View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2016) 1–8

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography

Fatma Turak [a], *Remziye Güzel* [b], *Erdal Dinç* [c,*]

[a] *Department of Chemistry, Faculty of Science and Art, Yıldız Teknik University, İstanbul, Turkey*
[b] *Department of Chemistry, Faculty of Education, Dicle University, Diyarbakır, Turkey*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Tandoğan, Ankara, Turkey*

ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form
9 September 2016
Accepted 12 September 2016
Available online xxx

Keywords:
ascorbic acid
caffeine
commercial soft drinks
quantification
reverse-phase ultraperformance
liquid chromatography

ABSTRACT

A new reversed-phase ultraperformance liquid chromatography method with a photodiode array detector was developed for the quantification of ascorbic acid (AA) and caffeine (CAF) in 11 different commercial drinks consisting of one energy drink and 10 ice tea drinks. Separation of the analyzed AA and CAF with an internal standard, caffeic acid, was performed on a Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.), using a mobile phase consisting of acetonitrile and 0.2M H_3PO_4 (11:89, v/v) with a flow rate of 0.25 mL/min and an injection volume of 1.0 μL. Calibration graphs for AA and CAF were computed from the peak area ratio of AA/internal standard and CAF/internal standard detected at 244.0 nm and 273.6 nm, respectively. The developed reversed-phase ultraperformance liquid chromatography method was validated by analyzing standard addition samples. The proposed reversed-phase ultraperformance liquid chromatography method gave us successful results for the quantitative analysis of commercial drinks containing AA and CAF substances.

Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Caffeine (CAF; 1,3,7-trimethylxanthine), which is a xanthine alkaloid, has widely been used in tea (black, white, and green), coffee, guarana, chocolate, cocoa, soft and energy drinks, and pharmaceutical products. In recent years, the use of CAF in energy drinks has increased significantly due to its excitation and analgesic properties. However, the use of a high dosage of CAF gives rise to some symptoms such as headache, slowness, fatigue, and depression. Ascorbic acid {AA; (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one} is one of the most important vitamins, which plays an important role for hydroxylation reactions and antioxidants. Symptoms of lack of AA are physical and mental infirmity, fatigue, weight loss, bruising, dry hair and skin, and increased sensibility of infections. Nowadays, the production of commercial drinks as a function of the developments in the food industry has

* *Corresponding author*. Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey. E-mail address: dinc@ankara.edu.tr (E. Dinç).

http://dx.doi.org/10.1016/j.jfda.2016.09.004

1021-9498/Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

increased tremendously. Taking such situations into account, quality control and routine analysis of commercial drinks have very vital importance for the human health and life quality. In this context, quantitative analysis and quality control of commercial drinks require new powerful analytical methods giving reliable, precise, and accurate results with short runtime and low cost of analysis.

Several analytical methods including spectrophotometry for CAF [1,2] and AA [3], high-performance liquid chromatography for CAF [4–16] and AA [17–22], liquid chromatography–mass spectrometry for CAF [23] and AA [24], voltammetry for CAF [25–27] and AA [28–32], Fourier transform infrared spectrophotometry for CAF [33–35] and AA [36], chemiluminescence for CAF [37], gas chromatography–mass spectrometry for CAF [38], ion chromatography for CAF [39], capillary electrophoresis for CAF [40], and ultra-high-performance liquid chromatography for CAF [41] and AA [42] have been reported for the analysis of the related compounds in drinks and pharmaceuticals. A literature survey revealed that there was no report about the simultaneous estimation of AA and CAF in the mentioned samples. Hence, the authors have attempted to develop a rapid, precise, and accurate method for the simultaneous determination of these active compounds in commercial drink samples. Some typical studies related to AA [43,44] and CAF [45] were reported.

Today, the ultraperformance liquid chromatography (UPLC) method is preferable to high-performance liquid chromatography for the analysis of raw samples, food products, drug preparations, and compounds in biological fluids due to short runtime and less solvent consumption. Moreover, the UPLC technique offers new possibilities in liquid chromatography, giving short analysis time and better chromatographic elution for the simultaneous determination of compounds in samples with adequate precision and accuracy.

In this study, a new reversed-phase UPLC (RP-UPLC) method was developed for the simultaneous quantitative analysis of AA and CAF in 11 different commercial drinks. The validation of the proposed UPLC method was carried out analyzing standard addition samples to evaluate its precision, accuracy, and selectivity. It was concluded that the UPLC method provided successful results for the quantitative estimation and quality control of the analyzed commercial drink samples containing CAF and AA. The analysis results provided by the developed and validated RP-UPLC method were compared with those obtained by the literature methods.

2. Experimental

2.1. Instrument and software

Chromatographic separation was carried out using the Waters ACQUITY UPLC H-Class system, including a quaternary solvent manager photodiode array detector, a cooling autosampler, and an oven enabling the control of column temperature. Chromatographic data collection and evaluation were made by Waters Empower2 chromatography software. Chromatographic elution of AA and CAF was performed via a Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.).

2.2. Chromatographic conditions

In the chromatographic analysis, the analytical column was the Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.). The mobile phase for the elution of AA and CAF in samples in the presence of an internal standard (IS) was a mixture of acetonitrile and 0.2M H_3PO_4 (11:89 v/v). The mobile phase was filtered through a 0.20 μm microfilter. The total runtime of AA and CAF with IS was 14 minutes, with a flow rate of 0.25 mL/min and column temperature of 50°C. AA and CAF were detected at 244.0 nm and 273.6 nm, respectively.

