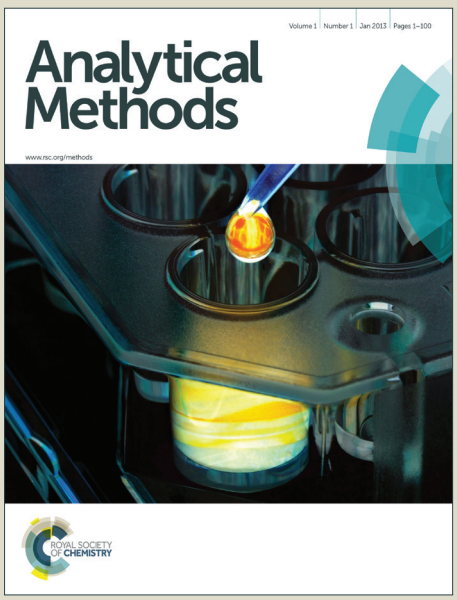
Analytical Methods

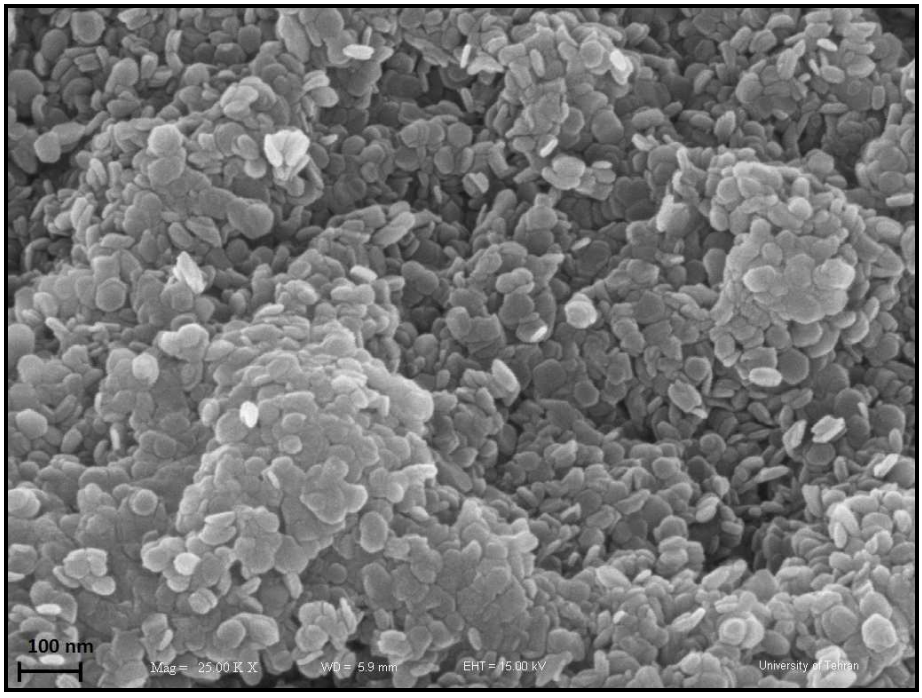
Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Graphical abstract



A simple solid phase extraction system based on nano-structured Ni-Al layered double hydroxide was developed for the separation of thiocyanate from human saliva and serum samples prior to determination by gas chromatography.

Monitoring of thiocyanate as a biomarker in saliva and serum samples by combination of solid-phase extraction based on a layered double hydroxide nano-sorbent with gas chromatography

Hossein Abdolmohammad-Zadeh*, Hakimeh Vasli

Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, 35 Km Tabriz-Marageh Road, P.O. Box 53714-161, Tabriz, Iran

* Corresponding author. Tel.: +98 4124327500, Fax: +98 4124327541

E-mail addresses: h.abdol@azaruniv.edu, h_abdol@yahoo.com

Abstract

Thiocyanate ion is a biomarker to environmental tobacco smoke exposure. In this research, we have used a simple solid-phase extraction method based on a nickel-aluminum layered double hydroxide (Ni-Al LDH) nano-sorbent for the extraction of trace levels of thiocyanate from biological samples followed by gas chromatography with flame ionization detection. Extraction is based on the adsorption of thiocyanate anions on the Ni-Al-nitrate LDH, and/or their exchange with the nitrate anions in the LDH interlayer. Several parameters affecting the extraction performance such as pH value, amount of nano-sorbent, eluent type and volume, sample loading flow rate and sample volume were investigated and optimized. Under optimized experimental conditions, good linearity was achieved ranging from 0.6–80 ng mL^{-1}. The limit of detection and relative standard deviation were 0.2 ng mL^{-1} and 4.6% for thiocyanate ion, respectively. The method was successfully applied to the monitoring of thiocyanate in human saliva and serum samples. It was confirmed that the thiocyanate concentration in saliva noticeably increased after smoking.

Keywords: Thiocyanate; Nano-sorbent; Layered double hydroxide; Solid phase extraction; Gas chromatography; Saliva and serum samples

1. Introduction

Anion profiling can help us to get a better sight of the physiology and biochemistry of many illnesses. Because, abnormal levels of many organic acids and inorganic anions are representative of diseases, inborn errors of metabolism, toxin exposure and even nutrient insufficiencies detrimental to health [1]. Saliva is the watery secret produced in the mouth of humans which carries a variety of important compounds including a group of inorganic anions such as chloride, phosphate, and bicarbonate [2]. Many substances used as biomarkers or indicators of biological conditions can be easily found in saliva. One example of the use of saliva as a bioindicator is the determination of thiocyanate, which is a product of detoxification of hydrocyanic acid present in cigarette smoke by an enzyme called rodanase, and can be regarded as a biomarker of smoke exposure [3]. Thiocyanate is also present as a normal constituent in mammalian tissues and body fluids. Accordingly, its extraction and monitoring in human saliva and serum can provide a useful probe for distinguishing between smokers and non-smokers. If the content of thiocyanate in the body is a little higher than normal, the protein dialysis will be affected and it may even result in coma [4]. Therefore, applying an accurate, simple and rapid method in the aspect of thiocyanate monitoring is significant in the field of medicine and life sciences [5].

