Development of a method for simultaneous analysis of caffeine and taurine in energy drinks by micellar electrokinetic chromatography with diode-array detector

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Abstract

The objective of this study was to develop, optimize and validate a fast and reliable method for the simultaneous determination of caffeine and taurine contents by micellar electrokinetic chromatography with diode array detector, using direct and indirect detection concomitantly. Multivariate statistical techniques were used as a central composite design and the simultaneous optimization method of responses of Derringer and Suich were used for optimization. The method was applied in the analysis of 73 samples of energy drinks commercialized in Brazil. The optimized method employed a capillary tube with an extended bulb of 50 μm i.d. x 72 cm total length, an electrolyte containing 16.20 mmol.L^{-1} of benzoic acid and 39.90 mmol.L^{-1} of SDS, a pH value of 7.26, + 30 kV voltage, direct detection of caffeine at 274 nm and indirect detection of taurine at 230 nm. Validation parameters have demonstrated the reliability and applicability of this method. It was found that more than 50% of the samples were out of the legal limits determined by the Brazilian government regarding the taurine content and 68 % contained caffeine below the value declared on the label. Therefore, the need for greater control concerning the composition of these drinks exists.

Keywords: multivariate optimization; added ingredients; capillary electrophoresis.

Practical Application: Development of method for simultaneous determination of caffeine and taurine content in energy drink.

1 Introduction

According to the Brazilian Health Regulatory Agency (ANVISA), energy drink is defined as a "liquid compound ready for consumption", and may present the following ingredients in its composition: inositol and/or glucuronolactone (maximum 20 mg.100 mL^{-1}), and/or taurine (maximum 400 mg.100 mL^{-1}), and/or caffeine (maximum 35 mg.100 mL^{-1}), vitamins and/or minerals (up to 100% of the Reference Daily Intake); other ingredients can be added, provided that there is no distortion of the product (Brasil, 2005).

Caffeine is considered one of the most common ingredients to any brand of energy drink (Paula Lima & Farah, 2019; McLellan et al., 2016; Rai et al., 2016; Turak et al., 2017). In the human body, it has a stimulation effect both in the central nervous system and the cardiac activities (Paula Lima & Farah, 2019; McLellan et al., 2016; Seifert et al., 2011). However, when ingested at doses above 400 mg/day (considering a 70 kg adult) it may cause anxiety and other undesirable symptoms (McLellan et al., 2016; Reid et al., 2016; Temple, 2019; Tran et al., 2016; Turak et al., 2017). From 1.000 mg/day, it presents toxicity to the organism, and it may be lethal in quantities of 5.000 to 10.000 mg/day (Seifert et al., 2011).

Taurine is a free amino acid which plays a fundamental role in the human body by absorbing fats, protecting the heart and acting as an antioxidant (Alzawqari et al., 2016; Catharino et al., 2011; Giles et al., 2012; Goron & Moinard, 2018; Heidari et al., 2016; Mele et al., 2019; Pansani et al., 2012; Pereira et al., 2012;

Rai et al., 2016; Wen et al., 2019). Regarding toxicity, it is generally well accepted by the body, without any adverse physiological effects. It is estimated that the world population consumes about 400 mg per day (Sanctis et al., 2017) and there are no reports of side effects regarding the therapeutic use of doses between 1.000 and 3.000 mg per day (Heckman et al., 2010; Jakopin, 2019).

Both caffeine and taurine content are usually determined by high-performance liquid chromatography with diode array detector (HPLC-DAD) (Bizzotto et al., 2013; Chirita Tampu et al., 2018; Paula Lima & Farah, 2019; Rai et al., 2016) and capillary electrophoresis, mainly zone or micellar electrokinetic chromatography (MEKC) with diode array detector (DAD), is also often employed. However, taurine does not absorb light in the UV - VIS region, being detected indirectly, either by DAD, or by laser - induced fluorescence, amperometric and conductometric detectors (Rai et al., 2016; Sawabe et al., 2008; Vochyánová et al., 2014; Zinellu et al., 2009).

The main difficulty in the development of a method for simultaneous analysis of caffeine and taurine consists of the detection mechanism to be applied. Simultaneous analysis of two compounds has been already described in studies that employed HPLC with detector by mass spectrometry and by MEKC with two connected detectors, being the DAD for caffeine and the conductometer for taurine (Vochyánová et al., 2014; Welch et al., 2015). The use of two methods makes this analysis slow and laborious, which highlights the importance of

the development of methods with the simple and simultaneous detection of the two compounds. The technique of capillary electrophoresis has been increasingly used due to high resolution, speed, low cost and low residual generation, which is the reason why this technique is an important tool for analyses such as caffeine and taurine.

Therefore, the objective of this study was to develop, optimize and validate a method for simultaneous determining of caffeine and taurine content, using MEKC-DAD and multivariate statistical techniques, applying the method in the analysis of 22 energy drink brands commercialized in Brazil.

2 Materials and methods

2.1 Samples

For the development and optimization of this method, a single brand of energy drinks was obtained in Campinas- SP, whose composition was considered the most complex in comparison with other brands.

For the evaluation of caffeine and taurine content, 22 brands of energy drinks were obtained in supermarkets in the city of Campinas, each one from three different batches of five brands, only two batches were acquired due to lack of availability, totaling 73 samples. The preparation of the samples consisted in degassing in ultrasound for 20 minutes and filtering in a 0.22 μm Millipore filter (Millipore, USA), before injection in the capillary electrophoresis system. All determinations were made in triplicate, and each replicate represented an average of three injections.

