Journal of Chromatography B, 875 (2008) 419–426

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Application of ion chromatography for the determination of inorganic ions, especially thiocyanates in human saliva samples as biomarkers of environmental tobacco smoke exposure

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ARTICLE INFO

Article history:
Received 22 May 2008
Accepted 19 September 2008
Available online 25 September 2008

Keywords:
Environmental tobacco smoke (ETS)
Active and passive smokers
Thiocyanate
Ion chromatography (IC)
Human saliva

ABSTRACT

Environmental tobacco smoke is a major factor influencing the indoor air quality. Various toxic compounds emitted during tobacco smoking into the environment have a significant influence on the chemical composition of human biological fluids. The thiocyanate concentration in saliva is a biochemical measure, frequently used as an objective indicator of tobacco consumption. The goal of this study was to find significant relationships between salivary thiocyanates and other inorganic ions, which are constituents of natural saliva (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-}) and to present the effectiveness of the proposed sample preparation procedure combined with ion chromatography technique for the determination of inorganic ions in human saliva samples collected from passive, moderate and heavy smokers.

1. Introduction

Tobacco smoking constitutes a significant source of indoor air pollution in many types of closed spaces. Some research centers conduct studies on the tobacco burning process, the composition of tobacco smoke and on how it affects the bodies of active and passive smokers. The analysis of human biological fluids (urine, whole blood, blood serum and plasma, saliva, breast milk) can be a valuable source of information about the risk level for humans from environmental tobacco smoke.

One of the biomarkers of environmental tobacco smoke exposure is thiocyanate ions (cyanide metabolites). The thiocyanate ion is usually present in low concentrations in biological fluids as a result of digestion of cassava foods (mostly in the tropical countries in Africa, Asia, Latin America [1–4]), some vegetables of the genus *Brassica* containing glucosinolates (cabbage, turnip, kale) or by intake of thiocyanate-containing foods such as milk and cheese [5–8]. When absorbed from digesta to blood, the thiocyanate ion can be transferred to the milk or transformed by liver enzymes. Chronically elevated levels of thiocyanate can inhibit the uptake of iodine by the thyroid gland, thereby, reducing the formation of thyroxine. Thiocyanate is also introduced into humans as a drug in the treatment of thyroid conditions and arterial hypertension [9]. Higher concentration of this ion, which is a metabolic product of cyanide, arises from tobacco smoke. This reaction is catalysed by the enzyme rhodanese. The level of thiocyanate is thus considered a good probe for distinguishing between smokers and non-smokers and its determination is useful for the evaluation of smoking behaviour.

Some methods have been reported for individual determination of thiocyanates in biological fluids, such as: capillary electrophoresis [5,10], capillary isotachophoresis [9], spectrophotometry [11,12], atomic absorption spectrometry [13], cathodic stripping voltammetry [14], ion chromatography [15–19]. Analyses of such samples characterized by complex composition of the matrix represent a difficult analytical task and require special sample pretreatment in order to obtain successful separation of analytes under investigation.

Based on the published literature [15], urinary thiocyanate concentration levels were determined by means of ion exchange chromatography. It should be underlined that there is still a lack of a similar analytical approach for the determination of salivary thiocyanate concentration levels. Additionally, information regarding relationships between salivary thiocyanates and other inorganic ions, which are constituents of natural saliva (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-}) are also rather limited.

There are two main goals of analytical work described here:

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doi:10.1016/j.jchromb.2008.09.018

- to present the effectiveness of the proposed sample preparation procedure (as a novel approach) combined with ion chromatography technique for the determination of inorganic ions, especially thiocyanates in human saliva samples (as biomarkers of environmental tobacco smoke exposure), collected from passive, moderate and heavy smokers;
- to check whether tobacco smoking has a significant impact on the chemical composition of natural saliva.

2. Materials and methods

2.1. Chromatographic conditions

Chromatographic separations were performed using ion chromatograph Dionex DX500 (Dionex, Sunnyvale, CA, USA) comprised of a GP50 gradient pump and a CD20 conductivity detector. Anions were determined on an IonPac AS9-HC (2 mm × 250 mm) anion exchange column using 9.0 mM Na_2CO_3 eluent at 0.25 ml/min and suppressed conductivity detection with ASRC®-ULTRA suppressor (2 mm) in recycle mode, 50 mA. Cations were determined on an IonPac CS14 (2 mm × 250 mm) cation exchange column using 4 mM methanesulfonic acid, 5.45 mM pyrophosphoric acid eluent at 0.25 ml/min and suppressed conductivity detection with CSRS®-ULTRA suppressor (2 mm) in recycle mode, 50 mA. The chromatographic separation was obtained within 42 min for anions and 18 min for cations. The sample injection volume was 7.5 μl for anions and 2.5 μl for cations.

