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Determination of methylxanthines in tea samples by HPLC method

Timotej Jankech[1,✉], Mária Maliarová[1] and Nicholas Martinka[2,3]

[1]*Department of Chemistry, University of SS. Cyril and Methodius in Trnava, Nám. J. Herdu 2, Trnava 91701, Slovak Republic*
[2]*Department of Clinical Biochemistry, Alexander Winter Hospital, Winterova 66, Piešťany 92101, Slovak Republic*
[3]*Department of Laboratory Investigation Methods, Trnava University in Trnava, Hornopotočná 23, Trnava 91843, Slovak Republic*

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Abstract

Methylxanthines such as caffeine, theophylline, theobromine are significant and widespread psychoactive substances. We developed the isocratic method with optimum composition of the mobile phase 90 % water: 10 % acetonitrile and confirmed repeatability of retention times and peak areas. The developed HPLC method was applied to determine the content of methylxanthines in selected types of black and green teas available on the market. Of the black teas (tea bags), the highest concentration of theobromine was found in Ceylon tea (18.98 $mg.L^{-1}$). The highest concentration of caffeine was in a cup of Earl Gray tea (254.09 $mg.L^{-1}$). Among loose black teas, the highest content of both theobromine and caffeine was found in Pu Erh Superior tea, where the theobromine content was 24.62 $mg.L^{-1}$ and the caffeine content was 520.67 $mg.L^{-1}$. Of green powder teas, highest caffeine content (306.46 $mg.L^{-1}$) was in Shizuoka Matcha Premium and the highest content of theobromine (8.45 $mg.L^{-1}$) was found in GABA Midori. From the loose green tea, the highest concentration of theobromine (12.85 $mg.L^{-1}$) was in Lung Ching West Lake. The highest caffeine content (484.85 $mg.L^{-1}$) was in Gyokuro Shizuoka Premium Tea. In both types of teas the amount of theobromine and caffeine was quantified, but the presence of theophylline was not proven. Data on contents of these metabolites in tea products are highly informative for consumers.

University of SS. Cyril and Methodius in Trnava

Introduction

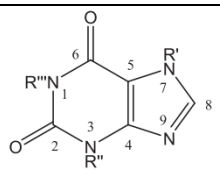
Several mild psychostimulants are derived from methylxanthines, including widely used and highly popular caffeine, theobromine and theophylline (Xia *et al*. 2013). The structures of these compounds (Fig. 1) with several health benefits are derived from the purine base xanthine and are obtained from plant secondary metabolism. Caffeine is the most routinely ingested bioactive substance throughout the world. It is a natural alkaloid found in more than 60 plants including coffee beans, tea leaves, cola nuts, and cocoa pods.

Its concentration varies depending on the type of product, agronomic and environmental factors, as well as processing (de Mejia and Ramirez-Mares 2014). Caffeine possess a number of effects: improves performance during sleep deprivation, in shift workers it leads to fewer mistakes caused by tiredness, moderate doses in athletics can improve sprit, endurance or tolerance and team sports performance. However, high dose of caffeine has adverse effect on health (Mufakkar *et al*. 2015).

Theophylline, also known as dimethylxanthine, is naturally found in tea and cocoa beans. For several decades, theophylline has been used as a therapeutic agent of chronic airway disease (asthma) with a narrow serum therapeutic range

Corresponding author: timotej.jankech@gmail.com

(Jafari *et al.* 2011). The main drawbacks of this active pharmaceutical ingredient (API) are related to its narrow therapeutic index and several side effects, i.e. nausea, headache, dizziness and vomiting. Increasing evidence shows that it has significant anti-inflammatory effects in chronic obstructive pulmonary disease at lower plasma concentrations (Zhu *et al.* 2015).



Compound	Substituent R′	R″	R‴
Caffeine	CH_3	CH_3	CH_3
Theobromine	CH_3	CH_3	H
Theophylline	H	CH_3	CH_3

Fig. 1. Structures of the methylxanthines analysed in this study (Gibson and Fowler 2014).

Theobromine is a bitter alkaloid of the cacao plant, providing specific flavour in chocolate as well as in a number of other foods (Xia *et al.* 2013). Although, chemically is very similar to other methylxanthines, such as caffeine and theophylline, theobromine stimulates the central nervous system to a lesser extent than these other methylxanthines. Ingestion of theobromine has demonstrated health benefits, including protection of the enamel surface of teeth, cough suppression and cardiovascular protection, increasing HDL and decreasing LDL cholesterol concentrations (Rodriguez *et al.* 2015). Different analytical techniques and sample preparation protocols have been proposed for the simultaneous determination of the methylxanthines in foods and biological fluids. Analytical techniques routinely used for the analysis of these components include high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) (Xia *et al.* 2013). An overview of published papers dealing with determination of methylxanthines in various matrices is provided in Table 1.

