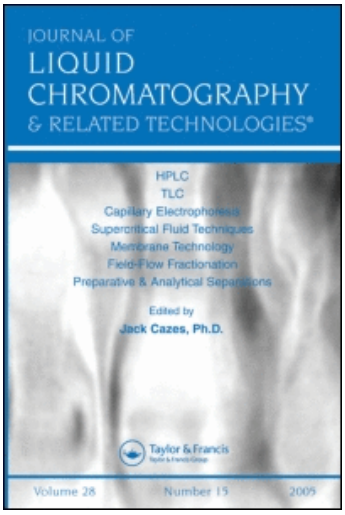

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Trace Amounts of Sulfide in Human Serum by High-Performance Liquid Chromatography with Fluorometric Detection After Derivatization with 2-Amino-5-n, n-Diethylaminotoluene and Iron (III)

K. Nagashima[a]; K. Fukushima[a]; M. Kamaya[a]
[a] Department of Applied Chemistry, Faculty of Engineering, Kogakuin University, Hachioji-shi Tokyo, Japan

To cite this Article Nagashima, K. , Fukushima, K. and Kamaya, M.(1995) 'Determination of Trace Amounts of Sulfide in Human Serum by High-Performance Liquid Chromatography with Fluorometric Detection After Derivatization with 2-Amino-5-n, n-Diethylaminotoluene and Iron (III)', Journal of Liquid Chromatography & Related Technologies, 18: 3, 515 — 526
To link to this Article: DOI: 10.1080/10826079508009253
URL: http://dx.doi.org/10.1080/10826079508009253

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(3), 515-526 (1995)

DETERMINATION OF TRACE AMOUNTS OF SULFIDE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUOROMETRIC DETECTION AFTER DERIVATIZATION WITH 2-AMINO-5-N,N-DIETHYLAMINOTOLUENE AND IRON(III)

K. NAGASHIMA, K. FUKUSHIMA, AND M. KAMAYA

Department of Applied Chemistry
Faculty of Engineering
Kogakuin University
2665-1 Nakano-machi Hachioji-shi
Tokyo 192, Japan

ABSTRACT

A fluorometric high-performance liquid-chromatographic method is described for the determination of sulfide in human serum. The sulfide ion had been derived into a fluorescent compound with 2-amino-5-N,N-diethylaminotoluene and Fe (III) under an acidic condition. The compound was extracted into 2-octanol as an ion-pair with sodium 1-octane sulfonate. The extract was separated by a reversed phase method (column, Inertsil ODS-2, 250mm $\times$4.6mm i.d.;mobile phase,90%(V/V) acetonitrile aqueous solution containing sodium 1-octanesulfonate as a counter ion reagent; flow rate 0.5cm^3·min^{-1} and detected fluorometrically (Ex. 640nm; Em. 675nm). The sulfide ion could be determined over the range from 4.4×10^{-10}M to 4.4×10^{-7}M. The coefficient of variation at 4.4×10^{-8}M of sulfide was 3.2%(n=7). The range of concentration of sulfide ion in human serum were from 3.04×10^{-8}M to 2.24×10^{-8}M by this method.

INTRODUCTION

A simpler and more specific colorimetric method based on the formation of Methylene Blue by the reaction of sulfide with N,N-dimethyl-p-phenylenediamine in the presence of an oxidizing agent. This method is widely used for the detection of sulfide in many samples since it was reported by Fisher in 1883(1- 5). Recently, Kloster and King (6) have obtained twice sensitivity of the Methylene Blue method by converting the methyl groups into ethyl groups and found that n-propylene blue method(7) was slightly higher sensitivity than that of Ethylene Blue method. But these methods were not successful to detect ppb levels of sulfide ion in bloods because of insufficiency of the sensitivity.

Reversed-phase HPLC determination of sulfide in an aqueous matrix was reported(8) using 2-indo-1-methylpyridinium chloride as a precolumn ultra-violet derivatization reagent.

The calibration graph(340nm) was rectilinear for 0.04～50ug of sulphide ion with 20ul of sample. The sensitivity of this method was poor for the detection of sulfide ion in bloods.

