

## Determination of Elements in Energy Drinks by ICP OES with Minimal Sample Preparation

*Anna Szymczycha-Madeja,\* Maja Welna and Pawel Pohl*

*Analytical Chemistry Division, Chemistry Department, Wrocław University of Technology,  
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland*

Foram comparados vários procedimentos de preparo de amostras, isto é, decomposição total de uma mistura  $\text{HNO}_3/\text{H}_2\text{O}_2$  em placa de aquecimento, decomposição parcial por meio de solubilização em *aqua regia* e diluição com solução de  $\text{HNO}_3$  de baixa concentração, para determinação do conteúdo total de Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Sr e Zn em bebidas energéticas utilizando espectrometria de emissão ótica por plasma indutivamente acoplado (ICP OES). O critério foi o desempenho de cada método no que refere precisão, exatidão dos resultados e limites de detecção dos elementos obtidos por ICP OES. Os melhores resultados foram fornecidos pela diluição mais simples e fácil das amostras analisadas através de solução de  $\text{HNO}_3$  diluída, com limites de detecção dos elementos dentro de 0,08-42,0  $\text{ng mL}^{-1}$ , precisão de 0,5-3% e exatidão melhor que 5%. Seis amostras comerciais de bebidas energéticas foram analisadas com o procedimento proposto.

The suitability of various sample preparation procedures, i.e., total decomposition in a  $\text{HNO}_3/\text{H}_2\text{O}_2$  mixture by hot-plate heating, partial decomposition by means of solubilization in *aqua regia* and dilution with low concentrated  $\text{HNO}_3$  solution, for determination of the total content of Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Sr and Zn in energy drinks using inductively coupled plasma optical emission spectrometry (ICP OES) was compared. The criterion was the performance of each method referred to precision, accuracy of results and limits of detection of elements obtained by ICP OES. Best results were provided by the simplest and the fastest dilution of analyzed samples through a diluted  $\text{HNO}_3$  solution, with limits of detection of elements within 0.08-42.0  $\text{ng mL}^{-1}$ , precision of 0.5-3% and accuracy better than 5%. Six commercial energy drinks were analyzed with the proposed procedure.

**Keywords:** energy drinks, sample preparation, multi-element analysis, ICP OES

### Introduction

The market of energy drinks has rapidly increased for last few years, especially among teenagers between 18 to 34 years old.<sup>1,2</sup> A special popularity of these beverages is related to their ability to increase the attention, endurance or fitness of the body, lose the weight and keep up the energy during an intense physical activity. These effects are caused by components of the energy drinks, including caffeine, guarana or taurine and other components, e.g., sugar, artificial sweeteners, physiological stimulants, and other food additives.<sup>1</sup> Although the quantity of the mentioned ingredients should be known by consumers, there is still lack of legal regulations concerning the concentration of active

ingredients in these drink products. In addition, health effects (beneficial or hazardous) associated with the consumption of energy drinks are not fully known.<sup>1,2</sup> So far, energy drinks have been categorized as “functional beverages” that increase the life energy, however, the information concerning nutritional value, in terms of their mineral composition, i.e., macro-, micro- and trace elements, is not established at all.<sup>1</sup> All effort put into the analysis of energy drinks is mostly concerned on the caffeine determination.<sup>3-7</sup>

Although the analysis of energy drinks seems to be an easy task, it has been not reported in the literature so far. It could be expected, however, that the direct introduction of such samples into a flame (F) atomizer or an inductively coupled plasma (ICP) excitation source, followed by the atomic absorption (AAS) or optical emission (OES) spectrometry detection of elements would be difficult due to

\*e-mail: anna.szymczycha@pwr.wroc.pl

their complex organic matrix present and possible chemical interferences. Such an effect was lately reported in case of the direct analysis of fruit juices.<sup>8</sup> Hence, the total wet oxidative decomposition of energy drinks in aggressive reagents, e.g., HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, HCl, HF or H<sub>2</sub>SO<sub>4</sub>, would be a desirable way of their preparation prior to the spectrochemical elemental analysis. Unfortunately, although effective, the total wet digestion is time-consuming, requires large amounts of concentrated reagents and can lead to losses of analytes and/or contaminations of samples. Hence, it is worth examining whether simpler alternative approaches to the sample preparation, considerably diminishing the time of the sample treatment and eliminating all inconveniences of the digestion step, would not find the application in this type of the analysis.

