CHEMIJA. 2006. Vol. 17. No. 2–3. P. 21–24

Thiocyanate determination by ion pair chromatography

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A simple and rapid ion pair chromatographic method has been developed for the determination of thiocyanate. Separation was carried out on a 25 cm × 4.6 mm Ascentis C_{18} column (5 μm particle size) with a mobile phase containing 2 mmol/l tetrabutylammonium (TBA)-phosphate (pH 6.5) in 40 (v/v) % acetonitrile at a flow rate of 0.5 ml/min. Detection was performed by direct UV absorption at 210 nm. This study investigated the effect of various experimental factors, such as acetonitrile content, pH, and ion-pairing cation concentration in the mobile phase on the retention behaviour of anions. Under optimized conditions, four anions (Br^-, NO_3^-, I^- and SCN^-) were effectively separated within 8 minutes. The calibration curve was linear in the thiocyanate concentration range from 5×10^{-6} to 5×10^{-4} mol/l with an excellent correlation coefficient of more than 0.999. The limit of detection was 1.5×10^{-6} mol/l (signal / noise = 3). The method was applied for determination of thiocyanate ions in human saliva. Saliva sample preparation involved only centrifugation and appropriate dilution.

Key words: ion pair chromatography, thiocyanate, human saliva

INTRODUCTION

Low levels of thiocyanate (SCN^-) normally present in human body fluids (e. g., serum, saliva, urine) are produced during digestion of some vegetables (cabbage, turnip, kale) [1] or by intake of thiocyanate-containing foods such as milk and cheese [2]. Higher concentrations of thiocyanate, which is a major metabolite of cyanide, in physiological fluids arise from exposure to cyanide, inhalation of fires or tobacco smoke [3]. The presence of thiocyanate in body fluids may indicate cyanide exposure. Furthermore, thiocyanate content in human body fluids (especially saliva) is considered as a biomarker for identification of nonsmokers and smokers [4]. Therefore, precise knowledge of thiocyanate concentration in such samples is mandatory.

Many methods have been developed for determination of thiocyanate ions in various samples, for example, spectrophotometric [5–7] and flow injection [8] methods based on the reaction with Fe^{3+} or on the König reaction, atomic absorption spectrophotometry [9], electrochemical methods (with ion selective electrodes) [10–12], gas chromatography with electron capture [13] or mass spectrometric [14, 13] detection, capillary electrophoresis [15–17] and micellar electrokinetic capillary chromatography [18]. The major difficulty in its quantitation in most methods is the potential interference of cyanide which usually accompanies thiocyanate in real samples [19]. Furthermore, many of these methods suffer from poor reproducibility, sensitivity and selectivity, are complicated, laborious to perform and require unpleasant or toxic reagents.

During the last two decades perhaps the most common analytical technique used for thiocyanate determination is ion chromatography (IC). For a long time IC with conductivity detection [4] had been the most popular method for the determination of inorganic anions. However, this detector is not selective and therefore not suitable for the determination of thiocyanate in real samples containing large amounts of other anions (Cl^-, SO_4^{2-}, etc.). On the other hand, thiocyanate is electroactive and absorbs in the UV region, therefore selectivity can be significantly improved by using amperometric or photometric detectors [20, 21]. However, even these selective detectors often do not provide accurate determination of thiocyanate in complex anion mixtures due to a relatively low efficiency of the conventional anion-exchange stationary phases.

An alternative method to the traditional IC technique is the reversed-phase ion pair chromatography (IPC). In IPC, many different parameters modify the retention behaviour of the analytes and, consequently, the selectivity of the separation. Furthermore, reversed-phase stationary phases used in IPC are usually much more efficient than the conventional ion-exchangers. However, only a limited number of studies have been reported on the determination of the thiocyanate anion by IPC. Miura et al. [22] described separation of thiosulfate, thiocyanate and polythionates in a hot-spring water samples by ion-pair chromatography with ultraviolet absorbance detection. Xu et al. [23] developed this method for the

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separation of thiocyanate, nitrite, thiosulphate, iodide and sulphide in lake water with amperometric detection. However, to our knowledge, no method for the determination of thiocyanate in saliva by ion pair chromatography with UV detection has been reported to date.

This paper describes a simple and rapid ion pair chromatographic method for determination of thiocyanate in saliva by ion pair chromatography with UV detection.

