Short Communication

High-performance liquid chromatographic determination of thiocyanate anion by derivatization with pentafluorobenzyl bromide

Xiaoyu Liu and Zihou Yun*

Department of Chemistry, Beijing Normal University, 100875 Beijing (China)

ABSTRACT

This paper describes, for the first time, a high-performance liquid chromatographic method for the determination of thiocyanate anion as its pentafluorobenzyl derivative. The method is based on pre-column derivatization of thiocyanate anion with α-bromo-2,3,4,5,6-pentafluorobenzyl (PFB $\cdot$ Br). The derivative and chromatographic conditions were investigated. It is found that of different derivative solvents, such as acetone, acetonitrile and dichloromethane, acetone is the best. The chromatographic conditions were optimized by using a Zorbax ODS column and methanol–water (80:20, v/v) as mobile phase at a flow-rate of 1.0 ml/min and the eluent was monitored by a UV detector operating at 254 nm. The detection limit is 2.7 ng, and the method is both sensitive and selective. It has been successfully applied to the analysis of waste water of an electroplate and the saliva of regular smokers. Recoveries thus obtained were 93.7–102% and 92.1–101%, respectively.

INTRODUCTION

Thiocyanate has been extensively used for various purposes, such as dyeing, medicine, photography, catalysis, prevention of erosion, etc. [1]. Several authors have demonstrated *in vivo* conversion of thiocyanate to cyanide by an erythrocytic enzyme, and suggested the physiological effects of its conversion [2]. In addition, since thiocyanate has a long plasma half-life, its concentration is a good index of long-term exposure to cigarette smoke [3]. Finally, the thyreostatic effect of the thiocyanate anion, produced by inhibiting iodine transport in the thyroid, is well known [4].

A number of publications have been devoted to the determination of thiocyanate by chromatographic methods, such as derivative gas chromatography, ion chromatography and ion-exchange chromatography [5]. In derivative gas chromatography, α-bromo-2,3,4,5,6-pentafluorobenzyl (PFB $\cdot$ Br), which is highly sensitive to electron-capture detection (ECD), is widely employed as the derivative reagent with which thiocyanate reacts to give a high yield and stable product, which is then quantitatively analysed by GC [5]. This method has high sensitivity and selectivity, and thus is of recent interest. However, its disadvantages are obvious: (1) in pre-column derivatization the thiocyanate anion

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* Corresponding author.

reacts with PFB · Br in dichloromethane, which is an unsuitable solvent for ECD because of the existence of the halide element and (2) because derivatization occurs in CH_2Cl_2 solvent, phase-transfer catalysts (PTCs) are required to transfer the thiocyanate anion from the aqueous solution to the organic phase, thus making the procedure both complicated and time-consuming.

In this report, we first present a novel procedure for derivative determination of thiocyanate by HPLC. Thiocyanate reacts with PFB · Br and acetone used as the solvent. After derivatization, the product in high yield and with high stability is applied to HPLC, and detected by UV detection. The principal advantage of the approach lies in the fact that the derivative reaction takes place in a homogeneous phase, therefore no PTC is required. In addition, the selectivity of the procedure is better than that of previous methods. The method is comparatively easy to operate and suitable for microanalysis. At the end of this paper, the present method is utilized to analyse the waste water of an electroplate and saliva samples from regular smokers.

EXPERIMENTAL

Reagents

Methanol, dichloromethane, acetone, acetonitrile and potassium thiocyanate were obtained from the Chemical Reagent Company of Beijing, α-bromo-2,3,4,5,6-pentafluorobenzyl was purchased from Aldrich. All reagents used were analytical grade. Distilled and deionized water was used in the preparation of all solutions.

Apparatus

A Varian 5060 liquid chromatograph equipped with a UV-100 detector, a 3090A integrator, a Zorbax ODS analytical column (5 μm, 250 mm × 4.6 mm) and a Spectra-Focus fast-scanning UV detector was used.

Standard solution

A solution of PFB · Br in dichloromethane (4 μl/ml) and a solution of PFB · Br in acetone (7 μl/ml) were stored in a refrigerator. Potassium thiocyanate standard solutions (0.1 mol/l) and hexadecyl trimethyl ammonium bromide

(HDTMAB) solution (0.1 mol/l) were prepared by dissolving solid potassium thiocyanate and HDTMAB in water.