2.2. Chemicals

All standards (Mg^{2+}, Ca^{2+}, NH_4^+, Na^+, K^+, SCN^-, SO_4^{2-}, NO_2^-, NO_3^-, F^-, Cl^-, Br^-, PO_4^{3-} 1000 mg/l), pyrophosphoric acid and anhydrous sodium carbonate were obtained from Merck (Darmstadt, Germany). Methanesulfonic acid was purchased from Sigma–Aldrich (Schnelldorf, Germany). Deionized water was obtained from a Millipore Gradient A10 (resistivity: 18.2 MΩ cm at 25 °C) water purification system (Millipore, Bedford, MA, USA).

2.3. Analytical procedure

Human saliva samples were collected in sterile glass bottles and transported immediately to the laboratory for analysis. In Fig. 1 schematic presentation of the analytical protocol is shown.

Metrological characteristics of analytical procedure used in the studies of human saliva samples are presented in Table 1. Seven replicate injections of each standard at seven concentration levels over the range of 0.2–20 mg/l were performed. The peak areas of the standards were plotted against the concentration and the linearity was evaluated by linear regression analysis. Limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio, using the following equations:

$$\mathrm{LOD} = \frac{3.3\sigma}{S} \quad (1)$$

$$\mathrm{LOQ} = \frac{10\sigma}{S} \quad (2)$$

where σ is the standard deviation of the response of the blank and S is the slope of the calibration curve.

Ion chromatography technique was previously applied for the determination of selected ions in human urine samples collected from active, moderate and passive smokers [15]. In Table 2 a comparison between validation parameters for ion chromatography technique used in the studies of human urine samples (literature data [15]) and human saliva samples (experimental data obtained in the presented research) was shown.

Table 1
Metrological characteristics of analytical procedure used in the studies of human saliva samples

Analyte	LOD[a] (× 10^{-5} mg/g)	LOQ[a] (× 10^{-5} mg/g)	Expanded uncertainty (%)
Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}	0.93	2.79	
SCN^-	1.85	5.55	
F^-, Cl^-, Br^-, SO_4^{2-}, NO_3^-	0.93	2.79	10
NO_2^-	4.63	13.9	
PO_4^{3-}	3.70	11.1	

[a] Calculated on the basis of experimental value of average salivary density = 1.08 g/cm^3.

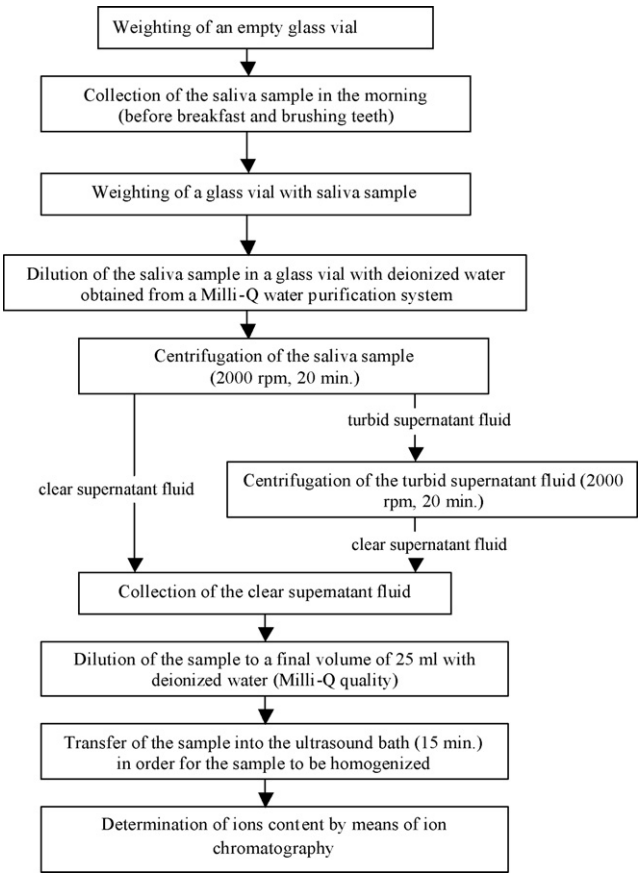


Fig. 1. Schematic presentation of the analytical protocol used at the step of saliva samples preparation for analysis.

3. Results and discussion

Saliva samples were collected from a selected population of passive, moderate and heavy smokers in order to demonstrate the effectiveness of this method as a means of evaluating smoking behaviour. The subjects were classified into the following groups by using their responses from the smoking habits questionnaire. The ranges of average analyte concentrations determined in saliva samples collected from heavy, moderate and passive smokers are presented in Table 3.

