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Analysis of thiocyanate in biological fluids by capillary zone electrophoresis

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Abstract

A new sensitive and simple method has been developed for the determination of thiocyanate in human serum, urine and saliva. The determinations were performed in a fused-silica capillary [64.5 cm (56 cm effective length)×75 μm] using 0.1 *M* β-alanine–HCl (pH 3.50) as a background electrolyte, separation voltage 18 kV (negative polarity), temperature of capillary 25°C and direct detection at 200 nm. Serum samples were 10-times diluted with deionised water and deproteinised with acetonitrile in the ratio 1:2. Urine and saliva samples need only 20-fold dilution with deionised water. The proposed method was successfully applied to the determination of thiocyanate in various human serum, saliva and urine samples.

Keywords: Tobacco; Thiocyanate; Inorganic anions

1. Introduction

The thiocyanate ion is usually present in low concentrations in human serum, saliva and urine as a result of the digestion of some vegetables of the genus *Brassica* containing glucosinolates (cabbage, turnip, kale) [1] or by intake of thiocyanate-containing foods such as milk and cheese [2]. Higher concentration of this ion, which is a metabolic product of cyanide, arises from tobacco smoke. This reaction is catalysed by the enzyme rhodanese (EC 2.8.1.1) [3]:

$$CN^- \xrightarrow{\text{rhodanese}} SCN^-$$

The level of thiocyanate is thus considered a good probe for distinguishing between smokers and non-smokers and its determination has been widely used in health screening programmes for the evaluation of smoking behaviour [4–8]. Moreover sodium nitroprusside often used as a hypotensive agent also contributes to serum thiocyanate [9,10].

Several methods have been reported for the determination of this ion in biological samples. These are spectrophotometric [11–13] or flow injection [14] methods based on the reaction with Fe^{3+} or on the König reaction, gas chromatography [15], ion chromatography [16–18] and atomic absorption spectrophotometry [19]. Many of these methods are complicated, laborious to perform and require unpleasant or toxic reagents.

Recently a new capillary zone electrophoretic method of thiocyanate analysis was developed for the assay of rhodanese enzymatic activity [20]. Because of better quantitative parameters in comparison with capillary zone electrophoresis (CZE)

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and micellar electrokinetic chromatography (MEKC) methods [21–24] published so far this method has a great potential for the thiocyanate determination in other applications. This paper reports results from investigations of its applicability for the analysis of thiocyanate in different biological fluids – human serum, urine and saliva. Special attention was paid to the procedures of sample preparation

2. Experimental

2.1. Materials and reagents

β-Alanine and sodium thiocyanate were obtained from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytical-reagent grade, supplied by Fluka (Buchs, Switzerland). All solutions were prepared using deionised water from a Milli-Q Academic system (Millipore, Milford, MA, USA) and filtered through a 0.45-μm membrane filter.

2.2. Capillary electrophoresis conditions

A Hewlett-Packard ^{3D}Capillary Electrophoresis system (Waldbronn, Germany) with a diode-array UV–Vis detector was used to carry out all CZE separations. Data were collected on a HP Vectra VL5 166 MHz personal computer using the Hewlett-Packard ^{3D}CE ChemStation software. A Polymicro Technology (Phoenix, AZ, USA) fused-silica capillary [64.5 cm (56.0 cm effective length)×75 μm] was used for all separations. The background electrolyte was prepared by adding hydrochloride acid to 0.1 *M* β-alanine solution up to pH 3.50. Injection was accomplished by an application of 50.0 mbar pressure to the inlet vial for 4.0 s. Separations were performed at 18 kV (negative polarity). Samples were detected using a diode-array detector at 200 nm with a bandwidth 20 nm. The capillary was washed with the background electrolyte for 3 min before each run. Peak identification was accomplished by comparing electrophoretic mobilities of suspected peaks with that of authentic standard.

2.3. Sample preparation

Serum samples were 10-times diluted with deionised water and deproteinised with acetonitrile in the ratio 1:2. Urine and saliva samples were 20-times diluted with deionised water. For quantification purposes, an internal standard was added to the samples and standards to correct for the changes in sample composition and sampling volume. Bromide anion was chosen as the internal standard since it migrates closely before the thiocyanate peak and its concentrations in biological fluids are below the detection limit of this method.

3. Results and discussion

Inorganic anions are almost always determined by capillary electrophoresis at an alkaline pH [25–27]. In order to reverse the direction of the electroosmotic flow a long-chain quaternary ammonium salt usually must be added as a flow modifier to the carrier electrolyte. In this work the analysis of thiocyanate was carried out at pH 3.5. By working at lower pH values, the capillary's silanol groups are hardly ionised and the electroosmotic flow is minimal [28]. No flow modifier is therefore needed. Direct UV absorbance detection at 200 nm was chosen because thiocyanate adsorbs reasonably well at this wavelength while other inorganic anions are not detected. Moreover most organic anions that may be present in these types of samples and adsorb at 200 nm, do not interfere as they are protonated at this pH.

