Talanta 96 (2012) 107–112

Contents lists available at SciVerse ScienceDirect

Talanta

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

Application of DV-SIA manifold for determination of thiocyanate ions in human saliva samples

Carolina Cecilia Acebal[a,b,*], Hana Sklenářová[a], Jana Škrlíková[a,c], Ivana Šrámková[a], Vasil Andruch[c], Ioseph S. Balogh[d], Petr Solich[a]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic
[b] INQUISUR (UNS-CONICET), Universidad Nacional del Sur, Bahía Blanca, Argentina
[c] Department of Analytical Chemistry, Faculty of Science, University of Pavol Jozef Šafárik, Košice, Slovak Republic
[d] Department of Chemistry, College of Nyíregyháza, HU-4400 Nyíregyháza, Hungary

ARTICLE INFO

Article history:
Available online 14 January 2012

Keywords:
DV-SIA
Thiocyanate
Extraction
Biological samples

ABSTRACT

An automated, simple and inexpensive double-valve sequential injection analysis (DV-SIA) spectrophotometic method with online liquid–liquid extraction, for the determination of thiocyanate has been developed. The method has been based on the formation of an ion associate between thiocyanate and Astra Phloxine in acidic medium, and the subsequent extraction with amylacetate. The absorbance of the extracted ion associate was measured at 550 nm.

The calibration function was linear in the range 0.05–0.50 mmol L^{-1} and the regression equation was $A = (1.887 \pm 0.053)[SCN^-\ mmol\ L^{-1}] + (0.037 \pm 0.014)$ with a correlation coefficient of 0.995. The precision of the proposed method was evaluated by the relative standard deviation (RSD) values at two concentration levels: 0.20 and 0.50 mmol L^{-1}. The obtained results were 1.0 and 2.8%, respectively, for the intra-day precision, and 4.2 and 3.8%, respectively for the inter-day precision. The calculated detection limit was 0.02 mmol L^{-1}.

The developed method has been successfully applied for determining thiocyanate ions in human saliva samples.

1. Introduction

Thiocyanate is usually present in human saliva in low amounts, but its concentration could be increased as the result of the digestion of glucosinolate-containing vegetables such as cabbage, turnip, tomato, or by the intake of thiocyanate-containing food such as milk and cheese [1,2]. It is also present in drugs that are used in the treatment of thyroid problems and arterial hypertension. Another important source of this ion arises from tobacco smoke, because thiocyanate is the main metabolic product of cyanide.

Though not as toxic as cyanides, thiocyanate in chronically increased levels can be harmful for life and its determination is of great interest [3,4].

A considerable number of methods for monitoring the level of thiocyanate are available in the literature in which different techniques were applied including amperometry [5], fluorimetry [6] and flame-atomic absoption spectrometry [7]. Several thiocyanate-selective electrodes have been reported over the last years [8,9]. Separation techniques such as electrophoresis and chromatography were applied to thiocyanate determination in biological samples [1,10].

Although some of these techniques have low detection limits and a very good selectivity along with the ability to perform multielemental analysis, spectrophotometry is still very popular because of its speed, simplicity and instrumentation availability. Many spectrophotometric methods have been developed to determine thiocyanate based on the Stugart reaction [11], on the well known Koning reaction [12] and on the quantitative oxidation of thiocyanate using permanganate [13]. Thiocyanate also forms colored complexes with copper and 2,2-dipyridyl-2-quinolylhydrazone that are extractable with chloroform [14], and with the ion-pairing reagent 1-(3,5-diamino-6-chloropyrazinecarboxyl) guanidine hydrochloride monohydrate that are extracted with 4-methyl-2-pentanone [15]. Another method that has been published was the reaction of the analyte with chloramine-T in the presence of iron (III) chloride as catalyst to give cyanogen chloride, which reacts with a mixture of γ-picoline (4-methylpyridine) and barbituric acid to form a soluble violet-blue product [16].