The objective of this work was to develop a precise and accurate method of the multi-element analysis of energy drinks by ICP OES without any need of a laborious and tedious sample preparation preceding spectrometric measurements. The selected straightforward and fast procedure was applied for the analysis of six energy drinks commercially available in Poland. To the best of our knowledge, the present work reports for the first time results of the analysis of such drink products in reference to their mineral composition. In addition to mineral content, concentration of caffeine in all analyzed energy drinks was determined and compared.

## Experimental

### Samples and reagents

Six samples of energy drink of different brands (Burn, Monster, N-gine, Power, Red Bull and Tiger) were purchased from a local grocery (Wroclaw, Poland). Before sampling, after opening, beverages were left under a laminar hood for 24 h to release CO<sub>2</sub>.

All chemicals were of analytical grade. Concentrated solutions of HNO<sub>3</sub> (Merck, Darmstadt, Germany) and HCl (POCh, Gliwice, Poland), and a 30% (v/v) solution of H<sub>2</sub>O<sub>2</sub> (POCh) were used for the sample preparation. Working standard solutions were prepared by stepwise dilutions of a multi-element (1000 µg mL<sup>-1</sup>) ICP standard (Merck). Deionized water (18.3 MΩ cm) was from an EASYpure™ water purification system (Baenstead, Thermolyne Corporation, USA).

### Sample preparation and determination of elements

Three different sample preparation procedures were tested. For the hot plate heating digestion (P1), 25.0 mL

of energy drink samples were placed into 150 mL Pyrex beakers and 10 mL of a concentrated HNO<sub>3</sub> solution were added. Solutions were heated on a hot plate at 85 °C for ca. 3 h. After cooling, 5 mL of a 30% (v/v) H<sub>2</sub>O<sub>2</sub> solution were added. The heating was continued until clear solutions were obtained and their volumes were reduced to about 2 mL. Resulting aliquots were quantitatively transferred into 25 mL volumetric flasks and made up to the volume with deionized water. In case of the solubilization in *aqua regia* (P2), 5 mL of energy drink samples were solubilized in 2 mL of *aqua regia*. Resulting mixtures were sonicated in an ultrasonic bath (UltrasonsH Selecta) for 15 min and then made up to 20 mL with deionized water. Finally, for the dilution with diluted HNO<sub>3</sub> procedure (P3), 10 mL of energy drink samples were diluted 1:1 with a 2.0% (v/v) HNO<sub>3</sub> solution.

Three parallel samples (n = 3) were prepared and analyzed for each energy drink brand. Monster samples were used for the comparison of the suitability of different sample preparation procedures. With each set of sample solutions, procedural blanks were prepared and subjected to the analysis to correct final results. Then, the chosen procedure was applied to the preparation of remaining energy drinks and the determination of 13 elements (Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Sr and Zn) by ICP OES against the external calibration with simple standard solutions. A JY38S (Jobin Yvon, France) instrument was used with the following operating parameters: a RF power of 1.0 kW, a plasma gas flow rate of 13 L min<sup>-1</sup>, a sheath gas flow rate of 0.2 L min<sup>-1</sup> and a nebulizer gas flow rate of 0.25 L min<sup>-1</sup>. Prepared sample solutions were introduced into the plasma using a Burgener (MiraMist) nebulizer and a cyclonic type spray chamber at a flow rate of 0.75 mL min<sup>-1</sup>. Analytical lines of Ba 233.5 nm, Ca 317.9 nm, Cd 228.8 nm, Cr 267.7 nm, Cu 324.7 nm, Fe 259.9 nm, Mg 285.2 nm, Mn 259.4 nm, Ni 221.6 nm, P 214.6 nm, Pb 220.3 nm, Sr 407.8 nm and Zn 213.9 nm were selected and measured.

### Caffeine determination

Caffeine was determined by UV spectrophotometry at 276 nm using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific) after the extraction of the target compound into chloroform from alkalized sample solutions (pH 12.5) according to the dependable method described by Shufen *et al.*<sup>9</sup> The caffeine content was measured against the calibration curve prepared with standard solution of the compound within 0-20 mg L<sup>-1</sup> concentration range and using chloroform as a blank.

