Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 1075–1081
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Analysis of potential adulteration in herbal medicines and dietary supplements for the weight control by capillary electrophoresis

Valeria Cianchino [a], Gimena Acosta [a,b], Claudia Ortega [a,c], Luis D. Martínez [a,b], María R. Gomez [b,d,*]

[a] Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, Química Analítica, Chacabuco y Pedernera, San Luis 5700, Argentina
[b] Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, CONICET, Chacabuco y Pedernera, San Luis 5700, Argentina
[c] Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, Farmacotecnia, Chacabuco y Pedernera, San Luis 5700, Argentina
[d] Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, Control de Calidad de Medicamentos, Chacabuco y Pedernera, San Luis 5700, Argentina

Received 16 November 2006; received in revised form 12 November 2007; accepted 18 November 2007

Abstract

Four different phytopharmaceutical dosage forms for use in weight control programs were analyzed. Two different ground herbal blends and their correspondent infusions, a capsule and a tincture were investigated for the presence of compounds used as adulterants in these products. A capillary electrophoresis (CE) method was developed and validated. The optimized experimental conditions were: BGE, sodium tetraborate buffer 20 mM, pH 9.2, voltage applied 30 kV, capillary temperature 25 °C, injection sample at 0.5 Psi during 5 s. Ephedrine, norephedrine, caffeine and furosemide were baseline separated in less than 7 min; the migration times were found to be 2.65, 2.90, 3.75 and 6.58 min, respectively. The analysis showed in sample 3 concentrations of 0.45 ± 0.03 mg g^{-1} (ephedrine), 0.33 ± 0.02 mg g^{-1} (norephedrine), 1.09 ± 0.41 mg g^{-1} (caffeine) and 0.80 ± 0.17 mg g^{-1} (furosemide). Caffeine content in samples 1, 2 and 4 was 0.61 ± 0.06 mg g^{-1}, 15.66 ± 1.05 mg g^{-1} and 2.27 ± 0.13 mg ml^{-1}, respectively. Linearity was obtained in the concentration range of 1–1000 μg ml^{-1}. Limits of detection (LOD) and quantification (LOQ) were determined as 0.42 μg ml^{-1} and 1.40 μg ml^{-1} (ephedrine), 0.47 μg ml^{-1} and 1.40 μg ml^{-1} (norephedrine), 0.12 μg ml^{-1} and 0.48 μg ml^{-1} (caffeine), 0.22 μg ml^{-1} and 0.73 μg ml^{-1} (furosemide).

The common constituents of the samples did not interfere with the potential adulterants. Repeatability was better than 0.24% RSD for the retention time and 1.43% for the peak area. Intermediate precision was tested by changing the capillary, the day of operation and the operator, in all the cases the %RSD was better than 3.06.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Capillary electrophoresis; Adulterants; Dietary supplements; Quality control; Weight control drugs

1. Introduction

Numerous and different botanical products used for maintain and/or promote health are known as Botanical Health Products (BHP) (Bast et al., 2002). Several vegetal drugs and their derivatives (dietary supplements and phytopharmaceuticals) can be considered BHPs and, in contrast with the conventional drugs are perceived as non-toxic products with therapeutic activity (Bauer, 1998). These BHPs are widely consumed because of their pharmacological activity in weight control programs (Anonymous, 2005).

In order to increase the therapeutic effect of BHPs, it has been reported that pharmaceutical active principles are included in the formula of products marketed as 'herbal

* Corresponding author. Address: Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia, CONICET, Chacabuco y Pedernera, San Luis 5700, Argentina. Fax: +54 2652 430224.
E-mail address: roxanag@unsl.edu.ar (M.R. Gomez).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.042

medicine' or 'dietary supplement' during the manufacturing process (Fugh-Berman & Ernst, 2001). When BHPs containing synthetic therapeutic drugs as adulterants are administered, pharmacokinetic and/or pharmacodynamic herb–drug interactions can occur (Fugh-Berman & Ernst, 2001). Adverse drug reactions and toxicity in heart, liver, blood, kidneys, central nervous system and skin and carcinogenesis have been reported (Bensoussan, Myers, Drew, Whyte, & Dawson, 2002; Ernst, 2000; Greensfelder, 2000; Kessler, 2000).

