

Comparison and Validation of Different Alternative Sample Preparation Procedures of Tea Infusions Prior to Their Multi-Element Analysis by FAAS and ICP OES

Anna Szymczycha-Madeja¹ · Maja Welna¹ · Pawel Pohl¹

Received: 25 May 2015 / Accepted: 18 September 2015 / Published online: 26 September 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract A simple, low-cost, and fully validated sample preparation procedure for the determination of 16 metals in tea infusions by flame atomic absorption spectrometry (FAAS) for Ca, Fe, K, Mg, Mn, and Na and inductively coupled plasma optical emission spectrometry (ICP OES) for Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, and Zn has been developed. Three different procedures, including the direct analysis (no pre-treatment), the acidification with HNO₃ or aqua regia, both at 0.25, 0.50, and 1.0 mol L⁻¹, have been tested. The reliability and the validity of examined procedures were verified considering significant validation parameters, i.e., the precision, the accuracy, and quantification limits (QLs). It has been evidenced that only the acidification of tea infusions with HNO₃ to 0.25 mol L⁻¹ prior to the spectrometric measurements produce dependable results, i.e., allows obtaining QLs of 9.9–108 µg L⁻¹ (FAAS) and 0.08–27 µg L⁻¹ (ICP OES), the precision within 0.2–6.9, and the accuracy ranged from -1.4 to +5.0 %. Moreover, concentrations of metals determined using this sample treatment agreed with those obtained using the hot plate wet digestion of tea infusions (the reference procedure). The chosen procedure was applied to the multi-element analysis of infusions of 20 various green and black teas commercially available in Poland. Based on results, the possibility of the classification of teas by using the principal component analysis (PCA) and the linear discriminant analysis (LDA) was investigated. Additionally, leachabilities of metals into infusions were calculated and compared.

Keywords Green and black tea · Infusions · Sample preparation · Multi-element analysis · Principal component analysis · Linear discriminant analysis

Introduction

Infusions prepared from leaves or bags of black and green made teas of the *Camellia sinensis* shrub are the most widely consumed beverages in the world (Szymczycha-Madeja et al. 2012; Welna et al. 2013). Tea can be classified into six basic categories, i.e., white, yellow, green, oolong, black, and pu-erh. Among them, green, oolong, and black teas are distinguished according to a degree of the fermentation of leaves. Leaves of green tea are dried and roasted but not fermented, whereas for black tea, leaves are additionally fermented. In case of partially fermented leaves, oolong tea is obtained. Black and oolong teas are typically produced from older leaves, while green tea is made from the flush containing only young leaves (Szymczycha-Madeja et al. 2012). When steeping tea leaves or brewing tea bags, different bioactive compounds are extracted into infusions making these beverages a valuable source of antioxidants and phytochemicals (Diniz et al. 2015; Jeszka-Skowron et al. 2015). Except for organic bioactive substances, infusions of black and green teas also contain various elements that are leached from tea leaves and enhance their pro-health activity (Jeszka-Skowron et al. 2015). In case of a regular drinking of tea, particularly in countries where it is a traditional and popular drink, tea brews are regarded as an additional source of some essential metals, e.g., Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, and Zn (Derun 2014; Jeszka-Skowron et al. 2015). Unfortunately, non-essential and toxic metals, e.g., Al, Ba, Cd, Ni, and Pb, present in made teas are extracted as well (Jeszka-Skowron et al. 2015; Ozdemir et al. 2014). The later metals could have a potentially negative

✉ Anna Szymczycha-Madeja
anna.szymczycha@pwr.wroc.pl

¹ Department of Analytical and Metallurgical Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Stanisława Wyspiańskiego 27, 50370 Wrocław, Poland

effect on the well-being of consumers of tea, particularly associated with the accumulation of these metals in the human body (Jeszka-Skowron et al. 2015; Ozdemir et al. 2014; Szymczycha-Madeja et al. 2012; Welna et al. 2013).

In this regard, studies on the multi-element analysis of infusions of teas appear to be an important part of the quality control and the safety management of the tea production. Such routine analyses can answer the question about the health risk of tea beverages (Jeszka-Skowron et al. 2015; Ozdemir et al. 2014). In addition, they can evaluate differences among black and green teas in reference to their nutritional value and the content of essential and other metals along with their contribution to recommended dietary intakes (RDIs) (Dash et al. 2008; Derun 2014; Jeszka-Skowron et al. 2015; Malik et al. 2008; Mehra and Baker 2007; Memic et al. 2014; Paz-Rodriguez et al. 2015; Ozdemir et al. 2014; Street et al. 2006; Schwalfenberg et al. 2013; Tahir Soomro et al. 2008).

The reliable assessment of the quality and the safety of tea infusions is, however, strictly dependent on accurate and precise analytical methods that enable to determine different metals. The most popular and extensively used in the analysis of tea infusions are spectrochemical methods, including flame atomic absorption spectrometry (FAAS) (Dambiec et al. 2013; Gallaher et al. 2006; Malik et al. 2008; Memic et al. 2014; Paz-Rodriguez et al. 2015; Polechonska et al. 2015; Tahir Soomro et al. 2008) and inductively coupled plasma optical emission spectrometry (ICP OES) (Altintig et al. 2014; Dash et al. 2008; Diniz et al. 2015; Derun 2014; Fernandez et al. 2002; Froes et al. 2014; Malik et al. 2008; Mehra and Baker 2007; Ozcan et al. 2008; Ozdemir et al. 2014; Salahinejad and Aflaki 2010; Street et al. 2006). In case of FAAS, it is particularly convenient in reference to selected metals that are highly abundant in tea infusions, i.e., Ca, Fe, K, Mg, Mn, and Na (Szymczycha-Madeja et al. 2012). In addition, graphite furnace atomic absorption spectrometry (GFAAS) (Jeszka-Skowron et al. 2015; Wrobel et al. 2000) and inductively coupled plasma mass spectrometry (ICP MS) (Milani et al. 2015; Schwalfenberg et al. 2013; Shen and Chen 2008; Sofuoglu and Kavcar 2008) were used. Among different sample preparation procedures reported so far for infusions of black (BTs) and green (GTs) teas before their spectrochemical analysis, it appears that no treatment and direct measurements of metals in them was preferred in case of FAAS (Dambiec et al. 2013; Malik et al. 2008; Memic et al. 2014; Polechonska et al. 2015), ICP OES (Altintig et al. 2014; Derun 2014; Diniz et al. 2015; Froes et al. 2014; Malik et al. 2008; Mehra and Baker 2007; Ozcan et al. 2008; Ozdemir et al. 2014; Street et al. 2006, and GFAAS (Jeszka-Skowron et al. 2015). In addition, infusions of teas were acidified with HNO_3 to a concentration of 0.28 mol L^{-1} (Salahinejad and Aflaki 2010) or 1.4 mol L^{-1} (Schwalfenberg et al. 2013) or HCl to a concentration of 0.12 mol L^{-1} (Paz-Rodriguez et al. 2015). Otherwise, infusions were evaporated to near dryness while

sample residues left were digested with concentrated solutions of HNO_3 and HClO_4 (Tahir Soomro et al. 2008) or HCl (Gallaher et al. 2006). The UV photolysis-assisted digestion in H_2O_2 , aimed at destructing the organic matrix of infusions of tea, was also carried out (Dash et al. 2008). Unfortunately, reported methods of the direct analysis of infusions of BTs and GTs (Altintig et al. 2014; Dambiec et al. 2013; Derun 2014; Jeszka-Skowron et al. 2015; Malik et al. 2008; Mehra and Baker 2007; Memic et al. 2014; Ozcan et al. 2008; Ozdemir et al. 2014; Street et al. 2006), or their acidification (Salahinejad and Aflaki 2010; Schwalfenberg et al. 2013) were not validated nor verified, therefore, the quality of the data achieved in above-cited works could be questioned. Although spike-and-recovery experiments were made in case of few direct analyses of infusions and quantitative recoveries were obtained, i.e., 91.9–113 % for infusions of GTs (Froes et al. 2014) and 85.2–113 % for infusions of BTs and GTs (Diniz et al. 2015), it should be noted that the recovery study is selectively used and does not have a high priority when another analytical method is available for comparison purposes. Paz-Rodriguez et al. (2015) compared concentrations of Ca, Co, Cu, Fe, Mn, Ni, Na, and Zn determined by FAAS in infusions of BTs and GTs acidified with HCl when using the standard addition method and simple standards for the calibration. By indicating the lack of systematic errors, they additionally checked that recoveries of added metals were also quantitative, i.e., 90.3–108 and 88.0–110 %, respectively, for infusions of BTs and GTs samples. Milani et al. (2015) evaluated the applicability of the acidification of tea and herbal infusions with a 0.2 % (v/v) HNO_3 for determination of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn by ICP MS. Results of this method were compared with those obtained using the microwave-assisted digestion of infusions. The accuracy and the precision of the simplified analytical method were additionally evaluated using the spike-and-recovery experiment and satisfactory results were obtained, i.e., 82 to 120 and 2 to 17 %, respectively.

The present work is a continuation of our recent study devoted to the development of alternative sample preparation procedures in the multi-element analysis of BTs and GTs by FAAS and ICP OES (Szymczycha-Madeja et al. 2015). The objective of the present contribution was to compare and verify the reliability of different simplified sample preparation procedures of infusions of BTs and GTs and particularly to verify the validity of the direct analysis of untreated infusions, commonly used but not validated in already published works. No such methodical comparison of different simplified sample preparation procedures of infusions of BTs and GTs has ever been reported before. The suitability of a selected, validated, and dependable sample preparation procedure, i.e., the acidification with HNO_3 to 0.25 mol L^{-1} , was shown by subjecting it to the multi-element analysis (16 metals, including Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, and

Zn) of infusions of 20 different bagged and leaf, black, and green teas, i.e., BTBs and BTLs, GTBs, and GTLs, marketed in Poland. The potential of the multi-element analysis and the information about concentrations of different metals in infusions of BTs and GTs was also investigated in reference to their classification by the principal component analysis (PCA) and the linear discriminant analysis (LDA). The percentage leachability of studied metals into infusions of analyzed BTs and GTs was evaluated and compared as well.