2.2 Reagents

Benzoic acid was purchased from Carlo Erba (Cornaredo, Ml, Italy) and sodium dodecyl sulfate (SDS) from Riedel-de-Haën (Seelze, NI, Germany). Caffeine and taurine standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared with ultrapure water (Milli-Q® system from Millipore Corporation (Jaffrey, NH, USA) and filtered in a 0.22 μm cellulose membrane porosity (Millipore, Jaffrey, NH, USA). Stock solutions of caffeine and taurine, as well as the solutions in this study, were stored under refrigeration temperatures (10 °C).

2.3 Equipment

The equipment used was the Agilent G1600AX capillary electrophoresis system (Agilent Technologies, Germany), equipped with diode array detector (DAD), automatic injector and temperature control system adjusted to 25 °C. A fused silica capillary with 50 μm of internal diameter and 72 cm of effective length, with extended bulb (Agilent Technologies, Germany) was used. Detection was performed at wavelengths of 230 and 274 nm, for taurine (indirect detection) and for caffeine (direct detection), respectively. The analysis and the treatment of the data were carried out on *HP ChemStation software*.

2.4 Method optimization (design) of the experiments and data processing

The choice of the electrolyte was based on the presence of a chromophore with absorption in the UV-VIS region, allowing indirect detection of taurine. On the other hand, it does not absorb in the UV light region (200 - 210 and 274 nm), enabling the direct detection of caffeine.

Then, a univariate study of the pH range was conducted. The range from 4.0 to 11.0 (1.0 interval) based on the pKa of compounds was studied. For such studies, we used a capillary with extended bulb of 50 μm i.d. x 72 cm in total length, an electrolyte containing 10 mmol.L^{-1} of benzoic acid and 50 mmol.L^{-1} of SDS, and the pH adjusted according to the matrix of the design, using NaOH (1 mol.L^{-1}) and HCl (0.1 mol.L^{-1}) solutions. The injection was hydrodynamic at 50 mbar for 5 s and + 30 kV voltage.

From the results obtained in the univariate tests, a central composite design 2^3, with central and axial points, was used to investigate the individual and interaction effects between pH variables, electrolyte and SDS concentrations. The central point was analyzed in triplicate, totaling 17 experiments. All experiments were conducted at random. The levels employed varied from 10 (-1.68) to 40 mmol.L^{-1} (+1.68) for electrolyte concentration; 10 (-1.68) to 50 mmol.L^{-1} (+1.68) for SDS concentration; and between 5.00 (-1.68) and 10.00 (+1.68) for pH. Table 1 shows the matrix of central composite design, with coded and decoded variables.

Experiments were conducted by injecting an added sample of standards, at concentrations of 0.5 mg.mL^{-1} for caffeine and 5 mg.mL^{-1} for taurine. All conditions of the central composite design were injected at 50 mbar of pressure for 5 s, at a temperature of 25 °C, and indirect detection at 230 nm for taurine and direct detection at 274 nm for caffeine. Before each condition of the design, a conditioning with 5 minutes of 1 mol.L^{-1} of sodium hydroxide, 5 minutes of water and 10 minutes of the electrolyte of the condition under study were performed, in order to maintain the original conditions of the capillary always the same. Each experimental condition was injected in duplicate, and between runs of the same condition, the capillary was conditioned for 2 minutes with the electrolyte.

Based on the results obtained in the tests, some responses were chosen to be optimized: (1) time difference between electroosmotic flow and caffeine (to evaluate the separation between the eak of caffeine and the peak of the system); (2) resolution between caffeine and taurine, calculated according to Equation 1; (3) symmetry of the taurine peak (due to the effects of dispersion resulting from the difference in conductivity of the electrolyte and the taurine molecule), calculated according to Equation 2; (4) height of the taurine peak (to improve the detection limit); and (5) baseline variation at 230 nm (very significant in indirect detections). For each response, linear and quadratic models were assessed regarding lack of fit, residual distribution and significance of regression by analysis of variance (ANOVA), at the confidence level of 95 %.

$$Rs = \frac{2(t_2 - t_1)}{w_2 + w_1} \qquad (1)$$

Table 1. Variables, levels and responses of the central composite design performed for the optimization in determining taurine and caffeine content in energy drinks.

