Ion Chromatographic Determination of Common Inorganic Anions in Tea Samples

Mustafa CİTTAN[1]*

[1] Department of Chemistry, Faculty of Science and Letters, Manisa Celal Bayar University, 45140, Manisa, Turkey

Abstract

A simple, rapid, and accurate technique based on ion chromatography coupled with a suppressed conductivity detector was described to determine the inorganic anions – fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulphate in tea bag samples. A Dionex IonPac AS9-HC 4×250 mm analytical column with a Dionex IonPac AG9-HC 4×50 mm guard column was used for the chromatographic separation of the analytes. The eluent was 12 mM KOH and the limit of quantification for the inorganic anions were in the range 0.02 - 0.20 mg L^{-1}. Tea infusions were prepared traditionally by pouring the tea bags with 200 mL of boiling ultra-pure water. Influence of the brewing time on the extraction of inorganic anions from the tea bags was examined and optimum brewing time was determined as 6 min according to the results obtained. Finally, the ion chromatographic technique was applied to analyze the inorganic anions in some black, green, and white tea bag samples and five anions were determined quantitatively. The nitrite and bromide amount in all the samples were well below the quantification limit of the method.

Keywords: Ion chromatography, inorganic anions, black tea, green tea, white tea

Yaygın İnorganik Anyonların Çay Örneklerinde İyon Kromatografik Tayini

Öz

Bu çalışmada, poşet çaylarda bulunan inorganik anyonları – florür, klorür, nitrit, bromür, nitrat, fosfat ve sülfat belirlemek için, iletkenlik detektörü ile eşleştirilmiş iyon kromatografisine dayalı basit, hızlı ve doğru bir teknik tanımlanmıştır. Analitlerin kromatografik ayrılması, Dionex IonPac AG9-HC 4×50 mm ön kolonu ile birleştirilmiş, Dionex IonPac AS9-HC 4x250 mm analitik kolon kullanılarak gerçekleştirilmiştir. Elüent olarak 12 mM KOH çözeltisi kullanılmıştır. İnorganik anyonlar için tayin limitleri, 0.02 - 0.20 mg L^{-1} aralığında belirlenmiştir. Çay infüzyonları geleneksel olarak poşet çayların 200 mL hacimli kaynar ultra saf su ile demlenmesi yoluyla hazırlanmıştır. Söz konusu anyonların ekstraksiyonuna demleme süresinin etkisi incelenmiş ve elde edilen sonuçlara göre optimum demleme süresi 6 dakika olarak belirlenmiştir. Son olarak tarif edilen kromatografik yöntem bazı siyah, yeşil ve beyaz poşet çay örneklerindeki inorganik anyonların analizleri için uygulanmış ve beş adet anyon numunelerde nicel olarak belirlenmiştir. Tüm örneklerdeki nitrit ve bromür miktarları ise yöntemin tayin limitlerinin oldukça altında tespit edilmiştir.

Anahtar Kelimeler: İyon kromatografi, inorganik anyonlar, siyah çay, yeşil çay, beyaz çay

1. Introduction

Tea is a caffeine-containing non-alcoholic beverage and gaining extra popularity as an important "health drink" (Mondal et al., 2004). Its consumption is very popular in the world, second only to water. Especially tea bags are frequently used to prepare the beverage practically in our country. Tea bags are simply brewed in approximately 200 mL

*Corresponding Author: mustafa.cittan@cbu.edu.tr

of boiling water for 2-6 minutes in this preparation technique.

Black, green, and white teas are produced from the leaves of *Camellia sinensis*. The differences between them are due to the processing technique of the leaves during harvesting and manufacturing (Mincă et al., 2013). The chemical composition of tea is complex (Michalski, 2006; Yashin et al., 2015). The major chemical compounds present in tea are given in Table 1.

