Comparison of capillary electrophoresis and high performance liquid chromatography methods for caffeine determination in decaffeinated coffee

Comparação de métodos por eletroforese capilar e cromatografia líquida de alta eficiência para a determinação de cafeína em café descafeinado

Carolina Schaper BIZZOTTO[1], Adriana Dillenburg MEINHART[1], Cristiano Augusto BALLUS[1], Gislaine GHISELLI[1], Helena Teixeira GODOY[1]*

Abstract

Decaffeinated coffee accounts for 10 percent of coffee sales in the world; it is preferred by consumers that do not wish or are sensitive to caffeine effects. This article presents an analytical comparison of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) methods for residual caffeine quantification in decaffeinated coffee in terms of validation parameters, costs, analysis time, composition and treatment of the residues generated, and caffeine quantification in 20 commercial samples. Both methods showed suitable validation parameters. Caffeine content did not differ statistically in the two different methods of analysis. The main advantage of the high performance liquid chromatography (HPLC) method was the 42-fold lower detection limit. Nevertheless, the capillary electrophoresis (CE) detection limit was 115-fold lower than the allowable limit by the Brazilian law. The capillary electrophoresis (CE) analyses were 30% faster, the reagent costs were 76.5-fold, and the volume of the residues generated was 33-fold lower. Therefore, the capillary electrophoresis (CE) method proved to be a valuable analytical tool for this type of analysis.

Keywords: *caffeine; capillary electrophoresis; HPLC; method comparison; decaffeinated coffee.*

Resumo

O comércio de café descafeinado constitui 10% das vendas mundiais de café, sendo preferido pelos consumidores que não desejam ou são sensíveis aos efeitos da cafeína. Este artigo apresenta uma comparação analítica de métodos por eletroforese capilar (CE) e cromatografia líquida de alta eficiência (HPLC) para a quantificação de cafeína residual em café descafeinado, quanto aos parâmetros de validação, custos, tempo de análise, composição e tratamento dos resíduos gerados, bem como quantificação de cafeína em 20 amostras comerciais. Ambos os métodos apresentaram parâmetros de validação adequados. O teor de cafeína não diferiu estatisticamente pelos dois métodos. A vantagem do método por HPLC foi o limite de detecção 42 vezes mais baixo. Não obstante, o limite de detecção da CE foi 115 vezes menor do que o permitido pela legislação brasileira. A análise por CE foi 30% mais rápida, os custos com reagentes foram 76,5 vezes mais baixos e o volume de resíduos gerados foi 33 vezes menor. Portanto, o método por CE mostrou-se uma valiosa ferramenta analítica para este tipo de análise.

Palavras-chave: *cafeína; eletroforese capilar; HPLC; comparação de métodos; café descafeinado.*

1 Introduction

Coffee is one of the most appreciated beverages in the world (FUJIOKA; SHIBAMOTO, 2008). Among the compounds present in this beverage, caffeine stands out (ESQUIVEL; JIMÉNEZ, 2012), mainly for acting as the central nervous system stimulant (ALI et al., 2012). Studies indicate that moderate coffee consumption has positive effects, such as psychoactivity responses (alertness and mood change), neurological conditions (infant hyperactivity, Parkinson's disease), metabolic disorders (diabetes), and gonad and liver functions (KERRIGAN; LINDSAY, 2005). However, when consumed in high doses, caffeine consumption can be associated with the following symptoms: nervousness, anxiety, restlessness, insomnia, gastrointestinal upset, tremors, tachycardia, and psychomotor agitation (REISSIG; STRAIN; GRIFFITHS, 2009).

Additionally, there are people that are sensitive to caffeine. In these cases, standard doses induce greater effects than normal. The half-life of caffeine varies widely among individuals, depending on factors such as age, liver function, pregnancy, some concurrent medications, and the level of liver enzymes needed to metabolize caffeine. In the human body, caffeine elimination time typically varies from three to seven hours. The half-life of caffeine is about 20-30% lower in women. Women taking oral contraceptives require about twice as long to process caffeine compared to women that are ovulating. The rate of elimination of caffeine progressively decreases during pregnancy, almost doubling the half-life of caffeine in the plasma during the third trimester. Furthermore, term or premature infants exhibit a markedly lower rate of caffeine elimination. Patients with hepatic disease may have a significantly lower rate of caffeine elimination (JAMES, 1991).