In recent years,the fluorescent properties of thionine were applied to the spectrofluorometric determination of sulfide(9). The thionine is the substuted compounds of the four methyl groups in methylene blue with hydrogen atoms Thus, the method for the detection of fluorescence of thionine produced in this reaction (9) and the method by the HPLC with fluorometric detection(10) were suggested. However, the former method was influenced considerably by excess iron ion. In the latter method, many disadvantages have been

pointed out, such as tailing problems and shorten the lifetime of columns (9) because of thionine has high adsorptivity onto the stationary phase in columns.

We had already reported a high sensitive methd for the determination of sulfide ion by HPLC with fluorometric tection. The method solve any problems of shorten of the li fetime of columns by using a 2-amino-5-N,N-diethylamino-toluene and the solvent extraction(11).

Thus, the applicability of the proposed method to the detection of sulfide ions in human sera using trace amounts of blood samples without any complicated pretreatments was studied.

EXPERIMENTAL

Reagent

All of the chemicals used were of analytical-reagent grade unless stated otherwise. Doubly distilled water was used in all experimentals. A standard sulfide solution was prepared by dissolving 6 g of crystals of sodium sulfide ($Na_2S \cdot 9H_2O$) in 10% zinc acetate with distilled water purged with N_2. The solution was stored in dark polyethylene bottle and was standardized by titration before use (11).

2-amino-5-N,N-diethylaminotoluene hydrochloride was purchased from Tokyo Kasei Kogyo (Tokyo) and the reaction solution was prepared by dissolving 2 g of it in 100 cm^3 of 0.70N sulfuric acid. This solution was effective for 2 weaks in cool and dark.

An approximately 1 $\times 10^{-4}$M iron (III) chloride solution was prepared by dissolving 0.135g of iron chloride ($FeCl_3 \cdot 6H_2O$) in 500cm^3 of 0.1N sulfuric acid. Counter-ion solution (0.1M) was pepared by dissolving 10.8g of sodium 1-octansulfonate in 500 cm^3 of water. The mobile phase was prepared by dissolving 1.08g of sodium 1-octansulfonate in 500cm^{3}of 90% (V/V) of acetonitrile aquaous solution. The acetonitrile was of HPLC-grade and purchased from Tokyo Kasei Kogyo (Tokyo). All mobile phase were de-gassed under vacuum in an ultrasonic bath just before use.

Apparatus

The HPLC with a fluorescent detector (Model F1050) was supplied by Hitachi Corporation with a pump (Model 576, GL Science Inc.) with connecting a loop injector obtained from Ryeodyne. A "Chromato-integrator"(Model 200) was supplied by Hitachi Corporation as a recorder. A separation column was supplied by GL Science Inc. A test tube mixer for extractions was supplied by Taiyo Science Industry Inc., and Centrifugal separator (SCT5BA) from Hitachi Corporation.

Sample pre-treatments

Human whole blood were obtained from the ante-cubital vein of healthy volunteers (subjects) in our laboratory. The human whole blood were immediately poured into centrifuge tubes, and were allowed to stand for two hours at room temperature. After the sample was solidified, the clots formed in the tube was removed with a thin glass stick. After that, those were centrifuged for 10 min at 2000G and classified as sera samples.

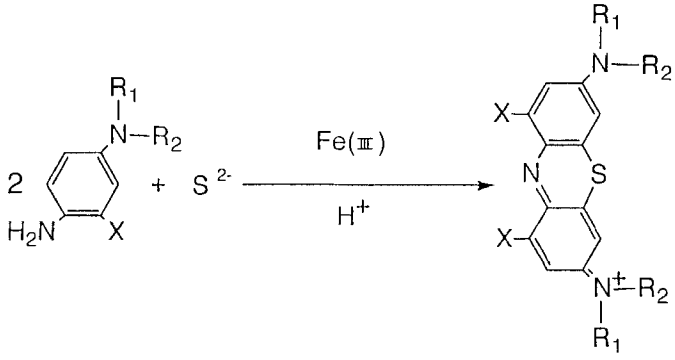

FIGURE 1 Fluorogenic reaction of sulfide with 2-amino-5-N,N-diethylamino toluene and Fe^{3+} to form thionine derivative

Recommended procedures

Pipete 4 cm^3 of sample solution containing sulfide ion, add 0.4cm^3 of a reaction solution and 0.2cm^3 of iron chloride solution in the measuring flask and dilute the solution to 5 cm^3 with pure water. Add 0.5cm^3 of counter-ion solution and 1 cm^3 of 2-octanol, and then extract the compound by mixing for 10 minutes. Since then, the compound was centrifuged at 1820G. HPLC analysis were performed at a 0.5cm^3 min^{-1} of flow rate of mobile phase, maximum excitation wavelength set at 640nm and fluorescent detection wavelengthset at 675nm(12).

