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# Determination of thiosulfate, thiocyanate and polythionates in a mixture by ion-pair chromatography with ultraviolet absorbance detection

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## Abstract

A sensitive ion chromatographic method has been developed for the determination of mixtures of thiosulfate, thiocyanate and polythionates (tri-, tetra-, penta- and hexathionate). The proposed method is based on the separation of the sulfur anions on an octadecylsilica (ODS) column with an acetonitrile–water mobile phase containing tetrapropylammonium salt (TPA) as an ion-pairing reagent and the ultraviolet absorption detection of the sulfur anions. When an acetonitrile–water (20:80, v/v) solution (pH 5.0) containing 6 mM TPA was used as a mobile phase at flow-rate of 0.6 ml min<sup>-1</sup>, the sulfur anions were resolved within 22 min. The detection limits defined at  $S/N=3$  and 230 nm were very low for all anions, except trithionate: 30 nM for thiosulfate, 60 nM for thiocyanate, 20 nM for tetrathionate, 15 nM for pentathionate and 18 nM for hexathionate. The proposed method gave recoveries ranging from 95.0 to 105.0% when applied to the determination of polythionates added to hot spring waters. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Water analysis; Thiosulfate; Thiocyanate; Polythionates; Sulfur compounds; Inorganic anions

## 1. Introduction

Polythionates ( $S_xO_6^{2-}$ :  $x=3, 4, 5$  and 6) are produced by the reaction of hydrogen sulfide with sulfite in an acidic medium, and are usually present as mixtures in hot spring waters and Wackenroder's solution. The analytical determination of polythionates in a mixture is important and increasingly desirable from the viewpoint of sulfur chemistry. But the analysis of the polythionate mixtures presents a difficult problem, as the individual polythionates are very similar in their chemical and physical properties. Ion chromatography (IC) has become one of the most powerful tools for the separation and the

quantitative analysis of various ions. Recently, several chromatographic methods have been proposed for the determination of polythionates ( $x=3-5$ ), except hexathionate, using UV detection [1–3], conductivity detection [4–6] and Ce(III)-fluorescence detection [7]. However, rapid and sensitive chromatography of a mixture of four polythionates ( $x=3-6$ ) becomes difficult because of the late elution of hexathionate due to its strong retention on the separation column; the retentions of polythionates are increased significantly with an increase in the number of sulfur atoms in the molecule. Steudel and Holdt [8] have separated thiosulfate and polythionates ( $x=3-6$ ) within about 15 mm, using detection at 254 nm. But these authors did not present any analytical determination of polythionates containing hexathionate. Takano et al. [9] used IC with polarographic detection for the determination of the four polythion-

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ates, in which trithionate could not be separated chromatographically from tetrathionate. Takano and Watanuki [10] also have proposed a method utilizing a microbore separation column for the determination of the polythionates in excess of 0.2 ppm. We have previously reported an ion-pair chromatographic method with conductivity detection for the determination of polythionates [11]. However, the sensitivity of these methods [10,11] was not sufficient to determine polythionates at concentration of  $<10^{-6}$  M level.

In this work, a sensitive method for the determination of thiosulfate, thiocyanate and polythionates in a mixture was established using photometric measurements at 230 nm, in which the sulfur anions were separated chromatographically within 22 min on an ODS separation column with an acetonitrile–water mobile phase containing an ion-pairing reagent of tetrapropylammonium salt. The detection limits defined as  $S/N=3$  were 30 nM for thiosulfate, 60 nM for thiocyanate, 20 nM for tetrathionate, 15 nM for pentathionate and 18 nM for hexathionate. Compared with earlier methods [9–11], the proposed method gave a higher sensitivity for the determination of polythionates ( $x=4-6$ ). This method was successfully applied to the determination of polythionates added to hot-spring water samples.