In Table 4, a comparison between the concentration levels of ions present in natural saliva (literature data [20]) with those determined in saliva samples collected from active and passive smokers (experimental data) is shown.

Table 2
A comparison between validation parameters for ion chromatography technique used in the studies of human urine samples (literature data [15]) and human saliva samples (experimental data obtained in the presented research)

	Precision CV (%)												
	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	SCN^-	F^-	Cl^-	Br^-	SO_4^{2-}	NO_2^-	NO_3^-	PO_4^{3-}
Urine (literature data [15])	–	–	–	–	–	1.10	–	–	–	–	0.87	1.44	–
Saliva (experimental data)	0.95	1.12	0.98	1.21	0.98	1.07	1.11	0.86	1.25	0.79	1.72	0.92	1.44
	Linearity (correlation coefficient R^2)												
	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	SCN^-	F^-	Cl^-	Br^-	SO_4^{2-}	NO_2^-	NO_3^-	PO_4^{3-}
Urine (literature data [15])[a]	–	–	–	–	–	>0.999	–	–	–	–	>0.999	>0.999	–
Saliva (experimental data)[b]	0.9994	0.9870	0.9994	0.9920	0.990	0.9980	0.9992	0.9993	0.9890	0.9994	0.9880	0.9970	0.9900
	LOD (mg/l)												
	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	SCN^-	F^-	Cl^-	Br^-	SO_4^{2-}	NO_2^-	NO_3^-	PO_4^{3-}
Urine (literature data [15])	–	–	–	–	–	0.02	–	–	–	–	0.02	0.04	–
Saliva (experimental data)	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.05	0.01	0.04
	LOQ (mg/l)												
	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	SCN^-	F^-	Cl^-	Br^-	SO_4^{2-}	NO_2^-	NO_3^-	PO_4^{3-}
Urine (literature data [15])	–	–	–	–	–	0.06	–	–	–	–	0.06	0.12	–
Saliva (experimental data)	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.15	0.03	0.12

[a] Linearity determined over the concentration range of 0.5–10 mg/l.
[b] Linearity determined over the concentration range of 0.2–20 mg/l.

Table 3
The ranges of average analyte concentrations determined in saliva samples collected from heavy, moderate and passive smokers (each sample was injected in triplicate)

Analyte (mg/g)	Type of the smoker		
	Heavy (more than 10 cigarettes smoked per day)	Moderate (1–10 cigarettes smoked per day)	Passive
Na^+	0.03–2.02	0.05–2.02	0.03–0.38
NH_4^+	0.008–0.979	0.067–0.381	0.1–0.5
K^+	0.24–3.51	0.48–3.51	0.53–1.18
Mg^{2+}	0.001–0.081	0.003–0.145	0.0001–0.0311
Ca^{2+}	0.02–0.15	0.04–0.21	0.03–0.11
SCN^-	**0.015–0.847**	**0.046–0.700**	**0.046–0.277**
F^-	0.001–0.141	0.003–0.342	0.0003–0.0864
Cl^-	0.23–3.91	0.11–1.04	0.20–1.20
Br^-	0.001–0.053	0.003–0.030	0.001–0.006
NO_2^-	0.009–0.279	0.021–0.983	0.03–0.12
NO_3^-	0.001–0.051	0.002–0.031	0.002–0.019
PO_4^{3-}	0.09–2.84	0.004–1.510	0.32–1.32
SO_4^{2-}	0.007–0.638	0.03–0.37	0.02–0.31

The increased concentration of individual ions (except for phosphate ions) in a group of both heavy and moderate smokers was noticed. The fact that such relationships exist can be treated as a proof that tobacco smoking has a significant impact on the chemical composition of natural saliva.

3.1. Thiocyanate ions as biomarkers of tobacco smoke exposure

The representative chromatograms obtained from the analysis of saliva samples collected from passive and heavy smokers are presented in Fig. 2. In Figs. 3–5 the average concentrations of

Table 4
A comparison between the concentration levels of ions present in natural saliva (literature data [20]) with those determined in saliva samples collected from active and passive smokers (experimental data)

Literature data		Experimental data		
Chemical ionic composition of natural saliva	Concentration levels of ions present in natural saliva (mg/g)	Type of the smoker		
		Heavy (more than 10 cigarettes smoked per day)	Moderate (1–10 cigarettes smoked per day)	Passive
Na^+	0.04–0.45	+	+	–
K^+	0.36–1.30	+	+	–
Mg^{2+}	0.02–0.11	–	+	–
Ca^{2+}	0.04–0.10	+	+	+
Cl^-	0.16–1.31	+	–	–
PO_4^{3-}	0.12–3.43	–	–	–

Applied abbreviations: "+" the increased concentration of analyte determined in saliva samples collected from a given group of smokers in comparison with its concentration in natural saliva was observed;"–" the increased concentration of analyte determined in saliva samples collected from a given group of smokers in comparison with its concentration in natural saliva was not observed.