The aim of this study was the simultaneous determination of methylxanthines (caffeine, theobromine and theophylline) on chromatographic column Luna Polar C18, which is suitable for separating polar analytes, such as methylxanthines. The versatile C18 ligand provides hydrophobic interactions while the polar modified surface provides enhanced retention of polar analytes and aqueous stability. The method was applied to analyze the content of methylxanthines in different types of commercially available tea, namely black and green loose teas as well as powdered green teas. In literature, data on the amount of methylxanthines are mostly associated with the type of tea (black-green).

Experimental

Chemicals and reagents

HPLC-grade acetonitrile was purchased from Central Chem (Banská Bystrica, Slovakia).

Table 1. Overview of published papers dealing with determination of methylxanthines in various matrices (Tzanavaras *et al.* 2010).

Sample	Column	Mobile phase	Analysis time [min]	Detection
Beverages, tea, coffee	RP-C18 (100 mm×3.2 mm i.d.×3 μm)	MeOH:buffer (pH = 3.5) (10:90, v/v)	16	Amperometric
Cocoa	Nova-Pak C18 (150 mm×3.9 mm i.d.×4 μm)	MeOH:H_2O (20:80, v/v)	10	UV at 274 nm
Coffee, tea	LiChrospher 100 RP-18 (244 mm×4.4 mm i.d.×5 μm)	H_2O:EtOH:AcH (75:24:1, v/v/v)	10	UV at 273 nm
Human urine	Supelcosil LC-18-DB (250 mm×2.1 mm i.d.×5 μm)	MeOH:buffer (pH = 5.0) (20:80, v/v)	10	UV at 280 nm
Human serum	Kromasil ODS C18 (150 mm×4.6 mm i.d.×5 μm)	MeOH:buffer (pH = 4.5) (30:70, v/v)	12	UV at 270 nm
Beverages, chocolate	Bondesil C18 (150 mm×4.0 mm i.d.×5 μm)	H_2O:Me(Et)OH:AcH (75:20:5, v/v/v)	12	UV at 273 nm

Table 2. An overview of the processing of tested tea samples.

Type	Product Name	Weight [g]	Volume [mL]	Leaching time [min]	Water temperature* [°C]
Black teas					
a	Earl Grey	2	250	4	100
a	Darjeeling	2	250	4	100
a	English breakfast	2	250	4	100
a	English Tea No.1	2	250	4	100
a	Ceylon	2	250	4	100
a	Decaffeinated	2	250	4	100
b	Pu Erh Superior	5	200	2	100
b	Darjeeling Gopaldhara Wonder Tea Muscatel	5	200	1	100
b	Darjeeling Gopaldhara Wonder Gold	5	200	1	100
Green teas					
c	Shizuoka Matcha Premium	1	100	-	70 – 80**
c	Japan Kate-Benifuki	1	100	-	80**
c	GABA Midori	1	100	-	70 – 80**
b	Zhu Cha	5	200	2	70 – 80
b	Lung Ching West Lake	5	200	2	70 – 80
b	Houjicha Shizuoka	5	200	2	70 – 80
b	Gyokuro Shizuoka Premium	5	200	2	60 – 65
b	Sencha Shizuoka Superior	5	200	2	70 – 80
b	Nepal Silver Needles	5	200	2	85 – 90

Tea type: *a* – bag, *b* – loose, *c* – powder;
* Ultrapure water; ** Boiled water, cooled down to the required temperature.

Standards of caffeine (≥ 99 % purity), theobromine (≥ 98 % purity) and theophylline (≥ 99 % purity) were purchased from Sigma-Aldrich (Darmstadt, Germany). Ultrapure water (0.055 μS.cm^{-1}) was prepared from distilled water using the Ultrapure Water (Type 1), Simplicity UV device.

