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Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 617–620
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

HPTLC determination of caffeine in stimulant herbal products and power drinks

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Received 20 February 2004; received in revised form 13 June 2004; accepted 15 June 2004
Available online 25 August 2004

Abstract

The caffeine content of selected herbal products and energy drinks available in the Saudi market was determined by HPTLC–UV densitometric analysis. Pre-coated HPTLC silica gel plates (20 cm × 10 cm) were used for the analysis. The solvent system consisted of ethyl acetate–methanol (85:15, v/v), and caffeine was detected at 275 nm. The developed method was validated for specificity, repeatability (C.V. < 5%), recovery (98.90 ± 3.46), and accuracy (99.84 ± 2.87). The levels of caffeine were 4.76–13.29% (w/w) and 0.011–0.032% (w/v), for the herbal products and the energy drinks, respectively.

Keywords: HPTLC; Caffeine; Dietary supplements; Herbal products; Energy drinks

1. Introduction

Caffeine, 1,3,7-trimethylxanthine, is the major alkaloid ingredient in about 60 herbs, including *Thea sinensis* (tea leaves), *Coffea arabica* (coffee beans), *Theobroma cacao*, *Paulinea coppana* (guarana seeds) and *Cola nitida* (kola nuts), to which their CNS stimulant is attributed [1]. The pharmacological effect of caffeine can be achieved when it is consumed in the form of herbal extract or pure ingredient added to various food products. Hot tea and coffee drinks are among the most popular sources for obtaining the desired effect of caffeine, providing ca. 50 and 100 mg caffeine per cup, respectively [2]. Caffeine is also a common ingredient in many painkillers and antimigraine pharmaceuticals. With the recent reemergence of medicinal herbs as a major player in the global dietary supplement market, such new products containing caffeine have been introduced. Of these, dry extracts of caffeine-containing herbs and carbonated beverages, known as power or energy drinks, enriched with pure caffeine/caffeine extracts are becoming popular in the Saudi market. The levels of caffeine in different matrices (e.g. biological, pharmaceutical and herbal) have been determined by numerous techniques, including spectroscopic and chromatographic methods [3–8]. Planar chromatography, and its high-performance version (HPTLC), coupled with densitometric detection, is among the various methods reported for the quality control of pharmaceutical products containing caffeine [9]. It has the advantages of simplicity, speed, reproducibility and cost effectiveness and can thus provide an affordable and reliable alternative to other analytical techniques, such as HPLC or GC [10]. As such, HPTLC may be utilized as an effective analytical tool for the quality control of caffeine-containing dietary supplements.

In this report, a developed HPTLC method was validated for specificity, linearity of calibration, recovery, accuracy and precision (repeatability) and was used to determine the levels of caffeine in stimulant herbal products and power drinks on the Saudi market.

2. Experimental

2.1. Materials and methods

Pure caffeine was obtained from Merck (Darmstadt, Germany). Three Herbal products and six energy drinks

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doi:10.1016/j.jpba.2004.06.029

were purchased from the local Saudi market. They were as follows: Kola, Laboratoire Boiron, Lyon, France (HP1); MegaRipped®, Weider Nutrition Group, Salt Lake City, Utah, USA (HP2); Extra Strength Guarana™, Natural Balance, Castle Rock, Illinois, USA (HP3); Magic® Energy Kick, Torgny Jahnsson's Magic House AB, Sweden (ED1); Power Horse®, Power Horse, Vienna, Austria (ED2); Shadow®, Abul Jadail Beverages, Jeddah, Saudi Arabia (ED3); Red Bull®, Red Bull Gmbh, Austria (ED4); Pepsi-Cola® X, Pepsi-Cola Co., USA (ED5); Pepsi-Cola®, PepsiCola Co., USA (ED6). HPTLC plates (silica gel 60 F254, 20 × 10 cm, Merck, Darmstadt, Germany) were used as supplied. TLC development system: EtOAc–MeOH (85:15, v/v). Plates were developed in glass chambers presaturated for 30 min with the development solvent, which was allowed to migrate to a height of 80 mm from the lower edge of the plate. Sample extraction and TLC development solvents were of analytical quality.

2.2. Standard solutions

All solutions were freshly prepared. Standard stock solution I (SS1): caffeine (10.7 mg/100 mL) in MeOH. Standard stock solution II (SS2): caffeine (21.4 mg/100 mL) in MeOH.