There are several pharmaceuticals active principles that have to be investigated like possible adulterants in products used in weigh control programs due to their potential toxic effects (anorexigens, diuretics, stimulants, laxative agents, etc.) (Bogusz, Al-Tufail, & Hassan, 2002).

Several analytical approaches were developed for adulterant determination in BHPs. A gas chromatography–mass spectrometry (GC–MS) method was applied to obtain an impurity profiling of ecstasy tablets seized in Hong Kong (Cheng, Chan, Chan, & Hung, 2006); two different high-performance liquid chromatography (HPLC) method were set up to verify the absence of hydroxyanthracene derivatives in commercial *Aloe vera* gel powders (Bozzi, Perrin, Austin, & Arce Vera, 2007) and to check components and purity in commercial saffron (Lozano, Castellar, Simancas, & Iborra, 1999). The use of capillary electrophoresis (CE) in BHP samples can have benefits in terms of low reagent and solvent consumption, simplicity and reduced time and cost of analysis (Sombra, Gómez, Olsina, Martínez, & Silva, 2005).

The present work consists in the development of a CE methodology and its application to the separation and determination of different active principles present as adulterants in weight control products. The potential adulterants studied show pharmacological properties corresponding with the claims of the analyzed remedies: caffeine (stimulant), furosemide (diuretic), norephedrine and ephedrine (stimulant, decongestant and anorexigen action); however, they can cause an unpredictable effect on the health of users.

2. Experimental

2.1. Instrumentation

The employed CE system consisted of a Beckman P/ACE MDQ instrument (Beckman Instruments, Inc., Fullerton, CA) equipped with a diode array detector and a data handling system comprising an IBM personal computer and P/ACE System MDQ Software. Detection was performed at 208 and 265 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 60 cm total length, 50 cm effective length, 75 μm ID, 375 μm OD. The temperature of the capillary and the samples was maintained at 25 °C. The pH of the electrolytes was measured by an Orion 940 pHmeter equipped with a glass-combined electrode.

The studied compounds were purchased from Sigma–Aldrich Co. (St. Louis, MO), sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) was acquired from Mallinckrodt (St. Louis, USA). The water used in all studies was ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultrapure water system. All other reagents and solvents were of analytical grade quality. All solutions were degassed by ultrasonication (Testlab, Argentina).

The electrolyte solution (background electrolyte, BGE) was prepared daily and filtered through a 0.45 μm Titan Syringe filters (Sri Inc., Eaton Town, NJ, USA). At the beginning of the day, the capillary was conditioned with 0.1 mol l^{-1} NaOH for 5 min, followed by water for 5 min, and then with running electrolyte for 10 min before sample injection. To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with sodium hydroxide for 2 min, followed by water for 2 min, then equilibrated with the running buffer for 4 min. Samples were pressure-injected at the anodic side at 0.5 Psi for 5 s. A constant voltage (30 kV) was used for all the experiments.