## Results and Discussion

### Comparison of sample preparation procedures

Unfortunately, the direct introduction of undiluted energy drinks into the ICP was established to extinguish it at relatively low forward powers (i.e., 1000-1100 W) likely due to an overloading with the organic matrix of these samples. Higher forward powers were avoided due to a fast increase in the background level in the vicinity of the analytical lines of the determined elements and hence, a deterioration of the detectability of the system for these elements. Therefore, three sample preparation procedures, aimed at simplifying the matrix of energy drinks, were tested and their analytical characteristic was compared by evaluating precision and accuracy of the ICP OES measurements to determine the element concentrations as well as their limits of detection achievable in these conditions. The relative standard deviation (RSD) was used to express the precision of the determination of element concentrations. The accuracy was evaluated by comparing total concentrations of elements obtained using procedures based on the *aqua regia* solubilization (P2) and the dilution with diluted HNO<sub>3</sub> (P3) to those achieved using the hot plate wet digestion procedure (P1). In addition, all studied elements were added to analyzed samples and respective recoveries were assessed. Limits of detection (LODs) were determined as concentrations corresponding to three times the standard deviation (3 × SD) of 10 consecutive measurements of the respective procedural blank.

### Precision and LODs

Average concentrations of elements measured in solutions of the Monster energy drink along with RSD and LOD values obtained for different sample treatments are listed in Table 1. As can be seen, the precision obtained for wet digestion (P1) and dilution with diluted HNO<sub>3</sub> (P3) procedures is comparable. Accordingly, RSDs vary from 0.49 to 3.6% (P1) and from 0.50 to 3.0% (P3). Only for Cd, much higher RSDs were obtained, i.e., 8.3 and 7.9%, respectively for P1 and P3, but the concentration of this element is extremely low. Unfortunately, for some elements, the precision achieved using the *aqua regia*-based procedure (P2) is nearly two times poorer than this achieved when two aforementioned procedures were used.

LODs of elements obtained using the wet digestion (P1) and the dilution with diluted HNO<sub>3</sub> (P3) are better than those achieved using the solubilization in *aqua regia* (P2). The use of both first procedures, i.e., the wet digestion (P1) and the simple dilution with diluted HNO<sub>3</sub> (P3),

**Table 1.** Concentrations and limits of detection (LODs) of elements determined in solutions of Monster energy drink using ICP OES and three different sample preparation procedures: the hot-plate digestion in a HNO<sub>3</sub> with H<sub>2</sub>O<sub>2</sub> mixture (P1), the solubilization in *aqua regia* (P2) and the dilution with a diluted HNO<sub>3</sub> solution (P3)

Element	Concentration / (µg mL <sup>-1</sup> )			LOD / (ng mL <sup>-1</sup> )		
	P1	P2	P3	P1	P2	P3
Ba	0.019 (2.6)	0.028 (2.8)	0.019 (2.6)	0.98	0.97	0.95
Ca	3.09 (3.6)	2.45 (5.2)	3.14 (0.91)	10	13	5.3
Cd	0.0016 (8.3)	0.0017 (8.9)	0.0016 (7.9)	0.93	1.5	0.97
Cr	0.014 (2.4)	0.013 (2.8)	0.014 (2.3)	0.80	0.83	0.77
Cu	0.047 (2.7)	0.045 (3.9)	0.047 (2.0)	1.2	1.0	1.0
Fe	0.064 (1.5)	0.053 (2.2)	0.064 (1.5)	1.3	1.3	1.2
Mg	0.436 (3.2)	0.564 (5.2)	0.440 (2.4)	2.5	2.5	1.6
Mn	0.021 (2.6)	0.024 (4.7)	0.021 (1.2)	0.45	0.47	0.43
Ni	0.025 (3.1)	0.027 (3.4)	0.025 (3.0)	3.6	6.6	3.0
P	1.90 (2.5)	2.42 (2.8)	1.93 (2.3)	42	55	42
Pb	0.046 (0.49)	0.042 (0.62)	0.046 (0.50)	11	12	11
Sr	0.019 (1.5)	0.015 (2.2)	0.020 (1.1)	0.09	0.09	0.08
Zn	0.030 (0.91)	0.029 (1.1)	0.030 (0.60)	0.75	0.84	0.73

results in quite comparable LODs for most elements. The only exception are Ca, Cu, Ni and Mg, which LODs obtained using the dilution with diluted HNO<sub>3</sub> (P3) are much better as compared to those achieved using the total decomposition (P1).