Materials and Methods

Reagents and Samples

EMSURE[®] ACS grade chemicals, i.e., concentrated HNO₃ (65 % (v/v)), HCl (36 % (v/v)) and H₂O₂ (30 % (m/v)) solutions, were supplied by Merck Millipore (Darmstadt, Germany). A concentrated solution of aqua regia was freshly prepared by mixing appropriate volumes of HNO₃ and HCl solutions at ratio 1:3. De-ionized water (18.3 MΩ cm) was obtained using an EASYpure[™] water purification system (Barnstead Thermolyne Corporation, Dubuque, IA, USA). A Merck Certipur[®] multi-elemental stock (1000 μg mL⁻¹) ICP standard solution IV was used to prepare matrix-matching standard solutions for the calibration of FAAS and ICP OES instruments.

Teas selected for the study were the finest loose leaf and bagged BTs and GTs commercially available in local markets of Wrocław (Lower Silesia Region, South-west Poland). BTs in bags (BTBs) or concurrently sold as loosely packed leaf teas (BTLs) included the following brands: BTB1 with BTL1 (Dilmah, Premium), BTB2 with BTL2 (Irving, Superior black), BTB3 with BTL3 (Twinings, English breakfast), BTB4 with BTL4 (Ahmad tea London, English breakfast), and BTB5 with BTL5 (Loyd tea, Ceylon). Corresponding bagged and loose leaf GTs (GTBs and GTLs), offered by the same producers/suppliers, were also selected for the study, i.e., GTB1 with GTL1 (Dilmah, Pure green), GTB2 with GTL2 (Irving, Pure green), GTB3 with GTL3 (Twinings, Gunpowder green), GTB4 with GTL4 (Ahmad tea London, Green tea original) and finally GTB5 with GTL5 (Loyd tea, Green tea).

Instrumentation

A single-beam flame atomic absorption spectrometer (Bodenseewerk Perkin-Elmer GmbH model 1100B, Überlingen, Germany) was used to measure concentrations of Ca, Fe, K, Mg, Mn, and Na by FAAS. Concentrations of major and minor metals were quantified in atomic absorption (Ca, Fe, Mg, Mn) and atomic emission (K, Na) modes. A bench-top optical emission spectrometer of an axially viewed

Ar-ICP (Agilent Technologies Inc., Santa Clara, CA, USA), model 720, was used to determine concentrations of trace metals, i.e., Al, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sr, and Zn by ICP OES. The most prominent atomic and ionic emission lines were selected for measurements. The information about experimental conditions of spectrometric measurements made by FAAS and ICP OES were exhaustively detailed in our recent research by Szymczycha-Madeja et al. (2015).

Tea Steeping Procedure

To prepare infusions of analyzed teas, de-ionized water was used. Steeping times and temperatures of water were used according to recommendations given by producers/suppliers for BTs and GTs. In case of the steeping of leaves of BTBs and BTLs, contents of bags (2.0 g) or portions of leaves (2.0 g) were placed in 400-mL glass beakers and poured with 220 mL of boiling (100 °C) de-ionized water. After mixing with a glass rod, infused liquids were left under the cover to steep for 5 min. Afterwards, infusions were separated from grounds by filtering them through 390-grade quantitative filter papers (Munktell & Filtrak GmbH, Bärenstein, Germany) and letting to cool down to a room temperature. In case of GTBs and GTLs, the same masses of samples (2.0 g) were taken but poured with 220 mL of hot (85 °C) de-ionized water, mixed and infused no longer than 3 min under the cover. Prior to the multi-element analysis by FAAS and ICP OES, portions (10.0 g) of resulting infusions of BTBs, BTLs, GTBs, and GTLs were placed in stoppered 10-mL PP tubes (Equimed, Wrocław, Poland).

Preparation Procedures of Tea Infusions Before Analysis

The suitability of three different alternative sample preparation procedures of tea infusions, including no treatment (P1) and the acidification with HNO₃ (P2) or with aqua regia (P3), prior to their multi-element analysis by FAAS and ICP OES was tested considering important validation parameters, i.e., the precision, the accuracy, and quantification limits (QLs) of metals. To evaluate the accuracy of results, the open-vessel wet digestion was taken as the reference sample preparation procedure (P4). These initial experiments were carried out on infusions of two different samples, i.e., BTB1 and GTB1.

In case of the procedure P1, prepared infusions (10.0 g) were directly analyzed by FAAS and ICP OES versus simple standard solutions. For the acidification of infusions prior to measurements, their 10.0 g portions, placed in 10-mL PP tubes, were acidified by adding appropriate amounts of concentrated HNO₃ (P2) or fresh aqua regia (P3) solutions. Final concentrations of HNO₃ or aqua regia in tea infusions were 0.25, 0.50, and 1.0 mol L⁻¹. Resulting sample solutions were analyzed by FAAS and ICP OES versus matrix-matching

standard solutions (containing appropriate amounts of HNO₃ or aqua regia).

Finally, for the open-vessel wet digestion of tea infusions (P4), their portions (20.0 g) were placed in 200-mL glass beakers and poured with 15 mL of a concentrated HNO₃ solution. Beakers were covered with watch glasses and sample solutions were heated on a hot plate to gently boil and reflux for 1.5 h. After reducing their volumes to less than 2 mL, 3.0 mL of a 30 % H₂O₂ solution were added and then, they were heated again almost to dryness. Resulting aliquots were quantitatively transferred to 30-mL PP screw-capped containers (Equimed, Wroclaw, Poland) and diluted with water to 25.0 g. Finally, they were analyzed by FAAS and ICP OES using matrix-matching standard solutions.

Total concentrations of studied metals in made teas were determined following the procedure described and validated in our recent study, i.e., the ultrasound-assisted extraction of tea samples in aqua regia (Szymczycha-Madeja et al. 2015). This paper also reports total concentrations of metals (in µg g⁻¹) quantified in all tea samples that are also analyzed in the present contribution, i.e., BTBs, BTLs, GTBs, and GTLs. In brief, 0.5 g samples of initially ground teas were placed in 30-mL PP centrifuge tubes and poured with 2.0 mL of fresh aqua regia. Resulting sample mixtures were immersed in an ultrasounds water bath and sonicated for 15 min at room temperature. Afterwards, they were diluted with water to 25.0 g and centrifuged at 12,000 rpm for 10 min. Supernatants were sampled using PE syringes and filtered through non-sterile 0.45-µm nylon membrane syringe filters. Filtrates were analyzed by FAAS and ICP OES against matrix-matching standard solutions.

Sample solutions resulted from all compared sample preparation procedures were prepared and analyzed in triplicate ($n=3$). For each procedure, respective blanks were also run, analyzed, and considered in final results. Concentrations of Ca, Fe, Mn, and Na (by FAAS) and Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, and Zn (by ICP OES) were determined in undiluted sample solutions. Concentrations of Ca (a single case), K, and Mg (by FAAS) were determined in appropriately diluted (from 5 to 100 times) sample solutions and analyzed against simple standard solutions. For the 7-point calibration of FAAS and ICP OES measurements, matrix-matching and simple standard solutions were used. Concentrations of studied metals in standards' solutions were in the range of 0.02 to 5 µg mL⁻¹.

Results and Discussion

Comparison of Alternative Sample Preparation Procedures

The suitability of alternative to the hot plate wet digestion treatments of infusions of BTs and GTs, i.e., procedures P1,

P2, and P3, was evaluated by comparing standard deviations (SDs) of measurement series ($n=3$) and means of concentrations of studied metals obtained with these procedures to SDs and means of concentrations of metals obtained using the open-vessel wet digestion procedure (P4). The latter procedure was treated as the reference giving dependable results, however, in a longer time (Szymczycha-Madeja et al. 2012; Welna et al. 2013). The comparison of SDs achieved using procedures P1, P2, and P3 and the reference procedure (P4) indicated differences in the precision of results. The statistical significance of these differences was tested using the Fisher-Snedecor F -test with the critical value ($F_{critical}$) of 19.0 ($p=0.05$) (Konieczka and Namiesnik 2009). When calculated values of the F -test ($F_{calculated}$) were lower than the $F_{critical}$ ($F_{calculated} < F_{critical}$), this indicated that SDs of results for compared procedures did not differ in a significant manner and that the precision of these results was at the same level. In this particular case, means of concentrations of studied metals were compared using the Student's t -test with the critical value ($t_{critical}$) of 2.776 ($p=0.05$) (Konieczka and Namiesnik 2009). Otherwise, when the precision of results achieved for compared sample preparation procedures statistically differed, the Cochran-Cox C -test was used with the critical value ($C_{critical}$) of 4.303 ($p=0.05$) (Konieczka and Namiesnik 2009).

Finally, when calculated values of both latter significance tests for means were lower than respective critical values, i.e., $t_{calculated} < t_{critical}$ and $C_{calculated} < C_{critical}$; this meant that results (means of concentrations) obtained with compared procedures (P1, P2, and P3) and those obtained using the reference procedure (P4) did not differ in a statistically significant manner.

Precision

Values of the $F_{calculated}$ are given in Table 1. As can be seen, in majority cases, they are lower than the $F_{critical}$, indicating that differences between SDs of results were insignificant. There are few exceptions observed for both analyzed samples. In case of the analysis of infusions of BTB1, it included Ca (P2 with 0.25 and 0.50 mol L⁻¹ HNO₃, P3 with 1.0 mol L⁻¹ aqua regia), Fe (P2 with 0.25 mol L⁻¹ HNO₃), Mg (P1, P2 with 0.50 and 1.0 mol L⁻¹ HNO₃, P3 with 0.25, 0.50, and 1.0 mol L⁻¹ aqua regia), Mn (P2 with 0.50 and 1.0 mol L⁻¹ aqua regia), Sr (P3 with 1.0 mol L⁻¹ aqua regia), and Zn (P1, P3 with 1.0 mol L⁻¹ aqua regia). In case of infusions of GTB1, the number of differences in the precision of results was lower, likely because infusions of GT had a relatively simpler matrix. Cases for which values of the $F_{calculated}$ were higher than the $F_{critical}$ were found for Ba (P2 with 0.50 and 1.0 mol L⁻¹ HNO₃, P3 with 0.25, 0.50, and 1.0 mol L⁻¹ aqua regia), Ca (P3 with 0.25 mol L⁻¹ aqua regia), Cu (P1, P3 with 0.25 and 1.0 mol L⁻¹ aqua regia), Mn (P2 with 0.50 mol L⁻¹ HNO₃), and Zn (P3 with 0.25 mol L⁻¹ aqua regia). Concentrations of Cd, Co, and Pb were established below their respective QLs.