Table 1. Chemical composition of tea

Group	Compounds
Polyphenols	Catechins, flavanols, theaflavins, theagallins
Amino acids	Asparagine, alanine, serine, proline, histidine, theanine, phenylalanine
Organic acids	Acetic acid, citric acid, malic acid, succinic acid
Enzymes	Polyphenol oxidase, peroxidase
Pigments	Chlorophyll, carotenoids
Carbohydrates	Glucose, fructose, saccharose
Alkaloids	Caffeine, theophylline, theobromine
Vitamins	C, tocopherols, riboflavin
Metals	Al, Cu, Fe, Ni, Zn
Cations	Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}
Inorganic anions	F^-, Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}

The toxicity level of inorganic anions is vital for human and environmental health. For example, fluoride is a significant micronutrient in low concentrations, and become toxic in high concentrations. Excessive fluoride consumption induces symptoms of acute and chronic fluoride toxicity (fluorosis) (Jagadish and Shanmugaselvan, 2018). Therefore, it becomes very important to clarify the amount of fluoride accumulation in tea plants (Ma et al., 2002). Chloride is the key anion in the extracellular fluid, and it is responsible for muscle irritability. Phosphorus compounds carry, store, and release energy and assist

numerous enzymes and vitamins in extracting energy from nutrients. Nitrites and nitrates induce paralysation of the vasomotor centre, causing vasodilatation of small calibre vessels associated with hypotension and collapse (Mincă et al., 2013). Therefore, it is very important to monitor the inorganic anion contents in tea samples to maintain product quality.

Ion chromatography (IC) method is being widely used in food analysis for the simultaneous determination of many anions, mainly at trace concentration levels. There are several works on the quantitative analysis of some anions in tea infusions (Alcázar et al., 2003; Balcerzak and Janiszewska, 2015; Ding et al., 1997; Kumar et al., 2008; Michalski, 2006; Minca et al., 2015).

As a result of the importance of the quantification of inorganic anions in tea samples, nitrite, nitrate, phosphate, sulphate, chloride, fluoride, and bromide contents of the tea infusions prepared by using commercially available black, green and white tea bags were determined simultaneously by ion chromatographic technique in present study.

2. Material and Methods

2.1. Reagents

All reagents were of analytical reagent grade. Millipore Elix 5 Water Purification System was used to obtain ultra-pure water. Standard solutions of nitrite, nitrate, phosphate, sulphate, chloride, fluoride, and bromide at 1000 mg L^{-1} were purchased from Merck (Darmstadt, Germany). Potassium hydroxide was obtained from Sigma-Aldrich (St. Louis, MO, USA). Calibration solutions were prepared freshly by diluting the stock solutions with ultra-pure water.

2.2. Apparatus

Ion chromatographic analyses were conducted by using Dionex ICS-5000 system coupled with a Dionex ASRS 300 4 mm suppressed conductivity detector. Millipore Elix 5 Water Purification System was used to produce ultra-pure water.

2.3. Sample collection

Three brands of black tea bags, three brands of green tea bags, and two brands of white tea bags were purchased from local markets in Manisa, Turkey.

2.4. Sample preparation

The tea bags (one bag contains between 1.2 and 2.0 g of tea) were poured traditionally with 200 mL of boiling ultra-pure water and allowed to infuse for 6 min (optimum time detected in the study). The infusions were then cooled to room temperature and filtered through 0.45 μM pore size, nylon syringe filter (Millipore) prior to analysis.

2.5. Ion chromatography method

The specifications of the ion chromatographic technique followed were presented in Table 2.

Table 2. The specifications of the ion chromatographic method

Parameter	
Guard column	Dionex IonPac AG9-HC 4×50 mm
Analytical column	Dionex IonPac AS9-HC 4×250 mm
Eluent	12 mM KOH
Flow rate	1 mL min⁻¹
Injection volume	20 μL
Column temperature	30 °C
Detection	Conductivity