### Accuracy

Considering the concentrations of elements determined in the Monster energy drink (Table 1), it can be seen that the differences between results obtained with the wet digestion (P1) and the dilution with diluted HNO<sub>3</sub> (P3) are lower than respective SDs. According to the *t*-test at the 95% level of the significance (see Table 2), it was found that differences between concentrations of all elements determined in sample solutions prepared with both procedures are statistically insignificant, i.e., for all compared elements, calculated values of the *t*-test ( $t_{\text{calculated}}$ ) are lower than the critical value ( $t_{\text{critical}}$ ) equal to 4.303. Therefore, it seems that the dilution of energy drink samples with diluted HNO<sub>3</sub> (P3) can be a reliable but simpler and faster alternative to the usually applied sample preparation procedure based on the wet acidic digestion. The solubilization using *aqua regia* (P2), although simple and fast as well, was found to be unsuitable to prepare energy drink samples before the multi-element ICP OES analysis. According to the *t*-test, its use seems to be justified but only in case of few selected elements, i.e., Cd, Cr, Cu, Ni and Zn. For

remaining elements (Ba, Ca, Fe, Mg, Mn, P, Pb and Sr), discrepancies between their concentrations achieved with both compared procedures, i.e., P1 and P2, are too high, i.e., from 10% (Pb) to about 200% (Ba). Hence, the application of this procedure can lead to analytical errors during the determination of 8 out of 13 elements investigated here.

The accuracy was also verified by performing the recovery test. Samples of Monster were spiked with a multi-element standard solution at two concentrations (0.5 and 1.0  $\mu\text{g mL}^{-1}$ ), subjected to compared sample preparation procedures, and resulting sample solutions were analyzed by ICP OES. As can be seen in Table 3, independently of the fortification level, recoveries of all elements are quantitative and span ranges within 98-106% (P1), 91-118% (P2) and 99-103% (P3). This is likely because all elements were added in the form of simple ions. Nonetheless, recoveries obtained for the solubilization in *aqua regia* (P2) have the greatest variance comparing to the remaining procedures.

Considering the precision and the accuracy of the concentrations measurements of all 13 elements and LOD values achieved for them with ICP OES, it was concluded that the dilution with a diluted  $\text{HNO}_3$  solution (P3) is the

**Table 2.** Calculated values of the  $t$ -test ( $t_{\text{calculated}}$ ) for the comparison of average concentrations of elements determined by ICP OES using the standard procedure (P1) and other alternative procedures: the solubilization in *aqua regia* (P2) and the dilution in  $\text{HNO}_3$  (P3)

Element	Procedure	
	P2	P3
Ba	16.40	0.940
Ca	6.514	0.785
Cd	0.922	0.323
Cr	1.299	0.192
Cu	2.218	0.305
Fe	12.40	0.742
Mg	6.844	0.446
Mn	4.560	0.460
Ni	2.038	0.288
P	10.76	0.851
Pb	18.31	0.373
Sr	18.33	0.999
Zn	4.261	3.256

Critical value of the  $t$ -test ( $t_{\text{critical}}$ ): 4.303 ( $p = 0.05$ ,  $n = 3$ ).

**Table 3.** Recoveries of elements determined in solutions of Monster energy drink prepared using the hot-plate digestion in  $\text{HNO}_3$  with  $\text{H}_2\text{O}_2$  (P1), the solubilization in *aqua regia* (P2) and the dilution with diluted  $\text{HNO}_3$  (P3)

