement (SAFEFOOD, 2002).

In Brazil, the National Agency of Sanitary Surveillance (ANVISA) defines an energy drink as a liquid compound ready for consumption containing inositol, glucuronolactone, taurine, or caffeine, either alone or combined, and with or without vitamins and/or minerals up to 100% of the daily recommended intake (DRI) (AGÊNCIA..., 2005). The maximum amount of caffeine allowed in this type of product is 35 mg.100 mL^{-1} (AGÊNCIA..., 2005). The minimum amount is not regulated. However, according to regulations of ANVISA (confirmed in protocol 2010391275, dated Dec. 06, 2010),

> "The quantity of caffeine present in food for consumption must correspond to that informed on the product label. There is no regulation that defines a margin of error for the caffeine value informed by the company on the product label in relation to its actual content."(AGÊNCIA..., 2005).

ANVISA establishes that the 20% variation limit set in resolution RDC nº 360/03 for other foods does not apply to caffeine levels in energy drinks.

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[1] *Department of Food Science, Faculty of Food Engineering, University of Campinas – UNICAMP, 13083-862, Campinas, SP, Brazil, e-mail: helena@fea.unicamp.br*
[2] *Water and Soil Department, Faculty of Agricultural Engineering, University of Campinas – UNICAMP, Cidade universitária Zeferino Vaz, Barão Geraldo, CEP 13083-970, Campinas, SP, Brazil*
**Correspondig author*

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Caffeine stimulates, in humans, the central nervous system and the heart, and it increases brain activity and has diuretic properties. However, the ingestion of high doses (10 to 15 mg.kg^{-1} body weight) can cause undesirable symptoms such as tremors, tachycardia, insomnia, irritability, anxiety, nauseas, and gastrointestinal discomfort (STEPHENSON, 1977; FERDHOLM, 1985; STAVRIC, 1988; JACOBSON; KULLING, 1989; FETT, 2000). Due to the growing energy drink market, it is extremely important to develop fast, reliable, and low-cost methods for the quality control of the final product and to determine the compliance of caffeine levels with the maximum permissible Brazilian limit regulations. Moreover, since the addition of caffeine is desirable in this kind of product, it is important verify if the added caffeine content is in accordance with the contents declared on the product labels nutritional information to obtain the stimulant properties.

Capillary electrophoresis (CE) is considered a fast, efficient, reproducible, and low-cost separation technique. It uses a small volume of sample and low-cost solvents (RONDA et al., 2008), and consequently, it produces small amounts of residues and has a low environmental impact. This technique, particularly micellar electrokinetic chromatography (MEKC), has been used with success in the determination of methylxantines in foods and vegetable products (THOMPSON; TRENERRY; KEMMERY, 1995; WALKER; ZAUGG; WALKER, 1997; FRAZIER; AMES; NURSTEN, 2000; MAESO et al., 2006; MEINHART et al., 2010).

The objective of this study was to validate a fast method for caffeine determination in energy drinks by micellar electrokinetic chromatography, as well as to evaluate commercial samples of these drinks to verify if their caffeine content is in accordance with that declared on the label and with legislation requirements.

2 Materials and methods

2.1 Samples

Samples of seven brands of energy drinks purchased in Campinas – SP were analyzed in three lots (total of 21 samples). Each brand was coded with letters from A to G. The sampling of each batch consisted in homogenizing two cans before removing the aliquot for the analysis. All samples were within the labeled expiration date.

2.2 Reagents

The caffeine standard was purchased from Sigma-Aldrich (USA). Sodium carbonate was purchased from Synth (São Paulo, Brazil) and sodium dodecyl sulfate (SDS) from Riedel-de-Haën (Germany). Ultrapure water obtained with a Direct-Q 3 Uv (Millipore Corporation, France) system was used in all experiments. Additionally, all solutions were degassed by ultrasonication (Microsonic SX-20, Arruda Ultra-sons Ltda, Brazil) for 5 minutes. The caffeine stock solution (1,000 mg.L^{-1}) was kept refrigerated until it was used to prepare the standard solutions.

