

## DETERMINATION OF COMMON INORGANIC ANIONS IN TEA SAMPLES BY ION CHROMATOGRAPHY

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(Received: 1 May 2013; accepted: 11 August, 2013)

Plants exposure to environmental sources of common inorganic anions (chloride, nitrate, phosphate, and sulphate) is discussed. Various commercial tea products were examined for the content of water soluble anions by the use of sample preparation procedure corresponding to the conditions generally applied for the preparation of tea infusions (2 g of tea, 5 min brewing time, 250 ml final volume) widely consumed by humans. Suppressed ion chromatography (IC) (Metrohm Metrosep A Supp 5 (150 mm × 4 mm) column) with conductometric detection was used for the determination. The IC technique allows isolation of the signals of particular anions and their detection (DLs of 0.012 (Cl<sup>-</sup>), 0.019 (NO<sub>3</sub><sup>-</sup>), 0.047 (PO<sub>4</sub><sup>3-</sup>), and 0.011 (SO<sub>4</sub><sup>2-</sup>) mg l<sup>-1</sup>) within 15 min.

**Keywords:** plants, tea, inorganic anions, ion chromatography

A common need for understanding biochemical processes occurring in the environment, in particular those affecting the uptake of various chemical substances by living organisms, requires continuous examination of a large variety of environmental materials for the content of chemicals originated from natural and anthropogenic sources. Monitoring of the levels of common inorganic anions: chloride, nitrates(V,III), phosphate, and sulphate, is necessary owing to their important biological role. Soluble inorganic anions are of particular significance to a number of biological processes occurring in the environment, among them those directly affecting plant growth and productivity. Plant life cycles are dependent on the availability of essential micro- and macroelements. Nitrogen, phosphorus, and sulphur make important macronutrients for a large variety of plants (EPSTEIN & BLOOM, 2005). Once assimilated, they directly contribute to essential components of different organs and organelles. They are involved in various metabolic processes resulting in synthesis of a large variety of organic derivatives of distinctive biological functions. The availability of nutrient elements is a limiting factor for plant growth. Plants absorb various minerals from soils, environmental waters, and air. Fertilizers are widely used in agricultural systems to fulfil plant mineral requirements. Balance between fertilizer nutrient load and required amount for particular plant is needed. High amounts of fertilizers used can negatively affect the quality of soils and environmental waters, which can result in deterioration of conditions for development and existence of living organisms, including plants.

The overdose of inorganic anions, in particular nitrate(V) and nitrate(III), generates a direct link between the environmental pollution and human health hazard owing to the possibility of entering the food chain (ROUACHED et al., 2010; BRYAN & LOSCALZO, 2011). Nitrate(III) can exhibit strong negative health effects known as methaemoglobinaemia associated with the oxidation of iron(II) in the haemoglobin molecule resulting in a lack of

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its capacity to bind and transport oxygen in the organisms. Vegetables and other plant foods provide the main (80–95%) sources of nitrates(V) for humans (MICHALSKI & KURZYCA, 2006). High possible rate, corresponding to 5–20%, of endogenous conversion of nitrates(V) into nitrates(III) in the gastrointestinal tract requires attention and careful examination of all nitrate dietary sources (SANTAMARIA, 2006; CORREIA et al., 2010). Chlorides are widely dispersed in the environment. The excess of chlorides can pose a threat to agriculture due to low tolerance of some crop species to saline conditions. Phosphorus is a key essential element for plants participating in vital metabolic processes and cell physiology. Plants exposure to various forms of phosphate (orthophosphate, polyphosphate, and organically bound phosphate) can originate from natural environmental processes, dissolution of phosphate minerals and mineralization of algae, as well as from anthropogenic sources such as fertilizers used, discharges of municipal and industrial effluents, from detergents and organic pesticides containing phosphates. Orthophosphate(V) ion is easy to assimilate and is most often used as active fertilizers component. Sulphate is an anionic solute making the main source of sulphur required for the synthesis of physiologically important compounds such as amino acids (cysteine and methionine) and numerous metabolites and coenzymes involved in various metabolic processes in plants.