2.3. Instruments

The HPTLC system (Camag, Muttanz, Switzerland) consisted of (i) TLC scanner connected to a PC running WinCATS software under MS Windows NT; (ii) Linomat IV sample applicator using 100 μL syringes and connected to a Nitrogen tank. Each plate accommodated 20 tracks of samples and standards, applied according to the following settings: band width 6 mm; distance between bands 3 mm; application volume 2–12 μL; gas flow 10 s/μL. The scanner was set for maximum light optimization and with the following settings: slit dimension, 4.00 mm × 0.30 mm, micro; scanning speed, 20 mm/s; data resolution, 100 μm/step. All remaining measurement parameters were left at default settings. Regression analyses and statistical data were generated by the WinCATS software.

2.4. Method validation

(A) Specificity (selectivity): a UV spectrum of caffeine was initially obtained from developed plate and the detection wavelength was chosen at λ_{max} 275 nm. In complex chromatograms, a UV spectrum was obtained for the caffeine peak to verify peak purity. Peak resolution was also calculated from the formula:
Peak resolution (R_s) = 1.18 × distance between two adjacent peaks/sum of two peak widths at half heights [11].
Spiking of HP1 with SS2 and quantitation of the fortified peak (see (D) below) was taken as an additional indication of specifity.

(B) Calibration curve: four concentrations of SS1 were applied in triplicates (2, 4, 8 and 12 μl equivalent to 214, 428, 856 and 1284 ng/spot, respectively). The plates were developed, dried and scanned at 275 nm. Peak areas were automatically stored in a computer file for regression analysis and curve generation.

(C) Precision: repeatability was determined by running a minimum of four analyses per sample and evaluating the coefficient of variability (C.V.%) for each sample. Repeatability was further confirmed from the C.V.% values of the standard addition at three concentration levels (Table 1) [12].

(D) Recovery: on the same plate in (A) above, 4 and 5 μL of SS2 were applied in quadruplicates. Their tracks were scanned simultaneously with (A).

(E) Accuracy: three samples of HP1 were spiked with SS2 as follows: (i) HP140.0 mg + SS21.00 mL; (ii) HP144.5 mg + SS22.00 mL; (iii) HP140.8 mg + SS23.00 mL. The mixtures were subjected to the general extraction procedure mentioned below, and the extract of each was analyzed in quadruplicates (3 μL/track). Accuracy was calculated from the formula:

$$\text{Recovery\%} = \frac{\text{Total caffeine} - \text{Caffeine in P1}}{\text{Caffeine in SS2}} \times 100$$

2.5. Sample preparation and analysis

2.5.1. Solid samples

The contents of six capsules of each herbal product were individually weighed, then mixed together. For each product, an accurate weight of the mixed powder (80.0 mg HP1, 40.0 mg HP2 and 25.7 mg HP3) was placed in a 15-mL centrifuge tube and ultrasonicated in 3 mL MeOH for 15 min (Transsonic 460/H, Barnstead/Lab-line, Illinois, USA). The resulting suspension was centrifuged at 1600 rpm for 5 min (Labofuge 200, Heraeus, Germany), and the supernatant was decanted into a 10-mL volumetric flask. The ultrosonication/centrifugation procedure was repeated two more times after which the combined supernatants were completed to 10 mL with MeOH and filtered. An accurate volume of the filtrate (4.00 μL) was applied to the plate in quadruplicate. Each applied band was dried by a stream of nitrogen at a speed of 6 s/μL.

2.5.2. Liquid samples

The contents of three cans of each product were mixed, vigorously shaken until effervescence ceased, and

Table 1
Validation of method accuracy by the standard addition method

Standard added (ng/spot)	Found (ng/spot), C.V. (%), n = 4)	Recovery (%)
64.20	64.78 (1.10)	100.90
128.40	131.00 (1.70)	102.02
192.60	186.03 (1.87)	96.59
	Mean ± S.D.	99.84 ± 2.87
	C.V. (%)	2.87