CH_2Cl_2 as derivative solvent

A 0.1-ml aliquot of 0.001 mol/l aqueous potassium hydroxide solution, 0.1 ml of standard solution of thiocyanate anion, 0.1 ml of HDTMAB solution and 0.5 ml of a solution of PFB · Br in dichloromethane were added in succession to a 5-ml glass-stoppered test tube. The reaction mixture was shaken mechanically at 30°C for 20 min. After reaction, 3 ml of water were added. The mixture was stirred, and then centrifuged at 3500 rpm for 10 min.

Acetone as derivative solvent

A 0.1-ml aliquot of 0.001 mol/l aqueous potassium hydroxide solution, 0.1 ml of standard solution of thiocyanate anion and 0.5 ml of a solution of PFB · Br in acetone were added in succession to a 5-ml glass-stoppered test tube. The reaction mixture was shaken mechanically at 30°C for 20 min.

Chromatographic conditions

Chromatographic separation was carried out on an ODS column at 30°C. The mobile phase was MeOH–water (80:20), the flow-rate was 1.0 ml/min, and the UV detector was set at 254 nm (see Fig. 1A).

Sample analysis

A 0.1-ml volume of waste water of an electroplate from Beijing Electroplate Plant and 0.1 ml of saliva from a smoker who had been smoking for 20 years were derivatized and determined according to the method for standard solution (see Fig. 1B and C); acetone was used as derivative solvent.

Recoveries

Recoveries were determined by analysis of samples spiked with 290, 407 or 581 ng of potassium thiocyanate.

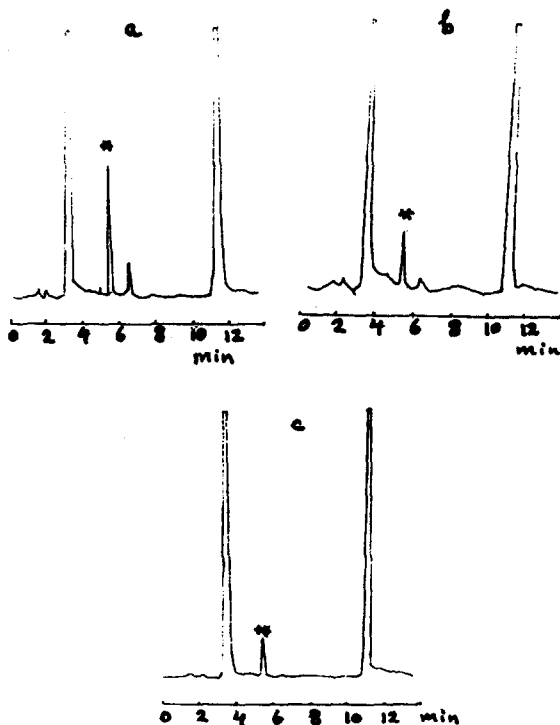Fig. 3. Optimization of the reaction time of the derivatization.

Fig. 1. Chromatogram of the standard solution and the samples. (a) Standard solution, (b) waste water of an electroplate, (c) saliva from a regular smoker. * = Derivative product.

RESULTS AND DISCUSSION

Optimization of derivative conditions

When the concentration of thiocyanate anion was about $1.0 \cdot 10^{-2}$ to $1.0 \cdot 10^{-2}$ mol/l, the volume of neat PFB · Br required to ensure a constant formation of thiocyanate derivative was 2 μl (see Fig. 2).
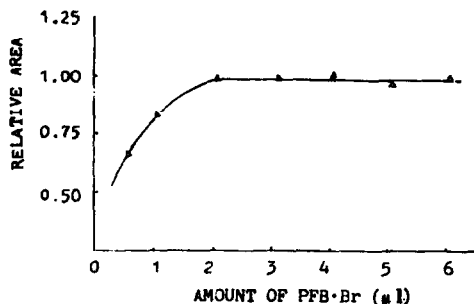
Thiocyanate reacts rapidly with PFB · Br, requiring only 15 min to reach constant yield (see Fig. 3). A reaction time of 20 min was enough for practical use. The derivative product, which was stored at room temperature for a day or in a refrigerator for a week, was still stable (see Fig. 4).

When the temperature of the derivative reaction was set at 20°C, 30°C or 40°C, the derivative yields were almost unchanged. Thus 30°C was selected as the reaction temperature.