2.3. Reagents

Acetonitrile was of high-performance liquid chromatography grade (Sigma-Aldrich, Germany), and H_3PO_4 (Merck, Germany), CAF (Sigma-Aldrich, USA), and AA (Merck, Germany) were of guaranteed reagent grade. Water purified with Milli-Q Gradient A10 Milipore System (Merck Milipore, ABD, USA) was used during chromatographic analysis. All solutions were filtered through a 0.20 μm hydrophilic PTFE syringe filter (Minisart, Germany).

2.4. Commercial drink products

A commercial energy drink (Burn energy drink) and 10 commercial ice tea drinks, consisting of Didi bargamot tea, Didi lemon tea, Fuse melon tea, Fuse lemon tea, Fuse pine-mango tea, Fuse peach tea, Lipton apple tea, Lipton green tea, Lipton lemon tea, and Lipton peach tea, were analyzed by the proposed RP-UPLC method. All the commercial drink products were purchased from local supermarkets.

2.5. Standard solutions

Standard stock solutions of AA, CAF, and caffeic acid were separately prepared by dissolving 10 mg of each compound in 100 mL of 0.1M HCl. A standard calibration set of five mixtures containing 2.5–40 μg/mL of AA and 4.0–44.0 μg/mL of CAF in the presence of 12 μg/mL of caffeic acid as an IS was

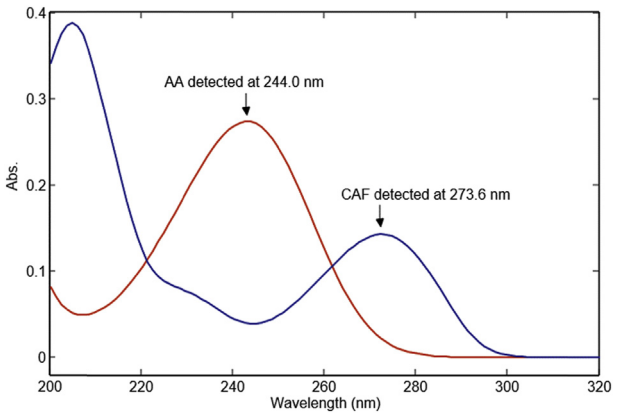

Figure 1 – UV spectra showing the detection wavelengths of the AA and CAF compounds. AA = ascorbic acid; CAF = caffeine.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

freshly prepared from the above stock solutions of the analyzed compounds. For method validation in the working concentration range of AA and CAF, standard addition samples were prepared by adding the mentioned standard stock solutions (at 3 different levels: low—2.5 μg/mL, medium—10 μg/mL, and high—30 μg/mL of AA, and low—4.0 μg/mL, medium—14 μg/mL, and high—34 μg/mL of CAF in the presence of 12 μg/mL IS in 3 replicates) to the solutions of each commercial drink. Under the optimized conditions, chromatograms of the calibration solutions, standard addition samples, and commercial drinks were recorded for the estimation of the amounts of AA and CAF in their related samples.

2.6. *Preparation of commercial samples*

For the analysis of the commercial drinks, sample solutions were degassed thoroughly in an ultrasonic bath for 25 minutes. An appropriate volume of samples was transferred to a 10 mL volumetric flask, and the flask was filled up with mobile phase to the mark and sonicated for 10 minutes. Prior to the UPLC analysis, the samples were filtered through a 0.2 μm membrane filter. This procedure was repeated nine times for each commercial sample. The resulting samples as three replicates were injected into the UPLC system for recording the chromatograms of AA and CAF samples.