2.2. Samples

Four different commercial products, BHPs, used in weight control programs were analyzed with the proposed methodology. The BHPs were: ground plant material containing *Marrubium vulgare* L., *Melissa officinalis* L., *Lippia fissicalyx* Tronc., *Maytenus ilicifolia* Reiss., *Prunus spinosa* L., *Hyssopus officinalis* L., *Equisetum arvense* L., *Cassia acutifolia* Delile y *Cassia angustifolia* Vahl., *Matricaria chamomilla* L. and *Fucus vesiculosus* L. as part of an herbal blend (sample 1); ground plant material containing *Ilex paraguariensis* St. Hil., *Fucus vesiculosus*, *Aloysia triphylla* L'Hér., *Camellia sinensis* L., *Equisetum arvense* L., *Minthostachys mollis* H.B.K. as part of an herbal blend (sample 2); a dietary supplement (capsule) claiming to contain *Garcinia cambogia* Desr. and *Centella asiatica* L. manufactured by "El Ceibo Laboratory" (San Luis, Argentina) (sample 3); a dietary supplement (tincture) claiming to contain *Fucus vesiculosus* L., *Hyssopus officinalis* L., *Equisetum arvense* L. and *Rhamnus purshiana* D.C L. manufactured by "El Ceibo Laboratory" (San Luis, Argentina) (sample 4). All the analyzed herbal products were taken randomly from drug shops and herbal markets in Argentina. These products were mainly from the central region of the country (four different batches) and were collected during 2004–2006.

2.3. Sample preparation

Samples consisted of both solid (ground plant material, capsule) and liquid (tincture) formulations. The water extracts (teas) of samples 1 and 2 were used for the examinations. The procedure adopted for the teas preparation

was: 200 ml boiling ultrapure water was poured onto 5 g of herbal blend, covered and left to infuse for 30 min, then filtered, and the volume made up to 200 ml with ultrapure water.

For capsule preparation (sample 3), the husks were removed and the powder contained in twenty capsules was mixed and homogenized; the quantity equivalent to one capsule filling was accurately weighed and extracted in an ultrasonic washer during 10 min with water–ethanol (10:1).

The tincture (sample 4) was prepared as follows: 10 ml of the commercial tincture were carefully measured into a volumetric flask and diluted to 100 ml with ultrapure water.

2.4. Statistical analysis

Mean values and standard deviation (SD) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values. P-value <0.05 was regarded as significant. The determinations were carried out in triplicate, results are given as means $\pm$ standard deviation (SD).

3. Results and discussion

In first place, optimization of CE conditions were performed, the effects of buffer concentration, pH and BGE composition, temperature and voltage on the analyzed samples were studied.

3.1. Effect of buffer pH

To investigate the effect of buffer pH on migration behavior, experiments were performed by using different BGEs (sodium tetraborate buffer, sodium phosphate buffer and their mixtures) within the pH range 7.5–10.0. This pH interval was selected considering the pK_a values of the studied analytes: ephedrine and norephedrine, 9.6 and 9.4, respectively are not totally dissociated in this pH range; by the other hand the carboxylic acid furosemide, pK_a 4, is fully dissociated in the selected pH range. The pK_a of caffeine, 14, indicates that it is both a weak acid and a weak base, we can deduce it is in neutral form.

The best results were achieved at pHs higher than 8.5, when the buffer pH increased from 8.5 to 9.2, the resolution of all the tested compounds was improved, whereas the resolution decreased when the pH was higher than 9.5. Thus, further studies were carried out at pH 9.2.

3.2. Effect of buffer composition and concentration

Sodium tetraborate buffer, sodium phosphate buffer and their mixtures were used as the running BGEs with all the samples. Considering reproducibility, current performance and BGE stability, sodium tetraborate buffer pH 9.2 was selected as the solution for the following experiments.

The effect of BGE concentration on the separation of the sample constituents was investigated at the concentration range of 10–75 mM. It was found that resolution improved with the increase of borate concentration, reaching an optimum at about 20 mM. The EOF mobility decreases with the increase of buffer concentration; thus, concentrations higher than 25 mM were not satisfactory due to the longer times of analysis. Therefore, tetraborate buffer 20 mM, pH 9.2 was chosen as the BGE.

3.3. Electrophoretic parameters

At the chosen BGE concentration the current met an acceptable value (80 μA) and remained constant when the study was performed under the optimal experimental condition.

The voltage applied was varied within the range 20–30 kV, good separations and acceptable analysis times were achieved with 30 kV.