Table 1 Calculated values of the F -test ($F_{\text{calculated}}$) and t -test ($|t_{\text{calculated}}|$) for the comparison of standard deviations of means and concentration means of metals determined in infusions of black (BTB1) and green (GTB1) teas by FAAS (Ca, Fe, K, Mg, Mn, Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn) using no treatment (the procedure P1); the

acidification with HNO_3 at 0.25, 0.50, and 1.0 mol L^{-1} (the procedure P2); and the acidification with aqua regia at 0.25, 0.50, and 1.0 mol L^{-1} (the procedure P3) versus the hot plate wet digestion in a mixture of concentrated HNO_3 and 30 % H_2O_2 solutions (the reference procedure P4)

Metal	$F_{\text{calculated}}$						$ t_{\text{calculated}} $							
	P1		P2		P3		P1		P2		P3			
			0.25	0.50	1.0	0.25	0.50	1.0	0.25	0.50	1.0	0.25	0.50	1.0
Infusion of BTB1														
Al	2.25	1.00	11.11	9.00	5.44	1.78	1.00	29.303	1.633	2.323	2.739	4.321	8.314	8.981
Ba	9.00	4.00	16.00	4.00	9.00	1.00	16.00	10.407	0.775	8.402	5.422	0.548	18.371	4.621
Ca	1.00	20.25	36.00	6.25	4.00	4.00	25.00	13.472	3.528 ^a	0.232 ^a	0.643	1.936	14.717	6.656 ^a
Cr	–	12.25	2.47	4.00	4.00	6.61	5.90	–	1.190	4.517	1.217	1.771	3.677	1.225
Cu	7.84	1.21	3.61	1.21	6.25	1.21	2.56	2.447	0.117	10.003	1.165	1.769	5.243	2.387
Fe	3.06	26.45	1.27	1.06	2.94	1.92	1.06	0.740	0.193 ^a	0.467	0.138	1.205	1.014	0.517
K	1.10	1.78	2.25	4.00	4.00	4.00	1.00	13.140	1.039	0.240	0.775	1.936	3.098	1.531
Mg	64.00	12.25	30.25	30.25	20.25	30.25	30.25	20.611 ^a	0.476	2.150 ^a	3.795 ^a	0.307 ^a	1.771 ^a	3.415 ^a
Mn	16.00	9.00	25.00	36.00	1.00	4.00	16.00	0.420	0.000	1.109 ^a	0.465 ^a	1.225	2.324	1.680
Na	6.25	2.25	1.00	2.25	1.00	4.00	2.25	5.468	2.402	1.225	1.441	1.225	1.549	9.608
Ni	2.42	1.65	2.09	1.78	2.78	4.00	5.44	4.267	0.456	5.149	2.540	4.951	11.705	6.216
Sr	2.94	2.51	1.78	1.19	16.67	5.76	35.01	19.325	0.231	10.161	2.128	4.086	6.395	3.064 ^a
Zn	342.25	4.00	9.00	18.06	16.00	7.56	56.25	0.668 ^a	1.743	4.382	3.769	15.543	6.363	1.215 ^a
Infusion of GTB1														
Al	2.04	2.78	4.00	6.25	6.25	1.23	2.04	6.385	0.297	0.310	1.608	1.608	0.515	1.277
Ba	16.00	16.00	100.00	169.00	36.00	25.00	100.00	7.562	0.420	0.985 ^a	2.495 ^a	1.395 ^a	2.219 ^a	8.443 ^a
Ca	7.56	3.36	1.74	5.17	30.25	1.40	4.84	8.509	0.484	2.189	3.805	0.416 ^a	1.424	12.184
Cr	–	1.56	4.00	2.25	1.00	1.00	3.61	–	0.271	1.782	1.922	1.592	0.980	1.210
Cu	100.00	16.00	4.00	16.00	81.00	16.00	121.00	4.503 ^a	1.260	82.882	23.945	2.967 ^a	3.781	2.305 ^a
Fe	2.34	1.51	2.64	1.41	4.00	1.41	1.94	1.509	1.428	0.567	1.534	0.629	0.174	2.154
K	1.49	3.64	3.64	13.22	4.84	1.21	1.00	30.467	1.461	2.192	4.175	0.000	4.660	2.227
Mg	4.00	5.06	1.78	3.06	5.06	1.78	3.06	2.324	0.176	2.771	4.082	0.528	2.078	3.437
Mn	16.00	4.00	64.00	1.31	7.11	1.31	4.00	1.680	0.581	0.877 ^a	1.629	0.405	1.629	1.549
Na	4.00	4.00	4.00	2.25	4.00	1.00	1.00	4.648	2.324	0.775	2.882	3.486	5.511	11.635
Ni	1.96	1.96	1.62	5.44	3.06	3.06	3.06	8.356	0.201	3.210	0.569	5.049	0.000	7.949
Sr	1.44	2.04	5.29	8.41	6.25	2.56	3.61	1.885	0.426	5.387	6.267	0.482	1.836	0.484
Zn	1.62	2.42	1.15	1.96	49.00	4.00	7.84	7.199	0.312	1.266	0.705	2.800 ^a	2.102	0.816

Not calculated for Cd, Co, and Pb due to concentrations of these metals below their respective LQs in infusions of BTB1 ad GTB1. The critical value of the F -test (F_{critical}) is 19.00 ($p=0.05$). The critical value of the t -test (t_{critical}) is 2.776 ($p=0.05$). Statistically significant differences are italicized

^a The C -test was used with the critical value (C_{critical}) of 4.303 ($p=0.05$)

Considering relative standard deviations (RSDs), the precision of results evaluated using alternative sample preparation procedures (P1, P2, P3) and the reference procedure (P4) for infusions of both types of teas are given in Table 2. In case of the hot plate wet digestion (P4), RSDs of mean concentrations of metals established for both tea infusions were within 0.2–6.9 %. The pooled RSD, reflecting the overall precision, was calculated from all results and equaled to 2.8 %. RSDs intervals and pooled RSDs (given in brackets) established for alternative procedures were as follows: 0.6–9.8 % (4.1 %) for

no treatment (P1), 0.7–5.6 % (2.2 %) for the acidification with 0.25 mol L^{-1} HNO_3 (P2), 0.4–12 % (4.4 %) for the acidification with 0.50 mol L^{-1} HNO_3 (P2), 0.7–9.1 % (4.0 %) for the acidification with 1.0 mol L^{-1} HNO_3 , 0.1–8.0 % (3.0 %) for the acidification with 0.25 mol L^{-1} aqua regia (P3), 0.6–9.0 % (3.2 %) for the acidification with 0.50 mol L^{-1} aqua regia (P3), and 0.7–15 % (4.9 %) for the acidification with 1.0 mol L^{-1} aqua regia (P3). Considering these values, it appears that among compared alternative sample preparation procedures, the best precision for all studied metals was achieved when

Table 2 Concentrations ($\mu\text{g mL}^{-1}$) of metals determined by FAAS (Ca, Fe, K, Mg, Mn, Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, and Zn) in infusions of black (BTB1) and green (GTB1) teas prepared before the analysis by no treatment (the procedure P1); the acidification with HNO_3 at 0.25, 0.50, and 1.0 mol L^{-1} HNO_3 (the procedure P2); the acidification with aqua regia at 0.25, 0.50, and 1.0 mol L^{-1} (the procedure P3); and the wet digestion in a mixture of concentrated HNO_3 and 30 % H_2O_2 solutions (the reference procedure P4)