Apparatus

The analysis was performed using Waters HPLC instrumentation, which consist of a dual solvent pump (Binary HPLC Pump 1525), thermostat (Column Heater 1500 Series), autosampler (Autosampler 2707) and PDA detector (PhotoDiode Array Detector 2998). Chromatograph was equipped with a column Luna Polar C18 100 Å, 100 × 4.6 mm, 3 μm (Phenomenex). The whole system was monitored by Empower 2 software. The column temperature was 35 °C, flow rate was set at 1 mL.min^{-1} and injection volume was 10 μL. The detection of methylxanthines was performed at 273 nm and UV scan 210 – 400 nm.

Green and black tea samples preparation

All green and black tea samples were prepared according to the preparing instructions for individual teas. Weights of both green and black tea samples, leaching time and water temperature were different depending on tea as shown in the Table 2. The samples were cooled down to room temperature. Subsequently, the samples were centrifuged for 5 minutes at 12.0 g. Supernatants were diluted as needed to keep analytes concentration within the linear range. The prepared sample infusions as well as the diluted sample infusions were analysed by HPLC-PDA using the mobile phase: 10 % acetonitrile : 90 % ultra-pure water. All samples were prepared and diluted in ultrapure water. Three samples were prepared from each type of tea. From each sample, triplicate portions were taken for analysis by HPLC.

Results and Discussion

Dependence of retention factors on mobile phase composition

The methylxanthines studied (theobromine, theophylline and caffeine) are structurally very similar, therefore it was necessary to choose the proper composition of the mobile phase for their separation. The retention factor is a qualitative

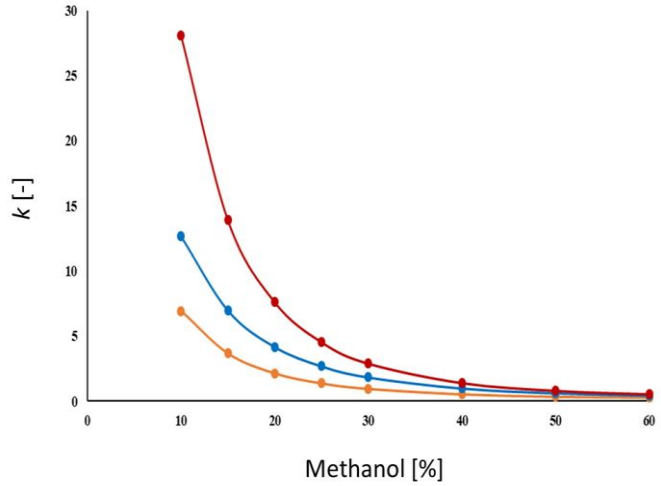


Fig. 2. Dependence of retention factors on content of methanol in the mobile phase [%] is shown for theobromine (orange line), theophylline (blue line) and caffeine (red line).

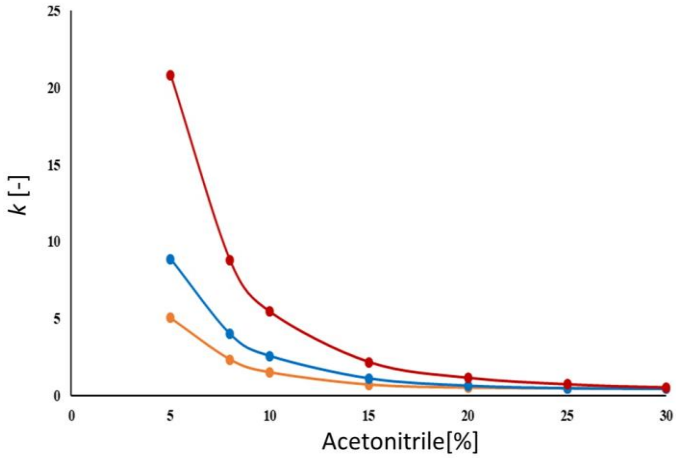


Fig. 3. Dependence of retention factor on content of acetonitrile in the mobile phase [%] is shown for theobromine (orange line), theophylline (blue line) and caffeine (red line).

parameter for characterizing the chromatographic properties of the separated substances. Retention factors of analytes were observed in two types of mobile phases: the first was water (A): methanol (B), and the second water (A) : acetonitrile (B). The dependence of analyte retention factors on the percentage of methanol in the mobile phase is presented on the Fig. 2.