The FDA (Food and Drug Administration) recommends a reduction of caffeine intake during pregnancy (GOYAN,

Received 13/9/2012
Accepted 10/12/2012 (005887)
[1] *Laboratory of Instrumental Analysis in Food, Department of Food Science, Faculty of Food Engineering, University of Campinas – UNICAMP, Rua Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, CEP 13083-862, Campinas, SP, Brasil, e-mail: helena@fea.unicamp.br*
**Corresponding author*

DOI: http://dx.doi.org/10.1590/S0101-20612013005000013

1980; FOOD..., 2007). Healthcare professionals recommend a caffeine-free diet to pregnant women and patients with various medical conditions such as hypertension and arrhythmia (McCUSKER et al., 2006). For many consumers, the use of decaffeinated coffee is the solution to avoid the supposed adverse effects of caffeine intake (COULTATE, 2004), which justifies the high consumption of decaffeinated coffee, which corresponds to 10% of the global coffee consumption (SILVAROLLA; MAZZAFERA; FAZUOLI, 2004).

Constant monitoring of caffeine levels in decaffeinated coffee available to the public is of paramount importance to ensure that caffeine content meets the legal limits allowed (MEINHART et al., 2010). Brazilian law allows up to 0.1% of residual caffeine in decaffeinated roasted-ground (powder) coffee (AGÊNCIA..., 2005). Therefore, various methods of caffeine analysis have been reported (ARESTA; PALMISANO; ZAMBONIN, 2005; POMILIO; TRAJTEMBERG; VITALE, 2005; BRUNETTO et al., 2007; DE MARIA; MOREIRA, 2007; FENSKE, 2007; KHANCHI et al., 2007; SHRIVAS; WU, 2007; PERRONE; DONANGELO; FARAH, 2008; MEINHART et al., 2010) highlighting the high performance liquid chromatography (ARESTA; PALMISANO; ZAMBONIN, 2005; BRUNETTO et al., 2007; PERRONE; DONANGELO; FARAH, 2008) and capillary electrophoresis methods (POMILIO; TRAJTEMBERG; VITALE, 2005; MEINHART et al., 2010).

Jimidar et al. (1993) compared HPLC and CE methods to determine caffeine, aspartame, and benzoic acid in diet cola soft drinks and in artificial sweeteners. They found that relative standard deviations for reproducibility were significantly higher in CE than in HPLC. The separation efficiency of CE was 65-110-fold higher than that of HPLC, while 10-20-fold lower detection limits were obtained in HPLC. In addition, the matrix effect was higher for CE. Lee and Ong (2000), in comparative studies of these two techniques to evaluate the catechins levels in tea, found that the CE method provided faster results, whereas the HPLC method showed lower detection limits. Kowalski et al. (2007), when comparing the same techniques in the analysis of cotinine in human plasma, verified that the CE method required smaller amounts of reagents, while the HPLC method resulted in lower detection limits. Furthermore, Sombra et al. (2005), while investigating synthetic adulterants in plant protection, also concluded that the CE method was faster, and that the HPLC method had lower detection limits. Conversely, Kowalski and Plenis (2007), in a quantification study on cetirizine di-hydrochloride in human plasma, obtained faster analysis using the HPLC, equivalent detection limits between the two methods, and lower solvent consumption with the CE method. In those studies, the costs, composition and volume of residues generated, and the required treatment and its cost were not measured and compared directly. In addition, there are no comparative studies available on the residual caffeine in decaffeinated coffee samples.