Thiocyanate ions interact with a poymethine dye, 1,3,3-trimethyl-2-[3-(1,3,3-trimethyl-1,3-*H*-indol-2-ylidene)propenyl]-3*H*-indolium chloride (Astra Phloxine, AP) to form an ion associate

* Corresponding author at: INQUISUR (UNS-CONICET), Universidad Nacional del Sur, Bahía Blanca, Argentina. Tel.: +54 291 4595100; fax: +54 291 4595159.
E-mail address: cacebal@uns.edu.ar (C.C. Acebal).

0039-9140/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2012.01.021

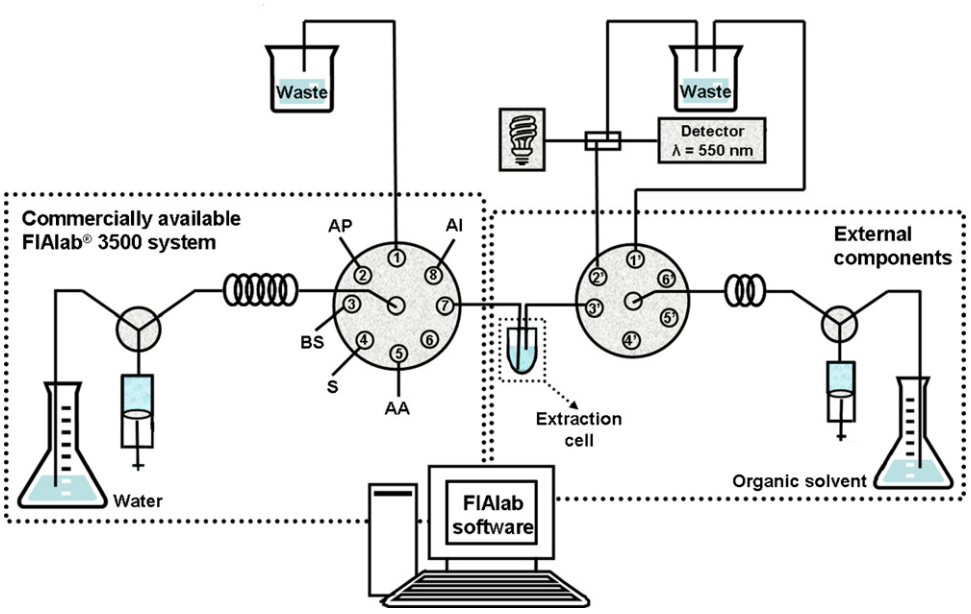

Fig. 1. Schematic view of the DV-SIA manifold for online liquid–liquid extraction and spectrophotometric determination of thiocyanate. AP, Astra Phloxine; BS, buffer solution; S, sample; AA, amylacetate; AI, air input.

in acidic medium. In general, the merits of polymethine dyes over other classes of analytical reagents include the stability of their solutions over time and their high values of absorbance [17]. AP has been applied in our laboratory as reagent for the determination of various analytes [18–20] but, to the best of our knowledge, it has not been previously employed in thiocyanate determination.

In spite of the different techniques that have been employed to determine SCN^-, often the pre-treatment of the sample is required. One of the most commonly and widely used sample pre-treatment techniques in analytical chemistry is liquid–liquid extraction (LLE), due to its simplicity, flexibility, selectivity and, in some cases, suitability to achieve the analyte preconcentration. Likewise other sample manipulation, the manual implementation of this technique introduces errors that affect the accuracy and precision of results. The application of the automation and miniaturization to perform LLE contributes greatly to improve the quality of the results and leads to significant reduction in solvent consumption and an inherent decrease in waste generation. In addition, the risks for the operator and sample contamination are minimized and the sampling throughput is significantly enhanced. The search for new and improved methods are thus still of great interest in analytical chemistry and has been the focus of many recent developments. In fact, several articles were published dealing with the use of LLE in flow systems [21].

A simple, user-friendly and universal dual-valve sequential injection (DV-SIA) system with the online incorporation of LLE cell into the SIA manifold has been designed [22,23]. The design of the SIA manifold was based on the separation of extraction and detection units, avoiding some common problems in such kind of flow systems due to the different affinity of the organic and aqueous phase to the walls of the PTFE tubing, and bubble formation. One of its main advantages is the variability of samples and organic solvents that could be used and analyzed in the same system. Furthermore, the system was constructed only with commercially available components, which makes it accessible and easy to be assembled in any other laboratory.