Metal	Concentration, $\mu\text{g mL}^{-1}$							
	P1	P2			P3			P4
		0.25	0.50	1.0	0.25	0.50	1.0	
Infusion of BTB1								
Al	3.36 (0.60)	3.93 (0.76)	3.83 (2.6)	3.82 (2.4)	3.78 (1.8)	3.73 (1.1)	3.75 (0.80)	3.97 (0.76)
Ba/ 10^3	19.8 (1.5)	17.8 (1.1)	15.9 (2.5)	17.2 (1.2)	17.8 (1.7)	16.4 (0.61)	19.0 (2.1)	17.9 (0.56)
Ca	3.09 (0.65)	3.08 (2.9)	3.29 (3.6)	3.33 (1.5)	3.36 (1.2)	3.69 (1.1)	3.79 (2.6)	3.31 (0.60)
Cd/ 10^3	<4.3 ^a	<0.71 ^a	<1.0 ^a	<1.0 ^a	<1.6 ^a	<1.7 ^a	<2.5 ^a	<1.1 ^a
Co/ 10^3	<4.8 ^a	<1.6 ^a	<2.7 ^a	<5.5 ^a	<4.8 ^a	<4.8 ^a	<3.3 ^a	<4.2 ^a
Cr/ 10^3	<3.4 ^a	1.53 (1.3)	1.24 (8.9)	1.69 (8.3)	1.74 (8.0)	1.99 (9.0)	1.45 (12)	1.58 (4.4)
Cu/ 10^3	50.8 (5.5)	55.1 (2.0)	42.6 (4.5)	56.0 (2.0)	53.9 (0.74)	50.5 (2.2)	57.6 (2.8)	55.0 (1.8)
Fe/ 10^3	64.5 (9.8)	61.9 (1.1)	60.1 (5.3)	61.8 (5.7)	58.5 (3.6)	58.8 (4.4)	62.9 (5.6)	61.4 (5.9)
K	97.0 (4.3)	138 (2.2)	142 (4.2)	143 (1.4)	136 (1.5)	133 (1.5)	136 (2.9)	141 (2.8)
Mg	4.73 (3.4)	7.06 (0.99)	7.25 (1.5)	7.38 (1.5)	7.10 (1.3)	7.22 (1.5)	7.35 (1.5)	7.08 (0.28)
Mn	1.13 (3.5)	1.14 (2.6)	1.10 (4.5)	1.12 (5.4)	1.13 (0.88)	1.11 (1.8)	1.18 (3.4)	1.14 (0.88)
Na/ 10^3	207 (2.4)	185 (1.6)	188 (1.1)	193 (1.6)	192 (1.0)	194 (2.1)	210 (1.4)	190 (1.0)
Ni/ 10^3	17.5 (8.0)	21.9 (3.2)	26.3 (4.9)	19.4 (6.2)	26.6 (5.6)	35.2 (5.1)	29.8 (7.0)	21.6 (4.2)
Pb/ 10^3	<27 ^a	<12 ^a	<28 ^a	<14 ^a	<18 ^a	<22 ^a	<34 ^a	<14 ^a
Sr/ 10^3	5.07 (1.4)	6.65 (2.8)	5.74 (1.6)	6.42 (1.7)	7.81 (6.3)	6.14 (0.81)	8.18 (8.7)	6.62 (1.8)
Zn/ 10^3	94.9 (7.8)	97.5 (0.82)	95.2 (1.3)	94.6 (1.8)	94.7 (0.11)	94.1 (1.2)	101 (3.0)	98.4 (0.41)
Infusion of GTB1								
Al	4.67 (1.5)	5.10 (1.2)	5.10 (0.98)	5.02 (0.80)	5.02 (0.80)	5.16 (1.7)	5.03 (1.4)	5.12 (2.0)
Ba/ 10^3	25.0 (1.6)	26.7 (1.5)	27.5 (3.6)	29.1 (4.5)	27.4 (2.2)	26.0 (1.9)	32.8 (3.0)	26.8 (0.37)
Ca	0.795 (1.0)	0.903 (1.3)	0.956 (3.0)	1.03 (4.8)	0.904 (0.44)	0.938 (2.8)	1.08 (0.93)	0.910 (2.4)
Cd/ 10^3	<4.3 ^a	<0.71 ^a	<1.0 ^a	<1.0 ^a	<1.6 ^a	<1.7 ^a	<2.5 ^a	<1.1 ^a
Co/ 10^3	<4.8 ^a	<1.6 ^a	<2.7 ^a	<5.5 ^a	<4.8 ^a	<4.8 ^a	<3.3 ^a	<4.2 ^a
Cr/ 10^3	<3.4 ^a	1.42 (5.6)	1.67 (12)	1.64 (9.1)	1.57 (6.4)	1.52 (6.6)	1.29 (15)	1.44 (6.9)
Cu/ 10^3	51.7 (1.9)	54.6 (0.73)	44.2 (0.45)	60.6 (0.66)	56.8 (1.6)	55.8 (0.80)	56.7 (1.9)	54.9 (0.18)
Fe/ 10^3	67.2 (7.3)	58.7 (4.4)	60.1 (8.7)	66.5 (5.7)	60.8 (2.6)	61.6 (6.2)	67.0 (3.4)	62.1 (5.2)
K	71.0 (1.3)	98.0 (2.1)	99.0 (2.1)	106 (4.0)	96.0 (0.52)	100 (1.0)	98.0 (1.1)	96.0 (1.1)
Mg	4.68 (1.7)	4.79 (1.9)	4.88 (0.62)	4.99 (1.4)	4.77 (1.9)	4.86 (0.62)	4.96 (1.4)	4.86 (0.83)
Mn	2.60 (0.77)	2.65 (1.5)	2.73 (0.37)	2.78 (2.5)	2.70 (1.1)	2.78 (2.5)	2.76 (1.4)	2.68 (3.0)
Na/ 10^3	118 (3.4)	127 (0.79)	129 (0.78)	136 (2.2)	139 (2.9)	139 (1.4)	149 (1.3)	130 (1.5)
Ni/ 10^3	26.9 (3.7)	35.0 (2.8)	31.9 (3.4)	34.7 (1.7)	39.9 (2.0)	35.2 (2.3)	42.6 (1.9)	35.2 (4.0)
Pb/ 10^3	<27 ^a	<12 ^a	<28 ^a	<14 ^a	<18 ^a	<22 ^a	<34 ^a	<14 ^a
Sr/ 10^3	4.79 (2.5)	4.93 (1.4)	5.74 (4.0)	6.07 (4.8)	4.99 (0.80)	4.76 (3.4)	4.90 (3.9)	4.96 (2.0)
Zn/ 10^3	68.5 (1.6)	75.6 (1.2)	74.4 (2.0)	75.2 (1.3)	73.1 (0.27)	74.0 (0.95)	75.2 (0.66)	75.9 (1.8)

Means ($n=3$) with relative standard deviations (RSDs) in brackets

^a Below the quantification limit (QL)

using the acidification with HNO_3 at the lowest concentration, i.e., 0.25 mol L^{-1} . The precision of results obtained using other sample preparation procedures was worse since ranges of RSDs were broadened and values of pooled RSDs were higher.

Accuracy

Means of concentrations of studied metals in infusions of BTB1 and GTB1, determined in sample solutions prepared using different procedures, are given in Table 2. Values of

the $t_{\text{calculated}}$ (in case of $F_{\text{calculated}} < F_{\text{critical}}$) and the $C_{\text{calculated}}$ (in case of $F_{\text{calculated}} > F_{\text{critical}}$) are given in Table 1. These tests were used to verify the significance of differences between mean concentrations of metals determined in differentially prepared infusions of teas.

According to both significance tests of means, it appears that only the procedure P2, based on the acidification of infusions of BTB1 and GTB1 with $0.25 \text{ mol L}^{-1} \text{ HNO}_3$, gave results that were statistically identical with those obtained using the procedure P4. Unfortunately, the direct analysis of infusions of both teas was useless since concentrations of 8 (Al, Ba, Ca, K, Mg, Na, Ni, and Sr in case of BTB1) and 9 (Al, Ba, Ca, Cu, K, Na, Ni, Sr, and Zn in case of GTB1) metals were biased from those evaluated when analyzing wet digested infusions of teas. The acidification with HNO_3 to higher concentrations (0.50 and 1.0 mol L^{-1}) resulted in a lower accuracy for several metals, i.e., from 2 (the case of Ba and Zn in BTB1 and the acidification with $1.0 \text{ mol L}^{-1} \text{ HNO}_3$) to 6 (the case of Ba, Cr, Cu, Ni, Sr, and Zn in BTB1 and the acidification with $0.50 \text{ mol L}^{-1} \text{ HNO}_3$ or Ca, Cu, K, Mg, Na, and Sr in GTB1 and the acidification with $1.0 \text{ mol L}^{-1} \text{ HNO}_3$). The acidification of infusions of teas with aqua regia at 0.25 mol L^{-1} neither provided satisfying results for all studied metals because statistically significant differences between mean concentrations was established for 2 (GTB1) and 4 (BTB1) metals. The use of higher concentrations of aqua regia resulted in obtaining even worse outcomes.

Because the acidification of infusions of both types of tea with HNO_3 at 0.25 mol L^{-1} was found to produce dependable results, the recovery test for infusions of BTB1 and GTB1 was additionally carried out. Infusions of both teas were spiked with known concentrations of studied metals, i.e., 0.10 , 0.20 , and $0.50 \mu\text{g mL}^{-1}$, acidified with HNO_3 to 0.25 mol L^{-1} and subjected to the analysis by FAAS and ICP OES. Because concentrations of Ca, K, and Mg in infusions of BTB1 and GTB1 were much higher than concentrations of other metals, and hence infusions required to be diluted from 10 to 100 times, the recovery of these metals were not examined. Recoveries of added metals were assessed by measuring spiked and unspiked sample solutions. Values established for BTB1 were in the following ranges (for 3 different levels of fortification): 100 – 103 % for Al, 99.5 – 101 % for Ba, 101 – 102 % for Cd, 98.6 – 100 % for Co, 103 – 104 % for Cr, 99.1 – 101 % for Cu, 101 – 105 % for Fe, 99.1 – 99.3 % for Mn, 99.1 – 101 % for Na, 103 – 104 % for Ni, 101 – 103 % for Pb, 99.2 – 100 % for Sr and 100 – 103 % for Zn. Similar quantitative recoveries were also achieved for infusions of GTB1, i.e., 99.0 – 100 % for Al, 99.2 – 101 % for Ba, 100 – 102 % for Cd, 100 – 101 % for Co, 99.0 – 101 % for Cr, 99.9 – 101 % for Cu, 98.9 – 102 % for Fe, 99.4 – 100 % for Mn, 99.1 – 101 % for Na, 100 – 101 % for Ni, 101 – 102 % for Pb, 101 – 102 % for Sr and 99.7 – 102 % for Zn. All these results additionally proved that the acidification of infusions of teas with HNO_3 to

0.25 mol L^{-1} led to precise and accurate results of their multi-element analysis by FAAS and ICP OES.

Quantification Limits

Using the acidification of infusions of BTB1 and GTB1 with HNO_3 to 0.25 mol L^{-1} , QLs were evaluated with FAAS (Ca, Fe, K, Mg, Mn, and Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, P, Sr, and Zn) as $10 \times \text{SD}_{\text{blank}}$, where the SD_{blank} is the SD of a blank adequate for a given procedure. As can be seen from Table 3, QLs assessed for the procedure P2 with $0.25 \text{ mol L}^{-1} \text{ HNO}_3$ give in the majority cases the lowest values among all procedures, particularly as compared to those assessed for the procedure P4 (18 – 196 ng mL^{-1} for FAAS and 0.14 – 14 ng mL^{-1} for ICP OES). Accordingly, QLs assessed using the selected procedure P2 were in the range of 9.9 – 108 ng mL^{-1} for FAAS and 0.08 – 27 ng mL^{-1} for ICP OES.