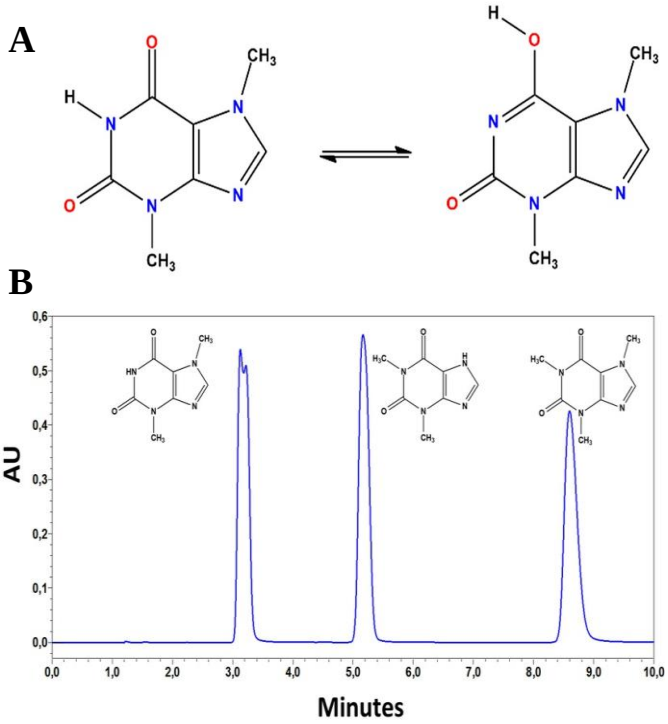


Fig. 4. Oxo-enol tautomerism of theobromine (**A**). Chromatogram of separation of standards of methylxanthines (the mobile phase 80 % water : 20 % methanol) with the split peak of theobromine (**B**).

At the first measurement, when the mobile phase contained 100 % of component B, all three methylxanthines eluted together at the same time. Gradually, we reduced the volume percentage of component B in the mobile phase to 10 %. With each reduction the time was prolonged, and at 10 % methanol, caffeine eluted ~ 30 min after the analysis started. The optimal resolution of the individual analytes was achieved when the mobile phase was 80 % water: 20 % methanol and the analysis time was acceptable (10 min). However, at this composition of mobile phase separation was not appropriate because the theobromine peak diffused. This could be caused by the oxo-enol tautomerism provided by theobromine (Fig. 3A). Chromatogram that depicts such behaviour of theobromine is shown on the Fig. 3B. Furthermore, as in the previous case, we carried out a series of measurements of the standards solutions (theobromine, theophylline, caffeine) in the acetonitrile/water mobile phase. The dependence of the retention factor on the percentage of acetonitrile in the mobile phase is shown on the Fig. 4. Since acetonitrile has a higher eluting power than methanol, the individual analysis times were shorter. We found that the retention time is the same for all components from 100 % ACN to 40 % ACN in the mobile phase. Optimal separation was achieved when the mobile phase composed 10 % of ACN, because under these conditions resolution between theobromine

Table 3. Basic analytical parameters of the HPLC method.

Analyte	Retention time [min]	Concentration range [μg.mL^{-1}]	Linear regression	R^2
Theobromine	2.59	0.506 – 8.096	y = 33915x + 4095.2	0.996
Theophylline	3.66	2.504 – 40.064	y = 32042x + 951.93	0.998
Caffeine	6.60	5.038 – 100.76	y = 29444x − 32335	0.999

and theophylline was 1.68 and resolution between theophylline and caffeine was 3.16, what we consider adequate. Total analysis time was 8 min. The developed HPLC method was validated in terms of linearity and within-day precision (repeatability). Linearity was evaluated in range of 5 – 100 μg.mL^{-1} for caffeine, 2.5 – 40 μg.mL^{-1} for theophylline and 0.5 – 8 μg.mL^{-1} for theobromine. Peak areas was used for signals evaluation. The obtained regression equations (n = 6) for all analytes as well as coefficients of determination are given in Table 3.

In order to evaluate the repeatability of the method (within-day precision) mixtures theobromine and caffeine was injected at two concentration levels for five replicates each. We determined the content of caffeine and theobromine in tea samples, thus we did not perform the method repeatability for theophylline. Results are shown in Table 4. Relative standard deviations for retention times were less than 1 %; only in a single case this value was above 1 %. Relative standard deviations for the areas of theobromine and caffeine, except of a single concentration of theobromine (1.21 μg.mL^{-1}), were up to 5 %. Same table also shows the relative standard deviations of averaged retention times and peak areas of individual analytes.

Determination of methylxanthines in selected tea samples

Prepared tea extracts were analysed by developed HPLC method and the peaks of the methylxanthines were subsequently identified by comparison of UV spectra and retention times with corresponding data of the standards. Table 5 provides an overview of the contents of theobromine and caffeine in individual bag teas. Concentrations are given corresponding to a single

Table 4. Within-day precision of developed HPLC method.