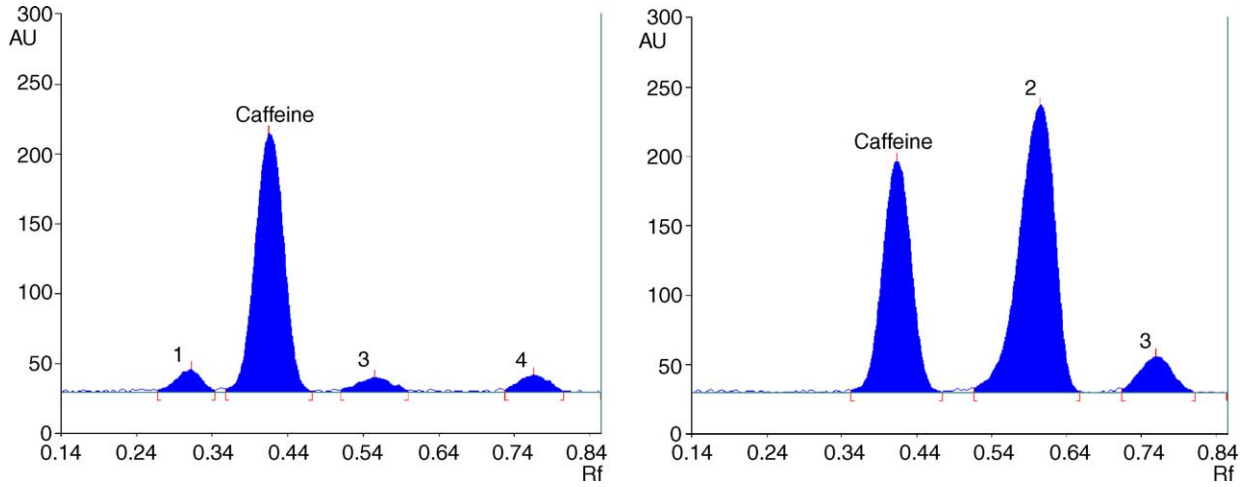

Fig. 1. Chromatograms of two samples showing baseline separation of the caffeine peak from other sample components at 275 nm.

2.00–3.00 μL were applied in quadruplicates directly to the HPTLC plate. Each applied band was dried by a stream of nitrogen at a speed of 12 s/μL.

3. Results

The R_f value of caffeine in the development solvent system was 0.38 ± 0.01, and there was no overlap with any other component in the analyzed samples at 275 nm. The chromatographic profiles of most samples were simple, showing caffeine as the main component; while baseline resolution between caffeine and adjacent peaks was obvious in the few remaining samples ($R_s \geq 1$), as shown in Fig. 1. For such samples, a UV spectrum was obtained for the caffeine peak to assure peak purity. Polynomial regression of the data points for standard caffeine resulted in a calibration curve with the equation $Y = 796.091 + 14.651x - 0.004x^2$ [regression coefficient (R) = 0.999, standard deviation (S.D.) = 1.33]. The calibration range was from 214.00–1284.00 ng/spot (Fig. 2 A). Validation of the curve by the standard recovery method returned a mean of 98.9 ± 3.46% and a coefficient of variance (C.V.) of 3.50% (Table 2). Determination of method accuracy by the standard addition method at three concentration levels returned a mean recovery value of 99.84 ± 2.87% (Table 2).

Table 2
Percentage recovery of pure caffeine at three concentration levels

Taken (ng/spot)	Found (ng/spot), C.V. (%), n = 3	Recovery (%)
642.00	655.11 (0.64)	102.04
856.00	851.46 (1.56)	99.47
1070.00	1018.62 (1.02)	95.20
	Mean ± S.D.	98.9 ± 3.46
	C.V. (%)	3.50

Analysis of three herbal products and six power drinks containing caffeine showed a concentration range of 4.76–13.29% (w/w) and 0.011–0.032% (w/v), respectively