In this work, the DV-SIA system was employed to the spectrophotometric determination of thiocyanate ions. The reaction between the analyte and AP, the extraction of the formed ion associate and the subsequent determination was carried out on-line. The automated method was successfully applied for the determination of SCN^- in saliva samples.

2. Experimental

2.1. Reagents

Solutions were prepared using analytical grade reagents and ultra pure water Millipore Milli-Q RG (Millipore, USA). Toluene 99.8% (HPLC grade, Fluka, Germany) and Amylacetate ≥99% (Sigma Aldrich, Germany) were used as organic solvents.

A 1.0×10^{-2} mol L^{-1} Astra Phloxine (AP) (Jiacheng-Chem Enterprise Ltd., China) solution was prepared by dissolving 0.1963 g of the dye in 0.5 mL of methanol and filled up with water to 50.0 mL. A 2.0×10^{-3} mol L^{-1} solution was prepared by diluting 5.0 mL of this solution to 25.0 mL with water.

A 1.0×10^{-2} mol L^{-1} NH_4SCN stock solution was prepared by dissolving 0.0381 g of ammonium thiocyanate purum p.a. (Chemapol, Czech Republic) in 50.0 mL of water. The working solutions were prepared by appropriate dilution of the stock solution with water.

The pH of the medium was adjusted with an ammonium acetate buffer solution, pH 3.0. The buffer solution was prepared by mixing 49.65 mL of a 1.0 mol L^{-1} CH_3COOH solution with 0.35 mL of a 1.0 mol L^{-1} NH_4OH solution.

2.2. Apparatus

The DV-SIA system divided into two parts (extraction and detection part) is depicted in Fig. 1. A commercially available FIAlab® 3500 system (FIAlab® Instrument Systems Inc., Bellevue, USA) consisting of a syringe pump (syringe volume 5 mL) and a central eight-port Cheminert selection valve belongs to the extraction part.

The central port of the selection valve was connected to a PTFE holding coil (0.75 mm i.d. and 150 cm length). The extraction cell consisted of a 1.5 mL polypropylene tube connected to both extraction and detection parts of the system.

External six-port Cheminert selection valve (Valco Instrument Co., Houston, USA), a holding coil 34 cm in length and an

Table 1
SIA procedure for the extraction and determination of thiocyanate ions in DV-SIA.

Step	Port selection	Flow rate ($\mu L\,s^{-1}$)	Operation	Description
1	–	200	Aspirate 600 μL	Syringe pump in the valve in position: aspiration of carrier
2	8	50	Aspirate 10 s	Syringe pump in the valve out position. Aspiration of air
3	2	50	Aspirate 50 μL	Aspiration of AP (2×10^{-3} M)
4	3	50	Aspirate 10 μL	Aspiration of buffer solution pH 3
5	4	50	Aspirate 50 μL	Aspiration of standard solutions/samples
6	5	80	Aspirate 350 μL	Aspiration of amylacetate
7	7	120	Empty	Empty syringe pump into the extraction cell for the extraction and self-separation of phases
8	–	100	Aspirate 600 μL	External syringe pump in the valve in position: aspiration of amylacetate as carrier
9	3′	100	Aspirate 30 μL	External syringe pump in the valve out position. Aspiration of the extracted ion associate to fill the port by the measured sample
10	1′	100	Dispense 100 μL	Cleaning the external holding coil
11	3′	50	Aspirate 100 μL	Aspiration of the extracted ion associate
12	2′	50	Empty	Empty the external syringe pump into the detector and measurement of absorbance

1-8–ports of selection valve in extraction part of the DV-SIA system.
1′–4′-ports of selection valve in detection part of the DV-SIA system.

external 5 mL syringe pump (FIAlab® Instrument Systems Inc., Bellevue, USA) constituted the detection part of the system. The absorption spectra were recorded by the fibre-optic charge-coupled detector USB 2000 (Ocean Optics Inc., Dunedin, USA), supplemented with a micro-volume Z-flow cell of 20 mm optical path length and the VIS light source LS-1 tungsten lamp (Ocean Optics Inc., Dunedin, USA).