Several methods, such as capillary electrophoresis [2,3], fluorimetry [6,7], electrochemical methods [5,8], spectrophotometry [9-11], ion chromatography [12,13], electrospray ionization tandem mass spectrometry [14] and gas chromatography-mass spectrometry [15,16] have been developed for the determination of thiocyanate ion. Among these, gas chromatography (GC) is the most widely used method for biological samples. However, as inorganic anions are not volatile, so directly analysis by GC is difficult. Therefore, GC methods for inorganic ions require

a derivatization step in order to conversion of the ion to a volatile compound such as a methyl, ethyl, butyl, pentafluorobenzyl (PFB) [17] and dimethyl sulfate [18] derivative. On the other hand, analysis of such samples characterized by complex composition of the matrix represents a difficult analytical task and require special sample pretreatment.

Sample preparation is important for isolating desired components from complex matrices and greatly influences their reliable and accurate analysis [19]. Solid phase extraction (SPE) is the most widely used separation and preconcentration technique mainly due to the variety of different materials employed as sorbents. The sorbent selected in SPE controls the analytical parameters such as selectivity, affinity and capacity [20]. In recent years, nano-structured materials have gained more attention due to their special physical and chemical properties. Layered double hydroxides (LDHs) are a class of synthetic two dimensional nano-structured inorganic materials, with general formula of $[M^{2+}_{1-x}M^{3+}_{x}\ (OH)_2]^{x+}\ [A^{n-}_{x/n}\cdot\ mH_2O]^{x-}$, where M^{2+} is a divalent metal ion like Zn, Mg, Cu, Co or Ni, M^{3+} is a trivalent metal ion like Al, Fe or Cr, *x* is the ratio of $M^{3+}/(M^{2+}+M^{3+})$ and A^{n-} is a *n*-valent anion [21]. LDHs consist of positively charged hydrotalcite-like layer of metal hydroxide and the excess positive charge of the layer is compensated by the exchangeable interlayer anions. LDHs act as potential ion-exchangers due to the presence of large interlayer spaces and the reasonable number of exchangeable anions [22].

To the best of our knowledge, so far, no attempt has been made to apply the LDHs in SPE of thiocyanate. Accordingly, in this research, a simple SPE system based on Ni-Al-nitrate LDH was developed for the separation of thiocyanate from biological samples prior to determination by GC. The main parameters influencing the extraction efficiency were investigated and optimized. The preconcentrated thiocyanate ions were derivatized by dimethyl sulfate prior to injection into

chromatographic column. The method was successfully applied to the determination of thiocyanate in saliva and serum samples as a biomarker of cigarette smoke exposure.

2. Experimental

2.1. Apparatus and instruments

Capillary gas chromatography (PHILIPS, USA) equipped with a split/splitless injector and flame-ionization detector (FID), T80 UV-Vis spectrophotometer (PG Instrument Ltd, England) with a wavelength range of 190–900 nm, Beckman centrifuge (model GS-6, USA) and N_2 gas (99.9995%, Azaroxide Co., Iran) were used. The pH values were measured with a Metrohm pH-meter (model 827, Switzerland), supplied with a glass-combined electrode. An electronic analytical balance (Mettler Toledo, PB303, Switzerland) was used for weighting the solid materials. A 2 mL polypropylene cartridge (30 mm×7 mm i.d.) (Shafa Co., Iran) packed with 0.2 g of Ni–Al(NO_3^-) LDH was used to pre-concentrate the analyte in SPE procedures. The flow rate of solution through the column was controlled with an air-driven fluid pump model P34112 (Taiwan).

2.2. Chemicals and solutions

All solutions were prepared with high purity deionized water (Shahid Ghazi Co., Tabriz, Iran). Potassium thiocyanate (KSCN), sodium hydroxide (NaOH) and methanol were analytical reagent grade. The derivatization agent, dimethyl sulfate, used was a commercial grade reagent (99% up). Dichloromethane used in this experiment was HPLC grade. All reagents and salts used for study of interferences and LDH precursors, i.e., $Ni(NO_3)_2 \cdot 6H_2O$ (99%) and $Al(NO_3)_3 \cdot 9H_2O$ (99%) were purchased from Merck (Darmstadt, Germany).

2.3. Preparation and characterization of nickel–aluminum layered double hydroxide

Ni–Al(NO_3^-) LDH was prepared by co-precipitation method with controlled pH, and followed by hydrothermal treatment as described in our previous works [23-25]. The synthesized Ni–Al(NO_3^-) LDH was characterized by powder X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The obtained results are given in the Electronic Supplementary Material, which are in good agreement with the previously results reported by the authors [23-25].

2.4. Solid-phase extraction procedure

The solid-phase extraction column was prepared by introducing 200 mg of Ni–Al(NO_3^-) LDH into a 2 mL polypropylene cartridge using the dry packing method. Ends of the column were plugged with a small portion of cotton to retain the nano-sorbent in the column. Before loading the sample, the column was cleaned with 1.5 mL of 1 mol L^{-1} NaOH solution and conditioned by passing only 5 mL of deionized water through the column prior to each use. For solid-phase extraction of thiocyanate, aliquots of sample or aqueous standard solution containing thiocyanate in the range of 0.6−80 ng mL^{-1} was passed through the column at a flow rate of 2.0 mL min^{-1}. After loading, the retained analyte on the micro-column was stripped by 1.5 mL of 1 mol L^{-1} NaOH solution.

2.5. Derivatization procedure

Derivatization procedure was performed according to the method reported by Funazo et al. [18] with some modifications. For this purpose, 0.1 mL of dimethylsulphate was added into 1.5 mL of an aqueous solution of the extracted thiocyanate ions in a reaction vessel. Afterward, the

vessel was placed in the ultrasonic bath and agitated by heating at 60 °C for 30 min. Then, after cooling to room temperature, 1.5 mL of dichloromethane was added to the reaction solution. Finally, the methyl derivative of thiocyanate was extracted by shaking the solution for 10 min at room temperature, and the organic layer was separated from the aqueous layer. An aliquot (2 µL) of the organic layer was injected into the gas chromatograph and the methyl derivative was determined with a FID.