When the concentration of potassium hydroxide was $1.0 \cdot 10^{-5}$ to 1.0 mol/l and the concentration of sulphuric acid $0.5 \cdot 10^{-5}$ to 0.5 mol/l, the derivative yield of the reaction system studied was almost invariable (see Fig. 5). In our study 0.1 ml of $1.0 \cdot 10^{-3}$ mol/l potassium hydroxide was added to the system. The pH value is 8.3.

When CH_2Cl_2 is used as the derivative solvent, PTCs, such as HDTMAB, etc., are required to transfer thiocyanate anion from water to the organic phase. This leads to a complicated

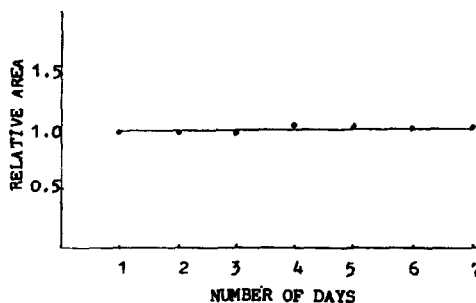Fig. 2. Optimization of the amount of PFB · Br used in the derivatization.

Fig. 4. Test of the stability of the derivative product stored in a refrigerator for a week.
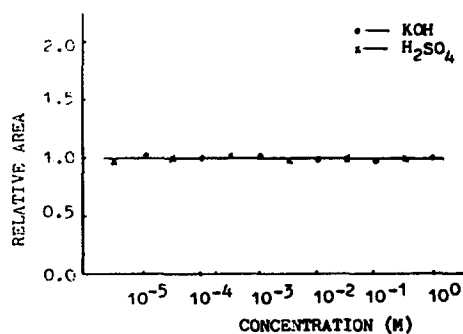
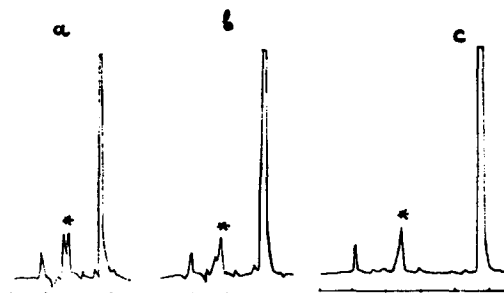Fig. 5. Influence of the basicity and acidity on the derivative reaction.

Fig. 6. Chromatogram of standard solution with different ratios of mobile phase (methanol–water) and dichloromethane as derivative solvent: (a) 87:13, (b) 85:15, (c) 80:20. * = Derivative product.

and time-consuming operation. In order to avoid using PTCs, we adopted two other solvents, *i.e.* acetone and acetonitrile, both of which are soluble in water. The different derivative yields of the reaction in the above three solvents are listed in Table I. it is seen from the table that the derivative yield in acetone is almost the same as in dichloromethane. This is why we selected acetone as the reaction solvent.

The other reason why we chose acetone lies in its selectivity. As is well known, thiocyanate anion is a dual nucleophilic reagent. When it reacts with PFB · Br, two products, namely PFB · SCN and PFB · NCS, are possible (see Fig. 6). However, it is found that the ratio of the two compounds in the derivative reaction varies as the solvent changes (see Fig. 7). Table II illustrates the results of this phenomenon. It shows that when acetone is employed only one chromatographic peak emerges, while in dichloromethane and acetonitrile solutions two chromato-
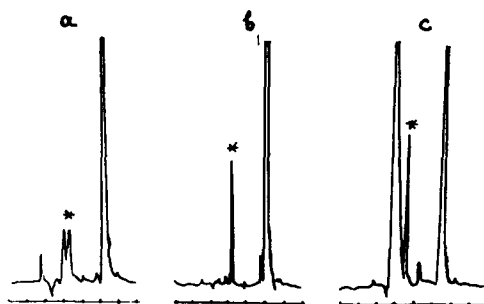

Fig. 7. Chromatogram of standard solution with different derivative solvents. (a) Dichloromethane, (b) acetonitrile, (c) acetone. * = Derivative product.

graphic peaks are formed with different yield ratios of the two possible products. The reaction selectivity thus increases in the order: $CH_2Cl_2 > CH_3CN > CH_3COCH_2$. This interesting phenomenon relates to the organic solvent effect, and can be accounted for in terms of the well-established hard and soft acid and base (HSAB) theory [6].