2.3 Equipment

The experiments were performed using an Agilent G1600AX capillary electrophoresis system (CE) (Agilent Technologies, Germany) equipped with a diode array detector. A fused silica capillary column (Agilent Technologies, Germany) with total length of 48 cm × 50 μm internal diameter was used in separation.

2.4 Sample preparation

Aliquots of homogenized samples were degassed by ultrasonication for 20 minutes and filtered with a 0.45 μm membrane. Next, the samples were injected in duplicate into the capillary electrophoresis system. All samples were analyzed in quadruplicate.

2.5 Capillary electrophoresis

The analyses were performed following the method developed by Meinhart et al. (2010) with modifications to reduce the run time. The CE operating parameters were as follows: detection at 274 nm, capillary temperature kept at 25 °C, voltage of +30 kV, and hydrodynamic injection of 50 mbar for 5 seconds.

The electrolyte consisted of a solution containing 10 mmol.L^{-1} of sodium carbonate and 50 mmol.L^{-1} of SDS (pH 11.0). After filtration with a 0.45 μm membrane, the buffer was centrifuged at 5,000 rpm for 10 minutes to remove air bubbles. The capillary was washed for 0.5 minutes with buffer between runs. The capillary was conditioned daily in the beginning of the analysis with 1 mol.L^{-1} sodium hydroxide for 5 minutes, with ultrapure water for 5 minutes, and with the run electrolyte for 10 minutes. The run time of each electrophoresis was only 2.0 minutes.

Caffeine in the samples was quantified in triplicate using a seven-point external calibration curve in the concentration range of 50 to 450 mg.L^{-1}.

2.6 Method validation

The following method parameters were validated: linearity, limits of detection and quantification, repeatability, intermediate precision, and recovery.

Linearity was tested in the range of 15.0-3,000.0 mg.L^{-1}. The limits of detection and quantification were estimated as being three and ten times the signal-to-noise ratio, respectively. Repeatability was determined by quantification of caffeine in the same sample ten times consecutively. The intermediate precision assays were carried out using the same sample on three different days.

In the recovery tests, standard solutions of caffeine were added to the energy drinks at two levels, 200.4 and 300.6 mg.L^{-1}, and the measured caffeine concentrations were compared with the labeled caffeine concentration of the sample. The recovery tests were performed in triplicate.

2.7 Statistical analysis

The caffeine content of the samples was submitted to analysis of variance (ANOVA), and the means were statistically

Table 1. Figures of merit of the method for the analysis of caffeine by MEKC.

Parameter		Value
Linearity range (mg.L^{-1})		15.0 – 3,000.0
Curve equation		y = 0.2171 × + 3.5414
r^2		0.9998
Limit of detection (mg.L^{-1})		4.06
Limit of quantification (mg.L^{-1})		13.55
RSD* (%) intraday (n = 10)		1.18
RSD (%) interday (n = 3)		1.89
Recovery (%)	Level 1	98.93
	Level 2	102.85

*RSD: relative standard deviation.

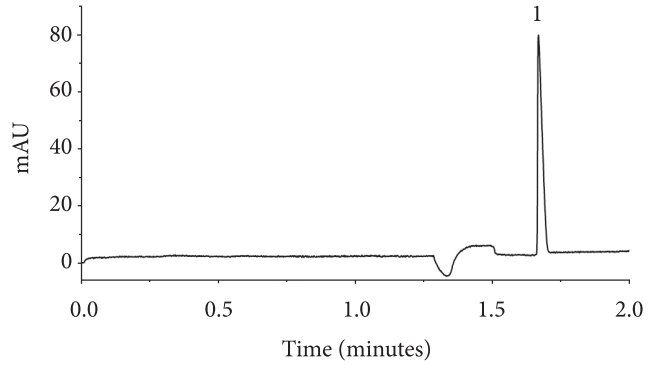

Figure 1. Electropherogram obtained in the determination of caffeine (1) in an energy drink sample. The electrophoretic conditions are described in the text.

compared with Tukey's test ($p < 0.05$) using the software Statistica 7.0 (Statsoft, USA).