2.6. Gas chromatography analysis

The quantitative calculations are based on the peak areas from GC–FID chromatograms. The column was a BP5 (non-polar, 5% phenyl, 95% dimethyl polysiloxane, SGE, USA) capillary column (25 m × 0.22 mm i.d., 0.25 µm film thickness) which nitrogen as a carrier gas passed through it at a flow-rate of 1.5 mL min^{-1}. The initial oven temperature was 60 °C. After 1 min, the oven temperature is increased at a rate of 5 °C min^{-1} and held at 120 °C for 1 min, then increased at a rate of 20 °C min^{-1} and held at 250 °C for 3 min. Detector temperature was 280 °C.

2.7. Real samples

All experiments on human subjects were performed in compliance with the relevant laws and institutional guidelines approved by the Medical Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran. The required consent was obtained for any experimentation with human subjects.

2.7.1. Preparation of saliva samples

Saliva is a cleaner matrix than plasma. For example, saliva is 98% water and typically contains <1 mg mL^{-1} protein, while plasma contains 55–80 mg mL^{-1} protein and, therefore, does

not need to protein precipitation as a sample preparation step [26]. Saliva samples from healthy volunteer smokers and nonsmokers were collected and centrifuged at 3000 rpm for 10 min. After centrifugation, 3 mL of the supernatant solution was transferred into 10.0 mL volumetric flask and after dilution to the mark with deionized water it was treated under SPE procedure described in Section 2.4.

2.7.2. Preparation of serum *samples*

The following procedure was used regarding the serum samples: 0.5 mL of each serum sample was mixed with 1.5 mL of methanol, and the obtained mixture was centrifuged at 4000 rpm for 15 min [27]. Then, the supernatant solution (around 2.0 mL) was transferred into 10.0 mL volumetric flask and after dilution to the mark with deionized water the SPE procedure was constructed as described in "Solid-phase extraction procedure" section.

3. Results and discussion

3.1. Optimization of solid-phase extraction conditions

To evaluate the capability of the presented SPE method for separation and pre-concentration of thiocyanate, several variables affecting the extraction efficiency including pH, sample loading flow rate, amount of the sorbent, sample volume and eluent conditions were studied and optimized. One at a time method was used for optimization of the parameters. A 25 ng mL^{-1} of thiocyanate solution was used for all the measurements and three replicates were performed for each experiment.

3.1.1. Effect of pH

In order to evaluate the pH dependency of the thiocyanate retention, the pH values of sample solution was varied in a range of 4–11 using diluted HNO_3 and/or NaOH solution. pH values lower than 4 were not tested due to the probability of the dissolution of the LDH in strong acidic media. The effect of pH value on the recovery of the analyte is shown in Fig. 1. It can be seen that the highest recovery was achieved between pH 5 and 9. An increase in the concentration of the competing OH^- anions at pH above 9.0 might be responsible for the observed decrease in the recovery at higher pH. Therefore, to achieve high efficiency and good selectivity, pH 6 was selected as optimum pH value for further experiments.

(Fig. 1 here)

3.1.2. Effect of sample loading flow rate

As the retention of analyte on adsorbent depends upon the flow rate of the sample solution, the effect of this parameter was examined under the optimum pH (pH 6.0) by passing 10.0 mL of sample solution through the column with the flow rate varying in the range 0.5–3.5 mL min^{-1}. According to the results depicted in Fig. 2, a constant analyte recovery (close to 100 %) could be achieved with flow rates up to 2.0 mL min^{-1}. At higher flow rates, recovery decreases. Thus, a flow rate of 2.0 mL min^{-1} was employed in this work.

(Fig. 2 here)

3.1.3. Effect of the amount of Ni–Al(NO_3^-) LDH nano-sorbent

In order to choose the optimum amount of nano-sorbent required for quantitative recoveries for thiocyanate ions, different quantities (50–300 mg) of Ni–Al(NO_3^-) LDH were examined. The results indicated that the quantitative recovery of analyte was obtained by using at least 200 mg of the nano-sorbent (Fig. 3). Therefore, 200 mg of nano-sorbent was used for further experiments.

(Fig. 3 here)

3.1.4. Effect of type and volume of eluent

LDHs consist of positively charged hydrotalcite-like layer of metal hydroxide and the interlayer region typically occupied by anionic species and water molecules. The interlayer bonding is relatively weak and, consequently, various anions could be introduced from aqueous solution into the hydroxide interlayer via three mechanisms; adsorption on external surface, intercalation by anion exchange, and intercalation by reconstruction of calcined products [28]. The nature and volume of the solution to be used for the elution stage are important to achieve the highest pre-concentration factor. Various alkaline and salt solutions or the mixture of these solutions have been successfully used to desorb and regenerate anion-loaded LDHs [28]. In order to choose the best eluent for stripping the retained thiocyanate ions from Ni–Al(NO_3^-) LDH, several reagent solutions such as NaOH, $HONH_2{\cdot}HCl$ and Na_2CO_3 were tested. It was observed that NaOH solution provided the best recovery. The concentration of the NaOH solution was also optimized for the quantitative elution of retained thiocyanate ions from the column. Based on the results given in Fig. 4A, 1 mol L^{-1} NaOH solution was sufficient for complete desorption of the retained analytes. Further experiments showed that quantitative recovery could be accomplished with at least 1.5 mL of eluent. So, to increase the pre-concentration factor, optimum volume of the eluent was chosen as 1.5 mL. The results are shown in Fig. 4B.

(Fig. 4 here)

3.1.5. Effect of the sample volume

Effect of sample volume on the retention of thiocyanate ions was studied in the range between 10 and 200 mL. In order to reach the maximum applicable sample solution, fixed volume (0.6 mL) of 1000.0 ng mL^{-1} thiocyanate was diluted to 10, 25, 50, 100, 150 and 200 mL,

then passed through the column. As shown in Fig. 5, quantitative recovery of thiocyanate was obtained up to 150 mL of sample solution. Above 150 mL, the recovery decreases for the analyte. So, by analyzing 1.5 mL of the final solution after the pre-concentration of 150 mL of sample solution, a pre-concentration factor could be found as 100.