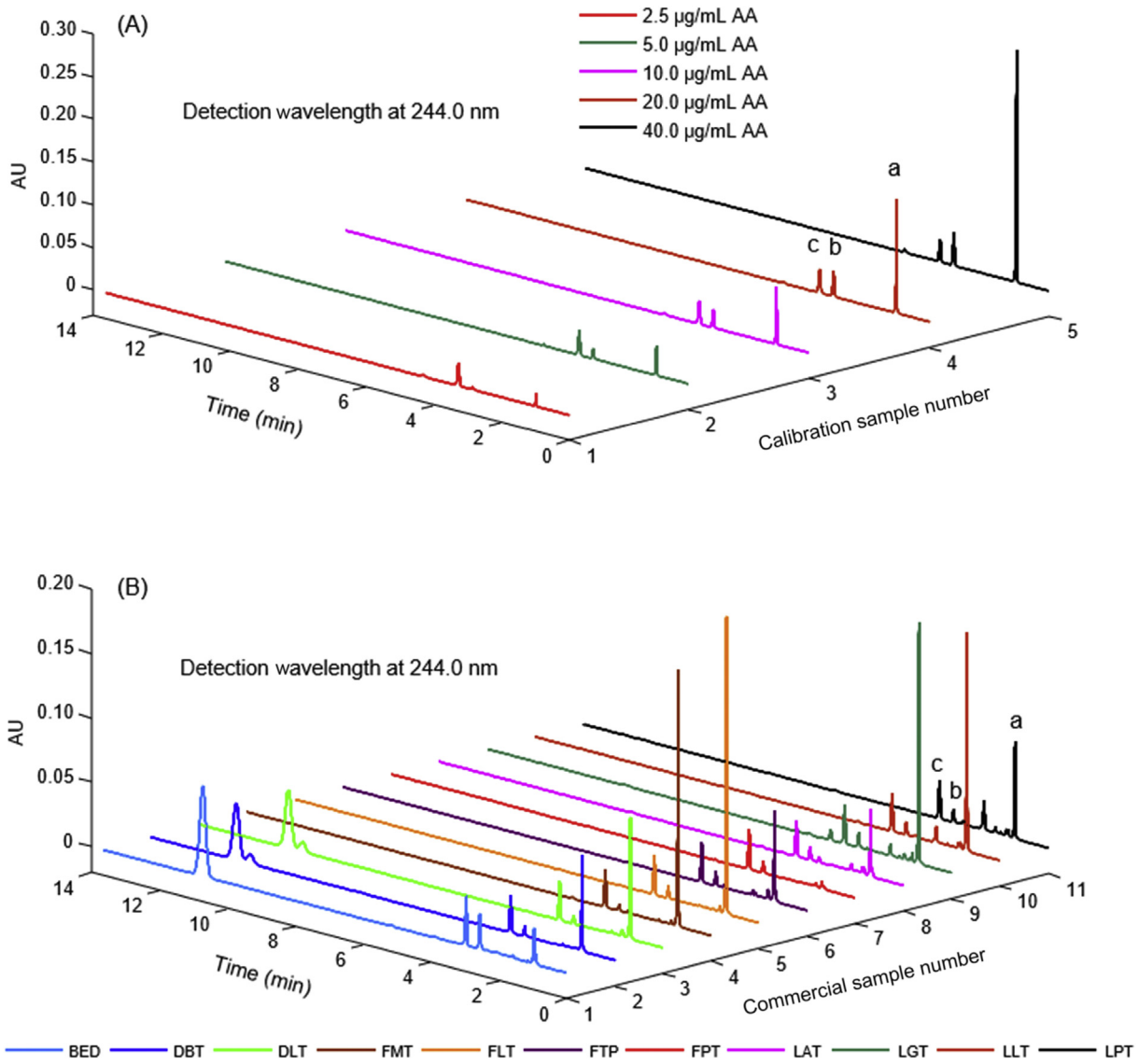

Figure 2 – Chromatograms of AA in (A) calibration set and (B) commercial drinks obtained by detection at 244 nm. In the chromatograms of Figures 2A and 2B, the letters a, b, and c correspond to AA, CAF, and IS, respectively. AA = ascorbic acid; BED = Burn energy drink; CAF = caffeine; DBT = Didi bargamot tea; DLT = Didi lemon tea; FMT = Fuse melon tea; FLT = Fuse lemon tea; FPMT = Fuse pine-mango tea; FPT = Fuse peach tea; IS = internal standard; LAT = Lipton apple tea; LGT = Lipton green tea; LLT = Lipton lemon tea; LPT = Lipton peach tea.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

3. Results and discussion

3.1. Method development and application

Several mobile phases in different compositions concerning organic solvents such as methanol and acetonitrile and different buffers such as acetate and phosphate with various pH levels were tested for finding the optimal conditions to get desirable elution of AA and CAF in the presence of IS. Based on the use of the Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.), a mobile phase consisting of acetonitrile and 0.2M H_3PO_4 (11:89, v/v), with a flow rate of 0.25 μL/min and column temperature of 50°C, was found to be very suitable for adequate elution of AA and CAF in samples with IS. Sample injection volume was 1.0 μL during chromatographic analysis.

As can be seen in Figure 1, the optimal chromatographic detection wavelengths for AA and CAF were chosen as 244.0 nm and 273.6 nm, respectively.

The calibration samples of AA and CAF in the concentration ranges of 2.5–40 μg/mL and 4.0–44.0 μg/mL containing 12 μg/mL IS were prepared by starting from the stock solutions of the analyzed compounds. The chromatograms of calibration samples for AA and CAF were recorded under optimized chromatographic conditions, as indicated in Figures 2A and 3A, respectively.

A similar chromatographic recording procedure was applied to standard addition samples and commercial samples. As can be seen in Figures 2A and 3A, the elution times of AA and CAF with IS were observed to be 2.5 minutes, 3.0 minutes, and 3.5 minutes, respectively. Calibration graphs for AA and CAF in the linear working concentration ranges of the