Formation reaction and calibration curve

The thionine derivative formation reaction(Fig.1) proceeded under an acidic and excess amounts of oxidizing agents such as, iron(Ⅲ).

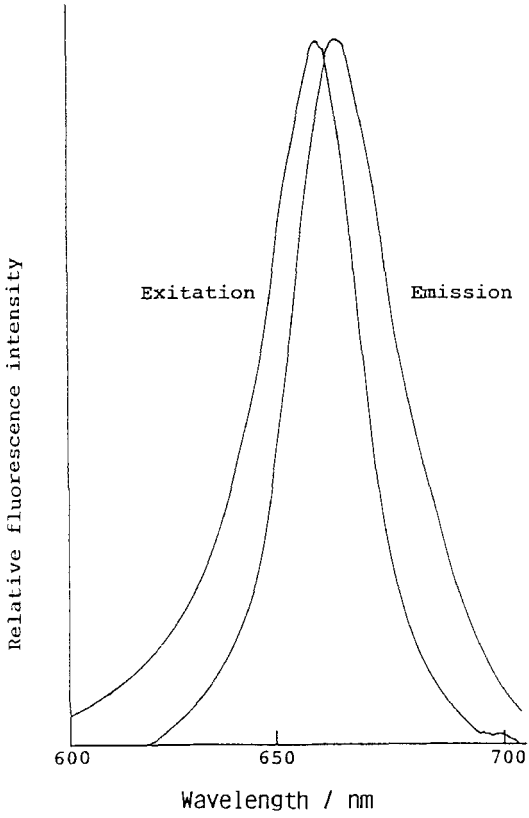

FIGURE 2 Fluorescence spectra of thionine in water
A:Excitation spectrum, B:Emission spectrum.

The detection wavelength was chosen from the fluorescence spectral data of a reaction solution we had already reported (12). The excitation and emission wavelengths maximum of the fluorescence using 2-amino-5-N,N- diethylaminotoluene appeared at 661nm, and 675nm, respectively (Fig.2).

The proposed method was studied using the excitation wavelength at 640nm and the emission wavelength at 675nm considering to obtain the maximum S/N ratio.

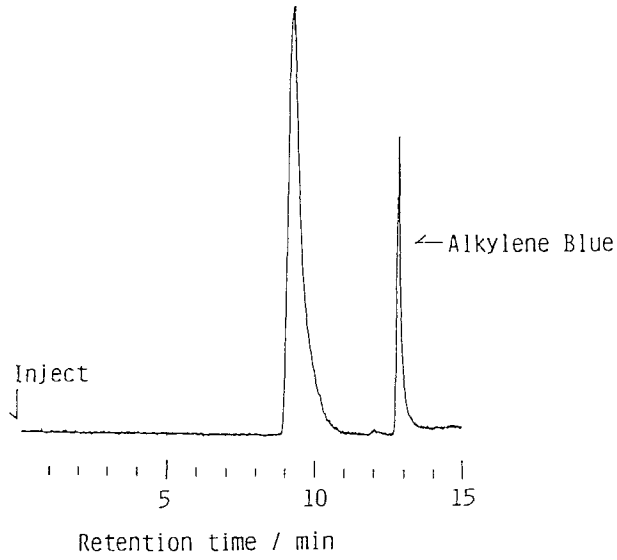

FIGURE 3 Typical chromatogram
Derivatized standard solution containing 0.056ng/10ul sulfide. Chromatographic condition; column: Inertsil ODS-2 (250mm ×4.6mmi.d.), mobile phase; 90% (V/V) acetonitrile aqueous solution containing sodium 1-octanesulfonate as a counter-ion reagent, flow rate: 0.5$cm^3 \cdot min^{-1}$, injection volume; 10ul.

The calibration curve using optimum conditions (12) was linear over the range from 4.4×10^{-10}M to 4.4×10^{-7}M sulfide (γ=0.999, Blank: 3.6×10^{-9} M). The precision for seven replicate determination at the 4.4×10^{-8}M level was 3.2% of relative standard deviation.