EXPERIMENTAL

The HPLC instrumentation consisted of a Waters Model 501 high-pressure pump, an injection valve equipped with a sample loop of 20 µl and a Waters Lambda-Max Model 481 variable wavelength UV detector set to absorb at 210 nm. The results and data were collected and plotted on a SP 4290 plotter/integrator (Spectrophysics, San Jose, CA, U.S.A.). The mobile phase flow rate was 0.5 ml/min. Chromatographic separations were performed on a 5 µm Ascentis C_{18} column (250 mm × 4.6 mm i. d.).

HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). H_3PO_4 (99%) was obtained from Labochema (Vilnius, Lithuania). Tetrabutylammonium (TBA) hydroxide (30% w/w in water) and all other reagents were of analytical-reagent grade from Reachim (Russia).

All mobile phases and standard solutions were prepared using doubly distilled water. Stock solutions (500 mg/l) of the anions were prepared from KSCN, KJ, KNO_3, KBr in water. All working solutions were prepared by suitable dilution. Acetonitrile-water mixtures containing TBA-phosphate (pH 6.5) were used as mobile phases. All mobile phase and sample solutions were filtered through a 0.45 µm nylon 66 membrane filter (Supelco, Bellefonte, PA, USA) and degassed by ultrasonication.

RESULTS AND DISCUSSION

Since thiocyanate has no significant absorption in the visible range, in this work the UV region was used for detection. The detection conditions were optimized by measuring the peak area of the 2×10^{-4} mol/l SCN^- standard at different wavelengths. The results are demonstrated in Fig. 1. As one can see, the SCN^- peak area gradually decreases as the detection wavelength increases. Although the highest detection sensitivity was achieved at 200 nm, a wavelength of 210 nm was selected for further experiments in order to reduce possible interferences from the sample matrix.

Several other inorganic anions also absorb at 210 nm and, consequently, may interfere in the determination of SCN^-. In consideration of the composition of physiological samples, IPC separation of thiocyanate and three singly charged anions (Br^-, NO_3^- and I^-) having similar chromatographic properties was optimized.

Four main parameters were chosen for separation optimization, namely the concentration of the ion-pairing reagent (TBA), the concentration of organic modifier (CH_3CN), mobile phase pH and ionic strength. Obviously

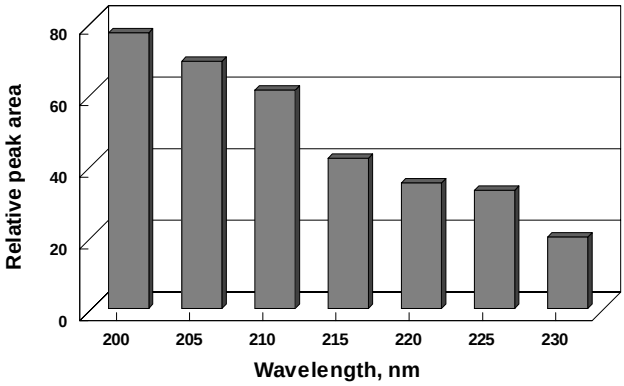

Fig. 1. Effect of detection wavelength on the relative peak area of the thiocyanate standard (2×10^{-4} mol/l)

the ion-pairing reagent plays a predominant role in ion-pair chromatography. In order to establish the optimal concentration of TBA in the mobile phase for complete and rapid separation, the effect of various concentrations (from 0 to 3 mmol/l) of TBA-phosphate (pH 6.5) on the chromatographic behaviour of anions was studied. As expected, the retention of all anions increased with increasing the amounts of TBA in the mobile phase (Fig. 2). If the ion-pairing reagent is omitted from the mobile phase, no retention of the anions is observed. According to the dynamic ion-exchange mechanism, the sorption of TBA cation on the stationary phase offers dynamic anion-exchange sites. Therefore with increasing the TBA-phosphate concentration in the mobile phase the ion-exchange capacity of the stationary phase and the retention of anions increases. Figure 2 also shows that the effect of TBA concentration is significant in the low concentration range (from 0 to 1 mmol/l) and less pronounced at higher concentrations of TBA. The explanation of this fact lies in the limited adsorption capacity of the stationary phase and / or in the increasing concentration of the counter-ion (phosphate) which competes