## 2. Experimental

### 2.1. Apparatus

The ion chromatographic system used in this study comprised a Model LC-10AD pump with dual pistons (Shimadzu, Kyoto, Japan), a Model 7725 injection-valve equipped with a 100- $\mu$ l sample loop (Reodyne, Berkeley, CA, USA), a silica ODS separating column (Ultaron VX ODS, 150 mm $\times$ 4.6 mm I.D., Shinwa Chemical Industries, Tokyo, Japan) and a Model SPD-10AV photometric detector (Shimadzu). The chromatograms were recorded with a Model U-135 recorder (Shimadzu). A Model C-R6A Chromatopack (Shimadzu) was employed for measurement of peak area.

### 2.2. Chemicals

The water used in this experiment was distilled twice and then deionized with a Model Milli-QII instrument (Nippon Millipore, Yamagata, Japan). All of the chemicals used, except the polythionates, were of analytical-reagent grade and were used without further purification.

An acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 6 mM tetrapropylammonium hydroxide (TPA) was prepared by adding 100 ml of acetonitrile to a mixture of 6.12 ml of 0.49 M TPA and small amounts of acetic acid, and then diluting it to 500 ml with water; the acetic acid was used to adjust the mobile phase pH to 5.0. The mobile phase so obtained was filtered through a membrane filter (pore size, 0.2  $\mu$ m) before use.

Polythionates were prepared as follows: potassium trithionate ( $K_2S_3O_6$ ) and potassium tetrathionate ( $K_2S_4O_6$ ) were prepared according to the procedure described by Stamm et al. [12] and potassium pentathionate ( $K_2S_5O_6 \cdot 1.5H_2O$ ) and potassium hexathionate ( $K_2S_6O_6$ ) as by Goehring and Feldmann [13]. The trithionate and tetrathionate salts were recrystallized with water at temperatures below 35 and 60°C, respectively. The pentathionate was purified by recrystallizing twice in 0.5 M hydrochloric acid at temperatures below 50°C. The hexathionate was recrystallized twice in 2 M hydrochloric acid below 60°C. These polythionates were stored at  $-10 \pm 2^\circ C$  after drying at room temperature. Stock solutions of standard polythionates (1.0 mM) were prepared by dissolving 68.3 mg of the trithionate, 75.6 mg of the tetrathionate, 90.4 mg of the pentathionate or 92.0 mg of the hexathionate, respectively, in water and diluting to 250 ml. The concentration of each standard polythionate solution was checked by their cyanolysis [14,15] and/or sulfitolysis [16]; the solutions of tri-, tetra-, penta- and hexathionate could be used in the present work for 6 weeks, 6 months, 4 months and 2 months, respectively, when stored at  $5 \pm 2^\circ C$  in a refrigerator after preparation. The working solutions were prepared by diluting each of 1.0 mM standard polythionate solutions.

A thiosulfate solution of about 0.1 M was prepared by dissolving sodium thiosulfate pentahydrate in water containing a small amount of sodium carbon-

ate (0.01%) as a stabilizer, and was standardized by iodometry 1 week after preparation. Working solutions were obtained by suitable dilution of the standard thiosulfate with oxygen-free water.

A thiocyanate solution (about 0.1 M) was prepared by dissolving potassium thiocyanate in water and was standardized by Volhard's method. Working standard solutions were obtained by appropriate dilution of the standard thiocyanate with water.

### 2.3. Procedure

An acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 6 mM TPA was allowed to flow at a rate of 0.6 ml min<sup>-1</sup>, and then a 100- $\mu$ l sample solution containing thiosulfate, thiocyanate and polythionates ( $x=3-6$ ) was injected into the separating column, which was maintained at 23 $\pm$ 2°C. The effluent was passed through a photometric detector cell. Chromatograms measured at 230 nm were recorded.