Analyte and its concentration [μg.mL^{-1}]	t_R [min]	t_R* ± SD [min]	RSD [%]	Y [μV/sec]	Y* ± SD [μV.sec^{-1}]	RSD [%]
Theobromine (1.21)	2.593	2.589±0.003	0.130	41910	45059±3852	8.549
	2.587			41531		
	2.586			48569		
	2.588			43509		
	2.593			49778		
Theobromine (2.48)	2.599	2.591±0.006	0.236	92921	88171±4275	4.848
	2.589			84447		
	2.582			85976		
	2.591			92704		
	2.592			84809		
Caffeine (8.80)	6.610	6.586±0.042	0.651	293843	291375±2163	0.742
	6.510			293068		
	6.603			291558		
	6.609			288819		
	6.600			289588		
Caffeine (45.71)	6.658	6.629±0.074	1.113	1389757	1378153±6765	0.491
	6.663			1373970		
	6.667			1374120		
	6.659			1374350		
	6.497			1378568		

t_R – retention time; SD – standard deviation; RSD – relative standard deviation; Y – peak area.

Table 5. Theobromine and caffeine contents in bag black teas.

Tea Product Name	Theobromine		Caffeine	
	c [mg/250 mL] ± SD	*c* [mg.L^{-1}] ± SD	*c* [mg/250 mL] ± SD	*c* [mg.L^{-1}] ± SD
Earl Grey	4.62±0.20	18.46±0.79	63.52±4.27	254.09±17.07
Darjeeling	2.63±0.18	10.52±0.73	62.05±1.76	248.19±7.03
Ceylon tea	4.74±0.14	18.98±0.56	56.97±2.56	227.90±10.24
English breakfast	3.34±0.24	13.36±0.98	53.13±3.55	212.51±14.21
English Tea No.1	3.12±0.37	12.49±1.50	52.68±1.15	210.72±4.58
Decaffeinated tea	3.32±0.14	13.26±0.55	2.94±0.08	11.76±0.34

cup of tea, prepared according to the instructions of the producer (mostly in mg/250 mL). As shown in Table 5. Theobromine content in all bag black teas ranges from 3 – 5 mg/250 mL, while the highest concentration was found in Ceylon tea (4.74 mg/250 mL). Caffeine content (excluding decaffeinated tea), varied in the range from 50 to 65 mg/250 mL with highest concentration in Earl Grey tea (63.52 mg/250 mL).

The second group of black teas analyzed was loose black teas. Table 6 shows a summary of their theobromine and caffeine contents. Concentrations are again presented as for single cup in mg/200 mL, for individual samples prepared according to the instructions for preparation. The highest content of both theobromine and caffeine can be found in Pu Erh Superior tea (Table 6), where the theobromine concentration was 4.92 mg/200 mL and of the caffeine it was 104.13 mg /200 mL. The lowest caffeine content (71.08 mg/200 mL) was measured in Darjeeling Gopaldhara Wonder Gold tea. The lowest theobromine content (1.51 mg/200 mL) was in Darjeeling Gopaldhara Wonder Tea Muscatel. An example of a chromatographic separation of methyl-xanthines in black tea samples is shown in Fig. 5. As it in evident from Tables 5 and 6, there is a clear difference between bag and loose tea, especially in terms of caffeine content. The visual comparison of contents of theobromine and caffeine in black teas are shown in Fig. 6, where teas are ranked by decreasing caffeine or theobromine content.

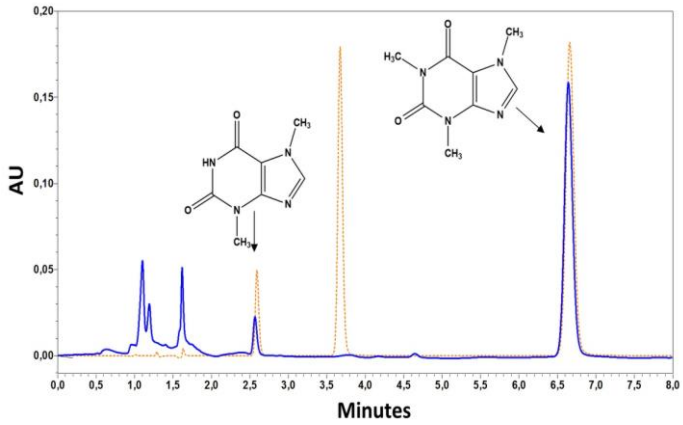


Fig. 5. Chromatographic separation of methylxanthines in black tea sample English breakfast (blue, solid line) and standards of methylxanthines (orange, dotted line).