Effect of capillary temperature was also studied, no appreciable improvements were observed when temperature was modify within the range 20–40 °C; trying to avoid high current the analyses were carried out at 25 °C.

Keeping other parameters constant (pH 9.2, 30 kV, 25 °C), sample injection was studied and the hydrodynamic mode was applied; after several tests the pressure selected was 0.5 Psi during 5 s.

3.4. Performance of the analytical method: method validation

Fig. 1 shows the obtained electropherograms under the optimized experimental conditions (BGE: sodium tetraborate buffer 20 mM, pH 9.2, voltage applied 30 kV, capillary temperature 25 °C, sample was injected at 0.5 Psi during 5 s). The migration times of ephedrine, norephedrine, caffeine and furosemide were found to be 2.65, 2.90, 3.75 and 6.58 min, respectively. The potential adulterants were baseline separated in less than 7 min. The migration times did not vary to any considerable degree during and in between analyses.

Previously, several approaches have been developed for furosemide, caffeine, ephedrine and norephedrine analysis. A micellar liquid chromatography (MLC) technique, a fast gas chromatography/electron impact mass spectrometry (fast GC/EI-MS) and a diffuse reflectance spectroscopy method were reported for the determination of furosemide (Carda-Broch, Esteve-Romero, & García-Alvarez-Coque, 2000; Gotardo, Gigante, Pezza, & Pezza, 2004; Morra et al. 2006). Respect to the analytical method performance, only GC/EI-MS seems to be more sensitive than ours although the equipment is more expensive. The same occurs with caffeine, two different chromatographic procedures (gas chromatography tandem mass spectrometry, GC–MS/MS and MLC) were applied for its quantification (Gómez et al., 2007; Martínez-Algaba, Bermúdez-Saldaña, Villanueva-Camañas, Sagrado, & Medina-Hernández,

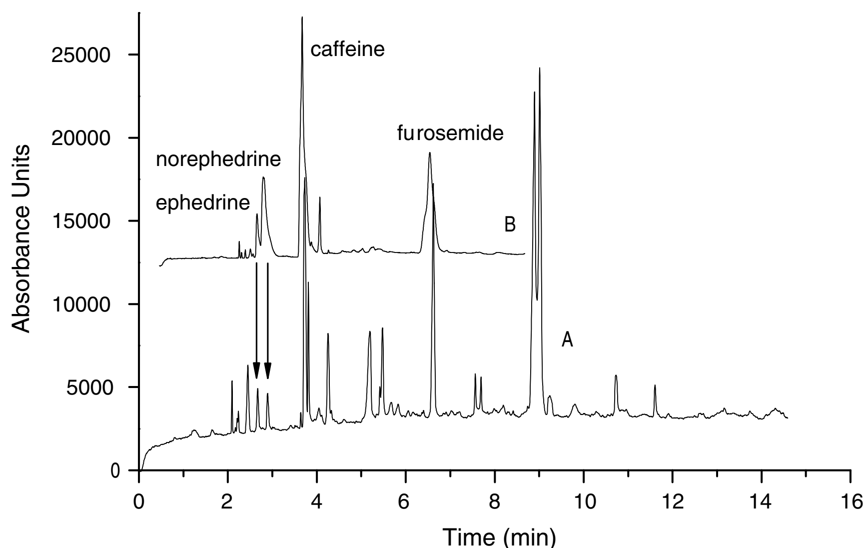

Fig. 1. Electropherograms obtained from: (A) capsule dilution (sample 3) in water and (B) capsule dilution spiked with a solution mixture of the possible synthetic adulterants. Conditions: BGE, sodium tetraborate buffer 20 mM, pH 9.2; capillary, 60 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 30 kV constant voltage applied; capillary temperature 25 °C; detection by UV absorbance at 208 nm.