## 3. Results and discussion

### 3.1. Calibration plots

A 100- $\mu$ l aliquot of standard solution containing thiosulfate, thiocyanate and polythionates ( $x=3-6$ ) was treated as described in Section 2.3. Chromatograms obtained are shown in Fig. 1. When peak heights vs. concentrations were plotted, each calibration graph of the five sulfur anions, except trithionate, was linear up to 7.00  $\mu$ M for thiosulfate, 9.00  $\mu$ M for thiocyanate, 3.00  $\mu$ M for tetrathionate, 3.00  $\mu$ M for pentathionate and 3.00  $\mu$ M for hexathionate, with correlation coefficients of >0.999. The line for trithionate plotted peak heights vs. concentrations was curved, whereas the graph showed a straight line up to 30  $\mu$ M when plotted peak areas vs. concentrations. From six results obtained for a mixture of thiosulfate (5.00  $\mu$ M, 0.560 ppm), thiocyanate (5.00  $\mu$ M, 0.290 ppm), trithionate (20.0  $\mu$ M, 3.84 ppm), tetrathionate (0.800  $\mu$ M, 0.170 ppm), pentathionate (0.800  $\mu$ M, 0.205 ppm) and hexathionate (0.800  $\mu$ M, 0.236 ppm), the proposed method gave a mean value of 4.95  $\mu$ M (0.554 ppm) for

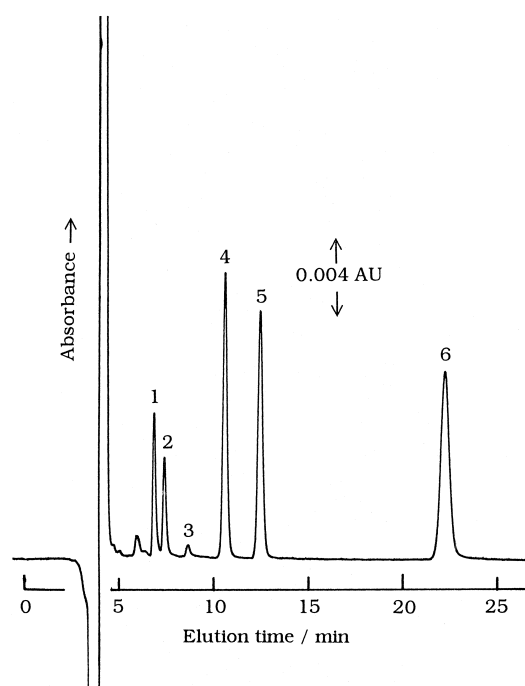


Fig. 1. Chromatograms of thiosulfate, thiocyanate and polythionates in a mixture. Peaks: 1= $S_2O_3^{2-}$  (1.5  $\mu$ M); 2= $SCN^-$  (1.5  $\mu$ M); 3= $S_3O_6^{2-}$  (5  $\mu$ M); 4= $S_4O_6^{2-}$  (1.5  $\mu$ M); 5= $S_5O_6^{2-}$  (1.0  $\mu$ M); 6= $S_6O_6^{2-}$  (1.0  $\mu$ M).

thiosulfate with a relative standard deviation (RSD) of 0.46%, 5.05  $\mu$ M (0.293 ppm) for thiocyanate (RSD=0.57%), 19.7  $\mu$ M (3.78 ppm) for trithionate (RSD=1.0%), 0.813  $\mu$ M (0.182 ppm) for tetrathionate (RSD=0.93%), 0.802  $\mu$ M (0.205 ppm) for pentathionate (RSD=0.84%) and 0.808  $\mu$ M (0.232 ppm) for hexathionate (RSD=1.1%).

### 3.2. Absorption spectra

Fig. 2 shows absorption spectra of each sulfur anion solution and an acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 6 mM TPA, in which a Model UV 3100-PC recording spectrophotometer (Shimadzu) with 10-mm quartz cells was used. The sulfur anions have high absorbance in the range 200–230 nm; absorbance of trithionate was relatively low. Absorbance of the mobile phase was high in the wavelength region below 210 nm, decreased gradually between 210 and 230 nm, and then became very low at 230 nm. Chromatograms of