In this work the contents of water soluble chloride, nitrate, phosphate, and sulphate in various tea commodities have been evaluated. Aqueous extracts of tea samples obtained under conditions commonly used for the preparation of tea infusions widely consumed by humans were examined. Tea infusions are the second most consumed beverages after drinking waters. They are sources of numerous biologically active compounds for humans, among them soluble inorganic anions. Ion chromatography (IC) with conductometric detection has been used for the identification and quantification of the determined anions. Ion chromatography is a technique widely applied for the determination of ionic species in a wide variety of samples (SMALL et al., 1975; HADDAD, 2004; WEISS, 2004). The technique is preferable for the determination of inorganic and small organic anions occurring, or generated under the conditions used, in the examined samples. Simultaneous detection of the analytes in a single run, speed of the determination, high sensitivity (detection limits at  $\text{ng l}^{-1}$  and  $\mu\text{g l}^{-1}$  levels) and selectivity, high reproducibility and robustness, as well as small sample volume required for the analysis, make IC procedures advantageous with respect to conventional wet-chemical and spectrophotometric methods widely used previously for the determination of inorganic anions (WILLIAMS, 1979; MARCZENKO & BALCERZAK, 2007).

## 1. Materials and methods

### 1.1. Apparatus

The 761 Compact IC System from Metrohm AG (Herisau, Switzerland) consisting of a MSM II (Metrohm Suppressor Module) suppressor and a conductometric detector was used. Anions separation was carried out on a Metrosep A Supp 5 (150 mm  $\times$  4 mm) Metrohm column and a mixture of 3.2 mmol  $\text{l}^{-1}$   $\text{Na}_2\text{CO}_3$ +1.0 mmol  $\text{l}^{-1}$   $\text{NaHCO}_3$  used as an eluent. The IC Net 2.3 Metrodata (Metrohm) software was used for data acquisition and evaluation of chromatograms.

Filters of 0.45  $\mu\text{m}$  pore size (MCE, Fisherbrand) and IC-RP (C18) from Metrohm were used for samples filtration throughout the experiments.

### 1.2. Reagents

All reagents used in the work were of analytical grade. Water purified in a Millipore Elix3/Simplicity UV system (a specific resistance  $>18.2 \text{ M}\Omega \text{ cm}$ ) was used in all experiments.

Standard solutions of fluoride, chloride, bromide, nitrate(V), phosphate, and sulphate anions (from Fluka) prepared from high purity sodium salts containing  $1000 \text{ mg l}^{-1}$  of each anion and dedicated to IC were used. Multi-anionic working solutions were prepared by mixing up single anion standards and an appropriate dilution. Acetate, formate, and oxalate solutions (from Fluka) prepared from high purity acids, dedicated to IC and containing  $1000 \text{ mg l}^{-1}$  of each analyte, were also used.

Certified Multianion Standard Solution dedicated to IC (from Fluka) containing an aqueous mixture of  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{SO}_4^{2-}$  ( $10 \text{ mg l}^{-1}$  each of anions) was used for the evaluation of the accuracy of the chromatographic procedure applied.

Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and sodium hydrogen carbonate ( $\text{NaHCO}_3$ ) from Fluka were used for the preparation of the eluent ( $3.2 \text{ mmol l}^{-1} \text{ Na}_2\text{CO}_3 + 1.0 \text{ mmol l}^{-1} \text{ NaHCO}_3$ ).

### 1.3. Tea samples examined

Black teas of various producers: Lipton, Loyd (Assam, Madras, Ceylon, and Yunnan), Tetley, and Tylos and of different forms (stick-shaped, granules, and paper bag-packages) available at the market were examined. Certified tea reference material NCS ZC73014 (NCS CRM, China National Analysis Center for Iron & Steel) was also examined.

### 1.4. Analytical procedure

Tea sample (2 g), preliminary dried at  $60 \text{ }^\circ\text{C}$  for 4 h, was treated with 200 ml of boiling deionised water. After 5 min, the sample was filtered, cooled to room temperature, transferred into a 250-ml calibration flask, and diluted to the volume with deionised water. The solution was injected into the chromatographic column after filtration through  $0.45 \text{ }\mu\text{m}$  membrane filter and IC-RP cartridge.