Test	Coded Variables			Decoded Variables			Responses				
	A	B	C	A pH	B Benzoic Acid (mmol.L^{-1})	C SDS$^{(1)}$ (mmol.L^{-1})	Time difference EOF$^{(2)}$/Caffeine (min)	Resolution Taurine and Caffeine	Symmetry Taurine	Height peak from Taurine (mUA)	Variation of baseline in 230 nm (mUA)
1	-1.00	-1.00	-1.00	6.00	16.10	16.10	0.25	13.3	9.2	453.3	181.6
2	-1.00	-1.00	1.00	6.00	16.10	39.90	0.55	11.4	10.1	505.9	85.6
3	-1.00	1.00	-1.00	6.00	33.90	16.10	0.07	27.5	7.6	296.2	156.2
4	-1.00	1.00	1.00	6.00	33.90	39.90	0.79	20.4	8.0	312.7	172.2
5	1.00	-1.00	-1.00	9.00	16.10	16.10	0.20	12.1	10.3	514.2	24.0
6	1.00	-1.00	1.00	9.00	16.10	39.90	0.53	10.4	11.3	492.6	43.9
7	1.00	1.00	-1.00	9.00	33.90	16.10	0.11	25.3	7.4	307.5	138.6
8	1.00	1.00	1.00	9.00	33.90	39.90	0.54	20.6	7.6	331.2	163.2
9	-1.68	0.00	0.00	5.00	25.00	25.00	1.83	8.4	10.1	612.3	1359.0
10	1.68	0.00	0.00	10.00	25.00	25.00	0.23	16.3	9.8	477.6	212.9
11	0.00	-1.68	0.00	7.50	10.00	25.00	0.85	8.6	13.1	445.6	208.5
12	0.00	1.68	0.00	7.50	40.00	25.00	0.35	28.2	6.9	195.5	114.1
13	0.00	0.00	-1.68	7.50	25.00	10.00	0.12	25.9	6.8	432.5	44.2
14	0.00	0.00	1.68	7.50	25.00	50.00	1.01	15.3	10.1	401.0	242.1
15	0.00	0.00	0.00	7.50	25.00	25.00	0.13	19.4	9.0	432.7	93.8
16	0.00	0.00	0.00	7.50	25.00	25.00	0.21	18.4	9.4	460.4	168.5
17	0.00	0.00	0.00	7.50	25.00	25.00	0.07	18.7	8.8	451.7	155.2

$^{(1)}$SDS = sodium dodecyl sulfate; $^{(2)}$EOF = electroosmotic flow.

where: Rs represents the resolution value; t_1 and t_2 represent the migration time of each analyzed pair compound; and w_1 and w_2 represent the width of each compound peak base (Collins et al., 2006).

$$S = \frac{T_{final} - T}{T + T_{inicial}} \qquad (2)$$

where: S = symmetry; T = time of the maximum peak height; T_{final} = time of the peak base end; and $T_{initial}$ = time of peak base start.

Then, to simultaneously evaluate the responses and determine the best condition of analysis, the desirability function of Derringer & Suich (1980) was used, which places a desirability value for each response, and, from that, combine them in an overall desirability (Derringer & Suich, 1980). Data processing was conducted with Design Expert 6.0.4 (Minneapolis, USA) software.

2.5 Method validation by capillary electrophoresis

The method was validated in accordance with the recommendations made by the Guide for Validation of Analytical and Bioanalytical Methods (Brasil, 2017). Detection and quantification limits were estimated as being 3 and 6 times the signal - to - noise ratio, respectively. The quantification limit was based on the lowest level that presented acceptable relative coefficient variation (≤ 20 %) for the concentration evaluated. Calibration curves were constructed in random triplicates, each with 7 concentration levels, equidistantly, considering the expected concentrations in the samples. The linearity of calibration curves was evaluated and the linear model was validated by the analysis of variance (ANOVA) for lack of fit, residual distribution and significance of regression.

In order to evaluate the repeatability, 10 determinations were carried out in a single day, including 3 concentrations of calibration curves (corresponding to the quantification limit – level 1, the intermediate curve point – level 2, and the maximum concentration – level 3). Intermediate precision (between days) was evaluated through five determinations, at the same concentration levels of repeatability, in five consecutive days. For the study of accuracy through recovery, the fortification of the samples with standards in known concentrations was carried out, also following 3 curve levels (at concentrations of the quantification limit, the intermediate point and the maximum concentration) through 3 determinations at each level. Robustness was evaluated for the pH of the electrolyte, in an univariate way. Between the days of analysis, the method was monitored by analyzing a reference commercial sample every 4 hours.

3 Results and discussion

3.1 Method optimization and experimental design

For the direct detection system of caffeine and indirect detection of taurine, the benzoic acid was the run electrolyte chosen because it presents a chromophore in its structure,

which has maximum absorptivity of 230 nm, allowing indirect determination of taurine content, but that does not absorb at the 274 nm region, which is the wavelength of caffeine absorption.

In the univariate study of the pH, it was observed that values above 10 increased the baseline noise substantially, and values below 5 notably extended the analysis time. As for the study of the SDS surfactant, it was proved to be effective, and it was observed that the higher the SDS concentration, the longer the analysis time.

Table 1 presents responses obtained in experiments of the central composite design 2^3. Table 2 presents tests for lack of fit, significance of regression and coefficients of the models.

The taurine peak models for responses of symmetry and height showed no evidence of lack of fit. On the other hand, models of time difference between electroosmotic flow and caffeine, of resolution between taurine and caffeine, and the variation in baseline at 230 nm showed a slight lack of fit. Regarding significance of the models, only the parameter of variation in baseline at 230 nm showed no significant regression model; however, the information is unreliable, since there was lack of fit.

When a model used for algorithms optimization presents lack of fit, the desirability function of Derringer & Such (1980) may not correctly estimate the optimal condition. However, it is possible to observe that the lack of fit for the responses "time difference between electroosmotic flow and caffeine", "resolution between taurine and caffeine" and "variation in baseline at 230 nm for taurine" was found to be small. The values of F calculated were 1.7, 1.2 and 3.7 times above the critical F $_{value}$, respectively. Other authors have demonstrated that models with a slight lack of fit can still be useful to predict analytical conditions. Dias et al. (2015), employed models with values of F $_{calculated}$ up to 10 times higher than the values of F $_{critical}$, obtaining satisfactory results in the optimization of responses. Similarly, in Meinhart et al. (2011) and Ballus et al. (2014) studies, models with a slight lack of fit significantly contributed to the optimization of the

analysis methods. Considering that such models presented random residual distribution, these were kept in the process of simultaneous optimization of responses.