Considering that Brazil is the largest coffee producer worldwide (FOOD..., 2012), and its derivatives are very important for the country's economy, there is constant search for faster, efficient, cost effective, and low environmental impact methods. This study was designed to evaluate the costs and benefits related to the use of two methods, reversed phase HPLC and CE with micellar electrokinetic chromatography (MEKC), for the analysis of residual caffeine in decaffeinated coffee. The study involved a detailed comparison including figures of merit, analysis time (running time, preconditioning, and cleaning of the capillary or column), cost of reagents, and residues generated by both methods.

2 Materials and methods

2.1 Reagents

Caffeine and theobromine (internal standard – IS) standards were purchased from Sigma Chemical Co. (USA); chloroform (pro analysis grade) was obtained from Merck (Brazil); sodium dodecylsulfate (SDS) from Riedel-de-Haën (Germany); sodium carbonate and sodium sulfate from Synth (Brazil); and methanol HPLC grade from J. T. Baker (USA). Caffeine and theobromine stock solutions were prepared by dissolving the compounds in ultrapure water, at concentrations of 1000 mg.L^{-1} and 100 mg.L^{-1}, respectively. Caffeine working solutions were prepared with final concentrations ranging from one to 100 mg.L^{-1}. Ultrapure water (18 MΩ.cm) obtained from a Direct-Q 3 UV ultrapure water system (Millipore Corporation, France) was used in the present study. All solutions were filtered through a 0.45 μm filter and stored under refrigeration. Before use, the standard solutions were degassed by ultrasonication for 5 minutes (Microsonic SX-20, Arruda Ultra-sons Ltda., Brazil).

2.2 Samples

The decaffeinated coffee samples consisted of one package of each twenty different brands of decaffeinated ground-roasted coffe purchased from a local market in Campinas (SP, Brazil).

Caffeine was extracted from the samples according to the procedure described by Meinhart et al. (2010). One gram of the coffee sample was added to a separatory funnel with 10 mL of 0.2 mol.L^{-1} NaOH and 30 mL of chloroform. The system was lightly shaken for 7 minutes, and the organic phase was collected in a 50 mL flask. The aqueous phase was then washed three times with 5 mL of chloroform, collecting the organic phase. Chloroform was evaporated from the extracts in a water bath at 60 °C. The residue was resuspended in 10 mL of ultrapure water and filtered through a 0.45 μm filter. After filtration, the samples were transferred to vials and were degassed by ultrasonication for 5 minutes. The resulting extract was analyzed using the two methods.

2.3 Instrumental parameters and conditions

Capillary electrophoresis

CE separation was performed using an Agilent G1600AX system (Agilent Technology, Germany) equipped with a diode-array detector (DAD), according to the method proposed by Meinhart et al. (2010). A fused-silica capillary (48 cm × 50 μm i.d) was used to carry out the separation. The buffer solution consisted of 10 mmol.L^{-1} sodium carbonate and 50 mmol.L^{-1}

SDS (pH 11.0). The electrolyte vials were changed after 20 successive injections. The detection was performed at 206 nm, capillary temperature was maintained at 25 °C, voltage at +15 kV, and hydrodynamic injection at 50 mbar for 7 s. After filtration through a 0.45 μm filter, the buffer was centrifuged at 5000 rpm for 10 minutes (for air bubbles removal). The capillary was washed with buffer between runs for 0.5 minutes. At the beginning of each day, the capillary was washed for 5 minutes with 1 mol.L^{-1} sodium hydroxide, followed by 5 minutes wash in ultrapure water and running buffer (10 minutes). At the end of the day, the capillary was cleaned for 5 minutes with 1 mol.L^{-1} sodium hydroxide, followed by 5 minutes wash in ultrapure water and stored in water. Each electrophoretic run lasted 5.2 minutes.

Caffeine in the samples was quantified using an internal calibration curve, and it was positively identified by comparing the migration time and UV-spectrum obtained to those of caffeine standard.

High performance liquid chromatography

HPLC analysis was performed using an Agilent system (Hewlett Packard, 1100 series) with quaternary pumping, column oven at 25 °C, diode-array detector (DAD) at 274 nm, and automatic injector. The compounds were separated using a 15 cm × 4.6 mm i.d., 5 μm particle reversed-phase C18 column (Varian, USA).