2006); with MLC, limits of detection are lower than 1 $\mu g\, ml^{-1}$ for caffeine while the proposed CE method is 0.12 $\mu g\, ml^{-1}$; on the other hand, the application of the MS/MS mode allowed better detection limits in the range of 0.20–16 $ng\, l^{-1}$, but the cost is high. Respect to ephedrine and norephedrine, a capillary zone electrophoresis (CZE) methodology was developed for separation and determination of ephedrine and related compounds in urine (Mateus-Avois, Mangin, & Saugy, 2003) in which method sensitivity was not evaluated and precision was determined by measuring repeatability and intermediate precision and expressed as RSD values for relative migration time and peak area ratio. Repeatability was 0.42% and 0.33% for the migration time and 3.13% and 4.09% for the peak area ratio of norephedrine and ephedrine respectively. Intermediate precision was found to be 2.61% and 2.28% for the migration time and 5.68% and 6.03% for the peak area ratio of norephedrine and ephedrine, respectively. Our method allows ephedrine and norephedrine quantification within the range 1.40–1000 $\mu g\, ml^{-1}$; respect to precision in all the cases our values showed to be lower than the above mentioned results.

The method of standard addition is considered as a validation method (ICH Harmonised Tripartite Guideline, 2005; Prichard, Mackay, & Points, 1996). With the aim of evaluating the accuracy of our method, a recovery study was carried out (Table 1). A capsule was appropriately diluted and after divided in ten aliquots, three of them (I–III) were analyzed and the compounds under study were determined according to the developed method; the obtained values were considered as the base value. Each sample was injected in triplicate. Other three aliquots (IV–VI) were spiked with accurately measured quantities of the analytes under study and the proposed method was applied. The results of the determination were considered as found value. The recovery was calculated as: 100 × [(found value − base value)/added value]. The recoveries were within the range 98.50–101.90%.

Table 1
Recovery test

	Base value[a] ($\mu g\, ml^{-1}$)	Quantity added ($\mu g\, ml^{-1}$)	Quantity found[a] ($\mu g\, ml^{-1}$)	Recovery (%)[b]
Aliquot I–III[c]				
Ephedrine	–	0	65.59	–
Norephedrine	–	0	48.51	–
Caffeine	–	0	158.3	–
Furosemide	–	0	115.5	–
Aliquot IV[c]				
Ephedrine	65.59	100	165.1	99.5
Norephedrine	48.51	40	88.99	101.2
Caffeine	158.3	0	–	–
Furosemide	115.5	0	–	–
Aliquot V[c]				
Ephedrine	65.59	0	–	–
Norephedrine	48.51	100	149.8	101.3
Caffeine	158.3	100	260.2	101.9
Furosemide	115.5	0	–	–
Aliquot VI[c]				
Ephedrine	65.59	0	–	–
Norephedrine	48.51	0	–	–
Caffeine	158.3	60	217.4	98.55
Furosemide	115.5	60	174.9	98.95

[a] Mean value ($n = 3$).
[b] 100 × [(found − base)/added].
[c] Capsule dilution.

Method precision was evaluated as repeatability (measurements over a short period of time) and intermediate precision (tested by changing the capillary, the day of operation and the operator). Repeatability was determined by injecting three times at three concentration levels the com-

bined solution containing furosemide, caffeine, ephedrine and norephedrine. The %RSD was better than 0.11% for the retention time and 1.43% for the peak area. Intermediate precision was evaluated by injecting the samples in triplicate. The %RSD values obtained after changing the capillary were 0.42% for the retention time and 3.06% for the peak area; the found values with different operators were 0.10% for the retention time and 1.48% for the peak area; finally, by performing the assay over different days (3 days) the estimated values were 0.44% for the retention time and 1.51% for the peak area.

Linearity was tested by determining standard solutions at five concentrations levels with the proposed method. Each sample was injected in triplicate. The calibration equations were calculated by the least-squares linear regression method, the analytical values obtained are shown in Table 2.