In this study, we also conducted experimental region restrictions to reduce the error of algorithm prediction due to the model's lack of fit, as was performed in Dias et al. (2015). The experimental responses of time difference between electroosmotic flow and caffeine, indicated that the conditions of the experiments 3, 5, 7, 10, 13, 15, 16 and 17 should be avoided, since the responses were unsatisfactory (difference less than 0.25 min). The other response with lack of fit (baseline variation) indicates that the conditions of the tests 9, 10, 11 and 14 presented a very high baseline variation (above 200 mUA), and such conditions should be avoided. The response for the resolution between caffeine and taurine, although presenting lack of fit, is not worrisome once all the resolutions among compounds were high. Thus, restrictions have been established to reject levels of variables that were experimentally harmful for time difference between electroosmotic flow and caffeine, and the baseline variation. As it can be seen in Table 3, the pH was limited to the levels from - 1.00 to 1.00, the concentration of benzoic acid from - 1.00 to 1.68 and the SDS concentration from - 1.68 to 1.00.

For the employment of the desirability algorithm proposed by Derringer & Such (1980), an individual desirability was established for each variable in order to obtain time difference between electroosmotic flow and caffeine above 0.8 and below 2.0 minutes, to obtain the smallest possible baseline variation, the highest taurine peak, and reduce the symmetry of taurine for values near 1.0 with minimum acceptable of 0.8. For the resolution between caffeine and taurine, the desirability criterion was to reduce it (since values were too high, from 8 to 28), with the minimum limit of 3.0, resolution considered excellent for quantification. With the reduction of the values closer to 3.0, there is a tendency of reduction of the analysis time.

Table 2. Mathematical models obtained from responses of the central compound design, tests of lack of fit and significance of regressions tests.

Responses	Coefficients of regression (errors)										Lack of fit and Regression tests	
	Intercept	A pH	B Benzoic Acid	C SDS[1]	A^2	B^2	C^2	AB	AC	BC	F$_{Calculated}$ Lack of fit[3]	F$_{Calculated}$ Regression[4]
Time Difference EOF[2] and Caffeine	0.46 (0.09)	-0.22 (0.10)	-0.06 (0.10)	0.24 (0.10)	-	-	-	-	-	-	32.87	3.47
ResolutionTaurine and Caffeine	18.78 (1.29)	0.66 (0.60)	5.82 (0.60)	-2.43 (0.60)	-2.16 (0.67)	-0.03 (0.67)	0.77 (0.67)	0.03 (0.79)	0.31 (0.79)	-1.04 (0.79)	22.76	14.17
SymmetryTaurine	9.16 (0.20)	0.07 (0.22)	-1.52 (0.22)	0.60 (0.22)	-	-	-	-	-	-	7.95	18.77
Height of peak of Taurine	449.88 (25.02)	-10.92 (11.76)	-83.44 (11.76)	1.33 (11.76)	28.81 (12.95)	-50.70 (12.95)	-16.62 (12.95)	-2.23 (15.36)	-8.37 (15.36)	1.15 (15.36)	12.80	8.92
Variation of Baseline in 230 nm	156.28 (163.77)	-157.68 (76.95)	10.00 (76.95)	21.75 (76.95)	171.84 (84.78)	-49.48 (84.78)	-55.91 (84.78)	21.59 (100.50)	15.56 (100.50)	14.59 (100.50)	70.91	1.24

[1]SDS = sodium dodecyl sulfate; [2]EOF = electroosmotic flow; [3]F$_{critical}$ for the test of lack of fit: 19.35 (values of F$_{calculated}$ higher than F$_{critical}$ indicate model with lack of fit); [4]F$_{critical}$ for the significance of regression: 3.55 (values of F$_{calculated}$ lower than F$_{critical}$ indicate significative regression).

Table 3. Conditions of desirability employed in simultaneous optimization of responses, values predicted by models and values observed.

Variable/ Response	Criterion	Lower Limit	Upper Limit	Importance	Predicted Value	Observed Value
Ph	within range	-1.00	1.00	3	7.26	-
Concentration of electrolyte	within range	-1.00	1.68	3	16.20	-
Concentration of SDS[(1)]	within range	-1.68	1.00	3	39.90	-
Time Difference EOF[(2)]/Caffeine	within range	0.80	2.00	5	0.80	0.31 ± 0.02
Resolution Taurine/Caffeine	Minimize	3.00	28.20	3	12.15	9.92 ± 1.04
Symmetry Taurine	Minimize	0.80	10.00	5	11.26	15.55 ± 1.47
Height of peak Taurine	Maximize	300.0	613.00	5	469.83	517.15 ± 15.63
Variation of baseline in 230 nm	Minimize	1.0	1359.00	3	79.81	76.00 ± 1.84

[(1)]SDS = sodium dodecyl sulfate; [(2)]EOF = electroosmotic flow.

Table 3 also presents the conditions of analysis suggested for each parameters, as well as the experimental responses provided. The analytical condition of pH 7.26, 16.20 mmol.L^{-1} of benzoic acid and 39.90 mmol.L^{-1} of SDS were tested in triplicate, and the experimental values observed are presented in the table with their respective deviations.