Element	Added / ( $\mu\text{g mL}^{-1}$ )	P1	P2	P3
Ba	0.5	102 $\pm$ 3	102 $\pm$ 3	102 $\pm$ 2
	1.0	99.4 $\pm$ 2.6	101 $\pm$ 3	99.6 $\pm$ 1.6
Ca	0.5	106 $\pm$ 4	108 $\pm$ 8	102 $\pm$ 2
	1.0	99.2 $\pm$ 3.2	97.1 $\pm$ 6.1	99.5 $\pm$ 1.8
Cd	0.5	99.5 $\pm$ 6.8	102 $\pm$ 9	100 $\pm$ 6
	1.0	99.5 $\pm$ 6.1	98.9 $\pm$ 7.4	100 $\pm$ 6
Cr	0.5	98.0 $\pm$ 1.5	102 $\pm$ 3	102 $\pm$ 1
	1.0	101 $\pm$ 1	99.4 $\pm$ 2.2	99.5 $\pm$ 0.8
Cu	0.5	98.5 $\pm$ 2.8	103 $\pm$ 6	99.9 $\pm$ 1.9
	1.0	100 $\pm$ 2	99.1 $\pm$ 5.1	100 $\pm$ 1
Fe	0.5	104 $\pm$ 1	105 $\pm$ 3	103 $\pm$ 1
	1.0	99.1 $\pm$ 1.4	97.2 $\pm$ 2.2	99.2 $\pm$ 0.7
Mg	0.5	103 $\pm$ 4	118 $\pm$ 8	101 $\pm$ 3
	1.0	99.4 $\pm$ 3.3	95.3 $\pm$ 5.6	99.8 $\pm$ 2.9
Mn	0.5	103 $\pm$ 3	95.1 $\pm$ 5.0	101 $\pm$ 1
	1.0	99.1 $\pm$ 2.3	101 $\pm$ 5	99.7 $\pm$ 0.8
Ni	0.5	104 $\pm$ 4	104 $\pm$ 4	98.6 $\pm$ 2.4
	1.0	99.0 $\pm$ 2.5	99.0 $\pm$ 3.2	100 $\pm$ 1
P	0.5	106 $\pm$ 3	110 $\pm$ 3	100 $\pm$ 2
	1.0	98.6 $\pm$ 2.7	97.6 $\pm$ 2.8	100 $\pm$ 2
Pb	0.5	103 $\pm$ 1	90.6 $\pm$ 2.4	99.1 $\pm$ 1.1
	1.0	98.1 $\pm$ 1.0	102 $\pm$ 2	100 $\pm$ 1
Sr	0.5	102 $\pm$ 2	106 $\pm$ 3	99.9 $\pm$ 1.2
	1.0	99.5 $\pm$ 1.7	98.4 $\pm$ 3.1	100 $\pm$ 1
Zn	0.5	102 $\pm$ 2	103 $\pm$ 2	101 $\pm$ 1
	1.0	99.4 $\pm$ 1.2	99.0 $\pm$ 2.7	99.8 $\pm$ 0.4

Average values ( $n = 3$ ) with standard deviations (SDs).

most suitable procedure for the preparation of energy drinks prior to their multi-element analysis. This procedure was chosen to prepare other energy drinks examined here. Additionally, for these procedures, slopes of standard addition calibration curves are comparable to those found by the external calibration curves with simple standard solutions. Hence, in practice, it can be concluded that matrix effects are not observed for these procedures.

#### Analysis of energy drinks

According to our best knowledge, the mineral content of energy drinks has never been reported in the related literature. Total concentrations of 13 elements (in  $\mu\text{g mL}^{-1}$ ) in six energy drinks prepared with the aid of a diluted  $\text{HNO}_3$  solution (P3) are presented in Table 4. In addition, results of the caffeine determination (in  $\text{mg } 100 \text{ mL}^{-1}$ ) are included.

As can be seen, the precision of measurements is good ( $< 5.0\%$  as RSD). The only exception is Cd, for which RSDs are higher (7.3-9.9%). Concentrations of individual elements in analyzed products are quite differentiated. Ratios of the smallest to the largest concentrations vary from about 2 (Fe) to 7 (Zn) times. The most significant discrepancy can be noticed for Ca and Mn (1 order), Sr (2 orders) and Mg (3 orders). In general, the highest concentrations of elements are found in Power (Ba, Cu, Fe, Mn, Zn), Red Bull (Ca, Mg, Ni, Sr) and Burn (Cd, P, Pb). In contrast, the lowest amounts of elements are found

in N-gine (Ca, Cr, Fe, Mg, Mn, P, Sr, Zn) and Tiger (Cd, Ni, Pb). Concentration of Cr and Cd in these two drinks are below their LODs.

Due to such high differences in concentrations of individual elements, their geometric means were used for the comparison of the mineral content of the analyzed energy drinks. Accordingly, Ca, Mg and P are major elements, while Ba, Cd, Cr, Cu, Fe, Mn, Ni, Sr, and Zn are trace elements. Concentrations of macro-elements fulfill the following order:  $\text{Ca} > \text{Mg} > \text{P}$ . The exception is Red Bull, for which a quite different relation is noticed, i.e.,  $\text{Mg} > \text{Ca} > \text{P}$ . The highest concentrations of Ca, Mg and P are determined in Red Bull (Ca, Mg) and Burn (P). Concentrations of trace essential elements, i.e., Cu, Fe, Mn and Zn, can be arranged as follows:  $\text{Fe} > \text{Mn} > \text{Cu} > \text{Zn}$ . Discrepancies between the highest, and the lowest concentrations of these elements can reach up to 4 times. Only in case of Power, the difference is more than 10 times. For non-essential elements, the following relation can be ascribed:  $\text{Sr} > \text{Pb} \sim \text{Ba} > \text{Ni} \gg \text{Cr} > \text{Cd}$ . Concentrations of Sr, Pb, Ba and Ni are close to those obtained for essential trace elements. Contents of Cd and Cr are significantly reduced (by about one order of magnitude) as compared to those established for aforementioned trace elements.