## 2. Results and discussion

### 2.1. Calibration

Synthetic mixtures containing the determined anions at concentrations given in Table 1 were used for the calibration procedure and the identification of the analytes in the examined tea samples. Fluoride and bromide anions were included in the calibration solutions owing to the necessity of the identification of their signals in tea samples examined under the conditions used. Figure 1 presents the chromatograms of the synthetic anion mixtures examined. Calibration curves of the determined anions generated by plotting the peak areas against their concentrations in the examined standard solutions are presented in Fig. 2. They are of quadratic functions characteristic for the conductometric detection with chemical suppression (EITZ et al., 2005). Detection and quantification limits (DL and QL, respectively) of the examined anions were evaluated in the analysis of their synthetic mixture containing minimum ( $0.1 \text{ (F}^-)$ ,  $0.5 \text{ (Cl}^-)$ ,  $0.1 \text{ (Br}^-)$ ,  $0.3 \text{ (NO}_3^-)$ ,  $0.2 \text{ (PO}_4^{3-})$ , and  $0.75 \text{ (SO}_4^{2-}) \text{ mg l}^{-1}$ ) concentrations of the analytes to be detected. Detection limit of each anion was calculated as triple standard deviation (SD) of the results ( $n=6$ ), quantification limit as triple DL value.

Coefficients of variation (CV) calculated as  $RSD \times 100\%$ , where RSD is a relative standard deviation, were in the range of 0.1–2.5%. The accuracy of the procedure applied was evaluated in the analysis of Certified Multianion Standard Solution (Fluka) containing  $10 \text{ mg l}^{-1}$  each of  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{SO}_4^{2-}$  anions. The recoveries (%) of particular anions were in the ranges of 99.2–99.4, 97.5–98.0, 96.5–97.4, 104.1–104.8, 99.0–99.6, and 100.4–101.2, respectively (six independent analyses were carried out).

Table 1. Concentrations of the anions in the calibration solutions, detection and quantification limits

Anion	No. of the calibration solution				DL	QL
	1	2	3	4		
	$\text{mg l}^{-1}$					
$\text{F}^-$	0.4	2	5	10	0.004	0.012
$\text{Cl}^-$	0.8	4	20	75	0.012	0.036
$\text{Br}^-$	0.4	2	5	10	0.009	0.027
$\text{NO}_3^-$	0.6	3	15	50	0.019	0.057
$\text{PO}_4^{3-}$	0.8	4	20	75	0.047	0.141
$\text{SO}_4^{2-}$	0.8	3	10	25	0.011	0.033

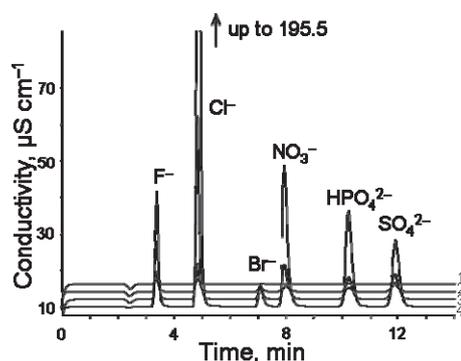


Fig. 1. The ion chromatograms of the calibration solutions used (details in Table 1)

## 2.2. The examination of tea samples

Portions of about 20 g of various commercial tea products were dried at  $60^\circ\text{C}$  for 4 h (tea from bag-packages after removing the paper bags and mixing their content). Samples of about 2 g (an average tea weight in popular tea bags) were analysed according to the above mentioned analytical procedure.

Figure 3 presents the examples of chromatograms of tea samples of the same form (paper bags) originating from various producers. Chromatograms of tea samples of different forms (sticks, granules, and bags) originated from one (Lipton) producer are given in Fig. 4. As can be seen in Figs 3 and 4, oxalate is the organic anion that can simultaneously be determined in tea samples under the conditions used.