FIAlab® for Windows software, version 5.9.290, was used to control the units of both parts of the SIA set-up and to perform the data acquisition.

PTFE tubes of 0.75 mm i.d. were employed in the SIA manifold, except for the aspiration of the carrier solution, which was made of 1.5 mm i.d. PTFE tubing.

2.3. Optimization of the DV-SIA system

The physical and chemical parameters of the DV-SIA system for the thiocyanate determination were optimized. The aspiration sequence, AP aspiration volume (30–70 μL) and AP concentration (1×10^{-3} to 5×10^{-3} mol L^{-1}) and the buffer aspiration volume (0–30 μL) were tested to find the most favourable extraction conditions. Additionally, the variables of the extraction procedure like the method of mixing, the aspiration time of the air used for mixing (2–14 s) and the flow rate to deliver the aspirated solutions into the extraction cell (80–140 $\mu L\,s^{-1}$) were thoroughly studied.

The sample volume was kept constant at 50 μL. All measurements were done in triplicate and average values with RSD (%) were evaluated.

2.4. DV-SIA procedure

Table 1 shows the SIA procedure employed to carry out the determination of thiocyanate. Firstly, 600 μL of water, used as the carrier, were aspirated at 200 $\mu L\,s^{-1}$ in the extraction part. After switching to the Valve Out position, an air plug was aspirated into the holding coil during 10 s at 50 $\mu L\,s^{-1}$, followed by 30 μL of a 2×10^{-3} mol L^{-1} AP solution, 10 μL of buffer solution (pH 3), 50 μL of NH_4SCN working solutions (0.05–0.50 mmol L^{-1}) or sample solution and 350 μL of amylacetate.

Next, all aspirated zones were delivered into the Extraction cell at 120 $\mu L\,s^{-1}$. The air was used for efficient mixing of the solutions and to speed up the extraction of the ion associate into the organic phase. The phases were then self-separated (3 s delay) due to their different densities, leaving the organic phase with the extracted compound in the upper part.

Then, the aspiration of 600 μL of amylacetate, using as a carrier, was carried out in the detection part. The valve position was changed and the aspiration of 30 μL of the extracted ion associate was performed in order to wash and fill port 3′ with the sample. The excess volume was pushed into port 1′, used as an auxiliary waste, by 100 μL of the organic solvent.

To carry out the measurement of the absorbance, 100 μL of the sample were aspirated and transferred to the detector by the port 2′ using the remaining volume of the carrier. The signal was recorded at 550 nm. The extraction of blank and sample solutions is depicted in Figs. 2 and 3, respectively, and a real-time procedure for the sample extraction is demonstrated with a short video file in Supplementary data (Video 1).

Finally, the extraction cell was emptied aspirating the residual solution through port 7 and discarding it through port 1 to the waste. In order to prevent sample cross-contamination, the extraction part of the SIA system was washed with water between determinations (washing step was included in the control program).

Fig. 2. Extraction procedure of blank solution measurement. The slight color of the upper organic phase is caused by low amount of extracted AP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 3. Extraction procedure of sample solution measurement. The ion associate formed is extracted into the upper organic phase.

2.5. Preparation of the samples

Human saliva samples were collected from 4 healthy volunteers between the breakfast and the lunch time in three non-consecutive days. The sample was taken in previously weighted plastic tubes and after that, the tube was reweighted to calculate the amount of sample to be analyzed. Then, the sample was diluted to 7.0 mL with water and centrifuged 10 min at 5000 rpm. The supernatant solution was collected and 50 μL was aspirated to the SIA system for the analyte determination.

For the recovery study, samples were spiked with 62.5 μL of 1×10^{-2} mol L^{-1} NH_4SCN solution using automatic pipette and the same protocol was followed. The recoveries were calculated according to the AOAC definition [24].

3. Results and discussion

3.1. Preliminary studies

To investigate the adequate conditions for the thiocyanate-AP interaction and the suitable extraction solvent, some preliminary studies were carried out in batch conditions.