Typical chromatogram

The chromatograms (Fig.3) obtained from 2-octanol phase showed a large peak at 9 minutes of retention time, and an another peak of thionine derivative compound (analyte) at 13 minutes of retention time.

The second peak was propotional to the concentration of sulfide ion injected, but the first peak was not proportional to one. So, the first peak was the reaction reagents oxidized and the second peak was the thionine.

RESULTS AND DISCUSSION

Effect of diverse ions

There are more than 20 kinds of amino acid compounds in a human body. We investigated the influences of some amino acids containing sulfur, especially methionine, cysteine, cystine.

The results are shown in Table 1.

The 10^4 times amino acids coexisted in the sample were determined by this method based on peak area which was proportional to the concentration of sulfide ion. It can be concluded that those amino acids did not react with the reaction reagents under this condition from the experimental

TABLE 1 Influences of sulfur-containing amino acids on HPLC measurement of sulfide ion

Amino acids	Molecular formula	Error (%)
l-Metionine	$CH_3S(CH_2)_2CH(NH_2)COOH$	-2.17
l-Cysteine	$HSCH_2CH(NH_2)COOH$	-0.45
l-Cystine	$HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$	+2.68

Amino acids: 1×10^{-4}M
Sulfide ion: 1×10^{-8}M

results that the measured values by coexistence agreed with the measured value by absence within±3%.

We examined the effect of some inorganic ions that might be present in a human blood. Van Hoff's artificial seawater (physiological saline) was used because the saline was used often as dilution solution for blood. As the relative error was less than 10% in the presence of 10^7-10^8 times ions, we have considered that there is no influences of inorganic ions on this method (Table 2).

Application to human sera

This proposed method is so useful to detect sulfide ion sensitively without any influenes of coexisting diverse ions. In order to make sure that the proposed method is practicable to determine the sulfide ion in a human blood. The human blood (sera) samples that obtained from healthy subjects of both sexes between the age of 21 and 24 years were analyzed by

TABLE 2 Effect of other ions on the determination of sulfide

Ion	Added as	Ion/S^{2-}	Error (%)
Cl^-	NaCl	10^9	-9
CO_3^{2-}	Na_2CO_3	10^3	+5
SO_4^{2-}	Na_2SO_4	10^8	-3
$H_2PO_4^-$	NaH_2PO_4	10^3	+6
NO_3^-	$NaNO_3$	10^3	-5
SiO_3^{2-}	Na_2SiO_3	10^3	-10
K^+	KCl	10^7	+7
Mg^{2+}	$MgCl_2$	10^8	+5
Ca^{2+}	$CaCl_2$	10^8	-6