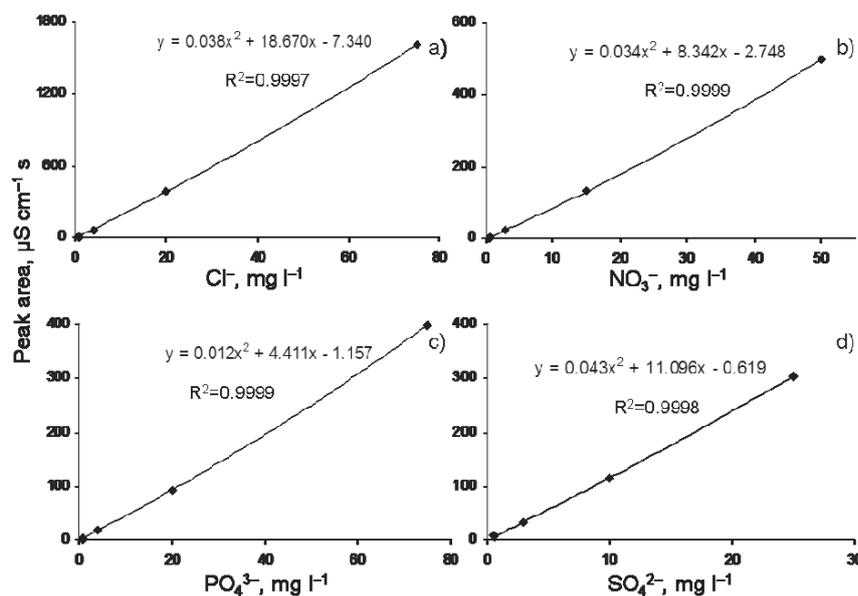


Fig. 2. Calibration curves for (a) chloride, (b) nitrate(V), (c) phosphate, and (d) sulphate determination by IC method (Metrosep A Supp 5 Metrohm column, 3.2 mmol l<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> + 1.0 mmol l<sup>-1</sup> NaHCO<sub>3</sub> (0.7 ml min<sup>-1</sup>) eluent)

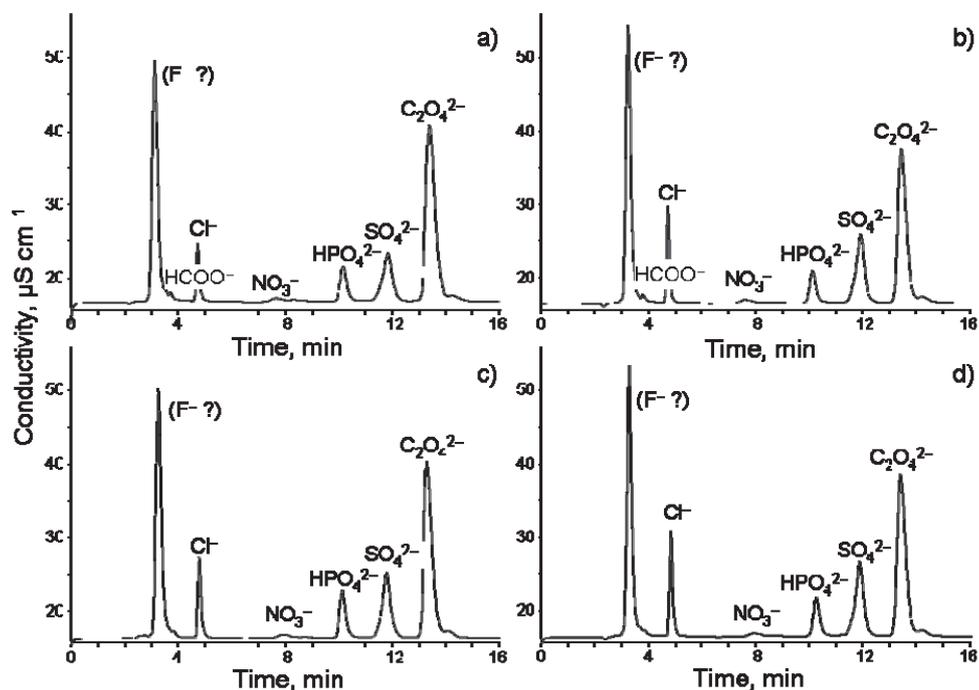


Fig. 3. The ion chromatograms of black (paper bag-packages) tea ((a) Lipton, (b) Loyd, (c) Tetley, (d) Tylos) infusions (2 g tea, 5 min brewing time, 250 ml H<sub>2</sub>O)