The HPLC method used was a modified version based on the studies by De Maria and Moreira (2007) and Gnoatto et al. (2007). The mobile phase was a binary mixture of water and methanol 60:40 (v/v). Isocratic elution was performed at a flow rate of 0.7 mL.min^{-1}. The injection volume was 20 μL. At the beginning of each day, the column was conditioned with mobile phase for 60 minutes with a flow of 0.5 mL.min^{-1}. After analyte elution, the mobile phase flow was maintained for one minute, for interference elution and column regeneration. At the end of the daily activities, the column was cleaned with the mobile phase (flow of 0.7 mL.min^{-1}, for 30 minutes), to avoid solutes adsorption, and stored in the mobile phase overnight. The analysis time was 6.5 minutes.

Caffeine in the samples was quantified using an external calibration curve, and it was positively identified comparing the migration time and UV-spectrum obtained to those of caffeine standard.

2.4 Comparison of the analytical methods

For figure of merit comparison, the parameters were evaluated according to the guidelines for validation issued by the National Health Surveillance Agency, Brazil (AGÊNCIA..., 2003).

The limit of detection (LOD) and limit of quantification (LOQ) were estimated as three and six times the signal-to-noise ratio, respectively. The linearity of both methods was investigated in the range of 1-100 mg.L^{-1}. Intraday repeatability was determined through quantification of caffeine in the same extract for 8 consecutive times. Interday precision assays were performed by analyzing this same sample 8 times for 5 different days.

The quantification of caffeine, extracted in triplicate, was performed in the twenty commercial samples using both methods. The results of each sample, obtained by CE and HPLC, were evaluated using analysis of variance (ANOVA, $p < 0.05$) to verify if there was difference between them using the Statistica 7.0 software (Statsoft, USA).

The following parameters, capillary and column initial stabilization, injection of a calibration curve (7 concentrations injected in triplicate), analysis of 10 samples (extracted in triplicate and injected three times), and capillary or column cleaning after the total sequence analysis (111 runs), were considered for the comparison between analysis time, amount of the reagents used and their costs, and the residues generated by the two methods. The costs with the reagents was based on the average of three cost estimates Brazilian companies, except for the ultrapure water, whose costs were based on the financial reports provided by the laboratory water purification system. The sample extraction phase was not considered since it was the same for both methods.

Moreover, the residue treatment was also considered. Laboratory treatment was provided to the residues requiring neutralization only. However, the costs of those needing incineration were also considered.

3 Results and discussion

Table 1 shows the figures of merit for the two methods. Both techniques exhibited good linearity in the analyzed range. All determination coefficients were better than 0.9997. The limits of detection and quantification were 42-fold lower using the HPLC. In the CE, the injected volumes and the optical path length were considerable smaller than those in the HPLC, thus leading to larger detection limits. It is noteworthy that the detectability of CE methods can be improved by on-line concentration techniques (MALÁ et al., 2009; MORAES et al., 2009). However, in this study, such procedure was unnecessary. Even with a larger detection limit in the CE method, the detection limit was 115-fold lower than the maximum residual content required by the Brazilian law, and therefore this analysis can be appropriately performed by the CE method.

Table 1. Quantitative parameters of the analysis obtained with CE and HPLC.

Parameter	CE	HPLC
Linearity range (mg L^{-1})	1.0-100.0	1.0-100.0
Slope	0.0767	81,42
Intercept	–0.0194	–39.902
Determination coefficient (r^2)	0.9997	0.9999
LOD[a] (mg 100 g^{-1}) – S/N ratio 3:1	0.87	0.021
LOQ[b] (mg 100 g^{-1}) – S/N ratio 10:1	1.74	0.042
Intraday precision (RSD, %), n = 8	1.42	0.31
Interday precision (RSD, %), n = 5	1.96	1.53
Separation time (min)	5.2	6.5

[a]LOD: limit of detection. [b]LOQ: limit of quantification.

The precision for both methods met the ANVISA validation parameters, resulting in relative standard deviations smaller than 1.96%.