Analytical Application

Considering validation parameters assessed for FAAS and ICP OES combined with examined sample preparation procedures, it appears that reliable results for studied metals were obtained only when infusions of teas were acidified with HNO_3 to a concentration of 0.25 mol L^{-1} . The use of other procedures, particularly the direct analysis of infusions with their no previous treatment, was found useless. Surprisingly, there are many contributions in the literature, in which the direct analysis of infusions of BTs and GTs was used (Altintig et al. 2014; Dambiec et al. 2013; Derun 2014; Diniz et al. 2015; Froes et al. 2014; Jeszka-Skowron et al. 2015; Malik et al. 2008; Mehra and Baker 2007; Memic et al. 2014; Ozcan et al. 2008; Ozdemir et al. 2014; Street et al. 2006). Unfortunately, in the overwhelming majority of cited papers (with exception for Diniz et al. 2015; Froes et al. 2014), no validation of sample preparation procedures used and results achieved was undertaken. Even when the accuracy of results was checked by the spiking experiment and recoveries obtained were quantitative (Diniz et al. 2015; Froes et al. 2014), this could not be a sufficient condition for obtaining accurate results. As a proof, in the present work, the recovery study at three different levels of fortification (0.10 , 0.20 , and $0.50 \mu\text{g mL}^{-1}$) was carried out for the direct analysis of infusions of BTB1 and GTB1 (the procedure P1). Recoveries determined for studied metals were quantitative, i.e., 104 – 107 % for Al, 103 – 114 % for Ba, 102 – 108 % for Cd, 103 – 107 % for Co, 103 – 105 % for Cr, 91.4 – 106 % for Cu, 87.4 – 109 % for Fe, 100 – 103 % for Mn, 93.3 – 106 % for Na, 102 – 106 % for Ni, 104 – 110 % for Pb, 102 – 107 % for Sr and 87.9 – 104 % for Zn. Nevertheless, as shown by the comparison of results obtained for the direct analysis (the procedure P1) and the reference procedure (P4), concentrations of many metals

Table 3 Quantification limits (ng mL⁻¹) of metals for FAAS (Ca, Fe, K, Mg, Mn, Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn) combined with different sample preparation procedures, i.e., no treatment (the procedure P1); the acidification with HNO₃ at 0.25, 0.50, and1.0 mol L⁻¹ (the procedure P2); the acidification with aqua regia at 0.25, 0.50, and 1.0 mol L⁻¹ (the procedure P3); and the wet digestion in HNO₃ with 30 % H₂O₂ solutions (P4)

Metal	Quantification limit, ng mL ⁻¹							
	P1	P2			P3			P4
		0.25 mol L ⁻¹	0.50 mol L ⁻¹	1.0 mol L ⁻¹	0.25 mol L ⁻¹	0.50 mol L ⁻¹	1.0 mol L ⁻¹	
Al	10	6.2	16	15	15	6.2	28	7.0
Ba	0.57	0.17	0.19	0.48	0.49	0.22	1.7	1.2
Ca	60	66	81	90	63	78	99	126
Cd	4.3	0.71	1.0	1.0	1.6	1.7	2.5	1.1
Co	4.8	1.6	2.7	5.5	4.8	4.8	3.3	4.2
Cr	3.4	1.1	4.2	3.2	1.5	3.6	6.7	3.8
Cu	4.2	2.6	4.1	2.8	4.2	4.6	4.7	3.1
Fe	108	108	120	129	96	117	135	195
K	13	13	16	15	12	12	14	25
Mg	10	9.9	10	12	11	10	14	22
Mn	22	21	30	36	25	30	45	36
Na	9.9	10	11	13	12	13	15	18
Ni	14	2.6	8.3	7.6	6.3	7.4	7.6	2.5
Pb	27	12	28	14	18	22	34	14
Sr	0.12	0.075	0.26	0.28	0.19	0.54	0.62	0.14
Zn	4.8	0.70	1.6	1.5	2.4	3.8	5.0	1.6

determined in untreated infusions were biased from those evaluated for digested infusions. This points out that a complex matrix of both types of infusions could be a serious source of systematic errors.

The validated sample preparation procedure prior to the multi-element analysis of infusions of BTs and GTs with the use of FAAS and ICP OES proposed in the present work is certainly a very attractive alternative to procedures based on high-temperature evaporations of infusions and wet ashings of residues in concentrated reagents (Gallaher et al. 2006; Salahinejad and Aflaki 2010; Tahir Soomro et al. 2008) or invalidated measurements of untreated infusions of BTs and GTs (Altintig et al. 2014; Dambiec et al. 2013; Derun 2014; Froes et al. 2014; Malik et al. 2008; Mehra and Baker 2007; Memic et al. 2014; Ozcan et al. 2008; Schwalfenberg et al. 2013; Street et al. 2006) or acidified (Salahinejad and Aflaki 2010). Considering a possibility of the analysis of a great number of infusions of BTs and GTs in a simple way, the described green analytical sample treatment prior to measurements of concentrations of different metals by FAAS and ICP OES seems to be particularly important for multi-element screening studies and the quality control monitoring of teas as served.

This validated procedure was applied for the multi-element analysis of infusions of different BTs (10) and GTs (10). Results (mean values along with SDs, $n=3$) of this analysis

are given in Table 4. In addition, means within both groups of teas (BTs and GTs) along with respective coefficients of variance (CVs) are given. Except for Cr, Mg, and Na, it was found that mean concentrations of studied metals in infusions of BTs were lower by about 10–20 % (Fe, K, Zn), 20–40 % (Al, Ba, Cu, Sr), or even more, i.e., 80 and 120 % in case of Mn and Ca, respectively, than mean concentrations of these metals evaluated for GTs. This was likely attributed to a stronger binding of metal ions by constituents of the matrix of BTs than this of GTs (Paz-Rodriguez et al. 2015). Mean concentrations of Cr, Mg, and Na in infusions of BTs were higher by about 10–20 % than those found in infusions of GTs. Concentrations of Cd, Co, and Pb in BTs and GTs were below their QLS, i.e., 0.71, 1.6, and 12 ng mL⁻¹, respectively. Descending orders of mean concentrations of studied metals determined in infusions of BTs and GTs were quite similar, i.e., K>Mg>Al>Ca>Mn>>Na>Zn>Fe>Cu>Ni>Ba>Sr>Cr (BTs) and K>Mg>Ca>Al>Mn>>Na>Zn>Cu>Fe>Ni>Ba>Sr>Cr (GTs) and followed general tendencies reported by other authors (Derun 2014; Malik et al. 2008; Mehra and Baker 2007; Memic et al. 2014; Ozcan et al. 2008; Schwalfenberg et al. 2013; Tahir Soomro et al. 2008). In addition, concentrations of studied metals found in the present contribution for infusions of BTs and GTs were within concentration ranges given in original works cited above. Here and there, K, Mg, Ca, Al, and Mn were major metals of

Table 4 Concentrations ($\mu\text{g mL}^{-1}$) of metals determined in infusions of black (BTs) and green (GTs) teas by using FAAS (Ca, Fe, K, Mg, Mn, Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn) combined with the previous acidification of infusions with HNO_3 to 0.25 mol L^{-1}