3 Results and discussion

3.1 Method validation

Table 1 shows the figures of merit of the proposed method. As can be seen, the method was proven adequate for the analysis of caffeine in energy drinks and was linear in the concentration range studied, with low relative standard deviations (RSD) for the precision assays, good recovery rates, and limit of quantification adequate to the samples analyzed. These parameters highlight the accuracy of the analytical methods for energy drinks quality control. Figure 1 presents the electropherogram of an energy drink. The good selectivity and absence of interferences can be observed.

The investigated capillary electrophoresis method is fast, simple, and low cost. Residue production was low and corresponded to less than 1.5 mL of the electrolyte solution per sample. The residual solution contained water, NaOH, SDS, and energy drink sample. This residue composition in the concentrations used requires only neutralization for its disposal, rather than burning, which is a costly process and has a large environmental impact.

Table 2. Mean content of caffeine (mg.L^{-1}) in the analyzed samples of energy drinks*.

Brands	Batches		
	1	2	3
A	267.8^{a} ± 2.13	272.6^{a} ± 3.21	271.0^{a} ± 1.75
B	256.8^{a} ± 1.18	276.0^{a} ± 5.93	263.9^{a} ± 8.86
C	115.4^{c} ± 1.59	130.3^{a} ± 0.84	122.9^{b} ± 0.17
D	327.7^{a} ± 2.21	295.9^{b} ± 2.25	315.5^{a} ± 6.02
E	288.5^{a,b} ± 1.79	273.2^{b} ± 3.51	291.6^{a} ± 6.02
F	279.0^{a} ± 2.44	267.5^{a} ± 5.86	289.7^{a} ± 11.20
G	309.7^{a} ± 3.15	320.7^{a} ± 3.57	325.4^{a} ± 9.34

*Mean ± standard deviation (n = 4). Equal letters on the same line indicate that there is not significant difference among batches by Tukey's test (95% confidence).

Table 3. Mean caffeine content in energy drink samples per brand with the respective interval of confidence.

Brand	Caffeine (mg.L^{-1})	Confidence interval (95%)*	Label value
A	270.5	264.4-276.5	320.0
B	265.6	241.4-289.7	320.0
C	122.9	104.4-141.4	148.0
D	313.0	273.2-352.9	320.0
E	284.4	260.0-308.9	320.0
F	278.7	251.2-306.3	320.0
G	318.6	298.6-338.6	320.0

*Confidence interval (95%) calculated for n = 3, t_2 = 4.303. Interval range in mg.L^{-1}.

3.2 Caffeine contents in energy drink samples

The mean caffeine contents of the samples of energy drinks and the Tukey's test results used in the evaluation of the differences among batches of a same sample are given in Table 2.

Out of the seven brands that were analyzed, three (brands C, D and E) had caffeine contents that were statistically different (95% confidence) among the batches. Because the energy drink is a formulated product, the intra batch variation can be related to a possible fault in the quality control of caffeine addition during processing.

It can be seen in Table 3 that after the calculation of the confidence intervals (n = 3, t_2 = 4.303), only two brands (D and G) had caffeine contents in agreement with the label information. Therefore, 72% of the samples (15 out of 21) had caffeine contents that were lower than those informed on the product labels. These levels disagree with the regulations of ANVISA since the amount of caffeine in this product must agree with the label information, without including the 20% variation admitted for other components.

Taking into consideration that the Brazilian regulation (AGÊNCIA..., 2005) allows for a maximum of 35 mg caffeine per 100 mL of product, all the samples had levels below the specified maximum levels.

4 Conclusions

The validation results of the method used to determine the caffeine content in energy drinks were adequate for the

parameters analyzed. Sample preparation was simple, analysis time was short (2 minutes), the reagents are inexpensive and were used in small amounts, residue generation is low, and the environmental impact, one of the most discussed aspects in the scientific community, is low. These characteristics allow for its use in the quantification of caffeine in the quality control of energy drinks in the industry.

Although the caffeine content of the samples was below the maximum limit set in Brazilian regulations for this kind of drink, most drinks (72%) had lower amounts (95% confidence) than those declared on the label, which is in disagreement with the regulations of ANVISA. This demonstrates the need for a more effective quality control by the industries that produce energy drinks to ensure the reliability of label information and do not mislead consumers.

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