To set the acidity of the medium to carry out the reaction, the optimum pH value was studied. For this, ammonium acetate buffer solutions of pH values between 3.0 and 8.0 were used, and varied in increments of 1.0 unit. Briefly, in a test tube, 0.5 mL of 1×10^{-3} mol L^{-1} NH_4SCN, 4.0 mL of ammonium acetate buffer solution and 0.5 mL of 1×10^{-3} mol L^{-1} AP were manually mixed with 3.0 mL of amylacetate. After the separation of the phases, the absorbance of the organic extract was measured at 550 nm against the reagent blank. The highest difference between the sample and the blank signals was obtained using the buffer solution of pH 3. Furthermore, at pH values lower than 3 the interference of some anions, such as nitrates, can be observed; and at pH values higher than 6, the absorbance of the blank increased. Thereby, pH 3 was chosen for further experiments.

In order to obtain the best extraction efficiency with the lowest blank signal, several organic solvents were tested. Amylacetate and toluene met these requirements, but in case of toluene the formation of air bubbles in the extraction cell was observed. Therefore, amylacetate was selected for the extraction in the DV-SIA system.

3.2. Optimization of the DV-SIA system

3.2.1. Optimization of the aspiration sequence

Different reagent sequences were tested at two thiocyanate concentration levels, keeping the aqueous phase (dye, buffer and sample solutions) together at the beginning or at the end of the aspiration sequence. The optimum values were selected taking into account the compromise between the highest value of the analytical signal and the lowest value of the % RSD, obtained for two standard concentrations (0.50 and 1.0 mmol L^{-1}).

Three possible combinations for the aqueous phase were tested when the organic solvent was aspirated at the end of the sequence: (1) sample–buffer–dye, (2) dye–sample–buffer, and (3) dye–buffer–sample. The best results were obtained with the last-mentioned combination, when the aspiration of the AP solution was carried out in the first place and buffer solution was aspirated between the dye and the sample solution.

Keeping the chosen combination of aqueous solutions, the aspiration of the solvent at the beginning of the sequence was tested, but an ineffective mixing between the solvent and the aqueous phase was observed. Thus the aspiration of the organic solvent after all aqueous solutions was the selected aspiration sequence (dye–buffer–sample–amylacetate).

3.2.2. Optimization of the volume of reagents

The optimization of the volume of reagents was carried out using three concentrations of the thiocyanate standard. To choose the optimal value of the reagent, a criterion was established to achieve the highest difference between three concentration levels (0.05, 0.25 and 0.50 mmol L^{-1}) and the best repeatability of the measurement.

The volume of the carrier was adjusted in order to keep the total volume to be transferred to the extraction cell constant.

The volumes of the ammonium acetate solution and the AP solution were studied in 5 μL increments, in ranges of 0–30 μL and 30–70 μL, respectively. The volumes of 10 μL of buffer and 30 μL of the dye were selected.

3.2.3. Optimization of the AP concentration

The effect of dye concentration was investigated in the range 1×10^{-3} to 5×10^{-3} mol L^{-1} of AP. In this case, the linearity of the calibration function was used as the criterion of choice and a 2×10^{-3} mol L^{-1} concentration was picked out. At lower concentrations, the linearity was affected and similar absorbance values were obtained for the higher standard concentrations, meaning that the concentration of the dye was not high enough to interact with the thiocyanate present in standard solution. At higher AP concentrations RSD % values were increased.

3.2.4. Optimization of the extraction procedure

To ensure the best mixing of aqueous and organic phases in the extraction part, three methods of mixing were studied: (1) unidirectional – sample and reagents passed directly through the holding coil to the extraction cell, (2) flow reversals in the holding coil and (3) mixing with air bubbles in the extraction cell. In the last case, zone of the air was aspirated followed by all reagents and bubbling in the extraction cell ensured effective mixing of all solutions [23]. The value of the slope and the linearity of the analytical curve were established as main criteria to choose among the methods.