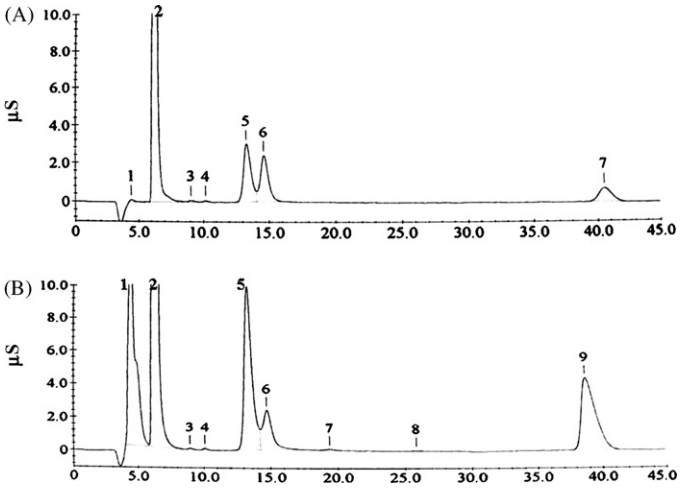


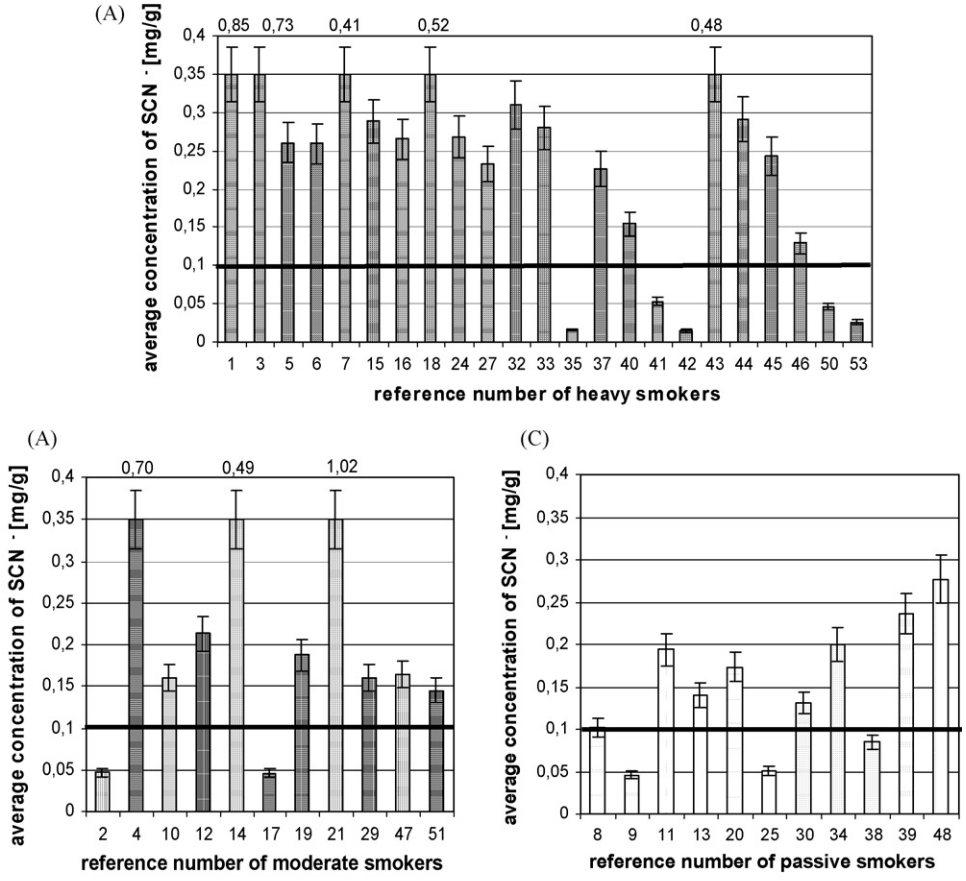
Fig. 2. The representative chromatogram obtained during the analysis of saliva samples collected from: (A) passive smokers, the peak numbered 2 (chloride ion), 5 (phosphate ion), 6 (sulfate ion), 7 (thiocyanate ion-biomarker of environmental tobacco smoke exposure); (B) heavy smokers, the peak numbered 1 (fluoride ion), 2 (chloride ion), 5 (phosphate ion), 6 (sulfate ion), 9 (thiocyanate ion-biomarker of environmental tobacco smoke exposure).

thiocyanate, nitrate and calcium ions determined in saliva samples corresponding to the different tobacco consumption categories established from the self-reports are shown. As tobacco consumption increased, a general increase in the SCN^-, NO_3^-, Ca^{2+} concentration values was observed.