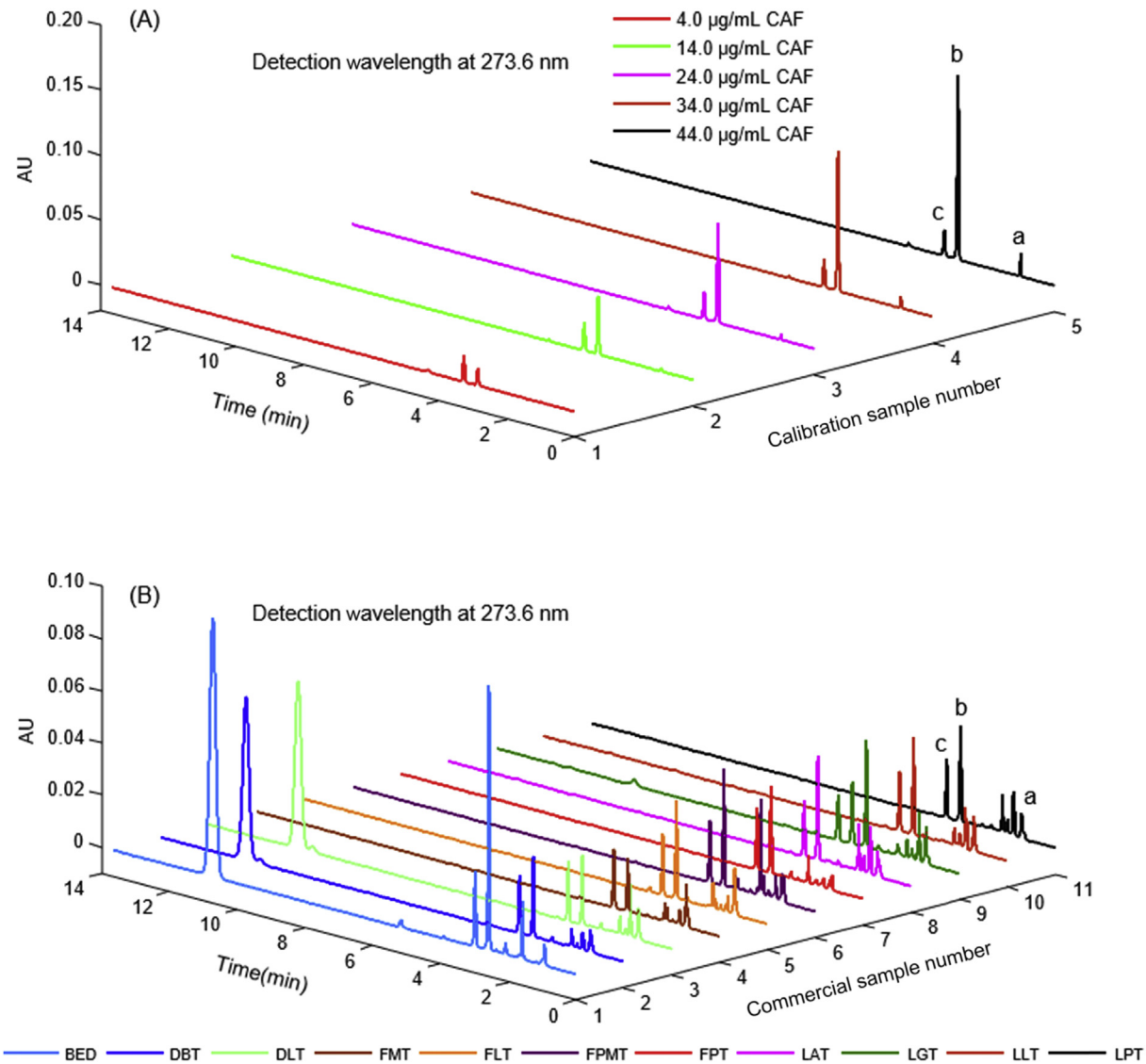

Figure 3 – Chromatograms of CAF in (A) calibration set and (B) commercial drinks obtained by detection at 273.6 nm. In the chromatograms of Figures 3A and 3B, the letters a, b, and c correspond to AA, CAF, and IS, respectively. AA = ascorbic acid; BED = Burn energy drink; CAF = caffeine; DBT = Didi bargamot tea; DLT = Didi lemon tea; FMT = Fuse melon tea; FLT = Fuse lemon tea; FPMT = Fuse pine-mango tea; FPT = Fuse peach tea; IS = internal standard; LAT = Lipton apple tea; LGT = Lipton green tea; LLT = Lipton lemon tea; LPT = Lipton peach tea.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

Table 1 – Least squares regression analysis and statistical results.

Parameters	AA	CAF
λ (nm)	244.0	273.6
m	0.2354	0.1512
n	0.0539	0.0095
r	1.0000	0.9999
SE(*m*)	0.0009	0.0012
SE(*n*)	0.0185	0.0323
SE(*r*)	0.0274	0.0367
LOD (μg/mL)	0.53	1.44
LOQ (μg/mL)	1.76	4.78

AA = ascorbic acid; CAF = caffeine; LOD = limit of detection (μg/mL); LOQ = limit of quantitation (μg/mL); *m* = slope of regression equation; *n* = intercept of regression equation; *r* = regression coefficient; SE(*m*) = standard error of slope; SE(*n*) = standard error of intercept; SE(*r*) = standard error of regression coefficient.

related compounds were computed from the mathematical relationship between concentration and peak-area ratio of AA/IS and CAF/IS at 244.0 nm and 273.6 nm, respectively. The regression analysis and statistical results were given in Table 1. The concentrations of AA and CAF in the analyzed commercial drinks were determined using the calculated calibration graphs.

3.2. *Method validity and applicability*

The validity of the developed UPLC method was assessed by estimating its precision, accuracy, linearity, selectivity, limit of detection, and limit of quantitation. Good linearity for AA and CAF was reported from their calibration curves, giving excellent correlation coefficients as shown in Table 1. The limit of detection, which corresponds to a signal/noise ratio of 3, and the limit of quantitation, which corresponds to a signal/noise ratio of 10, were computed from the slope and intercept's standard deviation of linear regression equation for each compound. Both the limit of detection and the limit of quantitation for the analyzed compounds are listed in Table 1. Accuracy and precision were evaluated by analyzing the standard addition samples using the proposed UPLC method. The standard addition samples were prepared by adding known amounts of AA and CAF (at 3 different concentration levels: 2.5 μg/mL, 10.0 μg/mL, and 30.0 μg/mL of AA and 4.0 μg/mL, 14.0 μg/mL, and 34.0 μg/mL of CAF) to the analyzed commercial drink samples, as indicated in Table 2. As can be seen in this table, percent recovery results, standard deviations, and relative standard deviations were calculated from the added and found amounts of AA and CAF in the standard addition samples. The results obtained for the validity and applicability of the developed UPLC method to analyze the related compounds were found to be within the acceptable limits.