Abbood and Aldiab (2017) analysed 3 samples of black tea using RP-HPLC/UV system, but determined only caffeine. In the black tea samples, the indicated amounts of caffeine were 16.5 mg.g^{-1}; 18.3 mg.g^{-1}; 33.4 mg.g^{-1}, which are comparable to those we found in loose black teas listed in Table 6. Similarly, the RP-HPLC/UV method was used by Bae *et al.* (2015) to determine the caffeine content of a black tea sample. The detected amount of caffeine was 20.09 mg.g^{-1}, which is also comparable with values reported in our study in Table 6 (Bae *et al.* 2015).

We divided green teas according to their character to powdered and loose green teas to powdered and loose green teas. The Table 7 shows the contents of theobromine and caffeine in powdered green tea teas. All three green tea powders contained

Table 6. Theobromine and caffeine content in loose black teas.

Name	Theobromine		Caffeine	
	c [mg/200 mL] ± SD	*c* [mg.L^{-1}] ± SD	*c* [mg/200 mL] ± SD	*c* [mg.L^{-1}] ± SD
Pu Erh Superior	4.92±0,31	24.62±1.54	104.13±1.13	520.67±5.63
Darjeeling Gopaldhara Wonder Tea Muscatel	1.51±0,26	7.54±1.32	76.87±1.39	384.33±6.96
Darjeeling Gopaldhara Wonder Gold	3.32±0,18	16.62±0.89	71.08±7.41	355.38±37.03

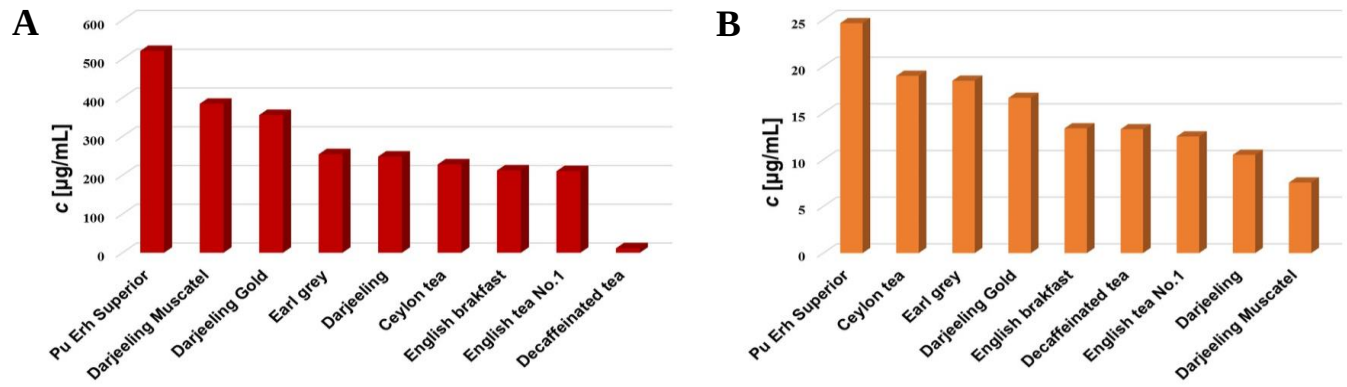


Fig. 6. Content of caffeine (**A**) and theobromine (**B**) in individual black teas.

relatively small amounts of theobromine, where the theobromine concentrations ranged from 0.20 to 0.90 mg/100 mL. The highest concentration of theobromine (0.85 mg/100 mL) was found only in GABA Midori. The highest content of caffeine (30.65 mg/100 mL) was found in Shizuoka Matcha Premium tea. The second group of green teas was loose green teas. The calculated concentrations for this group of teas is summarized in Table 7. An example of a chromatographic separation of methylxanthines in green tea sample is shown in Fig. 7. The highest concentration of theobromine was found in Lung Ching West Lake tea and it was 2.57 mg/200 mL.