Table 2 displays the results of the analysis time, costs, and generated residues for both techniques considering conditioning and cleaning of the column and capillary, construction of the analytical curve, and the analysis of ten samples.

Table 3 shows caffeine content for 20 samples of decaffeinated coffee obtained using the CE and the HPLC methods. Figure 1 shows a representative chromatogram and electropherogram obtained for a coffee sample. Through the analysis of variance, at 95% confidence level, there was not significant difference between the results for HPLC and CE. It was verified that differences between the two methods exhibited randomized distribution (Figure 2). Moreover, it was possible to observe that three of the twenty samples analyzed had caffeine levels above 0.1%, the maximum limit allowed by the Brazilian law (AGÊNCIA..., 2005).

Table 2. Comparison of analysis time, costs, and generated residues[a].

Description	HPLC	CE
Analysis time	14.6 hours	11.2 hours
Cost in reagents[c]	U$ 3.81	U$ 0.05
Residues composition	Methanol, water: 602.5 mL	SDS[b], Na_2CO_3, NaOH, water: 18 mL
Residues treatment	Incineration	Neutralization and disposal

[a]For calibration curve and 10 samples (extracted and injected in triplicate). [b]Sodium dodecylsulfate. [c]Mean of three budgets obtained in October 2008.

Table 3. Caffeine quantification by HPLC and CE.

Sample	Concentration (mg 100 g^{-1}) ± SD[a]		p[b]	Difference (mg)
	HPLC	CE		
1	14.0 ± 0.30	13.5 ± 0.31	0.063	0.5
2	12.1 ± 0.20	12.5 ± 0.28	0.058	–0.4
3	14.0 ± 0.15	13.9 ± 0.04	0.326	0.1
4	15.2 ± 0.27	15.4 ± 0.12	0.215	–0.2
5	76.7 ± 1.27	75.5 ± 0.93	0.174	1.2
6	26.7 ± 0.46	26.4 ± 0.41	0.329	0.3
7	26.5 ± 0.43	26.6 ± 0.62	0.828	–0.1
8	13.8 ± 0.35	13.6 ± 0.13	0.229	0.3
9	46.2 ± 0.77	45.4 ± 0.75	0.220	0.7
10	15.8 ± 0.13	15.9 ± 0.08	0.175	–0.1
11	204.4 ± 0.74	201.8 ± 2.87	0.132	2.6
12	242.8 ± 0.21	242.5 ± 4.44	0.894	0.3
13	93.2 ± 0.71	92.7 ± 0.93	0.500	0.4
14	25.8 ± 0.22	26.0 ± 0.56	0.589	–0.2
15	931.6 ± 0.77	930.9 ± 13.66	0.929	0.6
16	37.4 ± 0.35	37.4 ± 0.44	0.877	0.0
17	21.6 ± 0.12	21.8 ± 0.32	0.230	–0.2
18	26.8 ± 0.49	26.3 ± 0.18	0.088	0.5
19	47.1 ± 0.93	47.4 ± 0.66	0.607	–0.3
20	20.7 ± 0.40	20.2 ± 0.14	0.066	0.5

[a]Standard deviation (n = 4). [b]$p < 0.05$ indicates significant differences between the methods.

The HPLC method showed analysis time 30.4% higher than the time required by the CE method. The residual volume generated by the CE method was roughly 33-fold lower than that of the HPLC method. According to the Brazilian regulations, neutralization was the only requirement for the disposal of the solutions used by the CE (non-toxic solutions) (ASSOCIAÇÃO..., 2004; AGÊNCIA..., 2004; CONSELHO..., 2005). Conversely, the residues generated by HPLC require specific handling because under prolonged exposition, they can be harmful to health (McLEAN; JACOBS; MIELKE, 1980; FINKELSTEIN; VARDI, 2002; ASSOCIAÇÃO..., 2004). Moreover, the residues containing methanol are toxic, requiring special treatment including controlled incineration, which increases human resource costs associated with proper disposal and also requires more physical space for storage in the laboratory, thus increasing the costs associated with the analysis and the impact to the environment. The low consumption of solutions and reagents in CE, already verified by several other authors, is a result from the low internal volume of the capillary and from the ability to use the same buffer vials for the electrophoretic runs. From an environmental standpoint, the incineration of organic solvents, although performed according to the environmental protection procedures, still poses risks to health and threats to the sustainable development.