Selectivity of the proposed UPLC method was evaluated by comparing the slopes of the regression equations of the analyzed compounds and their standard addition samples containing commercial drinks. The comparison of the slopes of the regression lines of calibration and standard addition samples was carried out using the *t* test with 95% confidence interval. The statistical results of the *t* test with *p* values for AA and CAF are given in Table 3. From the *t* test results given in Table 3, no significant difference was observed between the

Table 2 – Analysis results obtained from the standard addition samples by the developed RP-UPLC method.

Added (μg/mL)	AA, found (μg/mL)										
	BED	DBT	DLT	FMT	FLT	FPMT	FPT	LAT	LGT	LLT	LPT
2.5	2.46	2.44	2.56	2.54	2.44	2.40	2.61	2.57	2.55	2.47	2.39
10	10.06	10.09	9.92	10.44	10.09	10.13	9.85	9.90	9.87	10.05	10.16
30	29.98	29.98	30.02	30.02	29.98	29.96	30.04	30.03	30.04	29.99	29.96
	AA, recovery (%)										
	98.3	97.4	102.3	101.6	97.4	96.1	104.3	102.9	102.1	98.7	95.4
	100.6	100.9	99.2	104.4	100.9	101.3	98.5	99.0	98.7	100.5	101.6
	99.9	99.9	100.1	100.1	99.9	99.9	100.1	100.1	100.1	100	99.9
Mean	99.6	99.4	100.5	102.0	99.4	99.1	101.0	100.7	100.3	99.7	99.0
SD	1.21	1.78	1.61	2.22	1.77	2.67	2.98	2.00	1.73	0.92	3.17
RSD	1.22	1.79	1.60	2.18	1.78	2.69	2.95	1.99	1.73	0.92	3.20
Added (μg/mL)	CAF, found (μg/mL)										
	BED	DBT	DLT	FMT	FLT	FPMT	FPT	LAT	LGT	LLT	LPT
4	3.92	3.89	3.92	4.17	4.08	4.20	3.52	4.06	3.88	4.06	4.14
14	14.13	14.17	14.12	13.74	13.89	13.69	14.71	13.91	14.19	13.90	13.79
34	33.96	33.94	33.96	34.09	34.04	34.10	33.76	34.03	33.94	34.03	34.07
	CAF, recovery (%)										
	97.9	97.2	98.0	104.3	101.9	105.1	88.1	101.6	96.9	101.6	103.5
	100.9	101.2	100.9	98.2	99.2	97.8	105.1	99.3	101.3	99.3	98.5
	99.9	99.8	99.9	100.3	100.1	100.3	99.3	100.1	99.8	100.1	100.2
Mean	99.6	99.4	99.6	100.9	100.4	101.1	97.5	100.3	99.4	100.3	100.7
SD	1.54	2.01	1.44	3.12	1.38	3.70	8.64	1.15	2.24	1.17	2.56
RSD	1.55	2.02	1.45	3.09	1.37	3.66	8.87	1.14	2.26	1.17	2.54

AA = ascorbic acid; CAF = caffeine; RP-UPLC = reversed-phase ultraperformance liquid chromatography; RSD = relative standard deviation; SD = standard deviation.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

Table 3 – Statistical comparison results of the regression slopes of the calibration and standard addition samples using t test.

Samples	AA				CAF			
	λ (nm)	Regression equation	t test	p	λ (nm)	Regression equation	t test	p
[a]	244.0	Y = 0.2354C + 0.0540	—	—	273.6	Y = 0.1512C + 0.0095	—	—
BED	244.0	Y = 0.2385C + 0.1802	2.47	0.07	273.6	Y = 0.1501C + 1.0713	0.67	0.54
DBT	244.0	Y = 0.2313C + 0.8660	2.59	0.06	273.6	Y = 0.1508C + 0.3532	0.32	0.77
DLT	244.0	Y = 0.2324C + 0.7250	2.04	0.11	273.6	Y = 0.1532C + 0.2830	1.27	0.27
FMT	244.0	Y = 0.2366C + 1.6270	1.03	0.99	273.6	Y = 0.1531C + 0.2359	0.76	0.49
FLT	244.0	Y = 0.2332C + 1.7825	1.39	0.24	273.6	Y = 0.1497C + 0.4139	0.98	0.38
FPMT	244.0	Y = 0.2376C + 0.5753	1.02	0.37	273.6	Y = 0.1477C + 0.5083	1.24	0.28
FPT	244.0	Y = 0.2384C − 0.0431	1.27	0.27	273.6	Y = 0.1450C + 0.3838	1.02	0.36
LAT	244.0	Y = 0.2365C + 0.4388	0.66	0.54	273.6	Y = 0.1490C + 0.4886	1.54	0.20
LGT	244.0	Y = 0.2385C + 1.5702	1.47	0.14	273.6	Y = 0.1508C + 0.4698	0.21	0.84
LLT	244.0	Y = 0.2363C + 1.4442	0.84	0.24	273.6	Y = 0.1494C + 0.4126	1.25	0.28
LPT	244.0	Y = 0.2419C + 2.0738	2.56	0.06	273.6	Y = 0.1494C + 0.4020	1.55	0.20

t table = 2.78 and $p = 0.05$.
AA = ascorbic acid; BED = Burn energy drink; CAF = caffeine; DBT = Didi bargamot tea; DLT = Didi lemon tea; FMT = Fuse melon tea; FLT = Fuse lemon tea; FPMT = Fuse pine-mango tea; FPT = Fuse peach tea; LAT = Lipton apple tea; LGT = Lipton green tea; LLT = Lipton lemon tea; LPT = Lipton peach tea.
[a] Calibration equations of AA and CAF are given in Table 1.

values of slopes of regression equations of the calibration and standard addition samples. This indicates that there is no matrix effect on the analysis of the marketed drinks using the proposed UPLC method.