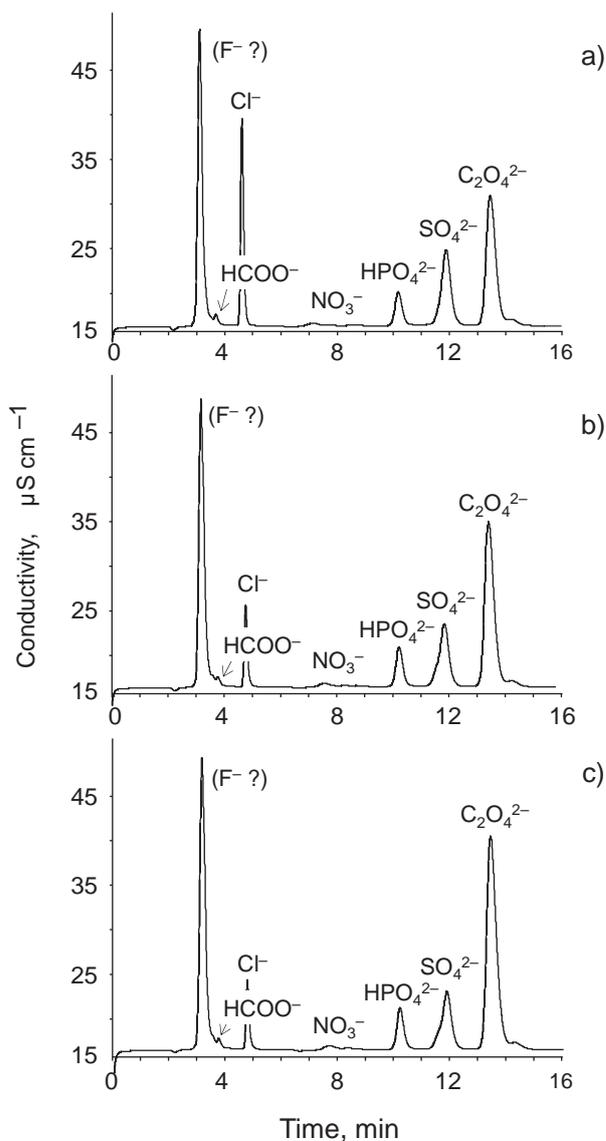


Fig. 4. The ion chromatograms of Lipton black tea ((a) stick-shaped, (b) granules, and (c) paper bag-packages) infusions (2 g tea, 5 min brewing time, 250 ml H<sub>2</sub>O)

The results obtained for the content of the determined anions in the examined samples, in tea infusions (mg l<sup>-1</sup>) and that of corresponding to dry mass (mg g<sup>-1</sup>), are given in Table 2. As can be seen from the results, phosphates and sulphates are the anions extracted at highest amounts from the majority of the examined samples. Bromide ions were not detected in any of the tea samples examined under the conditions used. Fluoride ions were not quantified due to possible interferences originating from small organic acids, which can occur in tea products (JANISZEWSKA & BALCERZAK, 2013). Statistical analysis (*t*-test) has shown that the differences

in the results obtained for the determined anions in various tea categories (sticks and bags, Assam and Ceylon, India and Sri Lanka) are not significant (the calculated *t*-values < table values).