TABLE I

COMPARISON OF THE DERIVATIVE YIELD IN THREE DIFFERENT SOLVENTS

Solvent	Reaction temperature (°C)	Relative yield[a]
CH_3COCH_3	30	101.0%
CH_3CN	30	90.3%
CH_3CN	70	97.3%
CH_2Cl_2	30	100.0%

[a] For the convenience of comparison, we suppose the yield of the derivatization in CH_2Cl_2 to be 100%.

TABLE II

SELECTIVITY OF THE PRODUCT IN DIFFERENT SOLVENTS

Solvent	Number of peaks	Product ratio[a]
CH_3COCH_3	1	0
CH_3CN	2	0.04
CH_2Cl_2	2	0.60

[a] The product ratio is defined as the ratio of the areas of the two peaks. The mobile phase is MeOH–water (87:13).

Chromatographic conditions

The maximum absorption wavelength of the derivative product was determined at 210 nm by the Spectra-Focus fast-scanning UV detector. Because there are interference peaks near this point, the detector wavelength chosen was 254 nm.

Because iodide, nitrite, sulphide and cyanide can react with PFB · Br, and acetone has a peak when detected at 254 nm, the optimal ratio of MeOH–H_2O is 80:20 (v/v) to avoid interference. When these anions do not coexist with thiocyanate, a ratio of MeOH–H_2O of 87:13 (v/v) can be chosen and the rate of analysis is quicker.

Interference

In our experiments, it is demonstrated that chloride, fluoride, sulphate, sulphite, thiosulphate, phosphate, acetate, carbonate, nitrate, chromate and oxalate anions do not react with PFB · Br. Thus, these anions do not interfere in the determination of thiocyanate. Iodide, nitrite, cyanide and sulphide anions do react with PFB · Br. However, their products can be separated by using a different mobile phase composition to avoid interference.

Linearity and detection limit

A linear relationship between the peak area and the amount of the thiocyanate anion was obtained for potassium thiocyanate standard solution concentrations of $1.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-3}$, $7.0 \cdot 10^{-3}$, $1.0 \cdot 10^{-2}$, $1.5 \cdot 10^{-2}$ and $2.0 \cdot 10^{-2}$ mol/l. The regression equation is:

$$y = -0.01938 + 0.4868x$$

The correlation coefficient is 0.9962.

When the signal-to-noise ratio is 2.0 and acetone is used as solvent, the detection limit is 2.7 ng.

Precision and accuracy

To assess the accuracy and precision of the method, the intra-day ($n = 10$) and inter-day ($n = 5$) reproducibility of the assay were determined at a concentration of $1.0 \cdot 10^{-2}$ mol/l potassium thiocyanate. The results are reported in Table III.

Repeatability of injection of the same system

TABLE III

ACCURACY AND PRECISION OF THE METHOD

	Intra-day ($n = 10$)	Inter-day ($n = 5$)
Mean peak area	27 609	27 965
S.D.	800.4	878.0
R.S.D.	2.90%	3.14%

($n = 10$) was acceptable. The relative standard deviation (R.S.D.) was 3.83%.

Samples analysis

Waste water of an electroplate and saliva from a smoker who had been smoking for 20 years were analysed by the method presented above. The concentration of thiocyanate was $1.402 \cdot 10^{-4}$ g/ml and $1.014 \cdot 10^{-4}$ g/ml, respectively.

Analytical recoveries were determined for the samples of waste water of an electroplate and of the saliva by spiking with standards of potassium thiocyanate at concentrations ranging from $5.0 \cdot 10^{-3}$ to $1.0 \cdot 10^{-2}$ mol/l. The recoveries of the added potassium thiocyanate are shown in Table IV.

CONCLUSIONS

Determination of thiocyanate anion at trace concentrations by derivatization HPLC using acetone as derivative solvent has been studied in this paper. It is found that, compared with

TABLE IV

RECOVERY OF SAMPLES

Sample	Labelled amount (ng)	n	Recovery		
			Mean	S.D.	R.S.D.
Waste water					
	290	4	96.3	2.42	2.51
	407	4	97.7	1.07	1.09
	581	4	99.2	2.14	2.16
Saliva					
	290	4	94.5	1.72	1.80
	407	4	98.6	2.34	2.37
	581	4	97.3	1.96	2.01

dichloromethane using acetone as derivative solvent, the method is simpler and more selective. From the results mentioned above, the present method is applicable to the determination of thiocyanate in real samples. It may be useful for the determination of thiocyanate anion in biological and environmental specimens.

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