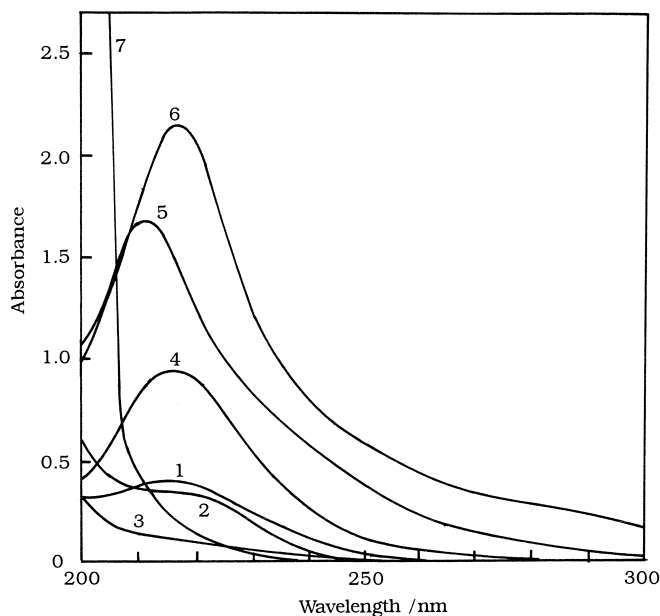


Fig. 2. Absorption spectra of sulfur anions and mobile phase. Curves: 1= $\text{S}_2\text{O}_3^{2-}$  (0.1 mM); 2= $\text{SCN}^-$  (0.1 mM); 3= $\text{S}_3\text{O}_6^{2-}$  (0.1 mM); 4= $\text{S}_4\text{O}_6^{2-}$  (0.1 mM); 5= $\text{S}_5\text{O}_6^{2-}$  (0.1 mM); 6= $\text{S}_6\text{O}_6^{2-}$  (0.1 mM); 7=acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 6 mM TPA.

three sulfur anions obtained at 200, 210 and 230 nm are shown in Fig. 3. At 200 and 210 nm, the baseline noise was increased significantly due to the high

absorbance of the mobile phase. Therefore, a wavelength of 230 nm was selected for measurement of the sulfur anions.

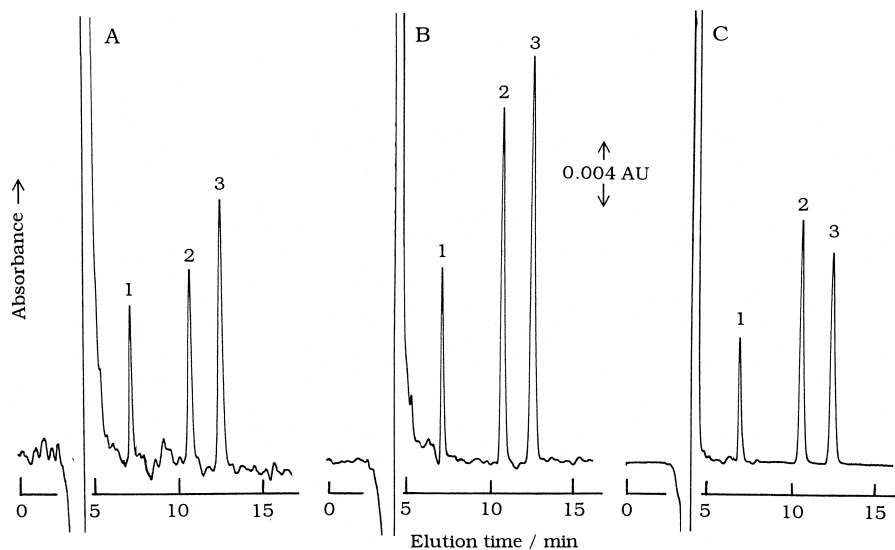


Fig. 3. Comparison of chromatograms of three sulfur anions measured at different wavelengths; (A) 200 nm (B) 210 nm (C) 230 nm. Peaks: 1= $\text{S}_2\text{O}_3^{2-}$  (1.5  $\mu\text{M}$ ); 2= $\text{S}_4\text{O}_6^{2-}$  (1.5  $\mu\text{M}$ ); 3= $\text{S}_5\text{O}_6^{2-}$  (1.0  $\mu\text{M}$ ).