S^{2-}: 1.25ng·cm^{-3}

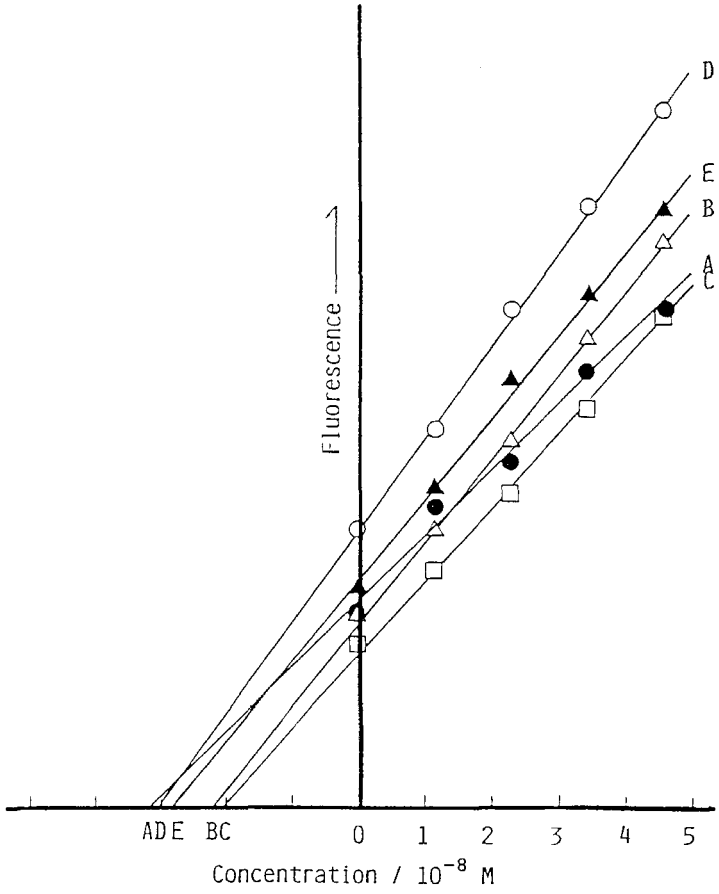

FIGURE 4 Determination of sulfide in human serum by standard addition method

this method with a standard addition method. To the 5 sulfide standard solutions (0 $\sim$4.4 $\times 10^{-8}$ S^{2-}M) 100ul of seram samples were added. And the 5 mixed solutions were analyzed without any complicated pretreatment(See Fig.4 and Table 3).

From these results it became clear that 10^{-8}M levels of sulfide ion in serum can be determined by this method.

TABLE 3 Determination of sulfide ion in human serum

Sample	Sex	Age(Y)	Sulfide concentration (M)
A	Male	24	3.04×10^{-8}
B	Male	23	2.15×10^{-8}
C	Male	22	2.04×10^{-8}
D	Female	22	2.93×10^{-8}
E	Female	21	2.76×10^{-8}

However, it's so difficult for us to obtain any diseased blood samples from AIDS, B-type hepatitis, and C-type hepatitis which are serious problems today. That's one of reasons we couldn't study the relation between concentrations of sulfide ion in human sera and physical conditions for human body.

Although the determination of sulfide in whole blood has been reported previously(13-16), no data exist for the sulfide concentration in human sera as published data. Furthermore, the concentration of sulfide in whole blood reported previously were obtained from human sera of hydrogen sulfide poisoned human.

Although the existence of endogenous sulfide as a normal microcomponent of mammals is evident, significant details of physiology are not clear because of lack of quantitative informations; in short, the normal level of sulfide ion in blood could not be estimated because of the sensitive limitations of measurment.

In conclusion, the HPLC method using 2-amino-5-N,N-diethylamino toluene method has described here is so

sensitive as to be able to determine the sulfide ion in biological materials like human sera without any complicated pretreatments.

REFERENCES

1. E.Fischer, Ber, 16, 2234-2238 (1983).
2. C.M.Johnson,,H.NishitaAnal. Chem.,24, 736-741 (1952).
3. L.Gustafsson, Talanta 4, 227-235, 236-243 (1960).
4. E.J.Skerrett, G.J.Dickes, Analyst,86, 69-71 (1961).
5. O.H.Kriege, A.L.Wolfe, Talanta, 9, 673-678 (1962).
6. M.B.Kloster, M.P.King, J.Am.Water Works Assoc. 69, 544-546 (1977).
7. M.Kamaya,K.Fukusima, K.Nagashima,E.Ishii,G.Sudo, Reseach Reports of Kogakuin Univ.,74, 101-104 (1993).
8. E.Bald,S.Sypniewski, J.Chromatogr.,641 184-188 (1993).
9. Y.Yamamoto, M.Umeda,K.Morishige,Y.Nishikawa, Bunseki Kagaku 37, 421-425 (1988).
10. Y.Ogasawara,K.Ishii,T.Togawa,S.Tanabe, Analyst,116, 1359-1363 (1991).
11. Japanese Standards Association (ed.)JIS K0102.Tokyo(1986).
12. M.Kamaya,K.Fukusima,K.Nagashima,E.Ishii, Bunseki Kagaku 42, 519-523 (1993).
13. B.H.Mc.Analley, W.T.Lowry, R.D.Oliver, J.C.Garriott, J. Anal. Toxicol. 3, 111-114(1979).
14. H.Lindell, P.Jappinen, H.Savolainen, Analyst 113, 839-840 (1988).
15. P.Jappinen, R.Tenhunen, Br.J.Ind.Med. 47, 283-285 (1990).
16. S.Koge, T.Nagata, K.Kimura, K.Kudo, J.Forensic. Sci. 33, 217-222 (1988).

Received: July 19, 1994
Accepted: August 29, 1994