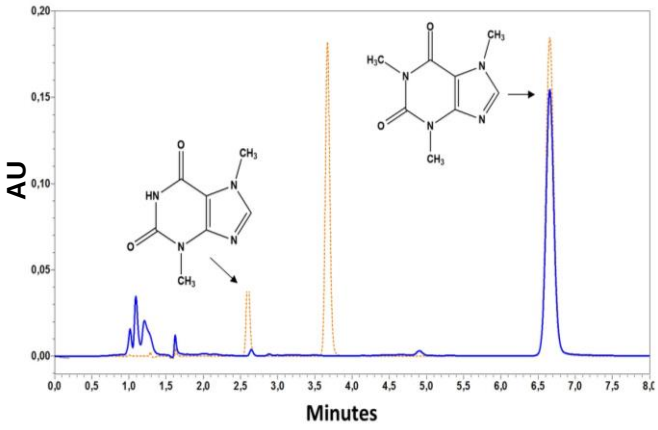


Fig. 7. Chromatographic separation of methylxanthines in green tea sample Gyokuro Shizuoka Premium (solid line) and standards of methylxanthines (dotted line).

The lowest concentration of theobromine was found in Nepal Silver Needles and it was 0.56 mg/200 mL. The highest caffeine content (96.87 mg/200 mL) was found in Gyokuro Shizuoka Premium Tea. By contrast, the lowest caffeine content (44.00 mg/200 mL) was found in Zhu Cha tea. Contents of caffeine and theobromine in green teas are shown in Fig. 8. In both, teas are ranked by decreasing content of methylxanthines. Grujić-Letić *et al.* (2016) quantified caffeine in different tea samples by HPLC/DAD. The caffeine content of samples was: 12.63 $mg.g^{-1}$ in black tea, 10.13 $mg.g^{-1}$ in green tea and 9.81$mg.g^{-1}$ in white tea. Table 7 shows that comparing to our analysis, the resulting caffeine content reported by authors is relatively low. On the other hand, caffeine contents in white tea coincide (caffeine content, which we found in the white tea sample – Nepal Silver Needles was 9.74 $mg.g^{-1}$) (Grujić-Letić *et al.* 2016). In other study, Srdjenovic *et al.* (2008) applied HPLC/UV method with detection at 273 nm to determine caffeine, theobromine and theophylline in a green tea sample; these authors reported 12.63 $mg.g^{-1}$ caffeine content, 0.32 $mg.g^{-1}$ theobromine content, and theophylline was below the limit of detection. The content of caffeine was comparable to some loose green teas (Table 7). The theobromine content reported by the authors was relatively

Table 7. Theobromine and caffeine content in powdered green teas.

	Theobromine		Caffeine	
Name	***c* [mg/100 mL] ± SD**	***c* [mg.L^{-1}] ± SD**	***c* [mg/100 mL] ± SD**	***c* [mg.L^{-1}] ± SD**
Shizuoka Matcha Premium	0.33±0.04	3.34±0.37	30.65±0.68	306.46±6.83
GABA Midori	0.85±0.28	8.45±2.79	25.87±8.55	258.65±85.53
Japan Kate-Benifuki	0.29±0.02	2.88±0.17	23.16±0.56	231.55±5.63

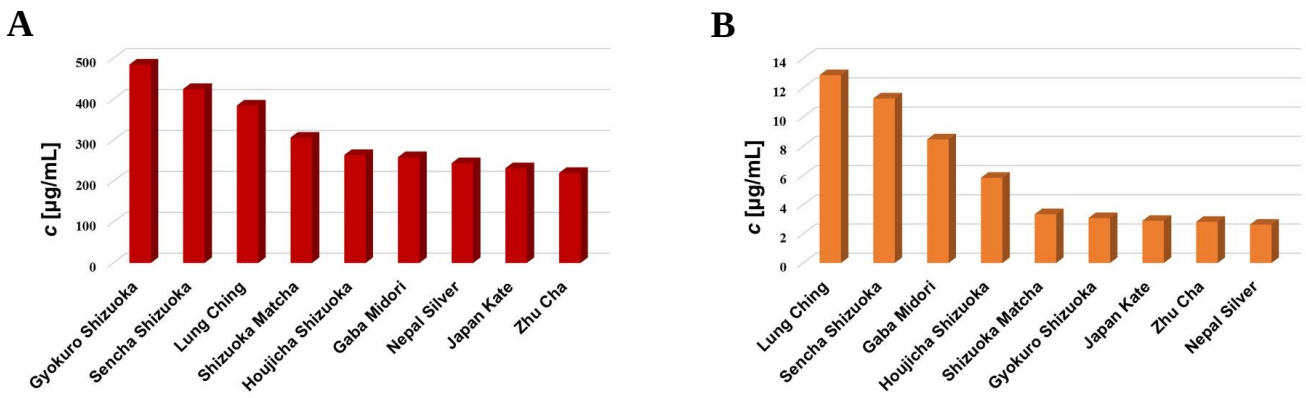


Fig. 8. Content of caffeine (**A**) and theobromine (**B**) in green teas.

low and more comparable to powdered green teas (the values in Table 7). Similarly to Srdjenovic *et al.* (2008) we failed to detect theophylline in the tested tea samples.