The average concentration level of SCN^-, NO_3^-, Ca^{2+} determined in saliva samples collected from a group of non-smoking subjects (control group) was 0.1, 0.01 and 0.03 mg/g, respectively. The percentage contribution of saliva samples, in which concentration levels of thiocyanate, nitrate and calcium ions were determined above the value obtained for the control group is presented in Fig. 6.

The strong influence of both mainstream and sidestream tobacco smoke was observed in the case of SCN^- and Ca^{2+} ions. However, the greatest impact on the content of NO_3^- in human saliva samples had mainstream tobacco smoke (in a smaller measure sidestream tobacco smoke). Additionally, a comparison between concentration level of selected ions determined in human saliva samples (research results described above) and human urine samples (literature data obtained by other group of scientists [15]) is presented in Table 5.

It was noticed that higher concentration levels of SCN^- were determined in human saliva samples, when compared with urine ones, whereas the opposite situation was observed for NO_3^- ion.



The average concentration level of SCN^- determined in saliva samples collected from non-smoking subjects, which were not exposed to the influence of environmental tobacco smoke at home or in the workplaces (experimental data) was marked in the figures by a **horizontal black line**.

Fig. 3. Average thiocyanate concentration values determined in saliva samples collected from: (A) heavy smokers, (B) moderate smokers and (C) passive smokers. Each sample was injected in triplicate.

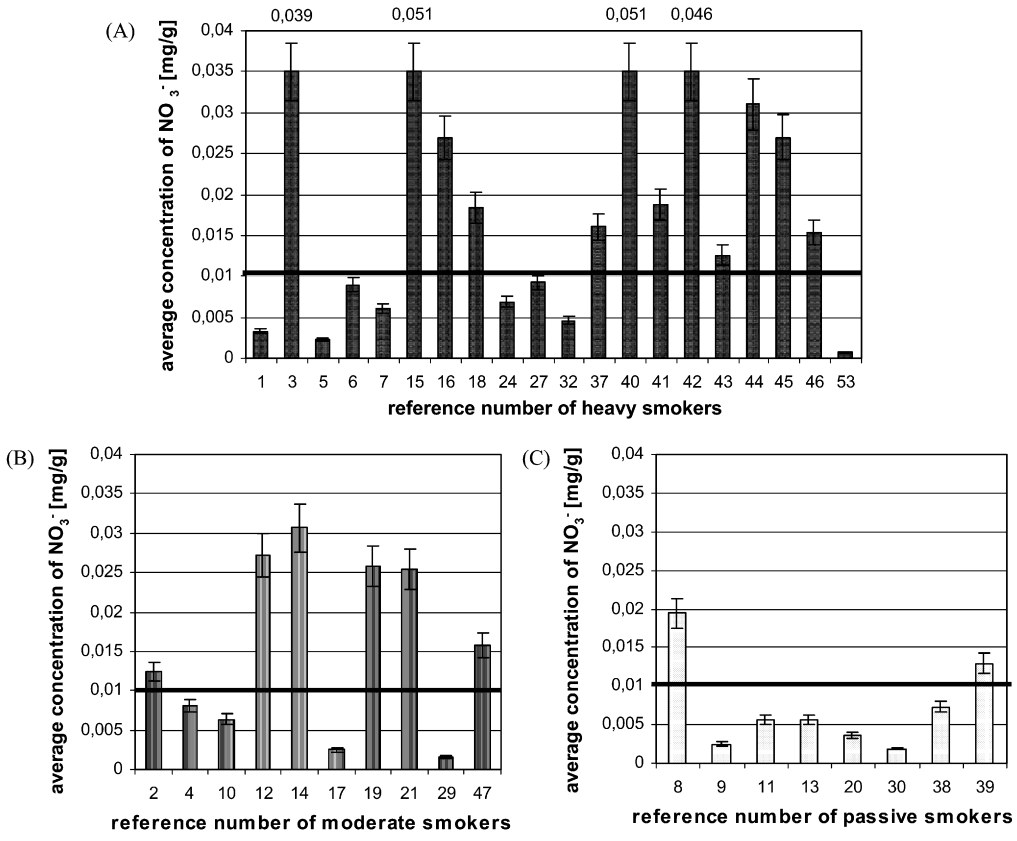


Fig. 4. Average nitrate concentration values determined in saliva samples collected from: (A) heavy smokers, (B) moderate smokers and (C) passive smokers. Each sample was injected in triplicate.