3.3. *Analysis of commercial drinks*

Samples of commercial drinks were prepared for chromatographic analysis as described in the 2.6. section, preparation

Table 4 – Determination results of AA and CAF in the commercial drinks analyzed by the developed RP-UPLC method.

Sample no.	AA (mg/L)										
	BED	DBT	DLT	FMT	FLT	FPMT	FPT	LAT	LGT	LLT	LPT
1	15.67	72.39	62.69	144.59	152.33	22.91	2.40	38.40	133.64	125.67	177.11
2	15.65	72.23	62.42	145.37	153.17	22.91	2.50	38.04	133.00	125.82	177.01
3	15.52	72.27	62.10	150.27	155.31	24.95	2.55	38.17	132.50	124.78	175.43
4	15.15	71.79	59.53	140.72	151.32	23.62	2.42	39.98	133.24	121.27	167.28
5	15.10	71.20	60.01	140.38	152.21	23.62	2.44	39.81	132.49	120.64	167.87
6	14.99	71.79	59.70	140.39	152.15	23.82	2.40	39.81	132.62	120.21	166.49
7	14.43	72.39	62.81	142.17	153.73	23.03	2.41	37.44	126.74	118.57	168.39
8	14.48	71.82	63.01	142.55	153.45	23.03	2.40	37.36	125.72	118.99	168.05
9	14.39	71.86	62.76	141.67	154.08	22.99	2.41	37.30	125.33	118.65	167.49
Mean	15.04	71.97	61.67	143.12	153.08	23.43	2.44	38.48	130.59	121.62	170.57
SD	0.52	0.39	1.47	3.21	1.22	0.67	0.05	1.11	3.53	3.00	4.52
RSD	3.43	0.54	2.38	2.24	0.79	2.86	2.17	2.88	2.70	2.47	2.65
Sample no.	CAF (mg/L)										
	BED	DBT	DLT	FMT	FLT	FPMT	FPT	LAT	LGT	LLT	LPT
1	139.98	45.61	38.12	31.10	52.93	34.31	31.76	64.75	58.28	53.69	52.98
2	140.60	45.73	38.31	30.86	53.17	34.45	31.92	64.60	58.56	54.07	52.85
3	140.10	45.88	38.24	31.26	53.40	34.23	31.88	64.87	58.58	54.01	53.19
4	146.88	47.65	40.16	32.06	54.96	36.12	33.91	63.55	61.80	57.02	55.00
5	146.91	47.53	39.95	32.30	55.11	35.83	33.98	62.99	61.76	57.58	54.91
6	146.44	21.66	39.94	32.32	54.97	35.83	33.98	62.68	62.41	57.40	55.57
7	149.17	48.94	40.49	32.21	56.74	35.78	34.57	63.35	63.46	57.89	55.57
8	149.23	48.70	40.45	32.35	56.58	36.00	34.29	63.43	63.55	57.89	55.69
9	149.40	48.73	40.43	32.50	56.37	35.97	34.64	62.93	63.12	57.96	55.55
Mean	145.41	44.49	39.57	31.88	54.92	35.39	33.44	63.68	61.28	56.39	54.59
SD	4.04	8.66	1.03	0.63	1.48	0.81	1.22	0.84	2.20	1.88	1.22
RSD	2.78	19.47	2.60	1.97	2.70	2.28	3.64	1.32	3.59	3.33	2.24

AA = ascorbic acid; BED = Burn energy drink; CAF = caffeine; DBT = Didi bargamot tea; DLT = Didi lemon tea; FMT = Fuse melon tea; FLT = Fuse lemon tea; FPMT = Fuse pine-mango tea; FPT = Fuse peach tea; LAT = Lipton apple tea; LGT = Lipton green tea; LLT = Lipton lemon tea; LPT = Lipton peach tea; RP-UPLC = reversed-phase ultraperformance liquid chromatography; RSD = relative standard deviation; SD = standard deviation.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

of commercial samples. Under optimized chromatographic conditions, chromatograms of the commercial drink samples for the analysis of AA and CAF were recorded, as indicated in Figures 2B and 3B, respectively. By replacing the peak area ratio of AA/IS and CAF/IS in the calibration equation of each compound, the amounts of AA and CAF in 11 different commercial samples were computed. Their analysis results are given in Table 4. As it can be seen in Table 4, a good accordance with the acceptable standard deviation and relative standard deviation was reported for the analysis results obtained by applying the developed RP-UPLC to the commercial drinks.