(Fig. 5 here)

3.2. Sorption capacity

To determine the sorption capacity, 200 mg of Ni–Al(NO_3^-) LDH was added to 50.0 mL of solution containing 400 μg mL^{-1} of thiocyanate (pH=6). The mixture was magnetically stirred at room temperature for 30 min and the supernatant was separated by centrifugation at 3000 rpm for 5 min. Loaded thiocyanate ions in the LDH nano-particles were stripped with 5 mL of 1.0 mol L^{-1} NaOH solution and concentration of the analyte was then determined after appropriate dilution by the presented GC–FID method. Eventually, capacity of the Ni–Al(NO_3^-) LDH for thiocyanate ion was found to be 86.8 mg g^{-1}.

3.3. Study of interferences

In order to evaluate the selectivity of the presented SPE system, the effect of some potentially interfering ions on the pre-concentration and determination of thiocyanate was investigated. In these experiments, different amounts of interfering ions were individually added to the test solutions containing 25 ng mL^{-1} of thiocyanate ion and then followed according to SPE procedure. The tolerance limits of the potentially interfering ions, defined as the maximum concentration of the foreign ion causing a change in the analytical signal less than ±5% are given in Table 1. As can be seen, most of examined cations and anions did not interfere with the extraction and determination. Therefore, the Ni–Al(NO_3^-) LDH has great potential for the selective extraction of thiocyanate ions from complex matrices.

(Table 1 here)

3.4. Figure of merits

Optimized experimental parameters and analytical characteristics of the method were given in Table 2. Under these experimental conditions, analytical features of the proposed method such as, linear range of calibration curve, limit of detection, accuracy and precision were examined. Calibration graph was linear in the range 0.6–80 ng mL^{-1} for a sample volume of 150.0 mL. The regression equation is $A = 42.08\ C – 1.86$ with a correlation coefficient of 0.9972, where A is the peak area and C is the concentration of thiocyanate in the sample solution in ng mL^{-1}, respectively. The relative standard deviation for six replicate measurements of 40 ng mL^{-1} thiocyanate was 4.6%. The limit of detection for thiocyanate, determined as the lowest concentration that produced a signal equal to three times the base line noise level, was found to be 0.2 ng mL^{-1}.

(Table 2 here)

3.5. Analysis of real samples and method validation

In order to evaluate the applicability of the presented procedure, the method was employed to monitor the amounts of thiocyanate in biological samples i.e., saliva and serum samples concerning smokers and non-smokers. In order to verify the accuracy of the established procedure, recovery experiments were also carried out by spiking the samples with different amounts of thiocyanate prior to sample preparation and analysis by GC−FID. Tables 3 and 4 show the obtained results regarding saliva and serum samples, respectively. The relative recoveries obtained for different concentrations of thiocyanate were within the range 94.0–101.4% and 94.2–99.4% for saliva and serum samples, respectively, indicating good selectivity of the method. Therefore, the established method has successfully applied to the detection of

thiocyanate in biological samples for distinguishing between smokers and non-smokers. The chromatogram of a human saliva sample (smoker) after solid phase extraction, and derivatization by dimethyl sulfate is shown in Fig. 6. To show the excellent efficiency of the presented SPE method for selective separation of thiocyanate from biological samples, chromatograms of a smoker sample with a non-smoker saliva sample are shown together in Fig. 7. It is clear that, the presented SPE cleanup system is very selective and there is no coelution of endogenous species in saliva samples along with thiocyanate ions. As the amount of thiocyanate in the tested non-smoker saliva sample may be less than limit of quantification of the presented GC−FID method, the thiocyanate related chromatogram is only appeared in the case of smoker sample (Fig. 7b).

The accuracy of the presented procedure was also evaluated by analyzing the samples using the standard spectrophotometric method [29] and comparing the results with those of achieved using the presented method. It was found that the presented procedure gave comparable results to the standard method. Applying the paired *t*-test, no significant difference at 95% confidence level was observed and it can be concluded that the presented method is accurate and could be used for determination of thiocyanate ion in human saliva and serum samples.

(Tables 3 and 4 here) (Fig. 6 here)

3.6. Comparison of the presented procedure with other methods

Table 5 compares the analytical characteristic data of the presented method with other techniques reported in the literature for pre-concentration and determination of thiocyanate [1, 11, 30-32]. In comparison with other reported methods, the presented method has low limit of detection and good enrichment factor. This method has some advantages such as; lower detection limit, higher pre-concentration factor, simplicity, low cost and environment-friendly.

(Table 5 here)

4. Conclusions

In this research, a nano-sorbent from layered double hydroxides group has been reported. Nanometer-sized Ni–Al(NO_3^-) LDH is a good choice for the separation and pre-concentration of anions in aqueous samples due to its simple synthesis procedure and low cost compared to commercially available sorbents. As far as we know, this is the first time that the Ni–Al(NO_3^-) LDH has been used as a packing material for the selective solid phase extraction of thiocyanate ions from aqueous solutions. The employed inorganic nano-sorbent has a high retention capacity regarding thiocyanate anion and the retained analyte can be easily stripped with NaOH solution. Different biological samples, such as saliva and serum of smokers and non-smokers were analyzed by the presented method and satisfactory results have been obtained. Although this system has been demonstrated specifically with thiocyanate anion, it can be translated to other anions in human saliva and serum samples, which will permit this SPE–GC–FID method to be used in clinical laboratories.

Acknowledgment

The financial support from the Research Council of Azarbaijan Shahid Madani University (ASMU, Iran) is gratefully acknowledged.