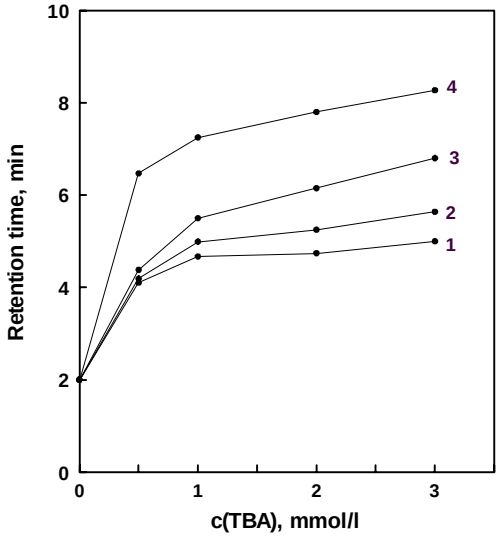

Fig. 2. Effect of TBA concentration in the mobile phase on anion retention. Mobile phase: TBA-phosphate, 40% CH_3CN, pH 6.5. *1* – Br^-; *2* – NO_3^-; *3* – J^-; *4* – SCN^-

with analyte anions. When the TBA concentration in the mobile phase is higher than 1 mmol/l, all anions are fully separated. For further investigation, a 2 mmol/l TBA concentration was chosen.

Another important parameter affecting the separation performance is the amount of organic solvent in the mobile phase. The acetonitrile concentration was varied from 30 to 60%. The plots of retention time versus acetonitrile concentration in the mobile phase are presented in Fig. 3. An increase in the acetonitrile concentration accelerates elution of the anions. One possible explanation is that acetonitrile competes with TBA cations for the sorption centers on the surface of the stationary phase. Therefore increasing the concentration of the acetonitrile reduces the ion-exchange capacity of the stationary phase, and the retention of anions decreases. Separation selectivity of anions increases with a decrease of CH_3CN concentration, and all anions are completely resolved when the concentration is less than 45%. Based on these results, the organic / aqueous solvent ratio of 40 : 60 (v/v) was selected for further separations, because it provided the shortest separation time with an acceptable resolution.

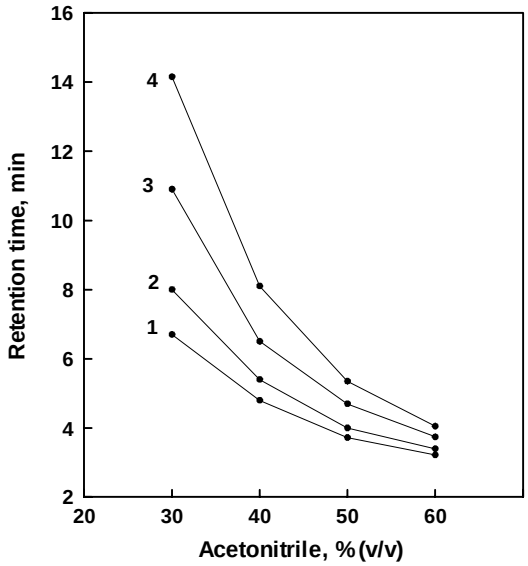

Fig. 3. Effect of acetonitrile concentration in the mobile phase on anion retention. Mobile phase: 2 mmol/l TBA-phosphate, CH_3CN/H_2O, pH 6.5. *1* – Br^-; *2* – NO_3^-; *3* – J^-; *4* – SCN^-

The influence of mobile phase pH on the resolution of anions was examined in the pH range 3.0–7.0. However, no significant changes in the retention and separation selectivity were observed.

Finally, the effect of mobile phase ionic strength (concentration of NaCl) on the retention time was briefly investigated. The retention times of all anions decreased with increasing the ionic strength. All anions carried the same charge, therefore ionic strength had only a minor effect on separation selectivity.

From the above optimization experiments, the optimum mobile phase conditions found were 2 mmol/l TBA-phosphate (pH 6.5) in 40% CH_3CN at a flow-rate of 0.5 ml/min. The chromatogram obtained under optimum conditions for a standard solution is show in Fig. 4. As one can see, an excellent separation of four compounds was obtained in about 8 min.

To evaluate the practical applicability of the proposed technique, several analytical performance characteristics such as linearity, detection limit and repeatability were investigated under optimized conditions. The linearity of the method was tested by preparing a calibration curve

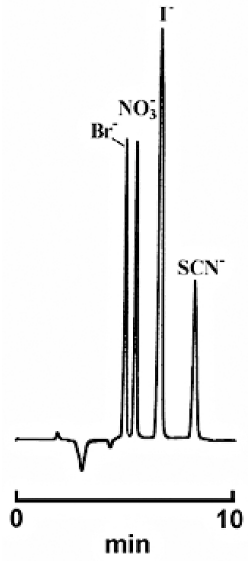

Fig. 4. Chromatogram of a standard anion solution under optimized conditions. Conditions: column 25 cm × 4.6 mm Ascentis C_{18} (5 μm). Eluent: 2 mmol/l TBA-phosphate, 40% CH_3CN, pH 6.5. UV detection at 210 nm

for thiocyanate with seven points. The concentration range tested was from 0.005 to 0.5 mmol/l and each concentration level was injected three times. The calibration curve was linear in the test concentration range, with an excellent correlation coefficient of more than 0.999. The limit of detection was defined as a concentration that produced a signal equal to three times the background noise level (signal/noise = 3). The main analytical characteristics are summarized in Table 1.