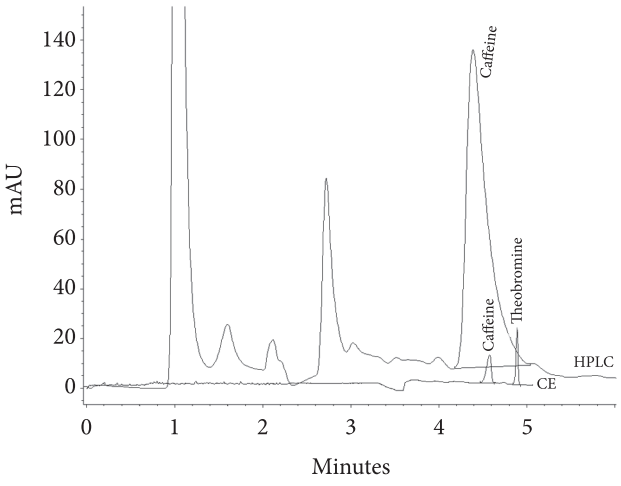

Figure 1. Chromatogram (HPLC) and electropherogram (CE) obtained for the determination of caffeine in a coffee sample. Electrophoretic conditions are described in the text.

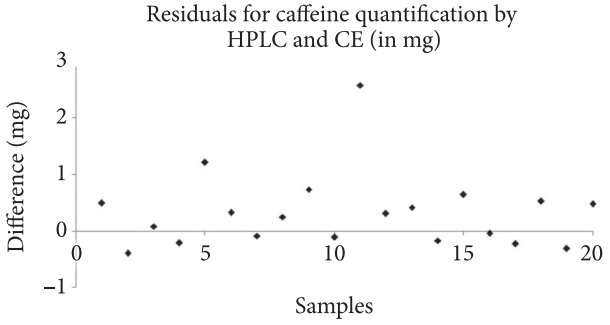

Figure 2. Residues of caffeine quantification by HPLC and CE (in mg).

4 Conclusions

The CE method proved to be a valuable tool for conducting routine determinations since it provided faster analyses, allowing the maximization of the equipment use with low cost reagents and reduced solvent consumption. The CE generated low levels of residues, constituted primarily of salts, which, after neutralization, can be naturally disposed. Therefore, the CE method, when compared to HPLC method, presented a good relationship between cost and benefits, while enabling monitoring of the residual caffeine content in decaffeinated coffee with the same reliability with a faster and more cost effective way.

Acknowledgements

This study was carried out with the financial support of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA – ANVISA. Resolução RDC nº 277, de 22 de Setembro de 2005. Aprova o Regulamento Técnico para Café, Cevada, Chá, Erva-Mate e Produtos Solúveis. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 23 set. 2005.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA – ANVISA. Resolução RDC nº 306, de 07 de dezembro de 2004. Aprova o Regulamento Técnico para o Gerenciamento de Resíduos de Serviços de Saúde. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 10 dez. 2004.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA – ANVISA . Resolução RE nº 899, de 29 de maio de 2003. Guia para Validação de Métodos Analíticos e Bioanalíticos. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 02 jun. 2003.

ALI, M. M. et al. Determination of caffeine in some Sudanese beverages by High Performance Liquid Chromatography. **Pakistan Journal of Nutrition**, v. 11, n. 4, p. 336-342, 2012. http://dx.doi.org/10.3923/pjn.2012.336.342

ARESTA, A.; PALMISANO, F.; ZAMBONIN, C. G. Simultaneous determination of caffeine, theobromine, theophylline, paraxanthine and nicotine in human milk by liquid chromatography with diode array UV detection. **Food Chemistry**, v. 93, n. 1, p. 177-181, 2005. http://dx.doi.org/10.1016/j.foodchem.2004.11.013

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS – ABNT. **NBR 10.004**: Resíduos Sólidos – Classificação. 2. ed. Rio de Janeiro: ABNT, 2004.