References

1 T.Y. Tana, C. Basheerb, K.P. Nga and H.K. Lee, *Anal. Chim. Acta*, 2012, **739**: 31–36.
2 Z. Xu, T. Doi, A. Timerbaev and T. Hirokawa, *Talanta*, 2008, **77**: 278–281.
3 L. Vitali, V.T. Favere and G.A. Micke, *J. Chromatogr. A*, 2011, **1218**: 2327–2333.
4 P. Yang, W. Wei and C. Tao, *Anal. Chim. Acta*, 2007, **585**: 331–336.
5 P. Singh and A.K. Singh, *Anal. Bioanal. Chem.*, 2011, **400**: 2261–2269.
6 G. Zhang, B. Li, J. Fan and S. Feng, *Talanta*, 1997, **44**: 1141–1147.
7 B. Gonga and G. Gong, *Anal. Chim. Acta*, 1999, **394**: 171–175.

8 A. Badri and P. Pouladsaz, *Int. J. Electrochem. Sci.*, 2011, **6**: 3178–3195.

9 M.N. Radhey, K. Basant and A. Abhas, *Spectrochim. Acta A*, 2010, **75**: 1152–1158.

10 H. Sulistyarti, S. D. Kolev and S. Lim, *Indo. J. Chem.*, 2010, **10**: 167–171.

11 C.C. Acebal, H. Sklenarova, J. Skrlikova, I. Sramkova, and V. Andruch, *Talanta*, 2012, **96**: 107–112.

12 I. Demkowska, Z. Polkowska and J. Namiesnik, *J. Chromatogr. B*, 2008, **875**: 419–426.

13 R.A. Niemann and D.L. Anderson, *J. Chromatogr. A*, 2008, **1200**: 193–197.

14 K. Minakata, H. Nozawa, I. Yamagishi and et al., *Forensic Toxicol.*, 2012, **30**: 45–50.

15 R.K. Bhandari, R.P. Oda, S.L. Youso, I. Petrikovics, V.S. Bebarta, G.A. Rockwood and B.A. Logue, *Anal. Bioanal. Chem.*, 2012, **404**: 2287–2294.

16 S. Kage, T. Nagata and K. Kudo, *J. Chromatogr. B*, 1996, **675**: 27–32.

17 M. Sakayanagi, Y. Yamada, C. Sakabe, K. Watanabe and Y. Harigaya, *Forensic Sci. Int.*, 2006, **157**: 134–143.

18 K. Funazo, M. Tanaka and T. Shonh, *J. Chromatogr. A*, 1981, **211**: 361–368.

19 H. Kataoka, *Anal. Bioanal. Chem.*, 2010, **396**: 339–364.

20 L. Vidal, M.L. Riekkola and A. Canals, *Anal. Chim. Acta*, 2012, **715**: 19–41.

21 D.G. Evans and R.C.T. Slade, Structural aspects of layered double hydroxides. In *Layered double hydroxides* (X. Duan and D.G. Evans, ed.), (2006), pp. 1–5. Springer, Berlin, Germany.

22 P.S. Braterman, Z.P. Xu and F. Yarberry, Layered double hydroxides (LDHs), in *Handbook of layered materials* (S.M. Auerbach, K.A. Carrado and P.K. Dutta, ed.), (2004), pp. 373–474. Marcel Dekker, Inc., New York.

23 H. Abdolmohammad-Zadeh, Z. Rezvani, G.H. Sadeghi and E. Zorufi, *Anal. Chim. Acta*, 2011, **685**: 212–219.

24 H. Abdolmohammad-Zadeh and Z. Talleb, *Microchim. Acta*, 2012, **179**: 25–32.

25 H. Abdolmohammad-Zadeh, A. Jouyban, R. Amini and G.H. Sadeghi, *Microchim. Acta*, 2013, **180**: 619–626.

26 F. Ahmadi, H. Rezaei and R. Tahvilian, *J. Chromatogr. A*, 2012, **1270**: 9–19.

27 S. Chinaka, N. Takayama, Y. Michigami, K. Ueda, *J. Chromatogr. B*, 1998, **713**: 353–359.

28 K.-H. Goh, T.-T. Lim, Z. Dong, *Water Res.*, 2008, **42**: 1343–1368.

29 M. Lahti, J. Vilpo and J. Hovinen, *J. Chem. Ed.*, 1999,**76**: 1281–1282.

30 A.S. Bashammakh, S.O. Bahaffi, A.A. Al-Sibaai, H.O. Al-Wael and M.S. El-Shahawi, *Anal. Chim. Acta*, 2007, **592**: 16–23.

31 D.G. Themelis and P.D. Tzanavaras, *Anal. Chim. Acta*, 2002, **452**: 295–302.

32 M. Ghaedi, H. Parham, M. Moghadam, K. Mortazavi, and M. Soylak, *Ann. Chim.*, 2006, **96**: 689–696.

Figure captions

Fig. 1: Effect of sample pH on the recovery of thiocyanate ions on the Ni-Al(NO_3^-) LDH nano-sorbent.

Fig. 2: Effect of sample loading flow rate on the recovery of thiocyanate ions on the Ni-Al (NO_3^-) LDH nano-sorbent.

Fig. 3: Effect of the amount of Ni–Al(NO_3^-) LDH nano-sorbent on the recovery of thiocyanate ions.

Fig. 4: Effect of NaOH concentration **(A)**, and its volume **(B)** on the elution of thiocyanate ions from the column containing Ni–Al(NO_3^-) LDH nano-sorbent.

Fig. 5: Effect of sample volume on the recovery of thiocyanate ions on the Ni-Al (NO_3^-) LDH nano-sorbent.

Fig. 6: Gas chromatogram of a human saliva sample after solid phase extraction with Ni–Al(NO_3^-) LDH under optimized conditions and derivatization by $(CH_3)_2SO_4$. Peaks: **(a)** CH_3OH (hydrolysis product of $(CH_3)_2SO_4$); **(b)** CH_2Cl_2 (solvent) and **(c)** CH_3SCN.

Fig. 7: Gas chromatogram of **(a)** a non-smoker sample and **(b)** a smoker saliva sample after solid phase extraction with Ni–Al(NO_3^-) LDH under optimized conditions and derivatization by $(CH_3)_2SO_4$.