Table 2. The concentrations of the determined anions in tea infusions and corresponding contents in dry samples (n=3)

Producer	Type	Geographical origin	Shape	Concentration (mg l <sup>-1</sup> )±SD/(mg g <sup>-1</sup> )±SD					
				Cl <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>		
Lipton	mixture	different countries (Kenya, Indonesia, India)	sticks	9.64±0.34/ 1.19±0.05	0.54±0.01/ 0.07±0.01	16.89±0.20/ 2.08±0.02	18.52±0.24/ 2.28±0.03		
			granules	4.21±0.13/ 0.54±0.02	0.70±0.02/ 0.09±0.01	18.60±0.10/ 2.30±0.02	15.83±0.13/ 1.96±0.02		
			bags	3.78±0.07/ 0.45±0.02	0.76±0.01/ 0.09±0.01	18.88±0.44/ 2.24±0.02	14.43±0.34/ 1.71±0.01		
		Loyd	Assam	India	sticks	9.07±0.27/ 1.12±0.03	0.91±0.03/ 0.11±0.01	15.29±0.31/ 1.89±0.03	17.18±0.27/ 2.12±0.02
					Madras	India	sticks	5.27±0.09/ 0.65±0.01	2.05±0.05/ 0.25±0.01
		Loyd	Assam	China	sticks	4.72±0.26/ 0.58±0.03	2.00±0.05/ 0.25±0.01	22.42±0.35/ 2.77±0.04	15.00±0.11/ 1.86±0.02
Sri Lanka	sticks				7.72±0.04/ 0.95±0.01	1.80±0.05/ 0.22±0.01	19.72±0.02/ 2.44±0.01	20.17±0.06/ 2.49±0.01	
	bags			5.66±0.16/ 0.69±0.01	2.24±0.01/ 0.27±0.01	16.02±0.21/ 1.96±0.03	17.17±0.42/ 2.10±0.02		
Tetley	Assam			India	bags	5.06±0.04/ 0.58±0.01	2.18±0.04/ 0.25±0.01	22.72±0.24/ 2.62±0.03	16.63±0.21/ 1.92±0.03
		Tylor	Ceylon		Sri Lanka	bags	6.49±0.15/ 0.79±0.01	2.18±0.07/ 0.26±0.02	18.99±0.31/ 2.30±0.03

Standard addition method was applied for the evaluation of the accuracy of the obtained results. Figure 5 presents the chromatogram of Loyd, Madras stick-shaped tea directly analysed (curve 1) and chromatogram of the same sample spiked with a synthetic mixture of 0.5 (Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>), 1.25 (F<sup>-</sup>), 2 (Cl<sup>-</sup>), 2.5 (PO<sub>4</sub><sup>3-</sup>, HCOO<sup>-</sup>), 5 (SO<sub>4</sub><sup>2-</sup>, C<sub>2</sub>O<sub>4</sub><sup>2-</sup>), and 12.5 (CH<sub>3</sub>COO<sup>-</sup>) mg (in 250 ml solution) of the determined anions (curve 2). Spike solution was introduced into the examined sample prior to the extraction with the water. The organic anions, acetate and formate, were added to the spike to show interferences with the selective isolation of fluoride signal from the signals of both of them under the conditions used. The recoveries (%) of the anions determined in the work were in the ranges of: 94.6–95.9 (Cl<sup>-</sup>), 109.0–109.8 (NO<sub>3</sub><sup>-</sup>), 99.1–101.5 (PO<sub>4</sub><sup>3-</sup>), and 96.1–98.9 (SO<sub>4</sub><sup>2-</sup>).

It can be noted that till now there is a lack of certified reference materials for the contents of the examined inorganic anions in tea infusions. In this work we have examined Certified Tea Reference Material NCS ZC73014 (NCS, China National Analysis Center for Iron & Steel) to estimate the parts of the given total element (Cl, N, P, and S) contents passing into tea infusions as soluble anionic species under the conditions used. The determined (n=6) concentrations of particular ions were as follows: 0.35±0.01 (Cl<sup>-</sup>), 0.30±0.01 (NO<sub>3</sub><sup>-</sup>),