Metal	Concentration, $\mu\text{g mL}^{-1}$										
	Infusions of BTs										
	BTB1	BTB2	BTB3	BTB4	BTB5	BTL1	BTL2	BTL3	BTL4	BTL5	Mean ^a (CV, %)
Al	3.93 (0.76)	3.30 (1.2)	3.94 (1.0)	2.22 (1.4)	1.67 (0.60)	1.75 (0.57)	1.32 (1.5)	3.03 (0.66)	1.46 (1.4)	1.97 (1.5)	2.46 (40.9)
Ba/ 10^3	17.8 (1.1)	49.0 (0.41)	27.1 (1.1)	11.0 (1.8)	7.28 (1.2)	3.92 (3.3)	5.45 (1.8)	6.25 (0.80)	3.38 (0.89)	3.80 (4.2)	13.5 (108)
Ca	3.08 (2.9)	2.20 (1.6)	2.74 (1.3)	1.62 (1.2)	1.67 (0.34)	1.28 (0.93)	1.60 (1.7)	2.98 (2.5)	1.75 (1.5)	1.66 (1.6)	1.96 (28.7)
Cd/ 10^3	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	–
Co/ 10^3	<1.6 ^b	<1.6 ^b	1.79 (3.9)	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	2.07 (2.4)	–
Cr/ 10^3	1.53 (1.3)	5.42 (0.74)	6.18 (2.9)	1.18 (4.2)	2.52 (3.6)	<1.1 ^b	1.79 (2.8)	1.52 (3.9)	2.02 (2.5)	1.43 (4.2)	2.47 (73.4)
Cu/ 10^3	55.1 (2.0)	41.3 (3.6)	55.5 (0.90)	47.7 (1.2)	34.6 (4.0)	36.8 (3.3)	41.2 (2.7)	69.6 (1.1)	28.4 (2.8)	29.1 (1.0)	43.9 (29.8)
Fe/ 10^3	61.9 (1.1)	29.7 (4.9)	77.8 (5.6)	39.4 (5.5)	37.9 (3.8)	46.6 (1.6)	33.3 (6.5)	39.6 (4.0)	53.8 (1.3)	43.0 (3.4)	46.3 (31.5)
K	138 (2.2)	77.5 (1.8)	109 (2.5)	61.4 (1.3)	60.0 (1.2)	102 (1.2)	118 (0.91)	116 (0.64)	111 (1.3)	108 (0.67)	100 (25.6)
Mg	7.06 (0.99)	6.66 (2.0)	9.14 (1.1)	4.09 (0.61)	5.33 (2.2)	4.30 (0.87)	6.59 (2.6)	7.04 (2.3)	7.44 (2.0)	5.48 (1.3)	6.31 (24.3)
Mn	1.14 (2.6)	1.23 (0.77)	2.04 (0.51)	0.918 (0.76)	1.24 (0.46)	0.611 (0.95)	1.00 (1.3)	0.339 (1.6)	1.22 (0.64)	0.748 (0.34)	1.05 (43.8)
Na/ 10^3	185 (1.6)	160 (0.71)	535 (0.74)	224 (0.85)	420 (1.8)	730 (0.37)	140 (2.8)	542 (1.0)	136 (1.2)	900 (0.41)	397 (68.7)
Ni/ 10^3	21.9 (3.2)	27.2 (4.8)	37.6 (0.80)	29.9 (1.3)	27.2 (0.74)	23.4 (0.86)	30.5 (3.0)	27.4 (2.2)	24.6 (4.1)	21.1 (3.3)	27.1 (18.0)
Pb/ 10^3	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	–
Sr/ 10^3	6.65 (2.8)	22.3 (0.45)	10.7 (0.94)	5.21 (0.77)	4.68 (1.5)	1.71 (1.8)	1.92 (3.1)	4.38 (1.4)	3.13 (2.6)	1.93 (2.1)	6.26 (99.9)
Zn/ 10^3	97.5 (0.82)	65.4 (1.1)	140 (0.29)	48.4 (0.41)	53.2 (2.6)	50.1 (3.8)	71.4 (3.4)	73.8 (3.0)	80.4 (0.75)	59.0 (0.85)	73.9 (37.5)
	Infusions of GTs										
	GTB1	GTB2	GTB3	GTB4	GTB5	GTL1	GTL2	GTL3	GTL4	GTL5	Mean ^a (CV, %)
Al	5.10 (1.2)	10.1 (0.99)	2.91 (0.69)	3.06 (0.98)	4.38 (0.46)	2.55 (0.78)	1.57 (0.64)	1.63 (0.61)	1.05 (0.95)	1.47 (0.68)	3.38 (79.8)
Ba/ 10^3	26.7 (1.5)	41.8 (1.2)	25.6 (0.39)	18.5 (1.6)	19.3 (2.6)	8.57 (0.82)	9.37 (1.8)	8.84 (1.9)	7.63 (2.8)	6.64 (0.75)	17.3 (66.2)
Ca	0.903 (1.3)	1.48 (2.1)	1.72 (2.2)	2.51 (0.61)	1.97 (0.66)	1.87 (1.3)	24.0 (1.7)	2.14 (1.6)	3.61 (0.74)	3.53 (1.0)	4.37 (159)
Cd/ 10^3	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	<0.71 ^b	–
Co/ 10^3	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	<1.6 ^b	–
Cr/ 10^3	1.42 (5.6)	7.17 (2.8)	1.18 (0.85)	1.15 (4.3)	1.55 (2.6)	<1.1 ^b	1.22 (4.9)	2.15 (4.6)	1.57 (4.5)	1.22 (4.1)	1.97 (93.9)
Cu/ 10^3	54.6 (0.73)	56.4 (2.5)	82.7 (1.2)	73.4 (1.5)	67.0 (3.1)	48.1 (3.5)	41.2 (0.48)	75.1 (1.5)	59.1 (1.4)	52.0 (1.3)	61.0 (21.6)
Fe/ 10^3	58.7 (4.4)	52.2 (5.5)	51.2 (5.6)	52.8 (1.4)	31.2 (2.3)	62.5 (2.3)	59.4 (7.3)	42.5 (1.7)	32.3 (6.7)	64.0 (3.4)	50.7 (23.2)
K	98.0 (2.1)	73.5 (1.3)	101 (1.9)	121 (1.4)	113 (1.2)	122 (1.3)	126 (1.4)	110 (0.39)	114 (1.0)	126 (1.2)	110 (14.6)
Mg	4.79 (1.9)	7.44 (0.38)	4.48 (1.6)	5.33 (0.44)	5.56 (0.51)	5.47 (0.74)	7.50 (0.38)	4.16 (0.54)	4.87 (0.81)	5.66 (1.0)	5.53 (20.5)
Mn	2.65 (1.5)	4.52 (0.80)	1.93 (1.7)	2.22 (0.91)	2.00 (1.5)	0.353 (3.8)	2.47 (1.0)	1.25 (1.3)	1.67 (1.6)	1.70 (0.50)	1.85 (65.2)
Na/ 10^3	127 (0.79)	368 (0.34)	460 (0.55)	134 (0.18)	233 (1.3)	144 (0.77)	320 (1.6)	137 (1.1)	1120 (0.65)	158 (0.84)	320 (95.0)
Ni/ 10^3	35.0 (2.8)	71.2 (1.4)	38.2 (1.8)	39.6 (1.8)	45.5 (2.0)	24.0 (3.3)	23.2 (0.43)	41.6 (1.4)	35.9 (2.0)	32.4 (4.6)	38.7 (34.8)
Pb/ 10^3	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	<12 ^b	–
Sr/ 10^3	4.93 (1.4)	12.1 (2.5)	9.52 (1.8)	7.01 (1.1)	7.67 (2.5)	9.44 (0.95)	15.4 (0.65)	4.47 (1.6)	4.02 (2.5)	4.35 (0.69)	7.89 (47.8)
Zn/ 10^3	75.6 (1.2)	52.1 (0.38)	81.5 (0.98)	98.0 (1.4)	81.6 (1.8)	117 (0.86)	93.2 (0.86)	101 (0.99)	102 (0.98)	86.4 (0.58)	88.8 (20.0)

Mean values ($n=3$) with relative standard deviations (RSDs) in brackets

^a Means within the group of tea samples with coefficients of variance (CVs) in brackets

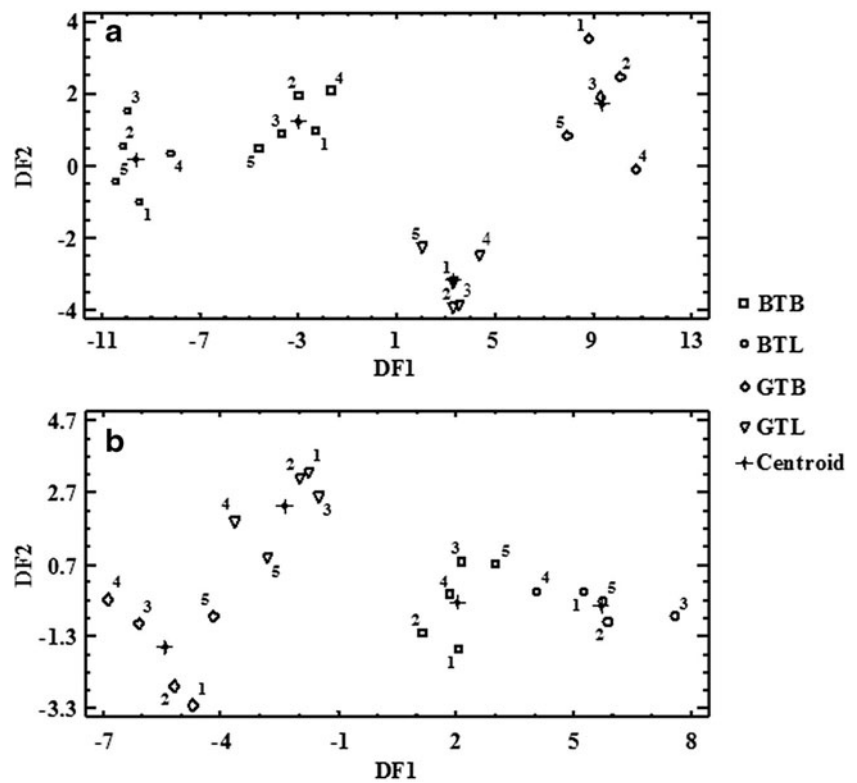
^b Below the quantification limit (QL)

infusions of BTs and GTs, while other metals, including Na, Zn, Cu, Fe, Ni, Ba, Sr, Cr, Cd, Co, and Pb, were only minor and trace constituents.

The variation of concentrations of studied metals within examined groups of teas (BTs and GTs) was quite different, and this was likely related to dissimilar metals by organic components of both types of teas. In case of BTs, the lowest values of the CV were determined for Ni, Mg, K, Ca, and Cu

(18–30 %); high CV values were established for Na (69 %), Cr (73 %), Sr (100 %), and Ba (108 %). In case of GTs, the lowest variation of concentrations in infusions was found for K, Mg, Zn, Cu, and Fe (CVs within 15–23 %). A higher variation of results was established for Mn (the CV of 65 %), Ba (the CV of 66 %), and Al (the CV of 80 %). The highest CVs were assessed for Cr (94 %), Na (95 %), and Ca (159 %).

Fig. 1 Results of the linear discriminant analysis (LDA). The 2-dimensional scatter plot of the two first discriminant functions (DFs) for (a) all selected variables (concentrations of Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, and Zn) and (b) variables selected by the stepwise regression with backward selection (concentrations of Ba, Ca, Mg, Mn, Sr, and Zn)



Chemometric Data Evaluation

To visualize the data into 2 or 3 dimensions (Diniz et al. 2015; Froes et al. 2014; Paz-Rodriguez et al. 2015), the principle component analysis (PCA) was applied to a data matrix that comprised all analyzed teas (objects) and concentrations of 13 metals (variables) determined in infusions of these teas, i.e., Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, and Zn (with the exception of Cd, Co, and Pb that concentrations were <QLs). Missing values in 2 cases for Cr were replaced with a respective QL value (Paz-Rodriguez et al. 2015). The PCA was based on a Pearson correlation table. Unfortunately, it was established that a model with 3 PCs explained only 68.5 % of the total variance, i.e., 34.3 % for the PC1, 20.2 % for the PC2, and 14.0 % for the PC3. Respective eigenvalues were equal to 4.46 (PC1), 2.62 (PC2), and 1.82 (PC3). In these conditions, no clear separation between 4 studied classes of teas (BTBs, BTLs, GTBs, and GTLs) was achieved. Variables with the highest contribution to the PC1 were Al (17.8 %), Ba (17.7 %), Cr (15.7 %), Mn (16.1 %), and Sr (14.8 %). In case of the PC2, it was Ca (12.2 %), Fe (19.6 %), K (18.2 %), Mg (15.6 %), and Zn (24.6 %). In the next trial, the PCA was repeated using only concentrations of Al, Ba, Cr, Mn, and Sr as variables. This time, the 2 first PCs were established to explain 88.0 % of the total variance, i.e., 68.5 % with the PC1 and 19.5 % with the PC2, while respective eigenvalues were equal to 3.43 (PC1) and 0.98 (PC2). Nevertheless, there was no clear separation between 4 groups of analyzed teas.