Fig. 1

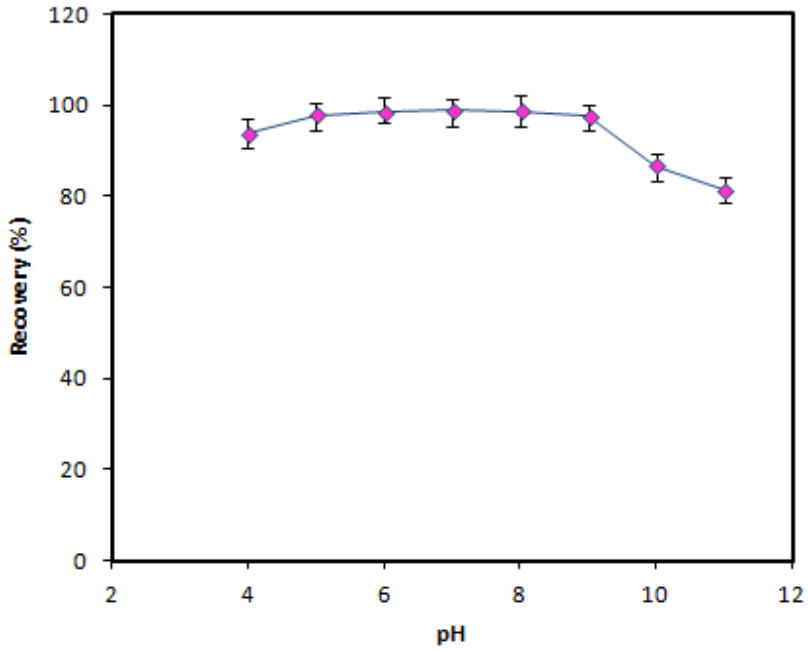


Fig. 2

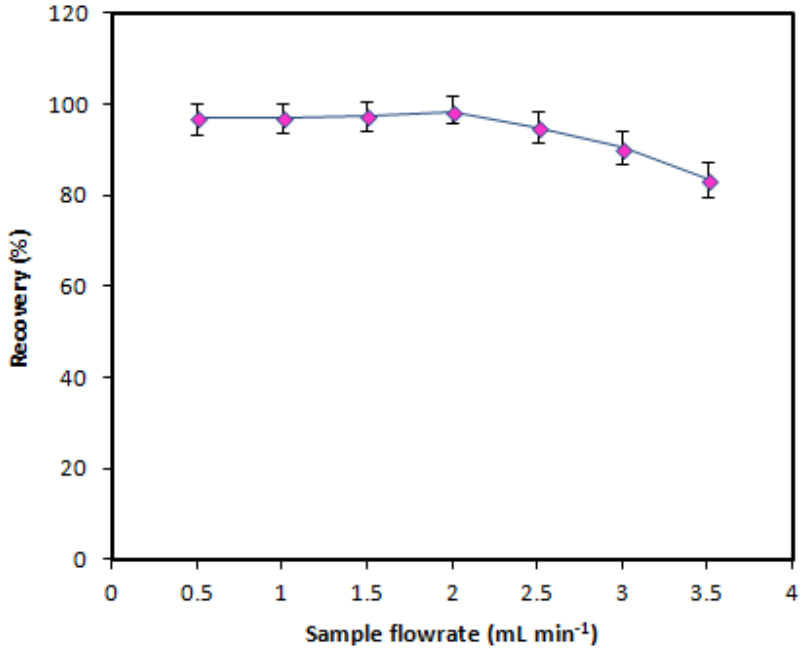


Fig. 3

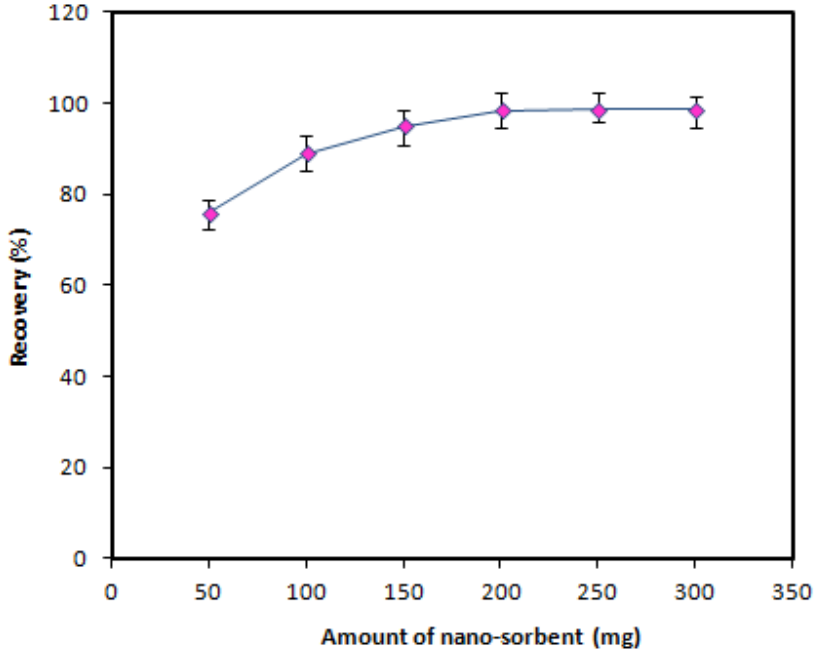


Fig. 4A

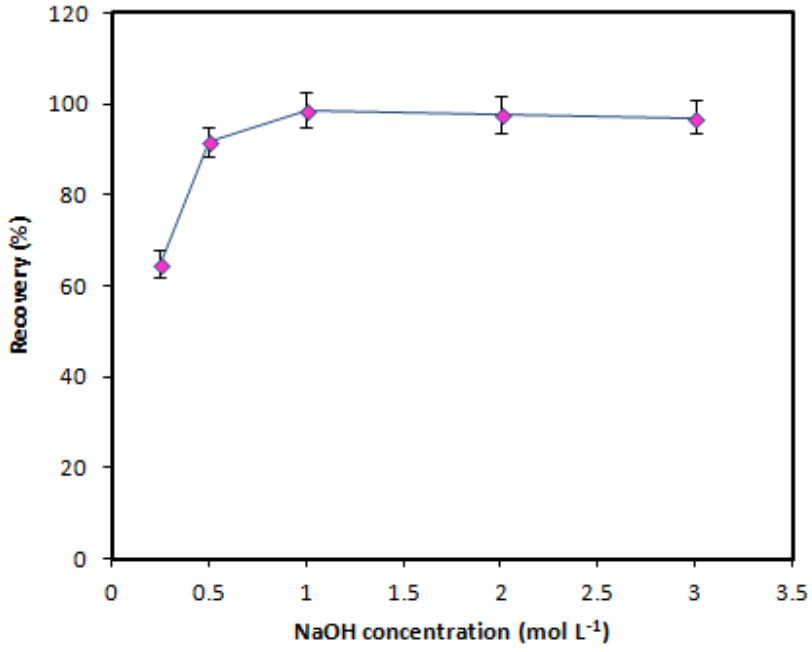


Fig. 4B

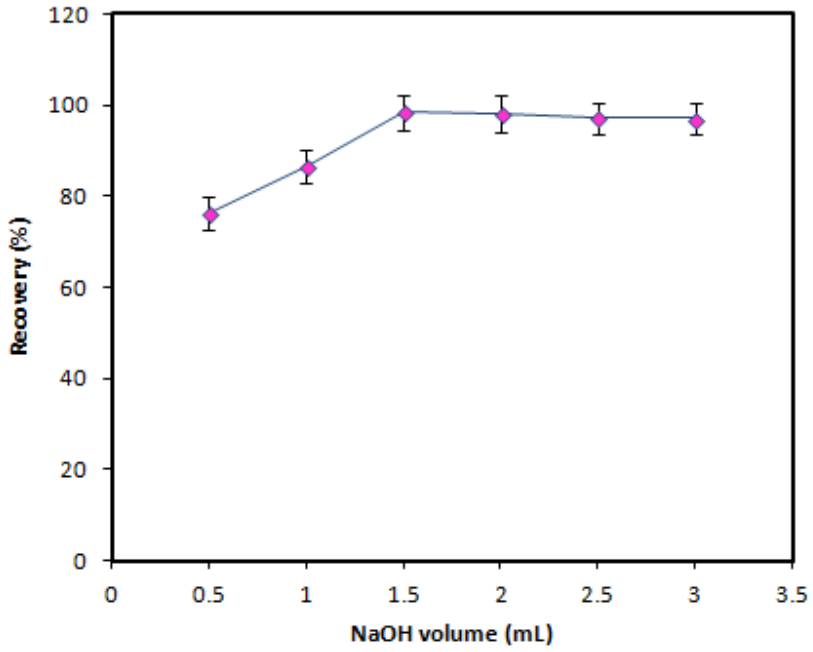


Fig. 5

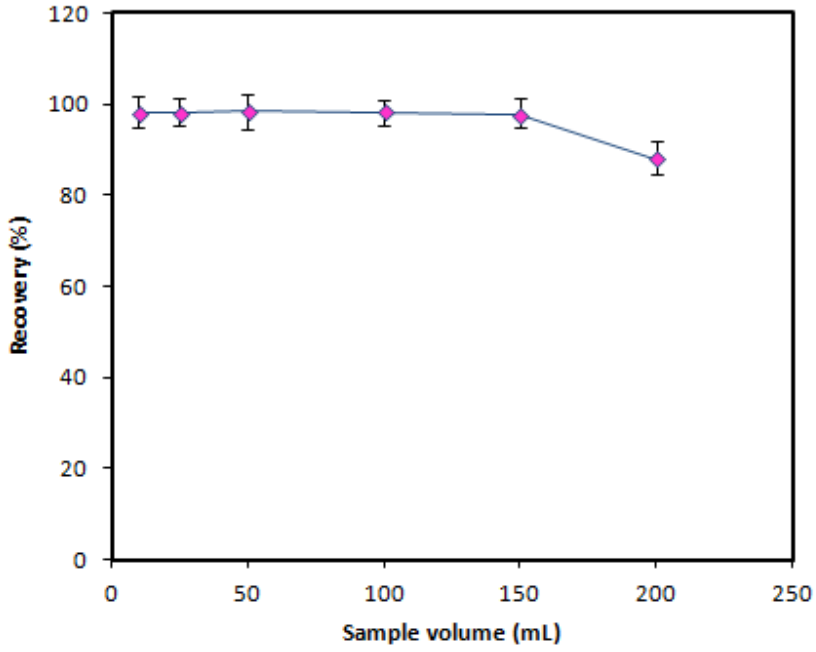


Fig. 6

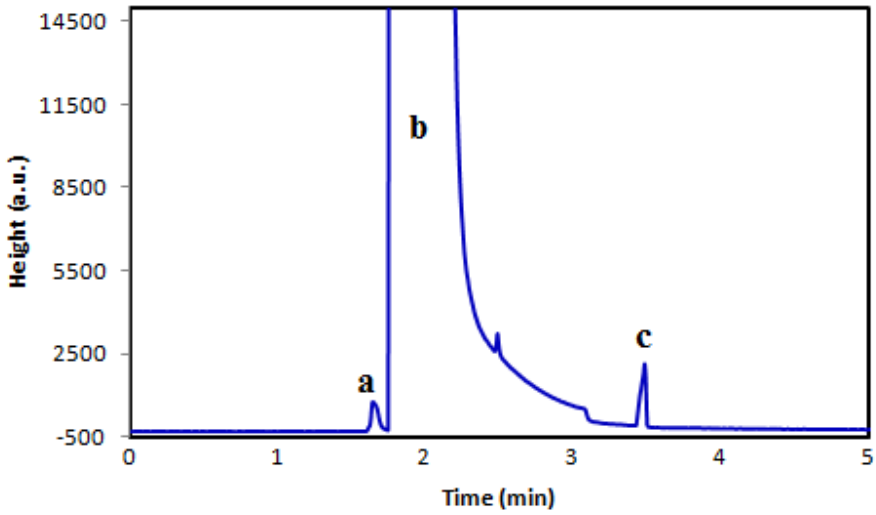


Fig. 7

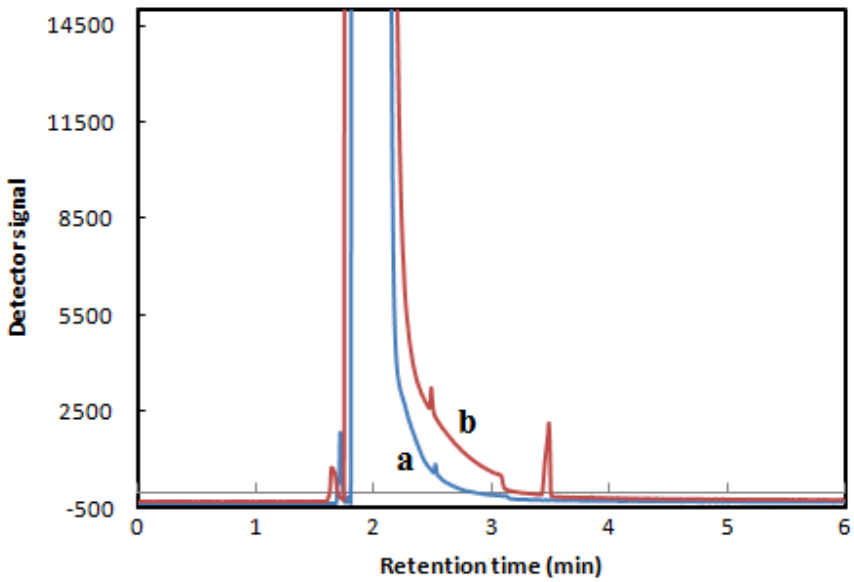


Table 1
Tolerance limits of potentially interfering ions in the determination of 4 ng mL^{-1} of thiocyanate

Interfering ions	Interferent to analyte ratio
Na^+ , K^+ , Li^+, Ca^{2+}, Mg^{2+}, NO_3^-	1000:1
CH_3COO^-, CO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}	500:1
Cl^-, I^-	200:1
SO_4^{2-},CN^-, Br^-	100:1
BrO_3^-,ClO_3^-, PO_4^{3-}, PO_3^{2-}, HCO_3^-	50:1
F^-	25:1

Table 2
Optimum extraction conditions and analytical performance of the presented method for thiocyanate ion determination.

Experimental conditions	
pH	4−11
Amount of sorbent (mg)	200
Eluent type	NaOH
Eluent concentration (mol L^{-1})	1.0
Eluent volume (mL)	1.5
Sample flow rate (mL min^{-1})	2.0
Eluent flow rate (mL min^{-1})	2.0
Maximum sample volume (mL)	150