3. Results and Discussion

3.1. Linearity, sensitivity, and selectivity

Sequential standard solutions of inorganic anions were analyzed by IC, respectively. All calibration standards were prepared by diluting the standard solutions of inorganic anions with ultra-pure water just before use. The correlation coefficients (R^2) of the calibration equations were higher than 0.9988 for all anions as shown in Figure 1.
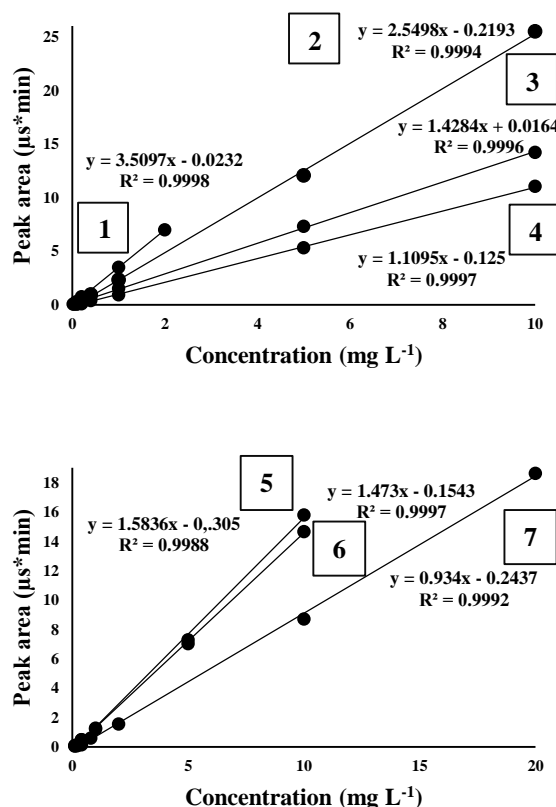

Figure 1. Calibration graphs of the inorganic anions. 1-7 represent the calibration curves of fluoride, chloride, nitrite, bromide, sulphate, nitrate, and phosphate, respectively.

The limit of quantifications (LOQs) were calculated by using the formula LOQ=10 S_a/b, where S_a is the standard deviation of the analytical response and b is the slope of the calibration equation (*Q2B Validation of*

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Analytical Procedures: Methodology | FDA, 1997). The linearity and the sensitivity properties were summarized in Table 3.

Although the tea infusions could contain some ionic compounds that respond to the conductivity detector such as anions of organic acids or other non-target anions, the selectivity of the analytical anion-exchange column was quite high. In other words, the selectivity of the analytical technique was provided by chromatographic separation of the anionic compounds. Therefore, there was no interference to the methodology with the determination of the target inorganic anions.

Table 3. Analytical features of the methodology

Analyte	R_t (min)	LOQ (mg L^{-1})	Linear range (mg L^{-1})	R-squared
Fluoride	4.097	0.02	LOQ-2.0	0.9998
Chloride	5.560	0.10	LOQ -10	0.9994
Nitrite	6.470	0.10	LOQ -10	0.9996
Bromide	7.514	0.10	LOQ -10	0.9997
Nitrate	8.297	0.10	LOQ -10	0.9997
Phosphate	13.110	0.20	LOQ -20	0.9992
Sulphate	19.750	0.10	LOQ -10	0.9988

R_t, retention time.

3.2. Effect of brewing time

In order to determine the change in the amount of inorganic anions in the infusions depending on the brewing time of the tea bags in boiling water, the amount of each anions in the extracts obtained by brewing a black tea bag sample for 2, 4, 6, and 8 minutes were determined. A graph was provided in Figure 2 indicating the total amount of inorganic anions determined versus brewing time of the tea bag. It was clear from the results that the total amount of anions in the tea infusions increased up to 6 min of brewing time. In addition, it remained nearly constant after 6 min. To this end, the infusion time was selected as 6 min.

3.3. Analysis of the tea samples

Inorganic anions in three brands of black and green tea samples, and two brands of white tea samples were quantified using the calibration equations of the anions as shown in Figure 1. The results obtained for the content of the determined anions in the tea infusions that of

Table 4. Contents of inorganic anions in tea samples

corresponding to dry mass (mg g^{-1}), were listed in Table 4. In addition, representative chromatograms obtained for a calibration standard and a black tea bag infusion were given together in Figure 3. The chromatographic technique provides well-defined peaks for all seven inorganic anions of interest in approximately 20 min. It was clear from the sample chromatograms obtained that the bromide and nitrite concentrations in the tea bag infusions were well below the quantification limits of the technique.
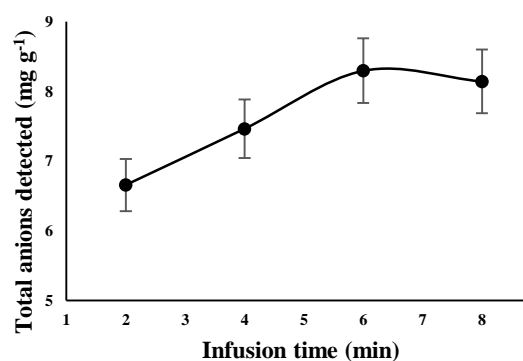

Figure 2. The influence of brewing time of tea bags on total anion content in the infusions.