Except for the response of time difference between electroosmotic flow and caffeine, other responses observed were very close to the responses provided. Differences may be attributed to the error in each one of the models due to lack of fit. However, the models were still useful for the optimization of the analytical method.

The method was validated and its parameters were: linear range from 4 to 84 mg.L^{-1} for caffeine and from 15 to 235 mg.L^{-1} for taurine; lack of fit of 6.76 for caffeine and 2.59 for taurine; detection limit of 2 mg.L^{-1} for caffeine and 7.5 mg.L^{-1} for taurine; quantification limit of 4 mg.L^{-1} for caffeine and 15 mg.L^{-1} for taurine; repeatability (n = 10) with coefficient of variation of 15.93% (level 1), 8.21% (level 2) and 8.75% (level 3) for caffeine, and for taurine the values were 13.80% (level 1), 13.25% (level 2) and 9.08% (level 3); intermediate precision (n = 5) with coefficient of variation of 10.57% (level 1), 5.59% (level 2) and 3.97% (level 3) for caffeine, and for taurine the values were 14.27% (level 1), 13.12% (level 2) and 10.57% (level 3). Recovery (n = 3) values were 105.11% (level 1), 111.26% (level 2) and 118.83% (level 3) for caffeine, and for taurine the values were 117.18% (level 1), 120.81% (level 2) and 115.40% (level 3). The concentrations for the repeatability, intermediate precision and recovery at levels 1, 2 and 3, were equivalent to 4, 40 and 84 mg.L^{-1} for caffeine and 15, 110 and 235 mg.L^{-1} for taurine, respectively. Regarding the robustness of the pH of the run electrolyte, it was observed that there was no significant difference in retention times and areas (in standard and control sample) when the electrolyte was prepared with pH variation from 7.21 to 7.29.

Through the analysis of variance, the linear regression was significant in the concentration ranges studied and the mathematical model for the caffeine presented a slight lack of fit(due to the low experimental error) and the mathematical model for taurine showed no lack of fit (p > 0.05), proving to be appropriate to perform quantifications. Quantification limits were suitable for energy drinks analysis with an extremely simple extraction method. The other parameters also presented suitable

results for the quantification according to the requirements of ANVISA (Brasil, 2003), demonstrating that the method is reliable for the simultaneous determination of caffeine and taurine contents in energy drinks.

In the literature, the caffeine content is generally determined by HPLC, using mobile phases containing water and methanol or acetonitrile (Rahim et al., 2014)or by capillary electrophoresis (Vochyánová et al., 2014), both with DAD detection. They are simple analysis methods and have a short running time (less than 10 minutes). Taurine content analysis, usually performed by HPLC, requires indirect detectors (Cao et al., 2003) or must be preceded by derivatization reactions (Mou et al., 2002; Zinellu et al., 2009) for detection by DAD or fluorescence. Derivatization reactions are expensive and may be incomplete (Mou et al., 2002). The method developed and validated in the present study has the advantage of quantifying both compounds in a single analysis, using only one detector, without the use of organic solvents, with a short time of analysis and an extremely simple sample preparation.

3.2 Determination of caffeine and taurine contents in energy drinks sample

Figure 1 presents an electropherogram obtained in the determination of caffeine and taurine contents in a sample of energy drink, and Table 4 presents the average concentrations of caffeine and taurine in each batch of 22 energy drink brands analyzed.

Regarding the caffeine content present in 22 analyzed brands, it ranged from 19.8 mg.100 mL^{-1} to 31.5 mg.100 mL^{-1}, and brand 16 presented the lowest level, which was also significantly different (p < 0.05) from the content found in brands 12c, 13, 19, 20, 21. These, in turn, were not statistically different from each other.

As for the taurine content, it was observed that the value ranged from 313.0 mg.100 mL^{-1} to 489.4 mg.100 mL^{-1}. All brands were statistically equal to each other (p < 0.05), except for brand 1 in relation to brand 16.

When the difference of the caffeine and taurine levels between batches of the same brand was investigated, it was verified that for caffeine, only 6 brands presented statistical equality between the batches and that 16 brands had at least one batch different from
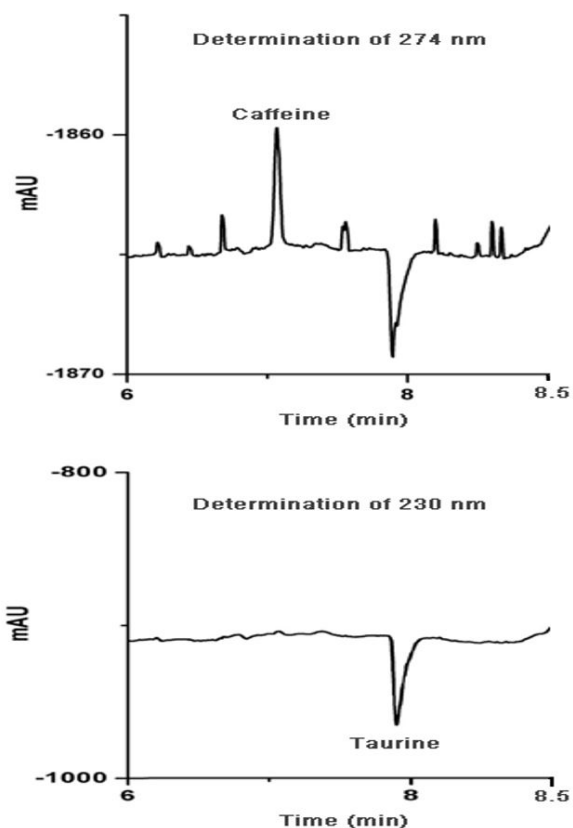
Figure 1. Electropherogram obtained in the determination of caffeine and taurine in a sample of energy drink. Capillary of 50 μm of i.d. x 72 cm of effective length with extended bulb, electrolyte benzoic acid 16.2 mmol.L^{-1} with sodium dodecyl sulfate (SDS) 39.9 mmol.L^{-1}, pH 7.26, 30 kV, 25 °C, injection of 50 mbar for 5 s and detection at 274 nm (caffeine) and at 230 nm (taurine).

the others. For taurine content, 9 brands presented statistical equality between the batches and 13 had different taurine content in at least one of the batches.