Analytical parameters	
Linear range (ng mL^{-1})	0.6−80
Intercept	1.86
Slope	42.08
Detection limit (ng mL^{-1})[a]	0.2
Correlation coefficient	0.9972
Relative standard deviation (RSD%) (n=6)[b]	4.6 (40)
Preconcentration factor[c]	100

[a]Calculated as three times the standard deviation of the blank signal divided by the calibration curve slope.
[b]Value in parentheses is the SCN^- concentration (ng mL^{-1}) for which the RSD was obtained.
[c]Calculated as the ratio between the volume of the initial aqueous solution and the final elution volume.

Table 3
Determination of thiocyanate in saliva samples (results of relative recoveries of spiked samples analysis).

Sample	Added SCN^- ($\mu g\ mL^{-1}$)	Found SCN^- [a] ($\mu g\ mL^{-1}$)	Recovery (%)[b]
Non-smoker	–	N.D.	–
	50	50.7± 3.6	101.4
	100	98.0± 3.2	98.0
Smoker 1	–	52.9± 3.8	–
	50	99.9± 4.4	94.0
	100	148.4± 4.7	95.5
Smoker 2	–	95.5± 3.4	–
	50	145.1± 4.8	99.2
	100	190.2± 4.1	94.7

N.D.: Not detected
[a] Mean of three experiments ± standard deviation.
[b] Relative recovery (%) = [(found-base)/added] × 100.

Table 4