| Sample	Inorganic anion content (mg g^{-1})						
	Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulphate
Black tea							
No.1	2.39±0.03	1.36±0.03	< LOQ	< LOQ	0.11±0.01	3.25±0.03	1.12±0.01
No.2	2.43±0.02	0.85±0.01	< LOQ	< LOQ	0.10±0.02	3.63±0.02	1.16±0.02
No.3	2.38±0.03	1.17±0.01	< LOQ	< LOQ	0.13±0.02	4.32±0.03	1.22±0.01
Green tea							
No.1	1.43±0.03	0.93±0.01	< LOQ	< LOQ	0.09±0.01	4.45±0.11	0.63±0.02
No.2	1.20±0.01	0.99±0.02	< LOQ	< LOQ	0.11±0.01	3.97±0.10	0.47±0.01
No.3	1.59±0.02	1.42±0.02	< LOQ	< LOQ	0.12±0.01	5.60±0.14	0.89±0.02
White tea							
No.1	1.83±0.10	0.45±0.02	< LOQ	< LOQ	0.09±0.01	3.80±0.34	0.67±0.01
No.2	0.58±0.03	0.20±0.01	< LOQ	< LOQ	0.11±0.02	1.17±0.06	0.56±0.01
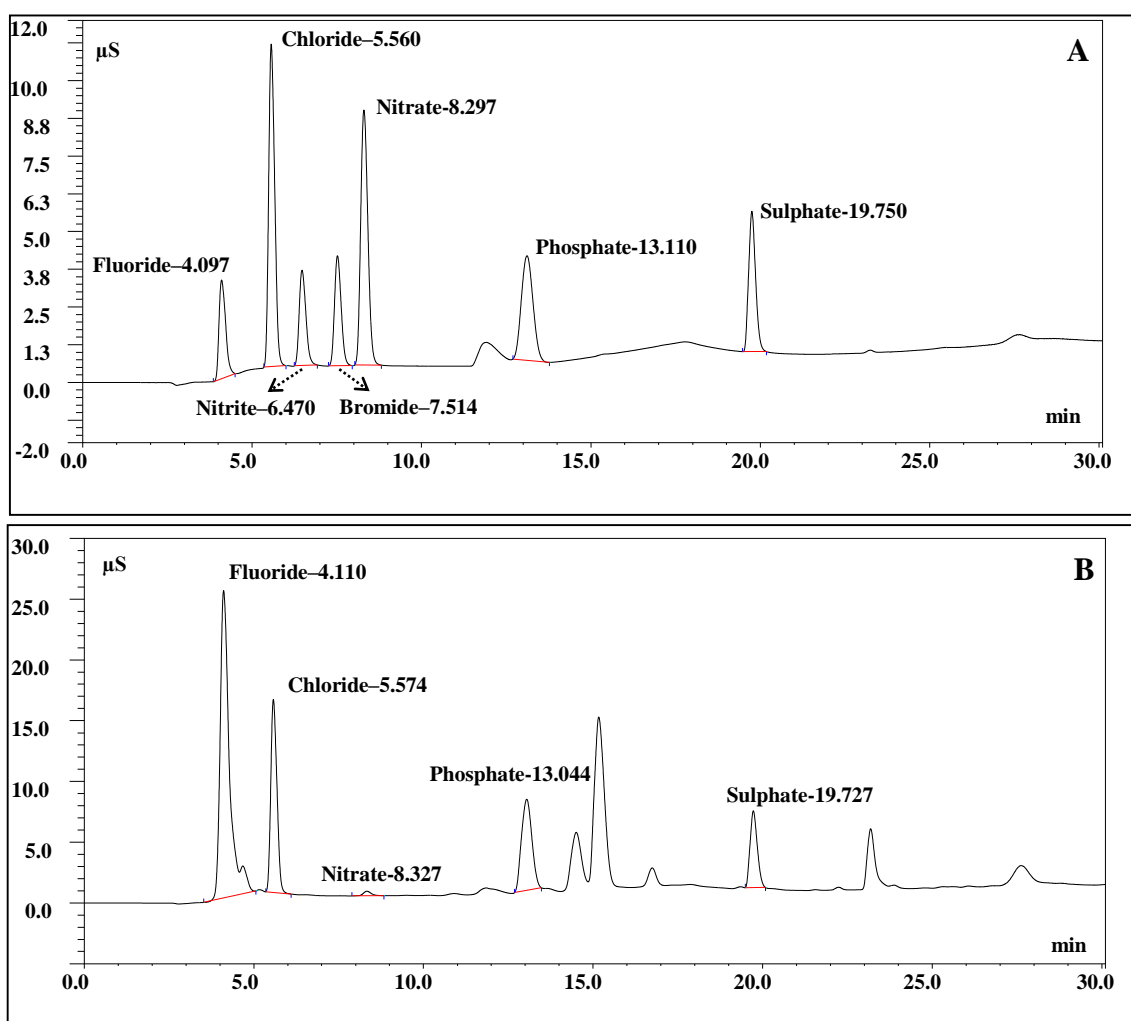
Figure 3. The representative chromatograms of (A) a calibration standard and (B) a black tea bag infusion.