In Accordance with ANVISA, the value declared on the label of energy drinks should be equivalent to the content present in the drink, without any tolerance (Brasil, 2005). Comparing the caffeine content with the value declared on the label and with values of confidence intervals, it was found that among the 22 brands analyzed, 21 presented at least one batch with caffeine content below the declared value, and from these, 9 brands had all batches with levels below the ones declared. Only the brand 20 presented all batches with the same concentrations of caffeine declared on the label.

Regarding taurine content, 11 brands presented at least one batch with value below the one declared on the label. Only 4 brands presented all batches with the same taurine concentrations as the ones declared on the label.

According to ANVISA (Brasil, 2005), the caffeine and taurine content present in samples must be below the legal limit, without tolerance, being 35 mg.100 mL^{-1} and 400 mg.100 mL^{-1}, respectively. From the confidence intervals (95 %), it was possible to observe that no brand presented the caffeine content above the maximum limit allowed. Regarding taurine content, 50% of the brands presented at least a batch above the maximum limit allowed. Similar results were found for caffeine by McCusker et al. (2006) using gas chromatography with nitrogen-phosphorus detector, and by Ballus et al. (2012) using the technique of micellar electrokinetic chromatography with DAD (MEKC) detection system.

Table 4. Average content of caffeine and taurine and the confidence interval (95%) of analysed energy drinks samples.

Brands	Batch	Caffeine			Taurine		
		Concentration (mg.100 mL^{-1})	CI (95%)	DV	Concentration (mg.100 mL^{-1})	CI (95%)	DV
1	A	24.9 ± 1.2[a]	21.9-27.9	32.0	614.2 ± 5.7[a]	525.4-703.0	400.0
	B	25.9 ± 1.9[a]	21.0-30.8		397.0 ± 9.9[b]	372.5-421.5	
	C	25.3 ± 1.4[a]	21.9-28.6		457.1 ± 13.1[b]	400.4-513.8	
	Average	25.4 ± 0.5			489.4 ± 112.2		
2	A	25.1 ± 0.8[a]	23.1-27.0	31.9	353.9 ± 10.4[a]	256.1-451.8	400.0
	B	25.6 ± 1.6[a]	21.8-29.5		407.6 ± 7.3[a]	389.4-425.7	
	C	26.1 ± 1.5[a]	22.3-30.0		412.1 ± 14.5[a]	376.1-448.0	
	Average	25.6 ± 0.5			391.2 ± 32.3		
3	A	27.6 ± 1.4[a]	24.1-31.0	32.0	361.0 ± 10.8[c]	334.1-387.9	400.0
	B	21.4 ± 1.6[b]	17.4-25.4		434.5 ± 4.3[b]	423.8-445.1	
	C	28.1 ± 0.2[a]	27.7-28.5		459.4 ± 6.8[a]	442.7-476.2	
	Average	25.7 ± 3.7			418.3 ± 51.1		
4[(1)]	A	25.0 ± 1.2[a]	22.1-27.8	29.7	405.0 ± 26.7[a]	338.7-471.3	371.7
	B	27.1 ± 2.4[a]	21.1-33.0		447.7 ± 10.9[a]	420.5-474.9	
	C	23.5 ± 0.2[a]	23.1-23.9		454.8 ± 29.8[a]	380.5-528.7	
	Average	25.2 ± 1.8			435.8 ± 26.9		

[(1)]traditional flavor; [(2)]zero sugar; [(3)]flavored. CI (95%) = confidence interval of 95% (n = 3, t$_2$ = 4.303); DV = Declared value on the label (mg.100 mL^{-1}). Identical lower case letters between batches of the same brand indicate that there is no significant difference between batches according to the Tukey test (95% of confidence).

Table 4. Continued...