Relationships between concentrations of elements using the Pearson's correlation coefficient ( $r$ ) were also investigated. As can be seen in Table 5, a very high positive correlation ( $r > 0.9$ ) was observed for concentrations

**Table 4.** Concentrations of elements in the analyzed energy drinks

Element	Concentration / ( $\mu\text{g mL}^{-1}$ )							Mean <sup>a</sup>
	Burn	Monster	N-gine	Power	Red Bull	Tiger	Min.-Max.	
Ba	0.030 (4.1)	0.019 (2.6)	0.029 (2.1)	0.082 (0.41)	0.058 (3.6)	0.033 (3.8)	0.019-0.082	0.037
Ca	13.3 (1.7)	3.14 (0.91)	2.39 (3.2)	120 (1.3)	63.9 (2.1)	20.6 (0.28)	2.39-120	15.8
Cd	0.0025 (7.3)	0.0016 (7.9)	0.0024 (8.0)	0.0017 (8.2)	0.0012 (9.9)	< LOD <sup>b</sup>	LOD-0.0025	0.0018
Cr	< LOD <sup>b</sup>	0.014 (2.3)	< LOD <sup>b</sup>	0.0067 (1.7)	0.0035 (3.3)	0.0052 (4.7)	LOD-0.014	0.0064
Cu	0.013 (1.9)	0.047 (2.0)	0.013 (3.8)	0.079 (1.3)	0.011 (2.2)	0.012 (2.0)	0.011-0.079	0.021
Fe	0.059 (4.1)	0.064 (1.5)	0.035 (2.3)	0.078 (4.2)	0.040 (1.7)	0.036 (3.2)	0.035-0.078	0.049
Mg	3.29 (0.61)	0.440 (2.4)	0.182 (2.7)	9.98 (2.6)	187 (3.1)	2.49 (1.4)	0.182-187	3.27
Mn	0.020 (2.4)	0.021 (1.2)	0.0094 (2.5)	0.586 (2.2)	0.011 (1.7)	0.031 (0.62)	0.0094-0.586	0.030
Ni	0.015 (2.8)	0.025 (3.0)	0.016 (2.5)	0.041 (0.87)	0.059 (3.0)	0.012 (2.4)	0.012-0.059	0.024
P	3.34 (3.0)	1.93 (2.3)	0.548 (1.7)	1.04 (2.2)	0.766 (2.9)	0.686 (3.3)	0.548-3.34	1.12
Pb	0.053 (1.0)	0.046 (0.50)	0.036 (2.3)	0.030 (2.1)	0.020 (1.1)	0.019 (1.3)	0.019-0.053	0.032
Sr	0.091 (4.1)	0.020 (1.1)	0.0018 (4.9)	0.185 (2.7)	0.608 (4.0)	0.027 (3.5)	0.0018-0.608	0.046
Zn	0.020 (3.9)	0.030 (0.60)	0.0077 (4.8)	0.058 (4.9)	0.012 (3.4)	0.0084 (4.4)	0.0077-0.058	0.0173
Caffeine / ( $\text{mg } 100 \text{ mL}^{-1}$ )	33.6 (3.8)	31.1 (5.4)	34.5 (3.2)	130 (4.2)	31.6 (3.8)	34.1 (2.6)	31.1-130	41.4
Package / mL	1000	500	250	60	250	250	–	–
Caffeine per serving / mg	336	156	86.3	78.0	79.0	85.3	78.0-156	115

Average values ( $n = 3$ ) with relative standard of deviations (RSDs) in brackets. <sup>a</sup>Geometric mean. <sup>b</sup>Below the limit of detection.