4. Conclusions

A new RP-UPLC method was developed and validated for the quantitative estimation of AA and CAF in 11 different commercial drinks without requiring additional chemical pretreatment. This study indicates that the proposed RP-UPLC approach gives us reliable, precise, and accurate results for the simultaneous quantification of the analyzed AA and CAF in commercial soft drinks with adequate runtime and low solvent consumption. In this study, the newly developed RP-UPLC method allowed for the simultaneous quantitation of AA and CAF in analyzed samples, whereas literature UPLC methods with electrospray ionization–multiple tandem mass spectrometry detection for the analysis of CAF alone [41] and photodiode array detection for the analysis of AA alone [42] require the use of pretreatment for sample preparation. In our case, after diluting commercial samples with the solvent system, filtered samples were directly injected into the UPLC system without using an initial extraction procedure. The mentioned arguments indicated that the newly developed RP-UPLC approach has advantages (simultaneous quantification of the related active compounds in samples without using preliminary extraction or chemical procedure) over literature UPLC methods. As a consequence, the developed and validated RP-UPLC method is a very useful technique for the quality control and routine analysis of marketed drinks containing AA and CAF substances.

Conflicts of interest

All authors declare no conflicts of interest.

REFERENCES

[1] Alpdoğan G, Karabina K, Sungur S. Derivative spectrophotometric determination of caffeine in some beverages. Turk J Chem 2002;26:295–302.
[2] Singh DK, Sahu A. Spectrophotometric determination of caffeine and theophylline in pure alkaloids and its application in pharmaceutical formulations. Anal Biochem 2006;349:176–80.
[3] Zhu M, Huang X, Li J, Shen H. Peroxidase-based spectrophotometric methods for the determination of ascorbic acid, norepinephrine, epinephrine, dopamine and levodopa. Anal Chim Acta 1997;357:261–7.
[4] De Camargo MCR, Toledo MCF. HPLC determination of caffeine in tea, chocolate products and carbonated beverages. J Sci Food Agr 1999;79:1861–4.
[5] Schreiber-Deturmeny E, Bruguerolle B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. J Chromatogr B 1996;677:305–12.
[6] Horie H, Nesumi A, Ujihara T, Kohata K. Rapid determination of caffeine in tea leaves. J Chromatogr A 2002;942:271–3.
[7] Bendriss E, Markoglou N, Wainer IW. Liquid chromatographic method for the simultaneous determination of caffeine and fourteen caffeine metabolites in urine. J Chromatogr B 2000;746:331–8.
[8] Martín MJ, Pablos F, González AG. Simultaneous determination of caffeine and non-steroidal anti-inflammatory drugs in pharmaceutical formulations and blood plasma by reversed-phase HPLC from linear gradient elution. Talanta 1999;49:453–9.
[9] Wang H, Provan GJ, Helliwell K. HPLC determination of catechins in tea leaves and tea extracts using relative response factors. Food Chem 2003;81:307–12.
[10] Holland DT, Godfredsen KA, Page T, Connor JD. Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation. J Chromatogr B 1998;707:105–10.
[11] Bispo MS, Veloso MCC, Pinheiro HLC, De Oliveira RFS, Reis JON, De Andrade JB. Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography. J Chromatogr Sci 2002;40:45–8.
[12] Srdjenovic B, Djordjevic-Milic V, Grujic N, Injac R, Lepojevic Z. Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. J Chromatogr Sci 2008;46:143–9.
[13] He Q, Lv Y, Zhou L, Shi B. Simultaneous determination of caffeine and catechins in tea extracts by HPLC. J Liq Chromatogr R T 2010;33:491–8.
[14] Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 2002;57:307–16.
[15] Aresta A, Palmisano F, Zambonin CG. Simultaneous determination of caffeine, theobromine, theophylline, paraxanthine and nicotine in human milk by liquid chromatography with diode array UV detection. Food Chem 2005;93:177–81.
[16] Thomas JB, Yen JH, Schantz MM, Porter BJ, Sharpless KE. Determination of caffeine, theobromine, and theophylline in standard reference material 2384, baking chocolate, using reversed-phase liquid chromatography. J Agr Food Chem 2004;52:3259–63.
[17] Ullah S, Hussain A, Ali J, Ullah K-A. A Simple and rapid HPLC method for analysis of vitamin-C in local packed juices of Pakistan. Middle East J Sci Res 2012;12:1085–91.
[18] Sawant L, Prabhakar B, Pandita N. Quantitative HPLC analysis of ascorbic acid and gallic acid in *Phyllanthus emblica*. J Anal Bioanal Tech 2010;1:2–4.
[19] Nováková L, Solich P, Solichová D. HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids. Trends Anal Chem 2008;27:942–58.
[20] Castro RN, Azeredo LC, Azeredo MAA, de Sampaio CST. HPLC assay for the determination of ascorbic acid in honey samples. J Liq Chromatogr R T 2001;24:1015–20.
[21] Nojavan S, Khalilian F, Kiaie FM, Rahimi A, Arabanian A, Chalavi S. Extraction and quantitative determination of