### 3.3. Mobile phase

#### 3.3.1. Selection of ion-pairing reagent

Tetraalkylammonium salts have been widely used as ion-pairing reagents of anions; it is known that the carbon-chain length of alkyl groups of these salts affects the separation of various anions. The effect of the ion-pairing reagents, tetraethylammonium (TEA), tetrapropylammonium (TPA) and tetra-*n*-butylammonium (TBA) salts, on the elution of the sulfur anions was first investigated. Various amounts of TEA, TPA and TBA were added to an acetonitrile–water mobile phase (pH 5.0). The results are shown in Table 1, in which concentrations of the ion-pairing reagents and an acetonitrile in the mobile phase were prepared to give good resolution of the sulfur anion mixtures. TEA and TBA were not useful for separations of the sulfur anions at short elution times; hexathionate required elution times of more than 60 min. However, when TPA was used, hexathionate could be eluted in a very short time, and the six sulfur anions could be separated completely within 22 min. Therefore, TPA was chosen as an ion-pairing reagent in this work.

#### 3.3.2. Effect of concentration of TPA on elution of sulfur anions

In order to establish the optimal concentration of TPA in the mobile phase for complete and rapid separations of the sulfur anions, various amounts (3–11 mM) of TPA were tested in acetonitrile–water

(20:80, v/v), pH 5.0. Increasing the TPA concentration gradually slowed the elution rate of the sulfur anions. At TPA concentrations of 3 and 4 mM, all six sulfur anions were eluted rapidly (<20 min); however, thiosulfate could not be separated from thiocyanate because thiocyanate eluted shortly after thiosulfate. A TPA concentration of 6 mM resulted in the complete separation of the six sulfur anions within 22 min. At high TPA concentrations, >9 mM, hexathionate required an elution time of more than 25 min.

#### 3.3.3. Effect of concentration of acetonitrile on elution of sulfur anions

An increase in the acetonitrile concentration in the mobile phase (pH 5.0) containing 6 mM TPA accelerated elution of the sulfur anions. When an acetonitrile–water (15:85, v/v) mobile phase was used, the anions, except for hexathionate, were eluted at times shorter than 23 min; hexathionate elution took 66 min, because of an insufficient amount of acetonitrile. By increasing the acetonitrile content to 20%, all of the six sulfur anions could be separated completely within 22 min. Use of 23% and 25% acetonitrile failed to completely separate thiosulfate from thiocyanate and thiocyanate from trithionate.

#### 3.3.4. Effect of pH of mobile phase on elution of sulfur anions

In measuring the effect of pH of the mobile phase on elutions of the sulfur anions, acetonitrile–water (20:80, v/v) containing 6 mM TPA was adjusted to various pH values by adding appropriate amounts of acetic acid. In the pH range 4.0–7.0, the elution times of any of the sulfur anions did not change measurably: thiosulfate was eluted at 6.8 min, thiocyanate at 7.2 min, trithionate at 8.4 min, tetrathionate at 10.4 min, pentathionate at 12.3 min and hexathionate at 21.5 min. Consequently, an acetonitrile–water (20:80, v/v) at pH 5.0 containing 6 mM TPA was used as a mobile phase in this work.

Table 1  
Effect of ion-pairing reagents TEA, TPA and TBA on the elution of sulfur anions

Anion	Elution time (min)		
	TEA <sup>a</sup>	TPA <sup>b</sup>	TBA <sup>c</sup>
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	Very fast	6.8	10.6
SCN <sup>-</sup>	7.0	7.4	28.3
S <sub>4</sub> O <sub>6</sub> <sup>2-</sup>	8.2	10.6	45.0
S <sub>5</sub> O <sub>6</sub> <sup>2-</sup>	9.8	12.4	50.0
S <sub>6</sub> O <sub>6</sub> <sup>2-</sup>	64.8	21.5	64.1

<sup>a</sup> TEA (30 mM) in acetonitrile–water (3:97, v/v) mobile phase (pH 5.0).