To evaluate the proposed method for physiological samples, it was applied for determination of thiocyanate in nonsmoker and smoker saliva samples. Saliva samples were prepared as follows: 1 g of saliva was diluted with 2 ml of distilled water, then 2 ml of acetonitrile was added and mixed for 15 min. The solution was centrifuged for 10 min in order to remove precipitated proteins, and then analyzed.

Figure 5 shows typical chromatograms for smoker (*a*) and nonsmoker (*b*) human saliva samples. The recoveries of thiocyanate were determined by adding known concentrations to saliva samples. As is shown in Table 2, the recoveries of thiocyanate from nonsmoker and smoker saliva were 96–102% and 102–104%, respectively. These results suggest that the matrix components do not interfere with the determination of thiocyanate.

Table 1. **Analytical performance characteristics**

Characteristic	Value
Linearity range (n = 3)	$5 \times 10^{-6} - 5 \times 10^{-4}$ mol/l (n = 3)
Correlation coefficient (r)	0.999
Detection limit (S/N = 3)	1.5×10^{-6} mol/l
Retention time RSD ($c = 5 \times 10^{-5}$ mol/l)	0.42 % (n = 5)
Peak area RSD ($c = 5 \times 10^{-5}$ mol/l)	2.6 % (n = 5)

Table 2. **Results of thiocyanate determination in saliva samples (n = 3)**

Sample	Found, mg/l	Added, mg/l	Found total, mg/l	Recovery, %
Saliva	88.2	5.0	93.3	102
(smoker)		10.0	98.6	104
Saliva	25.6	5.0	30.4	96
(nonsmoker)		10.0	35.8	102

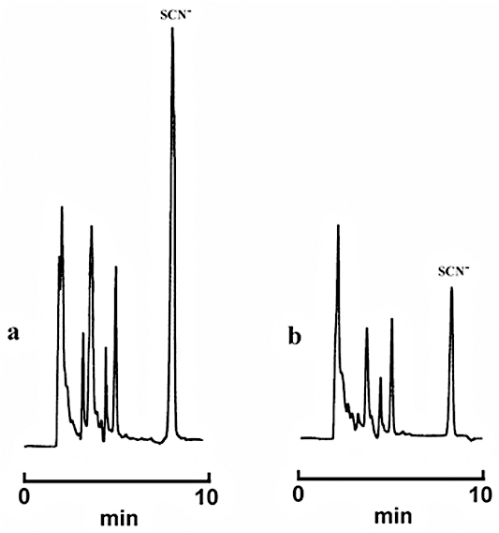

Fig. 5. Chromatograms of smoker (*a*) and nonsmoker (*b*) saliva samples. Chromatographic conditions as in Fig. 4

In conclusion, the developed ion pair chromatographic method is fully suitable for the determination of thiocyanate in human saliva. Thiocyanate concentrations in saliva were determined rapidly and easily by the proposed method without any special sample pretreatment, except centrifugation and appropriate dilution.

Received 28 June 2006
Accepted 28 August 2006

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TIOCIANATO NUSTATYMAS JONŲ PORŲ CHROMATOGRAFIJOS METODU

Santrauka

Ištirtos ir optimizuotos tiocianato nustatymo fiziologiniuose skysčiuose jonų porų chromatografijos metodu sąlygos: kolonėlė – 250 mm × 4,6 mm Ascentis C_{18} (5 μm); judri fazė – 2 mmol/l tetrabutilamonio fosfatas (pH 6,5), 40 (v/v) % acetonitrilo. UV detektavimas esant 210 nm bangos ilgiui. Ištirta judrios fazės pH, acetonitrilo kiekio joje bei jonų porų reagento koncentracijos įtaka keturių anijonų (Br^-, NO_3^-, I^- ir SCN^-) atskyrimui. Optimaliomis sąlygomis visi keturi anijonai yra puikiai atskiriami greičiau nei per 10 min. Tiocianatui išmatuotos pagrindinės analizinės charakteristikos: kalibracinė kreivė yra tiesinė koncentracijų intervale $5 \times 10^{-6} - 5 \times 10^{-4}$ mol/l, koreliacijos koeficientas – 0,999; aptikimo riba – $1,5 \times 10^{-6}$ mol/l. Metodas pritaikytas tiocianato nustatymui žmogaus seilėse.