3.2. Correlations between ions under investigation determined in saliva samples collected from active and passive smokers

In order to prove the influence of tobacco smoking on the type and concentration of individual ions determined in saliva samples collected from active and passive smokers, the relationships between these analytes were calculated. The obtained results in the form of correlation matrices for ion pairs are presented in Table 6.

For the whole population of saliva samples collected from heavy, moderate and passive smokers, it is characteristic that thiocyanates strongly correlate with one or even more ions present in natural saliva. The greatest number of the strongest correlations between thiocyanate ions and components in natural saliva, such as K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-} were observed for samples collected from heavy smokers (Cl^-/SCN^- (0.78), PO_4^{3-}/SCN^- (0.90), SCN^-/K^+ (0.80), SCN^-/Ca^{2+} (0.59), SCN^-/Mg^{2+} (0.67)). Such relationships were noticed only for SCN^-/Cl^- (0.60) and SCN^-/K^+ (0.72) for samples collected from moderate smokers and for SCN^-/Cl^- (0.67), SCN^-/PO_4^{3-} (0.66) and SCN^-/Ca^{2+} (0.55) for samples collected from passive smokers.

The greatest number of the strongest correlation coefficients (observed for a group of heavy smokers) between thiocyanate ions (biomarkers of environmental tobacco smoke exposure) and almost all of the ions present in natural saliva (Cl^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}) confirmed the fact that tobacco smoking has an important influence on the chemical composition of this biological fluid.

Table 5
A comparison between concentration level of selected ions determined in human saliva samples (research made within the framework of this scientific project) and human urine samples (literature data [15])

	SCN^- (mg/g)			NO_3^- (mg/g)			Total run time (min)
	Passive smokers	Moderate smokers	Heavy smokers	Passive smokers	Moderate smokers	Heavy smokers	
Urine[a] (literature data [15])	0.0065	0.014	0.030	0.14	0.20	0.22	10
Saliva[b] (experimental data obtained in the presented research)	0.16	0.37	0.43	0.010	0.016	0.026	42

[a] Calculated on the basis of water density = 1 g/cm^3.
[b] Calculated on the basis of experimental value of average salivary density = 1.08 g/cm^3.

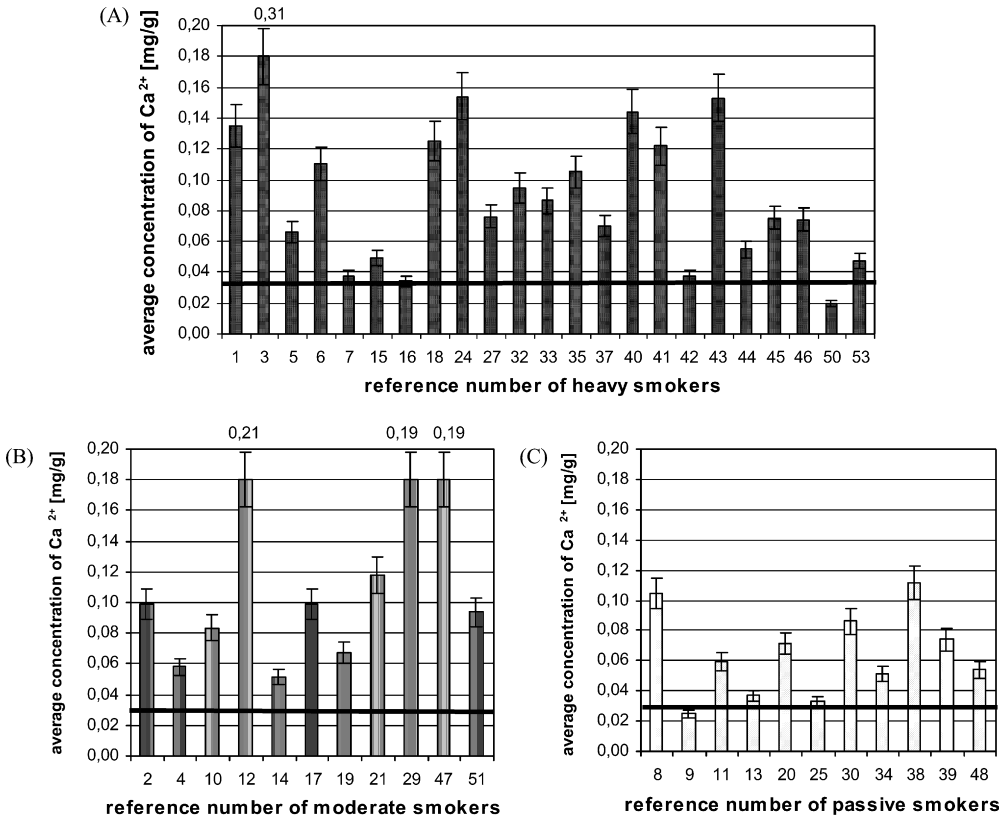


Fig. 5. Average calcium concentration values determined in saliva samples collected from:(A) heavy smokers, (B) moderate smokers and (C) passive smokers. Each sample was injected in triplicate.