Limits of detection (LOD) and quantification (LOQ) were determined as 0.42 μg ml^{-1} and 1.40 μg ml^{-1} (ephedrine), 0.47 μg ml^{-1} and 1.40 μg ml^{-1} (norephedrine), 0.12 μg ml^{-1} and 0.48 μg ml^{-1} (caffeine), 0.22 μg ml^{-1} and 0.73 μg ml^{-1} (furosemide) (Table 2).

Selectivity of the method was investigated by assessing the peak purity; this was achieved analyzing the UV spectra obtained for standard and sample analyte peaks from the diode array detector. The P/ACE System MDQ Software calculated resolution values of the peaks from each other.

Table 2
Validation parameters for the ephedrine, norephedrine, caffeine and furosemide quantification[a]

Analyte	Linearity range (μg ml^{-1})	LOD (μg ml^{-1})	LOQ (μg ml^{-1})	R^2	Regression equation $y = a + bx$[b] (95%; $n = 6$)
EP	1.40–1000	0.42	1.40	0.991	$y = -4968.17 + 152606.16x$
NEP	1.40–1000	0.47	1.40	0.991	$y = -5332.93 + 167771.47x$
CF	0.48–1000	0.12	0.48	0.997	$y = -3858.56 + 387841.96x$
FR	0.73–1000	0.22	0.73	0.998	$y = 654.16 + 216213.69x$

EP: ephedrine; NEP: norephedrine; CF: caffeine; FR: furosemide.
[a] Standard solution of the analytes.
[b] y and x stand for the peak area and the concentration (μg ml^{-1}) of the analytes, respectively.

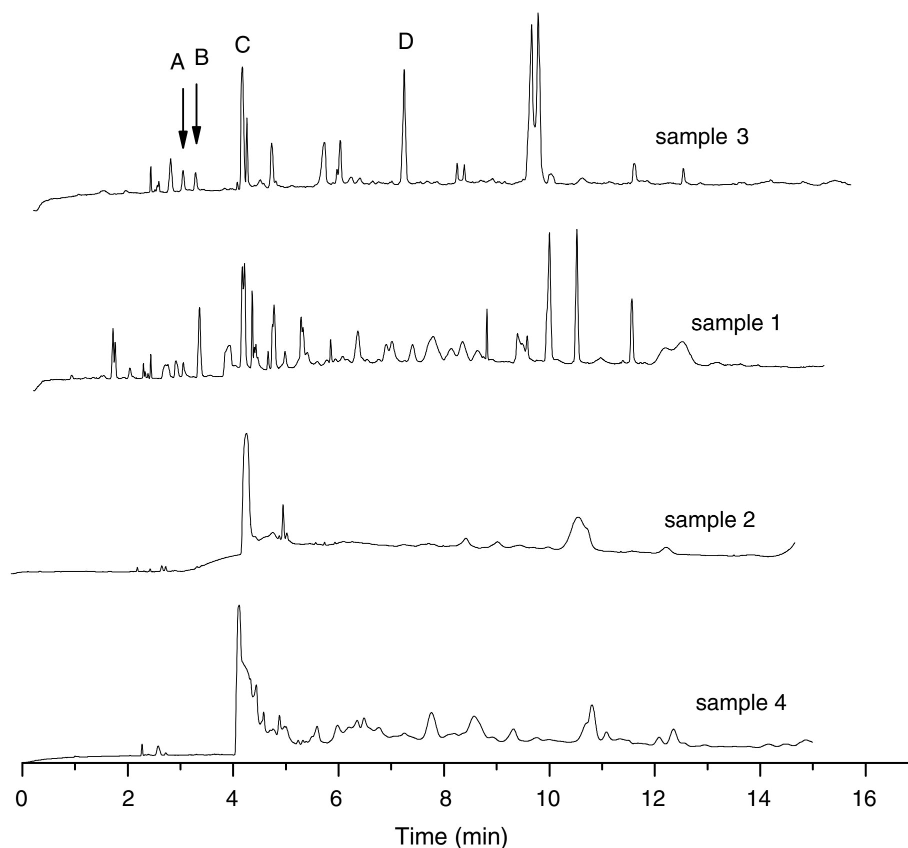

Fig. 2. Electropherograms obtained from samples 1–4. Peak identification: (A) ephedrine, (B) norephedrine, (C) caffeine, (D) furosemide. Conditions: BGE, sodium tetraborate buffer 20 mM, pH 9.2; capillary, 60 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 30 kV constant voltage applied; capillary temperature 25 °C; detection by UV absorbance at 208 nm.