Quantification of anionic amounts present in the set of tea bag samples studied showed variations for all anions: fluoride (ranging from 0.58 to 2.43 mg g^{-1}), chloride (ranging from 0.20 to 1.42 mg g^{-1}), nitrate (ranging from 0.09 to 0.13 mg g^{-1}), phosphate (ranging from 1.17 to 5.60 mg g^{-1}), and sulphate (ranging from 0.47 to 1.22 mg g^{-1}). The set of black and green tea bag samples were relatively homogeneous compared to the white tea bag samples in terms of the determined inorganic anions. However,

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significant variations were observed in the white tea samples set, especially in terms of fluoride, chloride, and phosphate amounts.

When the amount of inorganic anions in tea types were compared, the amount of fluoride present in the samples decreases in the order black > green > white and the amount of phosphate present in the samples decreases in the order green > black > white. While the highest sulphate content was obtained in black tea samples, there was no significant variation in sulphate content in white and green tea samples analyzed. On the other hand, the lowest amount of chloride was determined in white tea samples and no important variations were observed in black and green tea samples in terms of chloride contents. Finally,

approximately the same amount of nitrate was detected in all the tea samples examined.

Average amounts of inorganic anions for tea types obtained from present study were compared with several results available in the literature (Table 5). Table 5 indicated that some of the results obtained for the inorganic anion contents of tea samples were in agreement with the values obtained by earlier workers. On the other hand, there were some significant variances in anion contents of the samples between the present work and the literature data. This was attributed to the leaves harvesting, processing, and blending policies of the manufacturers and the origin of the soil in which the tea samples were grown.

Table 5. Comparison of the results obtained with previous data available in the literature

Work	Brewing time (min)	Tea type	Inorganic anion content (average results of the analyzed samples, mg g^{-1})						
			Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulphate
This work	6	Black	2.40	1.12	-	-	0.11	3.73	1.17
		Green	1.41	1.11	-	-	0.11	4.67	0.66
		White	1.21	0.32	-	-	0.10	2.49	0.61
Kumar et al., 2008	20	Black	1.20	3.12	-	0.04	0.34	0.08	4.20
Milovanovic et al., 2018	5-20	Green	1.47	0.37	-	-	0.18	1.92	1.28
Alcázar et al., 2003	-	Black	-	0.60	-	-	-	2.93	-
Ding et al., 1997	20	Green	-	1.78	-	-	-	7.88	4.58
Michalski, 2006	10-20	Black	14.86	11.19	-	-	6.56	23.14	34.33
Balcerzak and Janiszewska, 2015	5	Black	-	0.75	-	-	0.19	2.25	2.07
Spiro and Lam, 1995	-	Black	0.06	0.90	-	-	-	1.18	1.45
		Green	0.08	0.53	-	-	-	0.90	2.13

4. Conclusion

In present study, ion chromatography technique was used to analyze seven inorganic anions - fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulphate in commercially available black, green, and white tea bag samples. Tea infusions were

prepared traditionally by pouring the tea bags with 200 mL of boiling ultra-pure water. The optimum brewing time was determined as 6 minutes. Among inorganic anions, five of them were detected quantitatively in the tea infusions. The nitrite and bromide amount in all the samples were well below the quantification limit of the method. The

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method followed would be very useful for practical analysis of inorganic anions in tea infusions without any interferences and the results obtained from the study could contribute to the knowledge of inorganic anions in tea infusions that could be beneficial in human diet.

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