**Table 5.** Pearson's correlation coefficients (r)

Element	Ba	Ca	Cd	Cr	Cu	Fe	Mg	Mn	Ni	P	Pb	Sr	Zn	Caffeine
Ba	1													
Ca	<b>+0.989</b>	1												
Cd	-0.497	-0.541	1											
Cr	-0.560	-0.470	<b>+0.746</b>	1										
Cu	+0.567	+0.658	-0.318	+0.440	1									
Fe	+0.423	+0.520	-0.090	+0.520	<b>+0.868</b>	1								
Mg	+0.372	+0.323	<b>-0.710</b>	-0.590	-0.279	-0.302	1							
Mn	<b>+0.832</b>	<b>+0.870</b>	-0.218	-0.074	<b>+0.871</b>	<b>+0.728</b>	-0.171	1						
Ni	+0.698	+0.691	<b>-0.910</b>	-0.408	+0.269	+0.182	<b>+0.846</b>	+0.320	1					
P	-0.327	-0.279	+0.488	<b>+0.976</b>	-0.022	+0.465	-0.283	-0.152	-0.286	1				
Pb	-0.463	-0.424	<b>+0.740</b>	<b>+0.969</b>	+0.098	+0.461	-0.520	-0.131	-0.432	<b>+0.843</b>	1			
Sr	+0.561	+0.521	<b>-0.734</b>	-0.636	-0.103	-0.101	<b>+0.970</b>	+0.047	<b>+0.917</b>	-0.218	-0.491	1		
Zn	+0.639	<b>+0.722</b>	-0.245	+0.319	<b>+0.965</b>	<b>+0.942</b>	-0.235	<b>+0.902</b>	+0.302	+0.152	+0.184	-0.022	1	
Caffeine	<b>+0.835</b>	<b>+0.867</b>	-0.195	-0.092	<b>+0.857</b>	+0.712	-0.173	<b>+0.999</b>	+0.314	-0.163	-0.130	+0.044	<b>+0.890</b>	1

of the following pairs of elements: Ba-Ca, Cr-P, Cr-Pb, Cu-Zn, Fe-Zn, Mg-Sr, Mn-Zn and Ni-Sr. Only one pair of elements, i.e., Cd-Ni, was strongly negatively correlated. High correlations ( $0.7 < |r| < 0.9$ ) were also established and they were positive for Ba-Mn, Ca-Mn, Ca-Zn, Cd-Cr, Cd-Pb, Cu-Fe, Cu-Mn, Fe-Mn, Mg-Ni and P-Pb as well as negative for Cd-Mg and Cd-Sr pairs of elements. For other pairs of elements, positive or negative correlations were shown but they were moderate ( $0.4 < |r| < 0.7$ ), e.g., Ba-Cd, Ba-Cr, Ba-Cu, Ba-Fe, Ba-Ni, Ba-Pb, Ba-Sr, Ba-Zn, Ca-Cd, Ca-Cr, Ca-Cu, Ca-Fe, Ca-Ni, Ca-Pb, Ca-Sr, Cd-P, Cr-Cu, Cr-Fe, Cr-Mg, Cr-Ni, Cr-Sr, Fe-P, Fe-Pb, Mg-Pb, Pb-Sr, or low ( $0.2 < |r| < 0.4$ ), e.g., Ba-Mg, Ba-P, Ca-Mg, Ca-P, Cd-Cu, Cd-Mn, Cd-Zn, Cu-Mg, Cu-Ni, Cr-Zn, Fe-Mg, Mg-P, Mg-Zn, Mn-Ni, Ni-P, P-Sr. The relation between Cd-Fe, Cr-Mn, Cu-P, Cu-Pb, Cu-Sr, Fe-Ni, Fe-Sr, Mn-P, Mn-Pb, Mn-Sr, P-Zn, Pb-Zn and Sr-Zn pairs is practically negligible ( $r$  0 to  $\pm 0.2$ ).

The caffeine content (Table 4) is quite comparable in 5 out of 6 energy drinks (ca. 33 mg 100 mL<sup>-1</sup>). A 4 times higher caffeine concentration is found in Power and this agrees, because Power is called "energy shot", i.e., a concentrated energy drink, containing as much as permissible caffeine concentration. Noteworthy, results obtained here are in a very good agreement with declared values, i.e., 0.133% (m/v) for Power and 0.03% (m/v) for other drinks. It seems that analyzed energy drinks are more source of caffeine than inorganic constituents. Assuming their size (Table 4), they contain 78-336 mg of caffeine *per* serving. Safe limits of caffeine are 300-400 mg *per* day (ca. 3 coffee cups) for healthy adults and only up to 45-85 mg *per* day for children,<sup>10</sup> hence the consumption of such beverages by

children should be discouraged. On the other hand, in case of elements, a comparison of the measured and the dietary reference values (i.e., recommended allowances/adequate daily intakes, upper tolerable intake levels and maximum level of daily intake without detriment to health)<sup>11,12</sup> indicates that energy drinks are within safety levels for human consumption and the found values did not represent a health problem.