3.3. Statistical ANOVA test application

In order to compare the concentration levels of ions present in saliva samples collected from heavy and passive smokers, the statistical ANOVA test was conducted. The null hypothesis that average concentration values are equal ($\mu_1 = \mu_2$) against the alternative hypothesis that average concentration values are different ($\mu_1 \neq \mu_2$) in two investigated groups (heavy and passive smokers) was verified. All necessary calculations were made on the basis of the appropriate mathematical model.

If the calculated F_{sample} value is greater than the critical value of F, then the null hypothesis was rejected in favour of the alternative one at the 95% confidence level. In this case, the analyte concentration levels differed statistically significantly in two investigated groups of smokers. If the calculated F_{sample} value is lower than the critical value of F, then the null hypothesis was not rejected in favour of the alternative one at the 95% confidence level. In this case, the analyte concentration levels did not differ statistically significantly in two investigated groups of smokers. The calculated F_{sample} values for analytes determined in saliva samples collected from heavy and passive smokers are presented in Table 7.

Taking into consideration the values of appropriate statistical parameters calculated on the basis of ANOVA test, it was noticed that SCN^- (biomarkers of environmental tobacco smoke exposure) and NO_3^- ions differed statistically significantly in two investigated

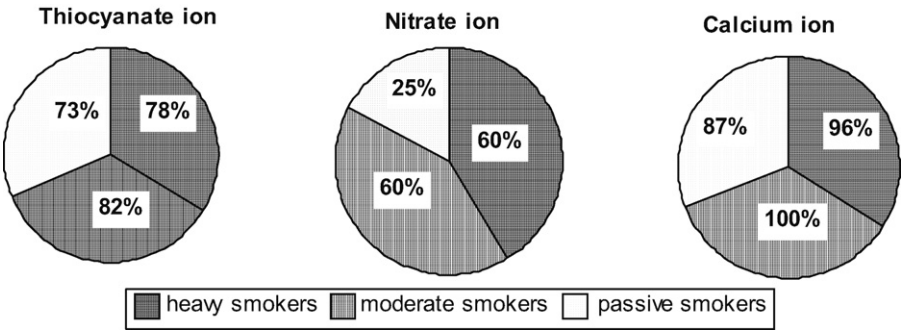


Fig. 6. Percentage contribution of saliva samples, in which concentration levels of thiocyanate, nitrate and calcium ions were determined above the value obtained for the control group.

Table 6
Correlation matrices for ion pairs[a]

	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	SCN^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+
Heavy smokers											
F^-	1.00										
Cl^-	0.24	1.00									
NO_3^-	−0.22	0.24	1.00								
PO_4^{3-}	0.34	**0.91**	0.03	1.00							
SO_4^{2-}	−0.21	0.34	0.48	0.17	1.00						
SCN^-	0.34	**0.78**	−0.14	**0.90**	0.20	1.00					
Na^+	0.24	**0.76**	0.36	**0.56**	0.28	0.33	1.00				
K^+	0.14	**0.88**	0.15	**0.94**	0.33	**0.80**	**0.53**	1.00			
Ca^{2+}	0.16	**0.87**	0.28	**0.70**	0.50	**0.59**	**0.73**	**0.72**	1.00		
Mg^{2+}	0.13	**0.93**	0.18	**0.83**	0.40	**0.67**	**0.76**	**0.84**	**0.89**	1.00	
NH_4^+	0.03	**0.89**	0.24	**0.76**	**0.57**	**0.62**	**0.72**	**0.79**	**0.90**	**0.96**	1.00
Moderate smokers											
F^-	1.00										
Cl^-	0.26	1.00									
NO_3^-	−0.45	0.43	1.00								
PO_4^{3-}	0.13	0.41	**0.53**	1.00							
SO_4^{2-}	−0.27	**−0.77**	**−0.54**	**−0.69**	1.00						
SCN^-	0.18	**0.60**	**0.71**	0.47	−0.50	1.00					
Na^+	**0.84**	0.25	**−0.60**	0.13	−0.34	−0.23	1.00				
K^+	0.28	**0.53**	0.47	**0.85**	−0.50	**0.72**	0.06	1.00			
Ca^{2+}	0.35	−0.28	−0.28	**0.59**	−0.14	−0.27	0.50	0.33	1.00		
Mg^{2+}	−0.15	**−0.77**	**−0.61**	**−0.70**	**0.99**	−0.49	−0.25	−0.49	−0.10	1.00	
NH_4^+	**−0.79**	−0.37	0.48	0.31	0.23	−0.01	**−0.76**	0.19	0.14	0.14	1.00
Passive smokers											
F^-	1.00										
Cl^-	−0.37	1.00									
NO_3^-	−0.41	0.09	1.00								
PO_4^{3-}	**−0.77**	**0.60**	**0.60**	1.00							
SO_4^{2-}	**−0.59**	0.13	0.23	0.12	1.00						
SCN^-	−0.26	**0.67**	−0.08	**0.66**	−0.40	1.00					
Na^+	**−0.71**	**0.69**	0.21	**0.54**	**0.78**	0.23	1.00				
K^+	−0.43	**0.87**	0.39	**0.66**	0.34	0.48	**0.79**	1.00			
Ca^{2+}	−0.03	−0.08	0.40	−0.12	**0.70**	**−0.55**	0.42	0.34	1.00		
Mg^{2+}	−0.29	0.30	**0.86**	0.48	0.41	−0.04	0.47	**0.62**	**0.67**	1.00	
NH_4^+	0.16	−0.24	0.28	−0.32	0.46	**−0.71**	0.11	0.15	**0.80**	0.35	1.00