At first, a comparison between (1) and (2) was made and better results were found by repeating flow reversals three times using 20 μL increments. Thus, different flow rates for the mixing in the coil, and the subsequent movement to the extraction cell were tested (80–140 μL s^{-1}). The optimal value (140 μL s^{-1}) was compared with mixing the solutions by aspirating an air zone (3). Significant difference was not found when evaluating the slope and

linearity. Taking into account the analysis time, mixing with air zone was chosen for further determinations.

The quantity of air that was necessary to accomplish the adequate mix of the solutions in the extraction cell was investigated. For this, the time of aspiration was optimized between 2 and 14 s with a flow rate of 50 μL s^{-1}, finding 10 s as the optimum value. Lower air aspiration times were insufficient to carry out mixing and extraction of the ion associate and some troubles with the separation of the phases were observed. At higher aspiration times, significant changes in the measured signal were not observed.

The flow rate used to dispense all aspirated zones into the extraction cell was also optimized between 80 and 140 μL s^{-1} in increments of 20 μL s^{-1}. The best results were achieved with a flow rate of 120 μL s^{-1}, and successive increase did not influence the obtained results.

The volume of the organic solvent used for the extraction was 350 μL following previous experience [22]. Lower volumes of solvent were not suitable owing to the SIA configuration.

3.3. Analytical performance

The analytical performance was evaluated by the calibration function, limit of detection (LOD), limit of quantitation (LOQ), sample throughput, intra-day and inter-day precision, and selectivity.

3.3.1. Analytical curve, LOD, LOQ and sample throughput

Using the proposed DV-SIA system and the optimized values for physical and chemical parameters, an analytical curve for the thiocyanate determination was constructed from seven data points over the range of 0.05–0.50 mmol L^{-1}. The regression equation was $A = (1.887 \pm 0.053)\,[SCN^-\ \text{mmol}\ L^{-1}] + (0.037 \pm 0.014)$ with a correlation coefficient of 0.995.

The LOD was 0.02 mmol L^{-1} and LOQ was 0.07 mmol L^{-1} calculated from the calibration function. The sample throughput was 5 h^{-1} for samples aspirated in triplicate and including the washing step.

3.3.2. Precision

The intra-day and the inter-day precision were checked by the RSD (%) values at two concentration levels: 0.20 and 0.50 mmol L^{-1}. The intra-day precision was carried out performing 10 determinations and the results were 1.0 and 2.8%, respectively. The inter-day precision was evaluated measuring the same concentrations by triplicate over 10 days. The results for both concentration levels were 4.2 and 3.8%, respectively.

The obtained RSD values showed good repeatability and inter-day precision of automated extraction procedure and were comparable with the values obtained when applying other methods found in the literature [25–27].

3.3.3. Selectivity

Human saliva is produced by salivary glands and it is composed of 98% water, but also contains macromolecules, antibacterial compounds, proteins and inorganic ions. The effect of various ions (possible interferents) on the extraction and determination of the analyte was tested. For this purpose, a 0.2 mmol L^{-1} SCN^- solution was used. The tolerable amount for each ion was evaluated as SCN^-: interferent ion ratio that resulted in an error that did not exceed ±5%. The tested species and the tolerance limits are summarized in Table 2. Nitrates significantly interfered with the determination of thiocyanate, but in case of real samples nitrate levels are about 100-times lower than thiocyanate concentrations, for this reason, in such conditions, it does not interfere with SCN^- determination [10]. Although phosphates can react with AP [20], they can barely be extracted with amylacetate giving a tolerance level higher than the quantity of phosphates that can be found in saliva samples.

Table 2
Effect of some possible interfering ions on the extraction and determination of SCN^-.

		Tolerance level (mM)	Error (%)
Anions	$S_2O_3^{2-}$	20	3.1
	Cl^-	60	2.3
	$H_2PO_4^-$	70	−1.6
	Br^-	2	−1.2
	SO_4^{2-}	200	−4.7
	F^-	300	−2.3
	HCO_3^-	200	−4.2
Cations	Ni^{2+}	2	−1.1
	Pb^{2+}	30	−1.9
	Zn^{2+}	2	−2.0
	Co^{2+}	2	−2.6
	Cu^{2+}	1	−0.7
	Ca^{2+}	30	−2.1
	K^+	200	−4.2
	Mg^{2+}	200	−2.3
	Fe^{2+}	2	−0.6
	Cr^{3+}	20	−3.1