Brands	Batch	Caffeine			Taurine		
		Concentration (mg.100 mL⁻¹)	CI (95%)	DV	Concentration (mg.100 mL⁻¹)	CI (95%)	DV
4[2]	A	26.4 ± 0.7ᵃ	24.7-28.1	29.7	355.1 ± 23.7ᵇ	296.2-414.0	371.7
	B	27.4 ± 1.3ᵃ	24.1-30.7		421.4 ± 26.0ᵃ	356.8-486.0	
	C	22.2 ± 1.4ᵇ	18.7-25.7		402.0 ± 8.3ᵃᵇ	381.4-422.6	
	Average	25.3 ± 2.8			392.8 ± 34.1		
5[1]	A	30.6 ± 0.5ᵃ	29.4-31.8	32.5	419.6 ± 27.5ᵃᵇ	351.3-487.9	400.0
	B	28.8 ± 2.6ᵃᵇ	22.4-35.2		467.7 ± 34.5ᵃ	381.9-553.5	
	C	25.6 ± 1.2ᵇ	22.5-28.6		357.5 ± 14.7ᵇ	320.9-394.1	
	Average	28.3 ± 2.6			414.9 ± 55.2		
5[3]	A	32.5 ± 0.7ᵃ	30.7-34.2	32.5	437.5 ± 35.1ᵃ	350.3-524.7	400.0
	B	31.0 ± 0.6ᵃ	29.5-32.5		452.4 ± 28.5ᵃ	381.6-523.2	
	C	25.8 ± 1.3ᵇ	22.6-29.0		411.7 ± 0.7ᵃ	410.0-413.3	
	Average	29.8 ± 3.5			433.8 ± 20.6		
6	A	33.0 ± 1.3ᵃ	29.9-36.2	32.0	414.0 ± 9.6ᵇ	390.1-437.9	400.0
	B	32.7 ± 1.3ᵃ	29.5-35.9		476.1 ± 11.5ᵃ	447.5-504.6	
	C	24.0 ± 1.2ᵇ	20.9-27.0		434.3 ± 8.5ᵇ	413.1-455.5	
	Average	29.9 ± 5.1			441.4 ± 31.6		
7	A	23.6 ± 0.6ᵇ	22.0-25.1	30.8	307.6 ± 21.6ᵇ	254.0-361.2	384.6
	B	30.4 ± 2.5ᵃ	24.2-36.6	32.0	440.0 ± 23.7ᵃ	381.3-498.8	400.0
	C	19.7 ± 1.0ᵇ	17.1-22.2	30.8	307.5 ± 3.4ᵇ	299.1-315.9	384.6
	Average	24.5 ± 5.4			351.7 ± 76.5		
8	A	24.2 ± 0.8ᵃ	22.1-26.3	32.0	285.6 ± 15.7ᵇ	246.5-324.6	400.0
	B	21.2 ± 1.3ᵃᵇ	17.9-24.6		347.0 ± 22.6ᵃᵇ	290.9-403.10	
	C	20.5 ± 1.7ᵇ	16.2-24.8		382.0 ± 39.4ᵃ	284.0-480.0	
	Average	22.0 ± 2.0			338.2 ± 48.8		
9	A	29.8 ± 0.6ᵃ	28.2-31.3	34.6	402.8 ± 24.9ᵃᵇ	341.0-464.5	400.0
	B	26.9 ± 0.3ᵇ	26.2-27.7		373.3 ± 12.2ᵇ	343.0-403.6	
	C	24.8 ± 1.0ᶜ	22.4-27.2	35.0	443.9 ± 22.6ᵃ	387.7-500.0	
	Average	27.2 ± 2.5			406.6 ± 35.5		
10	A	28.5 ± 0.7ᵃ	26.6-30.3	34.8	362.7 ± 5.5ᶜ	349.2-376.3	400.0
	B	22.0 ± 1.6ᵇ	18.0-26.0		333.2 ± 5.4ᵇ	319.8-346.6	
	C	27.0 ± 0.2ᵃ	26.4-27.5		453.1 ± 8.8ᵃ	431.3-475.0	
	Average	25.8 ± 3.4			383.0 ± 62.5		
11	A	29.7 ± 1.0ᵃ	27.2-32.1	32.0	404.3 ± 31.2ᵃ	326.8-481.8	400.0
	B	30.2 ± 0.4ᵃ	29.4-31.1		471.6 ± 37.1ᵃ	379.4-563.7	
	C	25.6 ± 1.4ᵇ	22.2-29.1		461.6 ± 25.0ᵃ	399.5-523.7	
	Average	28.5 ± 2.5			445.8 ± 36.3		
12[1]	A	28.6 ± 3.4ᵃ	20.3-37.0	32.0	395.0 ± 9.6ᵇ	371.1-418.9	400.0
	B	25.9 ± 1.6ᵃ	21.9-30.0		449.4 ± 6.3ᵃ	433.7-465.2	
	C	26.6 ± 1.4ᵃ	23.2-30.0	32.1	445.7 ± 28.0ᵃ	376.2-515.1	
	Average	27.1 ± 1.4			430.0 ± 30.4		
12[2]	A	28.8 ± 1.4ᵃ	25.3-32.4	32.0	381.5 ± 22.9ᵇ	324.6-438.5	400.0
	B	25.8 ± 2.3ᵃᵇ	20.2-31.5		369.8 ± 13.9ᵇ	335.3-404.3	
	C	24.4 ± 0.3ᵇ	23.5-25.2		458.9 ± 21.0ᵃ	406.8-511.0	
	Average	26.3 ± 2.3			403.4 ± 48.4		
12[3]	A	34.6 ± 2.3ᵃ	28.8-40.4	32.0	464.4 ± 8.8ᵃ	442.6-486.2	400.0
	B	32.8 ± 3.5ᵃ	24.0-41.5		475.0 ± 6.3ᵃ	459.4-490.7	
	C	25.1 ± 1.5ᵇ	21.4-28.7		461.8 ± 14.1ᵃ	426.7-496.9	
	Average	30.8 ± 5.1			467.1 ± 7.0		
13	A	34.0 ± 2.9ᵃ	26.9-41.1	35.0	387.6 ± 20.2ᵃ	337.3-437.8	400.0
	B	26.5 ± 0.6ᵇ	25.0-28.1		371.8 ± 3.9ᵃ	362.1-381.5	
	C	30.3 ± 1.6ᵃᵇ	26.3-34.3		418.3 ± 33.3ᵃ	335.6-501.0	
	Average	30.3 ± 3.7			392.5 ± 23.6		

[1]traditional flavor; [2]zero sugar; [3]flavored. CI (95%) = confidence interval of 95% (n = 3, t_2 = 4.303); DV = Declared value on the label (mg.100 mL⁻¹). Identical lower case letters between batches of the same brand indicate that there is no significant difference between batches according to the Tukey test (95% of confidence).