3.5. Search of adulterants

Several undeclared drugs were identified in "herbal" remedies, like ephedrine, norephedrine, caffeine and furosemide. Pharmacological properties of detected drugs always corresponded with the claims of the "natural" remedies, but the safety of the adulterated products has not been clinically tested and unpredictable effect on the health of users may be caused.

In Fig. 1, we can see the commercial capsule (sample 3) after spiking with the analytes the correspondence between the migration times of some capsule constituents with the possible adulterants. Peak purity was assessed analyzing the UV spectra for standard and sample analyte peaks by using the diode array detector (DAD). Ephedrine, norephedrine, caffeine and furosemide were found in sample 3 in concentrations of 0.45 ± 0.03 mg g^{-1}, 0.33 ± 0.02 mg g^{-1}, 1.09 ± 0.41 mg g^{-1} and 0.80 ± 0.17 mg g^{-1}, respectively.

In Fig. 2 we can observe the presence of caffeine in all the samples, this result is reasonable in the case of sample 2 considering its Ilex and Camellia content (Streit et al., 2007; Yang, Ye, Xu, & Jiang, 2007). Caffeine content in samples 1, 2 and 4 was 0.61 ± 0.06 mg g^{-1}, 15.66 ± 1.05 mg g^{-1} and 2.27 ± 0.13 mg ml^{-1}, respectively. In addition, in sample 1, norephedrine and traces of ephedrine were found.

4. Conclusions

Adulteration of "natural herbal medicines" with undeclared synthetic drugs is a common and dangerous phenomenon in alternative medicine. Methods that ensure the quality and safety of these products have to be developed. The presented methodology was found to be applicable to the investigation of synthetic drugs used as adulterants in phytopharmaceutics for the treatment of weight control. A capillary electrophoresis (CE) method was developed for the searching, separation and simultaneous determination of ephedrine, norephedrine, caffeine and furosemide in commercial preparations.

The present paper describes the development of a CE method that offers certain advantages in its simplicity and time saving. Successful separation and accurate results were obtained. The active compounds were determined with high efficiency in a short period of time (less than seven minutes). The proposed method was validated and showed a good performance with respect to selectivity, precision, linearity, LOD, LOQ and accuracy. The application of the CE method to pharmaceutical quality control may be considered for the routine analysis of a large number of samples.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID) and Universidad Nacional de San Luis (Argentina).

References

Anonymous (2005). *Dietary supplements: Safe, beneficial and regulated.* Council for responsible nutrition. http://www.crnusa.org/CRNRegQ-andA.html (accessed 02.02.05).

Bast, A., Chandler, R. F., Choy, P. C., Delmulle, L. M., Gruenwald, J., Halkes, B. A., et al. (2002). Botanical health products, positioning and requirements for effective and safe use. *Environmental Toxicology and Pharmacology, 12*, 195–211.

Bauer, R. (1998). Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standards be achieved. *Drug Information Journal, 32*, 101–110.

Bensoussan, A., Myers, S. P., Drew, A. K., Whyte, I. M., & Dawson, A. H. (2002). Development of a Chinese herbal medicine toxicology database. *Journal of Toxicology and Clinical Toxicology, 40*(2), 159–167.

Bogusz, M., Al-Tufail, M., & Hassan, H. (2002). How natural are 'Natural Herbal Remedies'?: A Saudi perspective. *Adverse Drug Reaction Toxicology Review, 21*, 219–229.