3.4. Analysis of real samples

The proposed method was applied for determination of thiocyanate in human saliva samples. To study the thiocyanate concentration levels at different day-times, saliva sample was collected from two volunteers early in the morning before breakfast and brushing teeth (1), before lunch (2), and after lunch (3). The results were expressed as milligrams of SCN^- per gram of saliva sample. The added amount of standard solution was recalculated considering the sample weight. As can be seen in Table 3, a significant decrease of the SCN^- concentration and the recoveries values occurred after lunch, probably because of the changes in the concentration of the different ions in the saliva as a result of the digestion process. On the other hand, sampling in the morning proved recovery values in the range acceptable for biological fluids (100 ± 10%).

Hence, the concentration of thiocyanate was determined in saliva samples collected between the breakfast and the lunch time.

Table 4 showed the obtained thiocyanate concentration for the samples and the results of the recovery study. The variation of the obtained results can be attribute to the strong relation between the SCN^- ions and the individual saliva composition (the thiocyanate concentration is different for each person). This fact can also be observed in the values found in the literature [2,8,27]. Additionally,

Table 3
Thiocyanate determination in real saliva samples at different day-times.

	Early morning		Before lunch		After lunch	
	Concentration (mg g^{-1})	R (%)	Concentration (mg g^{-1})	R (%)	Concentration (mg g^{-1})	R (%)
A	0.105 ± 0.011	101.0	0.178 ± 0.014	104.1	0.088 ± 0.010	77.3
B	0.141 ± 0.009	94.2	0.140 ± 0.008	84.3	0.133 ± 0.014	78.1

A, B: person A, person B; Confidence limit $x = st/\sqrt{n}$, where s means standard deviation, t is Student coefficient for $n-1$ degrees of freedom, $n = 3$; R%, recovery percentage, $\%R = ((Cf - C)/Ca) \times 100$, where Cf = measured analyte concentration in the spiked sample; C = measured analyte concentration in the non-spiked sample; Ca = added analyte concentration.

Table 4
Thiocyanate determination in real saliva samples.

		A	B	C	D
Day 1		0.102 ± 0.010	0.067 ± 0.024	0.116 ± 0.005	0.165 ± 0.041
	Added ($mg\,g^{-1}$)	0.026	0.022	0.065	0.033
	Found ($mg\,g^{-1}$)	0.134 ± 0.029	0.134 ± 0.024	0.206 ± 0.054	0.179 ± 0.066
	R (%)	104.5	86.8	113.4	110.8
Day 2		0.104 ± 0.009	0.102 ± 0.020	0.089 ± 0.009	0.127 ± 0.014
	Added ($mg\,g^{-1}$)	0.052	0.041	0.033	0.035
	Found ($mg\,g^{-1}$)	0.140 ± 0.004	0.119 ± 0.003	0.139 ± 0.013	0.137 ± 0.009
	R (%)	92.3	83.3	114.0	85.4
Day 3		0.107 ± 0.014	0.062 ± 0.007	0.140 ± 0.014	0.168 ± 0.030
	Added ($mg\,g^{-1}$)	0.034	0.052	0.028	0.050
	Found ($mg\,g^{-1}$)	0.161 ± 0.013	0.078 ± 0.006	0.142 ± 0.016	0.242 ± 0.042
	R (%)	114.0	80.3	84.3	111.0

Each measurement was done in triplicate.
A, B, C, D: person A, person B, person C, person D; R (%): recovery percentage.

a small variation could be observed for the same person at different days and this is possible to attribute to changes in the diet.

In spite of that, the obtained values are within the range of values found in the literature for the determination of this ion in saliva samples [2]. The recovery percentages varied between 80% and 114%. It is worth to notice that the values were almost equal for the same person, and it is related to the unique saliva composition of each person and the diet.

4. Conclusion

The DV-SIA system was successfully used for extraction and determination of thiocyanate ions in human saliva samples. Optimal chemical and flow conditions were found. Repeatability of the automated extraction procedure was 2.8%. Day-time of sampling was studied in detail to achieve accurate results proved by recovery tests.