Table 4. Continued...

Brands	Batch	Caffeine			Taurine		
		Concentration (mg.100 mL^{-1})	CI (95%)	DV	Concentration (mg.100 mL^{-1})	CI (95%)	DV
14	A	21.0 ± 1.3[b]	17.7-24.3	35.0	304.9 ± 6.9[b]	287.8-321.9	393.8
	B	21.8 ± 1.1[b]	19.0-24.6		365.8 ± 28.0[a]	296.1-435.4	
	C	27.6 ± 0.7[a]	26.0-29.3		406.6 ± 16.4[a]	365.8-447.3	400.0
	Average	23.5 ± 3.6			359.1 ± 51.2		
15	A	31.0 ± 2.6[a]	24.6-37.4	31.8	358.8 ± 21.5[b]	305.4-412.3	400.0
	B	31.4 ± 0.4[a]	30.3-32.4		461.0 ± 12.8[a]	429.3-492.7	
	C	25.5 ± 1.2[b]	22.5-28.6		189.0 ± 10.3[c]	163.4-214.6	
	Average	29.3 ± 3.3			336.3 ± 137.4		
16	A	19.8 ± 1.6[ab]	16.0-23.7	32.0	255.4 ± 16.8[b]	213.7-297.1	400.0
	B	21.2 ± 0.5[a]	19.9-22.5		333.1 ± 14.5[a]	297.0-369.2	
	C	18.4 ± 0.6[b]	17.0-19.9		350.6 ± 23.4[a]	292.5-408.7	
	Average	19.8 ± 1.4			313.0 ± 50.7		
17	A	32.7 ± 2.4[a]	26.7-38.7	32.0	380.2 ± 14.3[b]	344.6-415.8	400.0
	B	26.6 ± 1.3[b]	23.5-29.7		453.6 ± 16.6[a]	412.3-494.8	
	C	30.2 ± 1.6[ab]	26.2-34.3		470.0 ± 18.0[a]	425.4-514.6	
	Average	29.8 ± 3.1			434.6 ± 47.8		
18	A	24.8 ± 0.7[b]	23.0-26.5	30.0	384.8 ± 34.3[a]	299.5-470.0	372.5
	B	27.5 ± 1.2[a]	24.4-30.5		380.5 ± 3.2[a]	372.7-388.4	
	Average	26.1 ± 1.9			382.6 ± 3.0		
19	A	31.5 ± 1.1[a]	28.7-34.3	35.0	375.2 ± 33.8[a]	291.2-459.2	400.0
	B	30.9 ± 1.0[a]	28.5-33.3		384.2 ± 15.7[a]	345.3-423.1	
	Average	31.2± 0.4			379.7 ± 6.4		
20	A	31.6 ± 0.8[a]	29.7-33.6	32.0	338.8 ± 14.2[b]	303.6-373.9	400.0
	B	31.5 ± 1.4[a]	28.1-34.8		430.5 ± 14.5[a]	394.4-466.7	
	Average	31.5 ± 0.1			384.7 ± 64.9		
21	A	32.3 ± 0.5[a]	31.1-33.5	32.0	401.8 ± 32.1[a]	322.0-481.5	400.0
	B	28.7 ± 0.8[b]	26.9-30.6		438.9 ± 2.4[a]	433.0-444.9	
	Average	30.5 ± 2.6			420.4 ± 26.3		
22	A	32.3 ± 0.8[a]	30.3-34.2	32.0	400.6 ± 23.2[a]	343.0-458.2	400.0
	B	16.7 ± 0.7[b]	14.9-18.5		282.0 ± 11.2[b]	254.3-309.0	
	Average	24.5 ± 11.0			341.3 ± 83.8		

[1]traditional flavor; [2]zero sugar; [3]flavored. CI (95%) = confidence interval of 95% (n = 3, t$_2$ = 4.303); DV = Declared value on the label (mg.100 mL^{-1}). Identical lower case letters between batches of the same brand indicate that there is no significant difference between batches according to the Tukey test (95% of confidence).

4 Conclusion

The developed method showed appropriate validation parameters for the determination of the compounds in energetic samples, showing its applicability in the detection of caffeine and taurine contents by CE with low reagent costs, reduced analysis time, minimum residue generation, no exposure of the analyst to toxic solvents, and extremely simple sample preparation.

As for the evaluation of different samples of energy drinks, the caffeine content was always below the maximum limit allowed; on the other hand, the taurine content in 50% of the samples was above the maximum limit allowed in Brazilian legislation for energy drinks. From 73 samples, 68% showed an amount of caffeine statistically lower than the one declared on the product label, whereas for taurine, only 19% showed an amount lower than the one declared on the product label, being in disagreement with Brazilian legislation.

Most brands presented heterogeneity in the caffeine and taurine contents among analyzed batches from the same brand.

These differences between batches of the same brand show the need for a stricter control on standardization of added ingredients.

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