BRUNETTO, M. R. et al. Determination of theobromine, theophylline and caffeine in cocoa samples by a high performance liquid chromatographic method with on-line sample cleanup in a switching-column system. **Food Chemistry**, v. 100, n. 2, p. 459-467, 2007. http://dx.doi.org/10.1016/j.foodchem.2005.10.007

CONSELHO NACIONAL DO MEIO AMBIENTE – CONAMA. Resolução CONAMA nº 358, de 29 de abril de 2005. Dispõe sobre o tratamento e a disposição final dos resíduos dos serviços de saúde e dá outras providências. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 04 maio 2005.

COULTATE, T. P. **Alimentos – A química de seus componentes**. 3. ed. Porto Alegre: Artmed, 2004.

DE MARIA, C. A. B.; MOREIRA, R. F. A. Cafeína: Revisão sobre métodos de análise. **Química Nova**, v. 30, n. 1, p. 99-105, 2007. http://dx.doi.org/10.1590/S0100-40422007000100021

ESQUIVEL, P.; JIMÉNEZ, V. M. Functional properties of coffee and coffee by-products. **Food Research International**, v. 46, n. 2, p. 488-495, 2012. http://dx.doi.org/10.1016/j.foodres.2011.05.028

FENSKE, M. Caffeine determination in human saliva and urine by TLC and ultraviolet absorption densitometry. **Chromatographia**, v. 65, n. 3-4, p. 233-238, 2007. http://dx.doi.org/10.1365/s10337-006-0141-2

FINKELSTEIN, Y.; VARDI, J. Progressive Parkinsonism in a Young Experimental Physicist Following Long-Term Exposure to Methanol. **NeuroToxicology**, v. 23, n. 4-5, p. 521-525, 2002. http://dx.doi.org/10.1016/S0161-813X(02)00033-5

FOOD AND AGRICULTURE ORGANIZATION – FAO. **World Agricultural Production per Year**. FAO, 2012. Disponível em: <http://faostat.fao.org/site/339/default.aspx>. Acesso em: 17 abr. 2012.

FOOD AND DRUG ADMINISTRATION – FDA. **Center for Science and Public Interest Publisher of Nutrition Action Health Letter**. FDA, 2007. Disponível em: <http://www.fda.gov/OHRMS/DOCKETS/dailys/02/Apr02/042902/97p0329let6.pdf>. Acesso em: 11 jan. 2007.

FUJIOKA, K.; SHIBAMOTO, T. Chlorogenic acid and caffeine contents in various commercial brewed coffees. **Food Chemistry**, v. 106, n. 1, p. 217-221, 2008. http://dx.doi.org/10.1016/j.foodchem.2007.05.091

GNOATTO, S. C. B. et al. Influência do método de extração nos teores de metilxantinas em erva-mate (*Ilex paraguariensis* a. St.-Hil., Aquifoliaceae). **Química Nova**, v. 30, n. 2, p. 304-307, 2007. http://dx.doi.org/10.1590/S0100-40422007000200012

GOYAN, J. E. **Food and Drug Administration News Release, nº P80-36**. Washington: Food and Drug Administration, 1980.

JAMES, J. E. **Caffeine and Health**. London: Academic Press Limited, 1991.

JIMIDAR, M. et al. Comparison of capillary zone electrophoresis with high-performance liquid chromatography for the determination of additives in foodstuffs. **Journal of Chromatography**, v. 636, n. 1, p. 179-186, 1993. http://dx.doi.org/10.1016/0021-9673(93)80070-O

KERRIGAN, S.; LINDSEY, T. Fatal caffeine overdose: two case reports. **Forensic Science International**, v. 153, n. 1, p. 67-69, 2005. PMid:15935584. http://dx.doi.org/10.1016/j.forsciint.2005.04.016