Acknowledgements

This work was supported by the Charles University in Prague (Project SVV 263 002) and by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0226/11). In addition, C. Acebal would like to acknowledge financial support of her scholarship in research fellowship program between Ministries of Education of Argentina and the Czech Republic, and National Council of Scientific and Technical Research (CONICET).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.01.021.

References

[1] Y. Tanaka, N. Naruishi, H. Fukuya, J. Sakata, K. Saito, S. Wakida, J. Chromatogr. A 1051 (2004) 193–197.
[2] M. Mori, T. Iwata, T. Satori, S. Ohira, H. Itabashi, K. Tanaka, J. Chromatogr. A 1213 (2008) 125–129.
[3] Z. Glatz, S. Nováková, H. Sterbová, J. Chromatogr. A 916 (2001) 273–277.
[4] http://rais. ornl.gov/tox/profiles/cyanide_c_V1.html.
[5] K. Ozoemena, T. Nyokong, J. Electroanal. Chem. 579 (2005) 283–289.
[6] B. Gong, G. Gong, Anal. Chim. Acta 394 (1999) 171–175.
[7] S. Chattaraj, A.K. Das, Spectrochim. Acta Part B 47 (1992) 675–680.
[8] P. Yang, W. Wei, C. Tao, Anal. Chim. Acta 585 (2007) 331–336.
[9] W. Ju Xu, Y. Zhang, Y. Chai, R. Yuan, Desalination 249 (2009) 139–142.
[10] I. Demkowska, Z. Polkowska, J. Namiesnik, J. Chromatogr. B 875 (2008) 419–426.
[11] R. Stugart, Ind. Eng. Chem. Anal. Ed. 3 (1931) 390–393.
[12] T. Imanari, S. Tanabe, T. Toida, Chem. Pharm. Bull. 30 (1982) 3800–3802.
[13] M. Haque, J. Bradbury, Clin. Chem. 45 (1999) 1459–1464.
[14] D. Themelis, P. Tzanavaras, Anal. Chim. Acta 452 (2002) 295–302.
[15] A.S. Bashammakh, S.O. Bahaffi, A.A. Al-Sibaai, H.O. Al-Wael, M.S. El-Shahawi, Anal. Chim. Acta 592 (2007) 16–23.
[16] G. Giraudi, C. Grillo, Anal. Chim. Acta 128 (1981) 169–175.
[17] I.S. Balogh, P.P. Kish, A.A. Ishchenko, I.L. Mushkalo, V.A. Andruch, J. Anal. Chem. 45 (1990) 344–350.
[18] L. Kocúrová, I.S. Balogh, J. Škrlíková, J. Posta, V. Andruch, Talanta 82 (2010) 1958–1964.
[19] L. Rusnáková, V. Andruch, I.S. Balogh, J. Škrlíková, Talanta 85 (2011) 541–545.
[20] S. Khlyntseva, A. Vishnikin, M. Al-Shwaiyat, H. Sklenářová, P. Solich, Y. Bazel, V. Andruch, Talanta 84 (2011) 1355–1360.
[21] C. Silvestre, J. Santos, J. Lima, E. Zagatto, Anal. Chim. Acta 652 (2009) 54–65.
[22] J. Škrlíková, V. Andruch, H. Sklenářová, P. Chocholouš, P. Solich, I.S. Balogh, Anal. Chim. Acta 666 (2010) 55–61.
[23] J. Škrlíková, V. Andruch, H. Sklenářová, P. Chocholouš, P. Solich, I.S. Balogh, Anal. Methods 2 (2010) 1134–1139.
[24] AOAC Peer-Verified Methods Program, Manual on Policies and Procedures, Arlington, VA, USA, 1998.
[25] J.F. van Staden, A. Botha, Anal. Chim. Acta 403 (2000) 279–286.
[26] R. Naik, B. Kumar, A. Asthana, Spectrochim. Acta Part A 75 (2010) 1152–1158.
[27] W. Hu, H. Haraguchi, Anal. Chim. Acta 285 (1994) 335–341.