Analyses of biological fluids represent a difficult analytical task. Such complex matrices require special sample pretreatment for successful separation by CZE because of high protein concentrations and high ionic strengths. Various approaches have been used to overcome this problem. The direct injection of biological fluid was used in several clinical and pharmacological studies [29–34]. This approach could not be applied in this case considering low pH of background electrolyte. At this pH denaturation and subsequent precipitation of sample proteins could occur and cause the capillary clogging. The second possibility is using ultrafiltration for the preparation of protein-free samples. However special filtration membranes are rather expensive and require

long periods of centrifugation. Precipitation with acetonitrile is a simple, rapid and effective method for removal of serum proteins. In addition, the stacking effect of acetonitrile on sample has been documented [35,36]. Fig. 1A shows the analysis of thiocyanate in non-smoker serum after 10-fold dilution with deionised water and deproteinisation with acetonitrile. The spiking sample with the standard supported the identity of thiocyanate peak (Fig. 1B). As seen the thiocyanate peak is well resolved from preceded peak of nitrate, no other anions were detected. Fig. 2 illustrates the increasing concentration of thiocyanate in smoker serum. The same procedure could be also applied for plasma, urine and saliva samples (data not shown).

Since the concentration of proteins in urine from normal individuals is many times lower in comparison with serum, the possibility of direct injection of urine after dilution was also tested. As a result of experimentation with different dilution ratios, we found that a 20-fold dilution of urine samples is sufficient to keep the capillary performance. However, patients with proteinuria require sample treatment similar to that of serum. Fig. 3 gives the electropherogram obtained from the direct injection

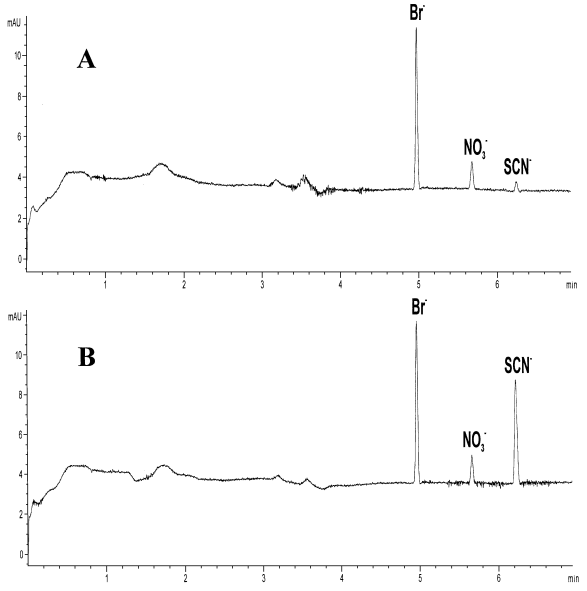

Fig. 1. Electropherogram of the non-smoker serum after 10-fold dilution with deionised water and deproteinisation with acetonitrile in the ratio 1:2 (A) and the same sample spiked with 500 μ*M* thiocyanate standard (B). Separation conditions: fused-silica capillary [64.5 cm (56.0 cm effective length)×75 μm], background electrolyte 0.1 *M* β-alanine–HCl (pH 3.50), injection 50 mbar for 4 s, separation voltage 18 kV (negative polarity), detection at 200 nm, temperature of capillary 25°C.

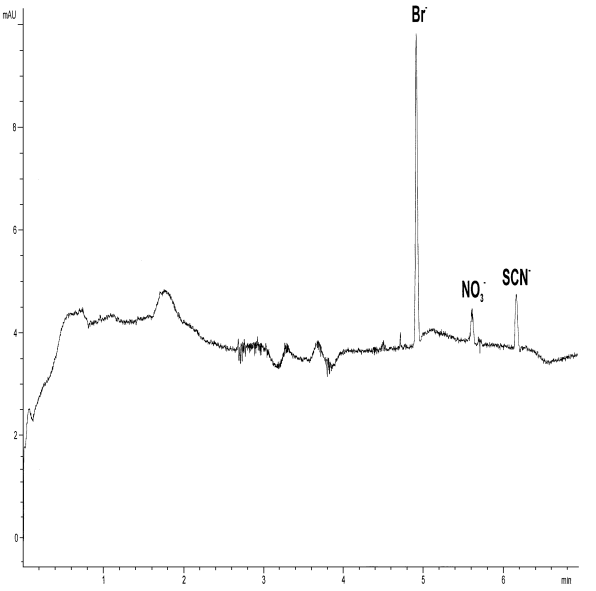

Fig. 2. Electropherogram of the smoker serum. Conditions as in Fig. 1.