<sup>b</sup> TPA (6 mM) in acetonitrile–water (20:80, v/v) mobile phase (pH 5.0).

<sup>c</sup> TBA (30 mM) in acetonitrile–water (25:75, v/v) mobile phase (pH 5.0).

### 3.4. Effect of flow-rate of mobile phase

An increase in flow-rate of the mobile phase was useful in accelerating the elution of hexathionate, but it caused an incomplete separation of the adjacent

peaks of thiosulfate and thiocyanate. In order to establish rapid resolution of the six sulfur anions, a mobile phase of an acetonitrile–water (20:80, v/v) (pH 5.0) solution containing 6 mM TPA was pumped at various flow-rates. At  $0.6 \text{ ml min}^{-1}$ , the six sulfur anions of thiosulfate, thiocyanate and polythionates were resolved completely at elution times below 22 min. At the higher flow-rates of 0.8 and  $1.0 \text{ ml min}^{-1}$ , separation of thiosulfate from thiocyanate became difficult. Therefore, the mobile phase was used at a flow-rate of  $0.6 \text{ ml min}^{-1}$ .

### 3.5. Detection limits of sulfur anions with UV and suppressed conductivity detection

Detection limits of the sulfur anions with a UV photometric detection, obtained as in Section 2.3, were compared with those with a conductivity detection in Table 2, and chromatograms for the sulfur anion mixtures using a suppressed conductivity detector are shown in Fig. 4. For the conductivity detection, a suppressor (Dionex HPIC-AMMS, Sunnyvale, CA, USA) with a strongly acidic cation-exchange membrane in the hydronium form was placed prior to a conductivity detector (Shimadzu CDD-6A). As can be seen in Table 2, the UV photometric measurement gave higher sensitivities (2.5- to 11.6-fold) for the detection of the sulfur anions than the conductometric method. This UV detection technique was found to be very useful for detection of sulfur anions at concentrations of  $<10^{-6} \text{ M}$ .

### 3.6. Application to polythionates in natural water samples

At first, the effect of foreign ions on the de-

Table 2

Detection limits of sulfur anions by UV and suppressed conductivity detections

Anion	Detection limit, $S/N=3$ ( $10^{-8} \text{ M}$ )	
	UV detection	Conductivity detection <sup>a</sup>
$\text{S}_2\text{O}_3^{2-}$	2.0	7.5
$\text{SCN}^-$	4.0	14.1
$\text{S}_4\text{O}_6^{2-}$	1.3	9.3
$\text{S}_5\text{O}_6^{2-}$	1.0	11.5
$\text{S}_6\text{O}_6^{2-}$	1.2	20.8

<sup>a</sup>Suppressor (Dionex, HPIC-AMMS) was used.

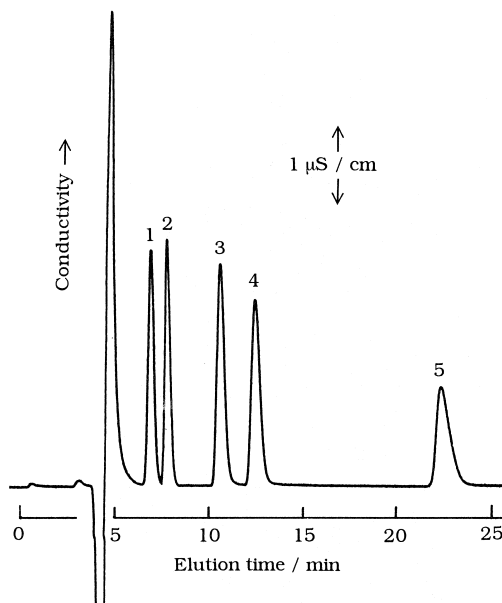


Fig. 4. Chromatograms of thiosulfate, thiocyanate and polythionates in a mixture by a suppressed conductivity detection. Peaks: 1 =  $\text{S}_2\text{O}_3^{2-}$  ( $100 \mu\text{M}$ ); 2 =  $\text{SCN}^-$  ( $50 \mu\text{M}$ ); 3 =  $\text{S}_4\text{O}_6^{2-}$  ( $60 \mu\text{M}$ ); 4 =  $\text{S}_5\text{O}_6^{2-}$  ( $60 \mu\text{M}$ ); 5 =  $\text{S}_6\text{O}_6^{2-}$  ( $60 \mu\text{M}$ ).