Reports on similar analyses of individual tea types in more detail are rather scarce in literature. The authors of various works that are available only mention whether it is black, green or white tea, in relation to given metabolites contents. Furthermore, the many of authors in their works mainly looked at the content of caffeine as a major component in various matrices. For these reasons, comparison of data on individual methylxanthines in different types of teas is difficult and is restricted to the tea group only (black, green and white).

Conclusions

This paper reports on development of HPLC method for simultaneous determination of methylxanthines in selected biological matrices. We worked only with isocratic elution, where we searched for appropriate type and composition of the mobile phase. We identified the mobile phase consisting of water : acetonitrile better than mixture of water and methanol because the obtained peaks were narrower and more symmetric.

In both types of tested teas (black and green) the presence of theobromine and caffeine was quantified. Presence of theophylline was not detected since it generally occurs in teas at very low concentrations and therefore its values were below our limit of detection. From a general comparison of theobromine content in green and black teas, we can conclude that theobromine content is higher in black teas. Concentration values of caffeine in green and black teas are overlapping. The content of theobromine and caffeine and differences between green and black teas are probably related to fermentation process apply ing to black tea preparation and on prolongation of enzymatic activity of xanthine methylases which produce caffeine form theophylline and theobromine. It means primary content will be depending on condition of fermentation process in black teas. Secondary content of methylxanthines found in tea is highly dependent on the sample preparation, especially leaching time and water temperature.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abbood A, Aldiab D (2017) HPLC determination of caffeine in some beverages and pharmaceutical dosage forms available in Syrian market. J. Chem. Pharm. Sci. 10: 1174-1179.

Bae IK, Ham HM, Jeong MH, Kim DH, Kim HJ (2015) Simultaneous determination of 15 phenolic compounds and caffeine in teas and mate using RP-HPLC/UV detection: Method development and optimization of extraction process. Food Chem. 172: 469-475.

de Mejia EG, Ramirez-mares MV (2014) Impact of caffeine and coffee on our health. Trends Endocrinol. Metab. 25: 489-492.

Gibson CM, Fowler PW (2014) Aromaticity of caffeine, xanthine and the dimethyl xanthines. Tetrahedron Lett. 55: 2078-2081.

Grujić-Letić N, Rakić B, Šefer E, Milanović M, Vujić I, Milić N (2016) Quantitative determination of caffeine in different matrices. Mac. Pharm. Bull. 62: 77-84.

Jafari MT, Rezaei B, Javaheri M (2011) A new method based on electrospray ionisation ion mobility spectrometry (ESI-IMS) for simultaneous determination of caffeine and theophylline. Food Chem. 126: 1964-1970.

Mufakkar M, Fatima K, Faheem H, Ahmad S, Khan MH (2015) Estimation of caffeine in different brands of energy drinks by Ultra-Violet spectroscopy. Int. J. Sci. Res. Pub. 5: 1-3.

Rodriguez A, Costa-Bauza A, Saez-Torres C, Rodrigo D, Grases F (2015) HPLC method for urinary theobromine determination: Effect of consumption of cocoa products on theobromine urinary excretion in children. Clin. Biochem. 48: 1138-1143.

Srdjenovic B, Djordjevic-Milic V, Grujic N, Injac R, Lepojevic Z (2008) Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks and herbal products. J. Chromatogr. Sci. 46: 144-149.

Tzanavaras DP, Zacharisa CK, Themelisa DG (2010) Rapid determination of methylxanthines in real samples by high-performance liquid chromatography using the new FastGradient® narrow-bore monolithic column. Talanta 81: 1494-1501.

Xia Z, Ni Y, Kokot S (2013) Simultaneous determination of caffeine, theophylline and theobromine in food samples by a kinetic spectrophotometric method. Food Chem. 141: 4087-4093.

Zhu B, Haghi M, Nguyen A, Goud M, Yeung S, Young PM, Traini D (2015) Delivery of theophylline as dry powder for inhalation. Asian J. Pharm. Sci. 10: 520-527.