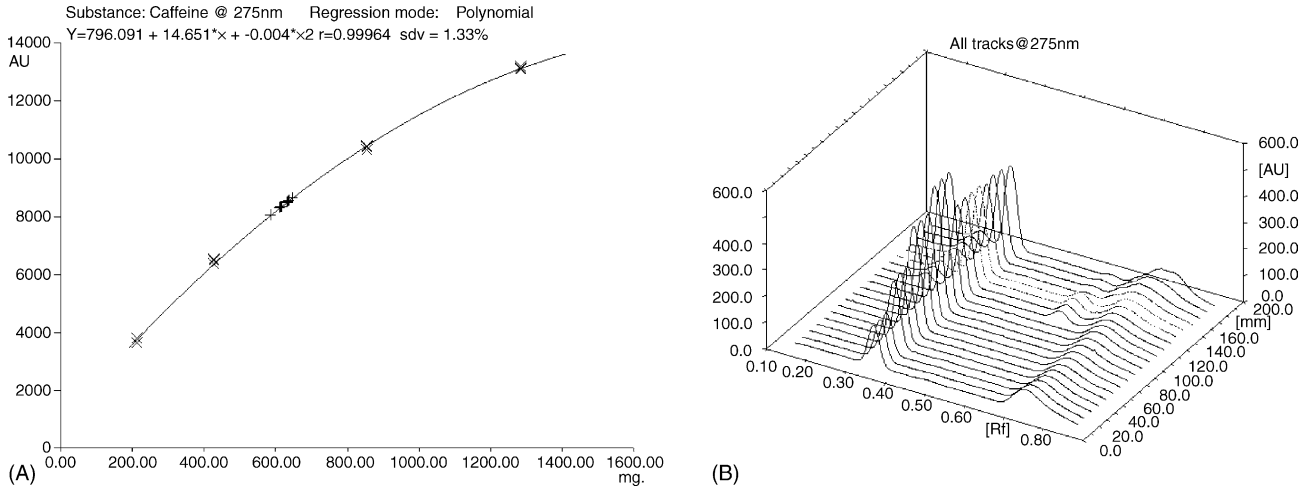

Fig. 2. (A) Calibration curve and (B) representative HPTLC chromatograms of caffeine on a 20-track plate; (×): standard concentration levels, (+): sample concentration, peaks at R_f 0.38 in (B) are for caffeine.

Table 3
Caffeine levels in herbal products and power drinks

Product	Product code	Found (ng/spot ± S.D., n = 4)	C.V. (%)	Concentration (%, w/w)	Caffeine/serving (mg)
Kola Extract	HP1	762.72 ± 10.61	1.39	4.76	33.32
Mega Ripped	HP2	1013.89 ± 9.66	0.95	12.68	198.06
Guarana Power	HP3	681.76 ± 19.48	2.86	13.29	70.60
Magic Energy Kick	ED1	393.42 ± 1.78	0.45	0.013	32.78
Power Horse	ED2	638.77 ± 7.34	1.15	0.032	79.85
Shadow	ED3	435.26 ± 6.59	1.51	0.022	54.4
Red Bull	ED4	608.00 ± 14.29	2.35	0.030	76.00
PepsiX Energy Cola	ED5	411.30 ± 6.61	1.61	0.021	52.50
Pepsi Cola	ED6	323.15 ± 9.34	2.89	0.011	26.93

(Table 3). A representative set of chromatograms is shown in Fig. 2B.

4. Discussion

The validation parameters for the developed method were the specificity, calibration curve, precision (repeatability), recovery, and accuracy. The detection wavelength was selective to caffeine and enabled its detection at R_f 0.38. Non-linear regression was applied for curve fitting, and the resulting equation was operational in the concentration range of 214.00–1284.00 ng/spot. This range was suitable for obtaining the results shown in Tables 1–3. The calibration curve was accurate within the specified concentration range with a mean recovery of 98.8 ± 3.46%. The method was also accurate 99.84 ± 2.87% after spiking the Kola sample with three different concentrations of standard caffeine (SS2). Spiking also served to further substantiate method specificity. Overall method repeatability was determined by calculating the coefficient of variance (C.V.%) in all the validation experiments and sample analyses, which was within 5% (Tables 1–3). This method is comparable to that published by Bebawy and El-Kousy for the analysis of caffeine in pharmaceutical products [9].

The current method was applied to the analysis of caffeine in two groups of stimulant products available in the Saudi market. The first group comprised of three herbal products formulated as dry extracts in hard gelatin capsules. The extracts were obtained from kola or guarana nuts, with additional ephedra extracts present in two of the products (HP2 and HP3). Products HP2 and HP3 clearly stated the amount of caffeine per serving, and the obtained values were slightly less than the stated ones (99.03 and 94.13% for HP2 and HP3, respectively). Product HP1 provided the least amount of caffeine per serving (33.32 mg), and it only stated the amount of kola extract included in each capsule, while HP2 provided the highest amount of caffeine, which was also much higher than that present in an average cup of coffee (Table 3). The second group of products comprised of six power drinks in the form of carbonated beverages. Four products (ED1-4) stated the amount of caffeine present and in all of these, the results of the analyses were in accordance with the label amount. The lowest amount of caffeine per serving was in ED6 (26.93 mg) while the highest, comparable to an average cup of tea or coffee, was present in ED2 (79.85 mg) (Table 3).

In conclusion, HPTLC is an analytical technique that can be utilized in the quality control of caffeine-containing dietary supplements, exemplified here in two types of products with high popularity in the Saudi market: stimulant herbal products and carbonated energy drinks.

Acknowledgements

This work was supported by the College of Pharmacy Research Center, King Saud University, Grant no. CPRC125.

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