Bozzi, A., Perrin, C., Austin, S., & Arce Vera, F. (2007). Quality and authenticity of commercial aloe vera gel powders. *Food Chemistry, 103*, 22–30.

Carda-Broch, S., Esteve-Romero, J., & García-Alvarez-Coque, M. C. (2000). Furosemide assay in pharmaceuticals by micellar liquid chromatography: Study of the stability of the drug. *Journal of Pharmaceutical and Biomedical Analysis, 23*, 803–817.

Cheng, J. Y., Chan, M. F., Chan, T. W., & Hung, M. Y. (2006). Impurity profiling of ecstasy tablets seized in Hong Kong by gas chromatography–mass spectrometry. *Forensic Science International, 162*, 87–94.

Ernst, E. (2000). Adverse effects of herbal drugs in dermatology. *British Journal of Dermatology, 143*(5), 923–929.

Fugh-Berman, A., & Ernst, E. (2001). Herb–drug interactions: Review and assessment of report reliability. *British Journal of Clinical Pharmacology, 52*(5), 587–595.

Gómez, M. J., Agüera, A., Mezcua, M., Hurtado, J., Mocholí, F., & Fernández-Alba, A. R. (2007). Simultaneous analysis of neutral and acidic pharmaceuticals as well as related compounds by gas chromatography–tandem mass spectrometry in wastewater. *Talanta, 73*(2), 314–320.

Gotardo, M. A., Gigante, A. C., Pezza, L., & Pezza, H. R. (2004). Determination of furosemide in pharmaceutical formulations by diffuse reflectance spectroscopy. *Talanta, 64*, 361–365.

Greensfelder, L. (2000). *Alternative medicine. Herbal product linked to cancer*. Science.

ICH Harmonised Tripartite Guideline (2005). Q2(R1): Validation of analytical procedures: Text and methodology. In *International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use*.

Kessler, D. A. (2000). Cancer and herbs. *New England Journal of Medicine, 342*(23), 1742–1743.

Lozano, P., Castellar, M. R., Simancas, M. J., & Iborra, J. L. (1999). A quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *Journal of Chromatography A, 830*(2), 477–483.

Martínez-Algaba, C., Bermúdez-Saldaña, J. M., Villanueva-Camañas, R. M., Sagrado, S., & Medina-Hernández, M. J. (2006). Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 40*, 312–321.

Mateus-Avois, L., Mangin, P., & Saugy, M. (2003). Development and validation of a capillary zone electrophoresis method for the determination of ephedrine and related compounds in urine without extraction. *Journal of Chromatography B, 791*, 203–216.

Morra, V., Davita, P., Capra, P., Vincenti, M., Di Stilo, A., & Botr, F. (2006). Fast gas chromatographic/mass spectrometric determination of diuretics and masking agents in human urine. Development and validation of a productive screening protocol for antidoping analysis. *Journal of Chromatography A, 1135*, 219–229.

Prichard, E., Mackay, G. M., & Points, J., 1996. *Trace analysis: A structures approach to obtaining reliable results* (p. 38). United Kingdom: The Royal Society of Chemistry.

Sombra, L. L., Gómez, M. R., Olsina, R., Martínez, L. D., & Silva, M. F. (2005). Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals. *Journal of Pharmaceutical and Biomedical Analysis*(5), 989–994.

Streit, N. M., Rychecki Hecktheuer, L. H., Weber do Canto, M., Mallmann, C. A., Streck, L., Parodi, T., et al. (2007). Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (*Ilex paraguariensis*). *Food Chemistry, 102*, 560–564.

Yang, X. R., Ye, C. X., Xu, J. K., & Jiang, Y. M. (2007). Simultaneous analysis of purine alkaloids and catechins in *Camellia sinensis*, *Camellia ptilophylla* and *Camellia assamica* var. kucha by HPLC. *Food Chemistry, 100*, 1132–1136.