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004

ascorbic acid during different maturity stages of *Rosa canina* L. fruit. J Food Comp Anal 2008;21:300–5.
[22] Gazdik Z, Zitka O, Petrlova J, Adam V, Zehnalek J, Horna A, Reznicek V, Beklova M, Kizek R. Determination of vitamin C (ascorbic acid) using high performance liquid chromatography coupled with electrochemical detection. Sensors 2008;8:7097–112.
[23] Gardinali PR, Zhao X. Trace determination of caffeine in surface water samples by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (LC–APCI–MS). Environ Int 2002;28:521–8.
[24] Frenich AG, Torres MEH, Vega AB, Vidal JLM, Bolaños PP. Determination of ascorbic acid and carotenoids in food commodities by liquid chromatography with mass spectrometry detection. J Agr Food Chem 2005;53:7371–6.
[25] Zhen JM, Ting Y-S. Simultaneous determination of caffeine and acetaminophen in drug formulations by square-wave voltammetry using a chemically modified electrode. Anal Chim Acta 1997;342:175–80.
[26] Ly SY, Jung YS, Kim MH, Han IK, Jung WW, Kim HS. Determination of caffeine using a simple graphite pencil electrode with square-wave anodic stripping voltammetry. Microchim Acta 2004;146:207–13.
[27] Brunetti B, Desimoni E, Casati P. Determination of caffeine at a nafion-covered glassy carbon electrode. Electroanalysis 2007;19:385–8.
[28] Huanga J, Liua Y, Houb H, You T. Simultaneous electrochemical determination of dopamine, uric acid and ascorbic acid using palladium nanoparticle-loaded carbon nanofibers modified electrode. Biosens Bioelectron 2008;24:632–7.
[29] Karimi-Maleh H, Moazampour M, Yoosefian M, Sanati AL, Tahernejad-Javazmi F, Mahani M. An electrochemical nanosensor for simultaneous voltammetric determination of ascorbic acid and Sudan I in food samples. Food Anal Methods 2014;7:2169–76.
[30] Zhang B, Huang D, Xu X, Alemu G, Zhang Y, Zhan F, Shen Y, Wang M. Simultaneous electrochemical determination of ascorbic acid, dopamine and uric acid with helical carbon nanotubes. Electrochim Acta 2013;91:261–6.
[31] Sun W, Yang M, Gao R, Jiao K. Electrochemical determination of ascorbic acid in room temperature ionic liquid BPPF6 modified carbon paste electrode. Electroanalysis 2007;19:1597–602.
[32] Sheng Z-H, Zheng X-Q, Xua J-Y, Baoa W-J, Wanga F-B, Xia X-H. Electrochemical sensor based on nitrogen doped graphene: Simultaneous determination of ascorbic acid, dopamine and uric acid. Biosens Bioelectron 2012;34:125–31.
[33] Bouhsain Z, Garrigues JM, Garrigues S, de la Guardia M. Flow injection Fourier transform infrared determination of caffeine in coffee. Vib Spectrosc 1999;21:143–50.
[34] Najafi NM, Hamid AS, Afshin RK. Determination of caffeine in black tea leaves by Fourier transform infrared spectrometry using multiple linear regression. Microchem J 2003;75:151–8.
[35] Daghbouche Y, Garrigues S, Vidal MT, de la Guardia M. Flow injection Fourier transform infrared determination of caffeine in soft drinks. Anal Chem 1997;69:1086–91.
[36] Yang H, Irudayaraj J. Rapid determination of vitamin C by NIR, MIR and FTRaman techniques. J Pharm Pharmacol 2002;54:1247–55.
[37] Khanchi AR, Mahani MK, Hajihosseini M, Maragheh MG, Chaloosi M, Bani F. Simultaneous spectrophotometric determination of caffeine and theobromine in Iranian tea by artificial neural networks and its comparison with PLS. Food Chem 2007;103:1062–8.
[38] Shrivas K, Wu H-F. Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent micro extraction with gas chromatography/mass spectrometry. J Chromatogr A 2007;1170:9–14.
[39] Chen Q, Mou S, Hou X, Ni Z. Simultaneous determination of caffeine, theobromine and theophylline in foods and pharmaceutical preparations by using ion chromatography. Anal Chim Acta 1998;371:287–96.
[40] Zhao Y, Lunte CE. Determination of caffeine and its metabolites by micellar electrokinetic capillary electrophoresis. J Chromatogr B 1997;688:265–74.
[41] Lee MS, Huong NL, Huang NH, Shrestha A, Park JW. Ultra-high performance liquid chromatography with electrospray ionization tandem mass spectrometry for the determination of caffeine in energy drinks. Anal Lett 2014;47:1852–61.
[42] Spínola V, Mendes B, Cámara JS, Castilho PC. An improved and fast UHPLC-PDA methodology for determination of L-ascorbic and dehydroascorbic acids in fruits and vegetables. Evaluation of degradation rate during storage. Anal Bioanal Chem 2012;403:1049–58.
[43] Nasirizadeh N, Shekari Z, Dehghani M, Makarem S. Delphinidin immobilized on silver nanoparticles for the simultaneous determination of ascorbic acid, noradrenalin, uric acid, and tryptophan. J Food Drug Anal 2016;24:406–16.
[44] Denardin CC, Hirsch GE, da Rocha RF, Vizzotto M, Henriques AT, Moreira JCF, Guma FTCR, Emanuelli T. Antioxidant capacity and bioactive compounds of four Brazilian native fruits. J Food Drug Anal 2015;23:387–98.
[45] Shehata AB, Rizk MS, Rend EA. Certification of caffeine reference material purity by ultraviolet/visible spectrophotometry and high-performance liquid chromatography with diode-array detection as two independent analytical methods. J Food Drug Anal 2016;24(4):703–15.

Please cite this article in press as: Turak F, et al., Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography, Journal of Food and Drug Analysis (2016), http://dx.doi.org/10.1016/j.jfda.2016.09.004