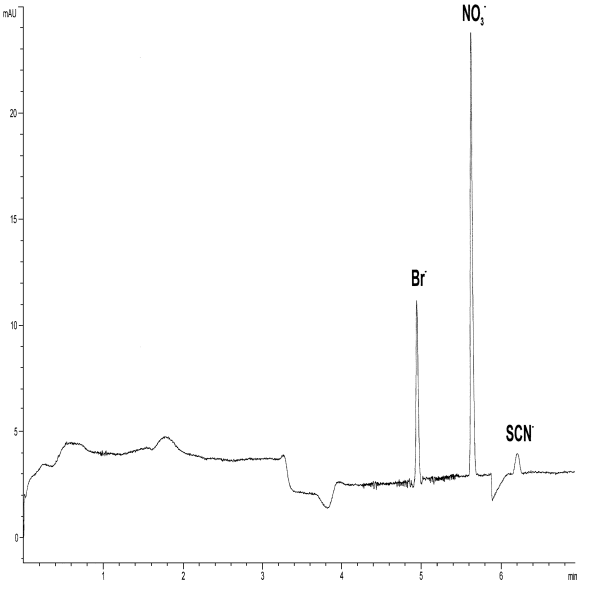

Fig. 3. Electropherogram of the 20-times diluted smoker urine. CZE conditions as in Fig. 1.

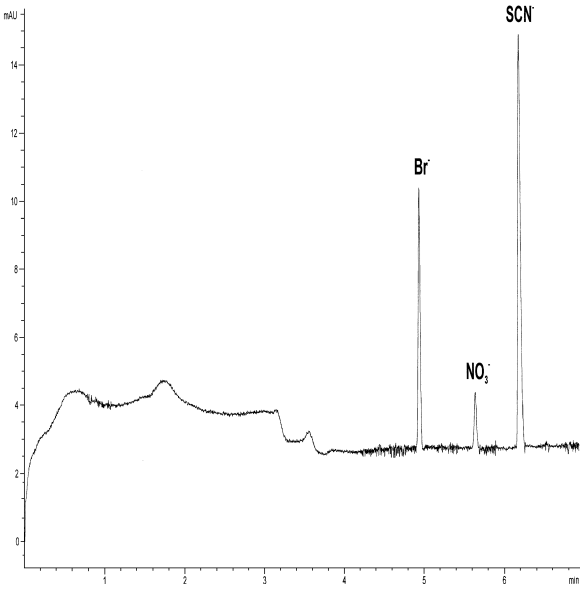

Fig. 4. Electropherogram of the 20-times diluted smoker saliva. CZE conditions as in Fig. 1.

of 20-times diluted smoker urine. This procedure of sample preparation is also applicable for analysis of thiocyanate in saliva (Fig. 4). As can be seen the saliva sample have much higher level of thiocyanate compared to serum and urine.

Reproducibility, linearity and sensitivity of the method were tested (Table 1) by analysing standards of thiocyanate prepared in blank serum and urine samples. The urine samples were prepared without deproteinisation. Table 1 shows excellent reproducibility obtained for migration time and peak area, which is probably result of the suppressed electroosmotic flow. The calibration graphs were linear over the range 25–500 μ*M* of thiocyanate with correlation coefficient better then 0.999, the detection limits were in the range 0.7–1.5 μ*M* at a signal-to-noise ratio of 3. The recoveries of thiocyanate by the CZE method described were tested by adding known amounts of thiocyanate to the serum and urine samples containing known level of this compound. The recoveries were reasonable (Table 1).

To demonstrate the application of the developed method for the analysis of thiocyanate, several serum, saliva and urine smoker and non-smoker samples were analysed as described. Table 2 lists the determined values of thiocyanate. All these values are close to those in the literature [7,8,17,18,37–39], which were determined with methods other than CZE. In addition the concentrations of thiocyanate in serum, urine and saliva were found to be different between the smokers and the non-smokers.

Table 2
Results of the thiocyanate determination in different biological fluids

Sample	Thiocyanate		
	Serum (μ*M*)	Urine (μ*M*)	Saliva (m*M*)
Non-smoker ($n=3$)	87.5±33.2	84.0±39.9	1.05±0.35
Smoker ($n=3$)	196.4±44.9	216.5±49.2	2.05±0.45

Table 1
Parameters of the developed method[a]

Parameter	Serum	Urine
Run time (min)	10[b]	10[b]
Migration time reproducibility (%, $n=10$)	0.04	0.08
Peak area reproducibility (%, $n=10$)	0.71	0.88
Linearity (μ*M*)	15–500[c]	25–500[c]
Correlation coefficient	0.99949	0.99992
Limit of detection (μ*M*; $S/N=3$)	1.5[c]	0.7[c]
Recovery (%, $n=5$)	92.2–105.7	94.7–101.9

[a] Samples: standards of sodium thiocyanate in blank serum or urine samples. Serum samples were 10-times diluted with deionised water and deproteinised with acetonitrile in the ratio 1:2. Urine samples were 20-times diluted with deionised water. Separation conditions: fused-silica capillary [64.5 cm (56.0 cm effective length)×75 μm], background electrolyte 0.1 *M* β-alanine–HCl (pH 3.50), injection 50 mbar for 4 s, separation voltage 18 kV (negative polarity), detection at 200 nm, temperature of capillary 25°C.

[b] Including a 3-min flush cycle.

[c] A final concentration of thiocyanate in sample.

4. Conclusion

A simple and rapid method for the determination of thiocyanate in human serum, saliva and urine has been developed. Compared to spectrophotometric and other assays, the capillary electrophoretic method is rapid, can be automated, and requires only a small amount of sample which is especially important in the case of serum analyses. In addition to that the method can be applied for determination of other ions such as nitrate.

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