Because the PCA did not lead to the unequivocal classification of analyzed teas (Diniz et al. 2015), therefore, the supervised linear discriminant analysis (LDA) was used to investigate the possible categorization of teas based on concentrations of metals and determine which metals are responsible for the eventual differentiation. At the beginning,

Table 5 Standardized coefficients of discriminant functions (DF1 and DF2) used to discriminate among four different groups (types) of teas, i.e., bagged and leaf, black and green teas (BTBs and BTLs, GTBs, and GTLs) marketed in Poland

Metal	All variables		Stepwise regression with backward selection	
	DF1	DF2	DF1	DF2
Al	1.95	-0.33	-	-
Ba	-3.33	3.69	1.94	-2.39
Ca	0.85	-0.41	-	-
Cr	-3.55	-3.31	2.52	1.17
Cu	0.70	-0.17	-	-
Fe	-0.75	0.32	-	-
K	-0.83	-1.62	-	-
Mg	-3.22	2.77	1.85	-1.15
Mn	5.55	-1.12	-3.86	0.18
Na	-0.51	0.27	-	-
Ni	-0.81	1.08	-	-
Sr	5.49	-2.47	-4.09	1.90
Zn	3.00	-0.19	-1.38	0.77

concentrations of 13 metals (Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, and Zn) were used as predictive variables (see Fig. 1a). It was found that 2 first discriminant functions (DFs), i.e., the DF1 and the DF2, explained 90.5 and 6.6 % of the total variance, respectively. Eigenvalues for the DF1 and the DF2 were correspondingly equal to 62.67 and 4.61. With respect to the classification model achieved (see standardized coefficients of linear combinations of concentrations of metals for both DFs in Table 5), the LDA differentiated and correctly classified 100 % of all analyzed teas. A stepwise regression with the backward selection of variables was additionally used to find the best discriminating model with only few

statistically significant variables. In this case, a linear combination of just 6 variables (concentrations of Ba, Ca, Mg, Mn, Sr, and Zn), being the most significant predictors of 4 classes of teas, gave DFs that 2 first (DF1 and DF2) explained 98.1 % of the total variance (see Fig. 1b), i.e., 88.1 % (DF1) and 10.0 % (DF2). Respective eigenvalues were equal to 22.39 (DF1) and 2.53 (DF2). Again, the established model correctly classified all 20 analyzed teas. All these outcomes proved that a reduced profile of the metal composition allows to discriminate teas in a unmistakable way according to their variety (BTBs, BTLs, GTBs, and GTLs). This finding is extremely important in terms of bromatological studies of teas because

Table 6 Percentage leachabilities of metals in infusions of black (BTs) and green (GTs) teas

Metal	Leachability, %										
	Infusions of BTs										Mean ^a (CV, %)
	BTB1	BTB2	BTB3	BTB4	BTB5	BTL1	BTL2	BTL3	BTL4	BTL5	
Al	47.0±0.6	37.0±0.4	41.0±0.4	39.5±0.6	20.5±0.1	33.3±0.2	38.0±0.6	39.5±0.3	41.4±0.6	43.1±0.6	38.0 (18.8)
Ba	8.43±0.04	8.20±0.03	6.91±0.08	3.56±0.06	2.34±0.03	1.99±0.07	1.86±0.03	3.07±0.02	2.34±0.02	2.70±0.11	4.14 (63.6)
Ca	5.95±0.17	3.10±0.05	3.82±0.05	2.69±0.03	2.61±0.01	2.16±0.02	2.83±0.05	4.32±0.11	4.07±0.06	3.26±0.05	3.48 (31.8)
Co	_b	_b	81.3±3.2	_b	_b	_b	_b	_b	_b	39.0±0.9	–
Cr	14.0±0.1	10.2±0.1	16.9±0.5	3.47±0.15	18.5±0.7	_b	40.0±1.1	42.8±1.7	48.5±1.2	52.3±2.2	27.4 (67.0)
Cu	27.4±1.0	38.3±1.4	42.4±0.4	42.8±0.5	21.8±0.9	18.0±0.6	22.8±0.6	32.2±0.4	22.3±0.6	22.0±0.2	29.0 (32.0)
Fe	6.19±0.07	1.82±0.09	4.60±0.26	4.03±0.22	3.46±0.13	8.68±0.14	7.14±0.46	5.06±0.20	6.62±0.09	6.69±0.23	5.43 (37.2)
K	92.5±1.7	51.7±0.9	74.5±1.9	42.8±0.6	41.1±0.5	68.1±0.8	87.6±0.8	91.0±0.6	79.8±1.0	75.8±0.5	71.1 (28.1)
Mg	45.1±0.4	33.7±0.7	47.6±0.5	22.7±0.1	29.3±0.6	27.2±0.2	37.6±1.0	37.6±0.9	47.8±1.0	35.5±0.5	36.4 (23.6)
Mn	28.0±0.7	17.4±0.1	23.8±0.1	11.4±0.1	15.2±0.1	14.4±0.1	19.4±0.2	17.7±0.3	24.2±0.2	17.5±0.1	18.9 (26.8)
Na	99.7±1.5	96.6±0.7	99.0±0.7	94.6±0.8	99.1±1.8	98.9±0.4	97.3±2.7	97.5±1.0	98.4±1.2	91.3±0.4	97.2 (2.6)
Ni	59.1±2.8	74.9±3.6	81.8±0.6	71.2±0.9	81.2±0.6	95.7±0.8	91.9±2.8	97.0±2.1	86.1±3.5	82.8±2.7	82.6 (14.8)
Sr	6.30±0.03	4.13±0.02	4.78±0.04	2.52±0.02	1.76±0.03	1.38±0.02	1.54±0.05	2.17±0.03	1.73±0.04	1.74±0.04	2.81 (59.9)
Zn	40.6±0.4	35.2±0.4	61.5±0.2	23.8±0.1	23.3±0.6	27.3±1.0	31.0±1.0	30.8±0.9	39.2±0.3	33.6±0.3	34.6 (32.0)
	Infusions of GTs										
	GTB1	GTB2	GTB3	GTB4	GTB5	GTL1	GTL2	GTL3	GTL4	GTL5	Mean ^a (CV, %)
Al	24.4±0.3	31.0±0.3	26.1±0.2	45.6±0.4	29.3±0.1	42.7±0.3	25.6±0.2	24.9±0.2	19.8±0.2	27.8±0.2	29.7 (27.6)
Ba	6.58±0.03	6.91±0.08	7.86±0.03	9.98±0.16	6.11±0.2	11.6±0.1	5.49±0.10	4.52±0.09	3.54±1.0	4.25±0.03	6.68 (38.2)
Ca	1.67±0.02	1.54±0.03	2.85±0.06	5.34±0.03	3.35±0.02	3.72±0.05	16.1±0.3	4.67±0.08	6.55±0.05	5.29±0.05	5.11 (82.0)
Cr	16.5±0.1	26.7±0.7	16.1±0.1	23.6±1.0	13.6±0.4	_b	20.9±1.0	53.9±2.5	34.4±1.5	24.5±1.0	25.6 (48.4)
Cu	53.0±1.9	57.2±1.4	56.2±0.7	66.4±1.0	59.2±1.8	50.7±1.8	26.5±0.1	68.0±1.0	54.8±0.8	41.5±0.5	53.4 (22.6)
Fe	4.96±0.22	1.17±0.06	1.79±0.10	5.89±0.08	1.49±0.03	5.57±0.13	5.91±0.43	2.86±0.05	2.21±0.15	6.86±0.23	3.87 (56.0)
K	99.7±2.1	99.7±1.3	99.5±1.9	98.8±1.4	99.8±1.2	99.0±1.3	97.2±1.4	89.9±0.4	99.8±1.0	91.3±1.1	97.5 (3.8)
Mg	32.1±0.6	37.7±0.1	29.2±0.5	38.1±0.2	34.8±0.2	39.4±0.3	52.0±0.2	32.3±0.2	39.2±0.3	39.2±0.4	37.4 (16.7)
Mn	17.8±0.3	22.8±0.2	18.2±0.3	28.8±0.3	18.9±0.3	25.4±1.0	36.0±0.4	20.7±0.3	24.1±0.4	22.2±0.1	23.5 (23.7)
Na	99.7±0.9	99.2±0.3	99.8±0.5	99.1±0.2	99.3±1.3	99.2±0.8	99.0±1.6	99.4±1.1	99.6±0.6	99.3±0.8	99.4 (0.3)
Ni	88.8±4.3	94.4±1.3	95.3±1.7	95.9±1.7	97.2±2.4	93.9±3.1	90.7±0.4	95.7±1.3	97.0±1.9	94.7±4.4	94.4 (2.8)
Sr	3.60±0.02	6.05±0.15	5.95±0.11	3.38±0.04	4.62±0.12	9.32±0.09	5.98±0.04	3.60±0.06	3.46±0.09	3.84±0.03	4.98 (37.9)
Zn	44.6±0.5	26.2±0.1	28.6±0.3	48.1±0.7	38.2±0.7	45.0±0.4	39.0±0.3	49.4±0.5	42.0±0.4	40.0±0.2	40.1 (19.1)

Mean values ($n=3$) with standard deviations (SDs)

^a Means within the group of tea samples with coefficients of variance (CVs) in brackets

^b Not calculated due to concentrations below quantification limits (QLs) in tea infusions

the proposed validated sample preparation procedure of infusions and the chemometric treatment of the data using the LDA could provide the fast and more affordable discrimination of teas.

Leachability of Metals into Infusions

Percentages of metals leached into infusions were assessed taking into consideration concentrations of studied metals in made teas (see results very recently reported by Szymczycha-Madeja et al. 2015). Leachabilities (in %) were calculated as ratios of masses of metals in infusions (final volumes were considered) to their masses in made teas (masses of teas taken for the steeping were considered). Mentioned leaching efficiencies (average values, $n=3$) and respective means within groups of both types of teas along with CV values are given in Table 6. As can be seen, highly extractable metals in BTs are only Na (97 %), Ni (83 %), and K (71 %), while moderately extractable metals are Al (38 %), Mg (36 %), Zn (35 %), Cu (29 %), and Cr (27 %). Poorly extractable metals were Sr (~3 %), Ca (~4 %), Ba (~4 %), and Fe (~5 %), probably because the matrix of BTs strongly binds these metals or their compounds formed are not well soluble in infusions. In case of infusions of GTs, leaching efficiencies evaluated for studied metals were relatively higher. As for BTs, highly extractable metals were Na (99 %), K (98 %), and Ni (94 %). Moderately extractable metals were Cu (53 %), Zn (40 %), Mg (37 %), Al (30 %), Cr (26 %), and Mn (24 %). As before, Fe (~4 %), Sr (~5 %), Ca (~5 %), and Ba (~7 %) were the poorest extractable. Observed differences in leachabilities of studied metals into infusions of BTs and GTs were previously reported in the literature (Dambiec et al. 2013; Dash et al. 2008; Gallaher et al. 2006; Malik et al. 2008; Mehra and Baker 2007; Salahinejad and Aflaki 2010; Street et al. 2006).