[a] Positive and negative correlation coefficients greater than 0.50 were indicated in bold.

groups of smokers. The obtained results confirmed the influence of the toxic constituents of environmental tobacco smoke on the content of ions in human saliva samples.

3.4. Clinical consequences of the concentration levels of selected inorganic ions present in human saliva samples

Salivary thiocyanate may have the antibacterial role in the mouth, decreasing the corrosion potential of amalgams [9,21]. In leukemia patients receiving chemotherapy treatment with cytotoxic agents resulted in granulocytopenia and a concomitant decrease in the thiocyanate concentration in saliva. The function of salivary peroxidase system is impaired by the decrease in thiocyanate concentration, which may be a contributing factor to some oral complications that occur in patients undergoing chemotherapy [9,22].

For various reasons nitrate and nitrite are considered to be important in relation to the human health. Nitrite ion, which in excessive amounts may induce methaemoglobinaemia in infants and form carcinogenic nitrosamines when present with secondary or tertiary amines and amides, is also present in saliva at lower levels [9,23].

Table 7
The calculated F_{sample} values for analytes determined in saliva samples collected from heavy and passive smokers[a]

Analyte	Calculated F_{sample} value	Critical value of F	Verification of the null hypothesis against the alternative one
SCN^-	**4.26**	**4.14**	$\mu_1 \neq \mu_2$
F^-	0.02	4.20	
Cl^-	0.49	4.14	$\mu_1 = \mu_2$
NO_2^-	0.12	4.54	
Br^-	3.88	4.26	
NO_3^-	**4.38**	**4.21**	$\boldsymbol{\mu_1 \neq \mu_2}$
SO_4^{2-}	3.07	4.14	
PO_4^{3-}	0.05	4.16	
Na^+	0.77	4.14	
K^+	0.76	4.14	$\mu_1 = \mu_2$
Ca^{2+}	2.94	4.14	
Mg^{2+}	0.17	4.38	
NH_4^+	0.008	4.14	

[a] SCN^- and NO_3^- ions were marked in bold, because they differed statistically significantly in two investigated groups of smokers.

4. Conclusions

A new successful approach for human saliva sample preparation was presented in this research. The proposed sample preparation procedure combined with ion chromatography technique is undoubtedly a valuable tool for studies of concentration level of a wide spectrum of inorganic ions in human saliva samples. Validation parameters for ion chromatography technique used in the studies of human urine samples (literature data [15]) and human saliva samples (experimental data obtained in this research) were compared.

Taking the obtained results into consideration, a strong influence of mainstream and sidestream tobacco smoke on the content of inorganic ions, especially thiocyanates (as biomarkers of environmental tobacco smoke exposure), was observed. In a group of heavy and moderate smokers, the concentration levels of almost all ions were determined above the value obtained for the control group (non-smoking subjects). The greatest number of the strongest correlations between thiocyanates (biomarkers of environmental tobacco smoke exposure) and other inorganic ions can be recognized as a proof that tobacco smoking has an important impact on the chemical composition of natural saliva. The obtained results can be treated as a source of information on the influence of intensity of smoking on the concentration of these constituents in saliva samples.

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