KHANCHI, A. R. et al. Simultaneous spectrophotometric determination of caffeine and theobromine in Iranian tea by artificial neural networks and its comparison with PLS. **Food Chemistry**, v. 103, n. 3, p. 1062-1068, 2007. http://dx.doi.org/10.1016/j.foodchem.2006.07.035

KOWALSKI, P. et al. Comparative Evaluation of CE and HPLC for Determination of Cotinine in Human Urine. **Chromatographia**, v. 66, n. 5-6, p. 357-361, 2007. http://dx.doi.org/10.1365/s10337-007-0331-6

KOWALSKI, P.; PLENIS, A. Comparison of HPLC and CE methods for the determination of cetirizine dihydrochloride in human plasma samples. **Biomedical Chromatography**, v. 21, n. 9, p. 903-911, 2007. PMid:17443836. http://dx.doi.org/10.1002/bmc.818

LEE, B.-L.; ONG, C.-N. Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. **Journal of Chromatography A**, v. 881, n. 1-2, p. 439-447, 2000. http://dx.doi.org/10.1016/S0021-9673(00)00215-6

MALÁ, Z. et al. Contemporary sample stacking in CE. **Electrophoresis**, v. 30, n. 1, p. 215-229, 2009. PMid:19101931. http://dx.doi.org/10.1002/elps.200800433

McCUSKER, R. R. et al. Caffeine content of decaffeinated coffee. **Journal of Analytical Toxicology**, v. 30, n. 8, p. 611-613, 2006. PMid:17132260.

McLEAN, D. R.; JACOBS, H.; MIELKE, B. W. Methanol poisoning: A clinical and pathological study. **Annals of Neurology**, v. 8, n. 2, p. 161-167, 1980. PMid:7425569. http://dx.doi.org/10.1002/ana.410080206

MEINHART, A. D. et al. Optimisation of a CE method for caffeine analysis in decaffeinated coffee. **Food Chemistry**, v. 120, n. 4, p. 1155-1161, 2010. http://dx.doi.org/10.1016/j.foodchem.2009.11.048

MORAES, M. L. L. et al. Preconcentration strategies in capillary electrophoresis (CE): part 1. Manipulation of the analyte electrophoretic velocity. **Química Nova**, v. 32, n. 4, p. 1041-1046, 2009. http://dx.doi.org/10.1590/S0100-40422009000400036

PERRONE, D.; DONANGELO, C. M.; FARAH, A. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography-mass spectrometry. **Food Chemistry**, v. 110, n. 4, p. 1030-1035, 2008. http://dx.doi.org/10.1016/j.foodchem.2008.03.012

POMILIO, A. B.; TRAJTEMBERG, S. P.; VITALE, A. A. Micellar electrokinetic capillary chromatography of methylxanthines-containing beverages: Discussion of the molecular species involved. **Journal of the Science of Food and Agriculture**, v. 85, n. 4, p. 622-628, 2005. http://dx.doi.org/10.1002/jsfa.2020

REISSIG, C. J.; STRAIN, E. C.; GRIFFITHS, R. R. Caffeinated energy drinks – A growing problem. **Drug and Alcohol Dependence**, v. 99, n. 1-3, p. 1-10, 2009. PMid:18809264 PMCid:2735818. http://dx.doi.org/10.1016/j.drugalcdep.2008.08.001

SHRIVAS, K.; WU, H.-F. Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent microextraction with gas chromatography/mass spectrometry. **Journal of Chromatography A**, v. 1170, n. 1-2, p. 9-14, 2007. PMid:17904565. http://dx.doi.org/10.1016/j.chroma.2007.09.020

SILVAROLLA, M. B.; MAZZAFERA, P.; FAZUOLI, L. C. Plan biochemistry: a naturally decaffeinated Arabica coffee. **Nature**, v. 429, n. 6994, p. 826, 2004. PMid:15215853. http://dx.doi.org/10.1038/429826a

SOMBRA, L. L. et al. Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals. **Journal of Pharmaceutical and Biomedical Analysis**, v. 36, n. 5, p. 989-994, 2005. PMid:15620524. http://dx.doi.org/10.1016/j.jpba.2004.08.026