Particularly, low extraction efficiencies of Ca and Fe were ascribed to the formation of compounds of these two metals that are strongly bound to the matrix of tea (Salahinejad and Aflaki 2010). In case of Ba and Sr, no data on the extraction efficiency of these two metals are available in the related literature although their measurements in infusions of different teas were reported in few works (Altintig et al. 2014; Ozcan et al. 2008).

In order to highlight significant differences between four types of analyzed teas (BTBs, BTLs, GTBs, GTLs) due to mean leachabilities of studied metals, the one-way analysis of variance (ANOVA) with the Welch correction of F values due to inequivalent variances was applied (Anderson 2003). It was found that differences in mean leachabilities of selected metals (Ba, Cu, Fe, K, Ni, Sr, and Zn) in analyzed four types of teas were statistically significant and allowed to unequivocally differentiate them according to the variety (see Table 7). To determine which groups of teas significantly differed from each other, a multiple comparison was used by means of Fisher's least significant difference test (LSD). Results of this test and the comparison of mean leachabilities of studied metals in one group of teas with respective means in another group are given in Table 7.

Conclusions

In this work, the reliability of various simplified sample preparation procedures of infusions of BTs and GTs was compared and evaluated. It was found, that the acidification of infusions of teas with HNO_3 (at a concentration of 0.25 mol L^{-1}) demonstrates the appropriate precision, accuracy, and sensitivity for reliable determinations of 16 metals by FAAS (Ca, Fe, K, Mg, Mn, Na) and ICP OES (Al, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn). This

Table 7 Results of the one-way ANOVA with the Welch correction and the post hoc Fisher's least significance difference test (LSD) carried out for leachabilities of studied metals assessed in infusions of black (BTs) and green (GTs) teas

	F^* ratio	p value	LSD test
Al	2.55	0.1276	BTLs-GTLs
Ba	16.75	<i>0.0012</i>	BTBs-BTLs, BTLs-GTBs, BTLs-GTLs
Ca	1.02	0.4329	BTLs-GTLs, GTBs-GTLs
Cr	2.94	0.0971	BTBs-BTLs
Cu	34.50	<i>0.0000</i>	BTBs-GTBs, BTBs-GTLs, BTLs-GTBs, BTLs-GTLs
Fe	4.56	<i>0.0344</i>	BTBs-BTLs, BTLs-GTBs
K	8.92	<i>0.0096</i>	BTBs-BTLs, BTBs-GTBs, BTBs-GTLs, BTLs-GTBs
Mg	0.85	0.5040	
Mn	1.55	0.2705	
Na	1.92	0.2056	BTLs-GTBs, BTLs-GTLs
Ni	7.00	<i>0.0120</i>	BTBs-BTLs, BTBs-GTBs, BTBs-GTLs
Sr	11.74	<i>0.0039</i>	BTLs-GTBs, BTLs-GTLs
Zn	4.43	0.0390	

Statistically significant differences for p values <0.05 are italicized

F^* Welch corrected value of the F -ratio of the between-group estimate to the within-group estimate, p value probability of the F^* -test

methodology was significantly improved in comparison to conventional sample treatments for measurements of different elements based on the wet digestion in concentrated HNO_3 and 30 % H_2O_2 solutions, which are effective and dependable but time-consuming and requiring large amounts of hazardous reagents and laborious preparations. The proposed and validated procedure is much simple, reduces the sample handling, and minimizes the time investment along with the consumption of reagents. It can be carried out at room temperature; hence, losses of volatile analytes are completely eliminated. All of these make this sample preparation procedure a very good alternative to time-consuming, laborious, and inconvenient wet digestion procedures.

Interestingly, it was verified that other tested sample preparation procedures, including the acidification with HNO_3 (at a concentration of 0.50 and 1.0 mol L^{-1}) or aqua regia (at a concentration of 0.25, 0.50, and 1.0 mol L^{-1}) and the direct analysis of untreated infusions, led to erroneous results.

The proposed validated procedure was applied for the multi-element analysis of infusions of 20 bagged and leaf BTs and GTs commercially available in Poland. It seems that it could be successively applied to multi-element analyses of infusions of teas focused on the examination of the quality and the safety of tea products due to their mineral characteristic. The simplicity of the proposed procedure could also be helpful in investigations dealing with the discrimination of teas of different types and origins based on the multivariable data analysis in reference to their distinct multi-element composition.

Acknowledgments

Funding This study was funded by the National Science Centre of Poland (grant number 2013/09/B/NZ9/00122X).

Compliance with Ethical Standards

Conflict of Interest Anna Szymczycha-Madeja declares that she has no conflict of interest. Maja Welna declares that she has no conflict of interest. Pawel Pohl declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Informed consent is not applicable for this study.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Altıntig E, Altundag H, Tuzen M (2014) Determination of multi element levels in leaves and herbal teas from Turkey by ICP-OES. *Bull Chem Soc Ethiop* 28:9–16
- Anderson TW (2003) Introduction to multivariate statistical analysis. Wiley, New York
- Dambiec M, Polechonska L, Klink A (2013) Levels of essential and non-essential elements in black teas commercialized in Poland and their transfer to tea infusions. *J Food Comp Anal* 31:62–68
- Dash K, Manjusha R, Thangavel S, Arunachalam J (2008) UV photolysis-assisted digestion of tea (*Camellia sinensis*) and tulsi (*Ocimum sanctum*) and their infusions: comparison of available elements. *At Spectrosc* 29:56–62
- Derun EM (2014) Determination of essential mineral concentrations in some Turkish teas and the effect of lemon addition. *Food Sci Biotechnol* 23:671–675
- Diniz PHGD, Pistonesi MF, Alvarez MB, Alvarez MB, Band BSF, de Araujo MCU (2015) Simplified tea classification based on a reduced chemical composition profile via successive projections algorithm linear discriminant analysis (SPA-LDA). *J Food Comp Anal* 39: 103–110
- Fernandez PL, Pablos F, Martin MJ, Gonzalez AG (2002) Multi-element analysis of tea beverages by inductively coupled plasma atomic emission spectrometry. *Food Chem* 76:483–489
- Froes RES, Neto WB, Beininger MA, Nascentes CC, da Silva JBB (2014) Determination of inorganic elements in teas using inductively coupled plasma optical emission spectrometry and classification with exploratory analysis. *Food Anal Method* 7:540–546
- Gallaher RN, Gallaher K, Marshall AJ, Marshall AC (2006) Mineral analysis of ten types of commercially available tea. *J Food Comp Anal* 19:53–57
- Jeszka-Skowron M, Krawczyk M, Zgola-Grzeskowiak A (2015) Determination of antioxidant activity, rutin, quercetin, phenolic acids and trace elements in tea infusions. Influence of citric acid addition on extraction of metals. *J Food Com Anal* 40:70–77
- Konieczka P, Namiesnik J (2009) Quality assurance and quality control in the analytical chemical laboratory. A practical approach. CRC Press, Boca Raton
- Malik J, Szakova J, Drabek O, Balik J, Kokoska L (2008) Determination of certain micro and macroelements in plant stimulants and their infusions. *Food Chem* 111:520–525
- Mehra A, Baker CL (2007) Leaching and bioavailability of aluminium, copper and manganese from tea (*Camellia sinensis*). *Food Chem* 100:1456–1463
- Memic M, Mahic D, Zero S, Muhic-Sarac T (2014) Comparison of different methods of green and black tea at the Sarajevo market for the determination of the heavy metal content. *Food Measure* 8:149–154
- Milani RF, Morgano MA, Saron ES, Silva FF, Cadore S (2015) Evaluation of direct analysis for trace elements in tea and herbal beverages by ICP-MS. *J Braz Chem Soc* 26:1211–1217
- Ozcan MM, Unver A, Ucar T, Arslan D (2008) Mineral content of some herbs and herbal teas by infusion and decoction. *Food Chem* 106: 1120–1127
- Ozdemir OD, Kipcak AS, Derun EM, Piskin S (2014) Determination of the effect of lemon addition upon element concentrations in tea, instrument. *Sci Technol* 42:153–160
- Paz-Rodriguez B, Dominguez-Gonzalez MR, Aboal-Somoza M, Bermejo-Barrera P (2015) Application of high resolution-continuum source flame atomic absorption spectrometry (HR-CS FAAS): determination of trace elements in tea and tisanes. *Food Chem* 170:492–500
- Polechonska L, Dambiec M, Klink A, Rudecki A (2015) Concentrations and solubility of selected trace metals in leaf and bagged black teas

- commercialized in Poland. *J Food Drug Anal.* doi: <http://dx.doi.org/10.1016/j.jfda.2014.08.003>
- Salahinejad M, Aflaki F (2010) Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. *Biol Trace Elem Res* 134:109–117
- Schwalfenberg G, Genus SJ, Rodushkin I (2013) The benefits and risks of consuming brewed tea. Beware of toxic element contamination. *J Toxicol.* ID 370460. doi: [10.1155/2013/370460](https://doi.org/10.1155/2013/370460)
- Shen FM, Chen HW (2008) Element composition of tea leaves and tea infusions and its impact on health. *Bull Environ Contam Toxicol* 80:300–304
- Sofuoglu SC, Kavcar P (2008) An exposure and risk assessment for fluoride and trace metals in black tea. *J Hazard Mater* 158:392–400
- Street R, Szakova J, Drabek O, Mladkova L (2006) The status of micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *Czech J Food Sci* 24:62–71
- Szymczycha-Madeja A, Welna M, Pohl P (2012) Elemental analysis of teas and their infusions by spectrometric methods. *Trends Anal Chem* 35:165–181
- Szymczycha-Madeja A, Welna M, Pohl P (2015) Determination of essential and non-essential elements in green and black teas by FAAS and ICP OES simplified—multivariate classification of different tea products. *Microchem J* 121:122–129
- Tahir Soomro M, Zahir E, Mohiuddin S, Nisar Khan A, Naqvi II (2008) Quantitative assessment of metals in local brands of tea in Pakistan. *Pakistan J Biol Sci* 11:285–289
- Welna M, Szymczycha-Madeja A, Pohl P (2013) A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. *Food Res Int* 53:922–930
- Wrobel K, Wrobel K, Urbina EMC (2000) Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (Mate) by ET-AAS. *Biol Trace Elem Res* 78:271–280