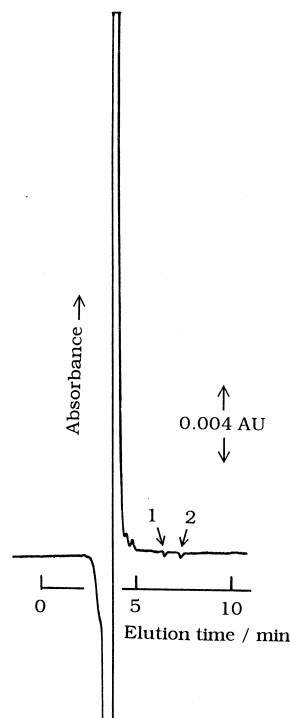


Fig. 5. Chromatograms of sulfate and dithionate at 230 nm. Peaks: 1 =  $\text{SO}_4^{2-}$  ( $50 \mu\text{M}$ ); 2 =  $\text{S}_2\text{O}_6^{2-}$  ( $50 \mu\text{M}$ ).

Table 3  
Determination of polythionates added to hot-spring waters

Sample	Dilution (fold)	Added ( $\mu M$ )				Found ( $\mu M$ )				Recovery (%)			
		$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$	$S_6O_6^{2-}$
A	0	0	0	0	0	0	0.58	0	0				
	1.25	0	0	0	0	0	0.47	0	0				
	1.25	25.0	1.50	1.50	1.50	25.0	1.99	1.46	1.53	100.0	101.2	97.3	102.0
	1.25	45.0	1.00	1.00	0	46.0	1.46	0.98	0	102.2	99.0	98.0	–
B	0	0	0	0	0	0	0	0	0				
	2	50.0	1.13	0.50	1.00	51.0	1.17	0.50	0.95	102.0	103.5	100.0	95.0
	2	0	0.60	2.00	0	0	0.63	1.89	0	–	105.0	94.5	–
C	0	0	0	0	0	0	0	0	0				
	2	50.0	1.49	1.50	1.50	48.0	1.46	1.54	1.49	96.0	98.0	102.7	98.0

termination of the sulfur anions was investigated. Anions of fluoride, iodate, bromate, chloride, nitrite, sulfide, phosphate, carbonate, bromide, nitrate, iodide, arsenite, lactate, formate, acetate, propionate, malate, butylate and valerate and cations of potassium(I), sodium(I), ammonium(I), calcium(II), magnesium(II), manganese(II), nickel(II), copper(II), iron(II), iron(III) and aluminum(III) were eluted prior to thiosulfate. These ions at concentrations as high as 0.005 M did not interfere with the separation of thiosulfate, thiocyanate and polythionates ( $x=3-6$ ). Sulfate and dithionate were eluted at 6.6 and 7.4 min, respectively, which times were close to those of thiosulfate and thiocyanate. However, sulfate and dithionate gave very small negative peaks based on a decrease in absorbance from baseline level of the mobile phase, because these anions did not have any absorbance at 230 nm. The chromatograms of sulfate and dithionate obtained at 230 nm are shown in Fig. 5. Therefore, the presence of sulfate and dithionate up to 50  $\mu M$  did not interfere with the determination of thiosulfate and thiocyanate, and furthermore sulfate and dithionate could be tolerated in amounts of 0.005 M for the determination of polythionates.

The determination of polythionates added to hot-spring waters is shown in Table 3. The proposed method was successfully applied to the determination

of the four polythionates ( $x=3-6$ ) contained in the hot-spring water samples with recoveries ranged from 95.0 to 105.0%.

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