Determination of thiocyanate in serum samples (results of relative recoveries of spiked samples analysis).

Sample	Added SCN^- ($\mu g\ mL^{-1}$)	Found SCN^- [a] ($\mu g\ mL^{-1}$)	Recovery (%)[b]
Non-smoker	–	N.D.	–
	50	48.2± 4.3	96.4
	100	95.9± 3.1	95.9
Smoker 1	–	N.D.	–
	50	47.1± 4.2	94.2
	100	99.4± 4.9	99.4
Smoker 2	–	N.D.	–
	50	48.6± 4.2	97.2
	100	95.1± 5.8	95.1

N.D.: Not detected
[a] Mean of three experiments ± standard deviation.
[b] Relative recovery (%) = [(found-base)/added] × 100.

Table 5
Comparison of the presented method with other reported methods for thiocyanate determination.

Technique	Detection method	Linear range (μg mL^{-1})	Enrichment factor	LOD (μg mL^{-1})	RSD (%)	Reference
EME	IC	0.1-10	4.2	0.03	6.6	1
LLE	DV-SIA	2.9-29	-	1.16	-	11
LLE	Spectrophotometry	0.1-20	-	0.02	2.3	30
SE	Spectrophotometry	0.0-4.0	-	0.007	1.7	31
SPE	Spectrophotometry	0.4-2	150	0.0028	-	32
SPE	GC-FID	0.0006-0.08	100	0.0002	4.6	This work

EME: Electro membrane extraction; LLE: Liquid-liquid extraction; SE: Solvent extraction; SPE: Solid phase extraction; IC: Ion chromatography; DV-SIA: Double-valve sequential injection analysis; GC-FID: Gas chromatography-flame ionization detection