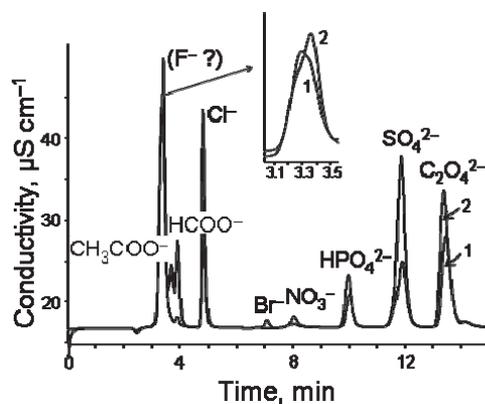


Fig. 5. The ion chromatograms of black (Loyd, Madras, sticks) tea infusions: directly analysed (curve 1) and spiked (0.5 (Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>), 1.25 (F<sup>-</sup>), 2 (Cl<sup>-</sup>), 2.5 (PO<sub>4</sub><sup>3-</sup>, HCOO<sup>-</sup>), 5 (SO<sub>4</sub><sup>2-</sup>, C<sub>2</sub>O<sub>4</sub><sup>2-</sup>), and 12.5 (CH<sub>3</sub>COO<sup>-</sup>) mg into 250 ml solution) (curve 2)

1.94±0.01 (PO<sub>4</sub><sup>3-</sup>), and 2.47±0.02 (SO<sub>4</sub><sup>2-</sup>) mg g<sup>-1</sup>. The amounts of particular elements extracted in the form of anionic species corresponded to 79.5%, 0.1%, 14.1%, and 27.4% of total contents of Cl ((0.044±0.003) × 10<sup>-2</sup>), N ((5.1±0.3) × 10<sup>-2</sup>), P ((0.45±0.03) × 10<sup>-2</sup>), and S ((0.30±0.03) × 10<sup>-2</sup>) g g<sup>-1</sup> certified for the examined material.

It can be noted that till now limited data on the content of chloride, nitrate, phosphate, and sulphate in tea products are available (ALCÁZAR et al., 2003; MICHALSKI, 2006; KUMAR et al., 2008). The amounts (mg g<sup>-1</sup>) of the anions earlier reported were as follows: 2.01 (Lipton) – 17.41 (Chai Tea) (Cl<sup>-</sup>), 4.03 (Lipton) – 9.47 (Earl Grey) (NO<sub>3</sub><sup>-</sup>), 8.52 (Lipton) – 35.78 (Chai Tea) (PO<sub>4</sub><sup>3-</sup>), and 25.80 (Lipton) – 61.23 (Earl Grey) (SO<sub>4</sub><sup>2-</sup>) (MICHALSKI, 2006) and 3.12±0.13 (Cl<sup>-</sup>), 0.34±0.02 (NO<sub>3</sub><sup>-</sup>), 0.08±0.01 (HPO<sub>4</sub><sup>2-</sup>), and 4.20±0.17 (SO<sub>4</sub><sup>2-</sup>) (KUMAR et al., 2008); and 0.60±0.02 (Cl<sup>-</sup>), and 2.93±0.08 (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) (ALCÁZAR et al., 2003). Direct comparison of the obtained results with those already published is, however, not possible, owing to different samples analysed (a large variety of the available tea products) and differences in the applied extraction conditions.

### 3. Conclusions

The differences in the contents of chloride, nitrate(V), phosphate, and sulphate anions in tea infusions of commercial tea products examined were determined by ion chromatographic method. Ion chromatography is a technique allowing rapid simultaneous multiple anionic analysis of the complex samples. The evaluated concentrations of particular anions in the examined tea samples were in the ranges of 3.78–9.64 mg l<sup>-1</sup> Cl<sup>-</sup>, 0.54–2.24 mg l<sup>-1</sup> NO<sub>3</sub><sup>-</sup>, 15.05–22.72 mg l<sup>-1</sup> PO<sub>4</sub><sup>3-</sup>, and 14.43–20.17 mg l<sup>-1</sup> SO<sub>4</sub><sup>2-</sup>, which corresponded to 0.45–1.19 mg g<sup>-1</sup> Cl<sup>-</sup>, 0.07–0.27 mg g<sup>-1</sup> NO<sub>3</sub><sup>-</sup>, 1.86–2.77 mg g<sup>-1</sup> PO<sub>4</sub><sup>3-</sup>, and 1.71–2.49 mg g<sup>-1</sup> SO<sub>4</sub><sup>2-</sup> of dry mass. The obtained values can help with the evaluation of the contribution of particular ions to the total human diet.

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Financial support of the work by the Warsaw University of Technology is kindly acknowledged.

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