According to previous study of Kolaylı *et al.*,<sup>13</sup> who found that caffeine would bind metal ions, i.e., Ca, Mg, Fe, Zn, Pb, Mn, Co and Cr, the such relations using the Pearson's correlation coefficient (r) between element and caffeine concentrations in analyzed here coffees were studied (see Table 5). Based on the results, a very high positive correlation was found for Mn ( $r = 0.999$ ) and high correlations ( $0.8 < r < 0.9$ ) were also established for Ba, Ca, Cu and Zn. It indicates the interactions between caffeine and these metal ions, suggesting a possible chelating activity of caffeine to complex formation.

## Conclusions

This work demonstrates for the first time results on the mineral content of energy drinks determined by ICP OES. Considering the reliability of results and the simplicity of the sample treatment, the analysis after dilution with a diluted HNO<sub>3</sub> solution demonstrates the highest simplicity, time-saving, reproducibility and dependability. This simpler sample handling is a vital alternative to the wet digestion procedure that can be adequately used for routine ICP OES analyses of energy drinks on the concentration of up to 13 elements.

Based on the content results of elements and caffeine, it can be concluded that energy drinks supply human organisms with higher amounts of caffeine than of minerals and hence, they are not an important source of elements in the human diet. In the light of limits estimated for caffeine, an excessive consumption of energy drinks may cause health hazard.

The complex formation capacity of caffeine was determined for Ba, Ca, Cu, Mn and Zn metal ions.

## Acknowledgements

The work was financed by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocław University of Technology.

## References

1. Heckman, M. A.; Sherry, K.; de Mejia, G.; *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 303.
2. Reissig, C. J.; Strain, E. C.; Griffiths, R. R.; *Drug Alcohol Depend.* **2009**, *99*, 1.
3. Aranda, M.; Morlock, G.; *J. Chromatogr.* **2006**, *1131*, 253.
4. Armenta, S.; Garrigues, S.; Guardia, M.; *Anal. Chim. Acta* **2005**, *547*, 197.
5. Liotta, E.; Gottardo, R.; Seri, C.; Rimondo, C.; Miksik, I.; Serpelloni, G.; Tagliaro, F.; *Forensic Sci. Int.* **2012**, *220*, 279.
6. McCusker, R. R.; Goldberger, B. A.; Cone, E. J.; *J. Anal. Toxicol.* **2006**, *30*, 112.
7. Srdjenovic, B.; Djordjevic-Milic, V.; Grujic, N.; Injac, R.; Lepojevic, Z.; *J. Chromatogr. Sci.* **2008**, *46*, 144.
8. Juranović-Cindrić, I.; Zeiner, M.; Kröpl, M.; Stinger, G.; *Microchem. J.* **2011**, *99*, 364.
9. Shufen, L.; Berger, J.; Hartland, S.; *Anal. Chim. Acta* **1990**, *232*, 409.
10. <http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/food-aliment/caffeine-eng.php> accessed in July 2013.
11. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine; *Dietary Reference Intakes for Calcium; Institute Phosphorous, Magnesium, Vitamin D, and Fluoride*; National Academies Press: Washington, DC, 1997, [http://www.nap.edu/openbook.php?record\\_id=5776&page=325](http://www.nap.edu/openbook.php?record_id=5776&page=325) accessed in March 2013; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine; *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; National Academies Press: Washington, DC, 2001, [http://www.nap.edu/openbook.php?record\\_id=10026&page=772](http://www.nap.edu/openbook.php?record_id=10026&page=772) accessed in March 2013.
12. Joint FAO/WHO Expert Committee on Food Additives; *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956-2003 (First through Sixty-First Meetings)*; Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), ILSI Press International Life Sciences Institute: Washington, DC, USA, 2004.
13. Kolaylı, S.; Ocak, M.; Kucuk, M.; Abbasoglu, R.; *Food Chem.* **2004**, *84*, 383.

Submitted: May 28, 2013

Published online: August 23, 2013