TECHNICAL NOTE

Shigetoshi Kage,[1,2] *B.S.; Takeaki Nagata,*[1] *M.D.; Kojiro Kimura,*[1] *M.D.; and Keiko Kudo,*[1] *Ph.D.*

Extractive Alkylation and Gas Chromatographic Analysis of Sulfide

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ABSTRACT: A sensitive analysis of sulfide in blood was established, using an extractive alkylation technique. Pentafluorobenzyl bromide was used as the alkylating agent, tetradecyldimethylbenzylammonium chloride as the phase-transfer catalyst, and potassium dihydrogenphosphate as the buffer to suppress the formation of sulfide. Mass fragmentography was used to identify the sulfide derivative and gas chromatography with an electron capture detector was used for quantitative determination, with the lowest limit of detection being about 0.01 μg/g. The blood level of rats exposed to hydrogen sulfide was also determined.

KEYWORDS: toxicology, hydrogen sulfide, extractive alkylation, chemical analysis, pentafluorobenzyl bromide, tetradecyldimethylbenzylammonium chloride, potassium dihydrogenphosphate, gas chromatography, mass spectrometry, phase-transfer catalyst, counter-ion, blood level

Abbreviations

GC	Gas chromatography
MS	Mass spectrometry
IS	Internal standard
TBB	1,3,5-Tribromobenzene
PFBBr	Pentafluorobenzyl bromide
TDMBA	Tetradecyldimethylbenzylammonium chloride
BPFBS	Bis(pentafluorobenzyl)sulfide
TsO	Toluenesulfonate

To elucidate factors related to poisoning, environmental pollution, or industrial accidents, as induced by hydrogen sulfide, a sensitive and reliable method to detect the sulfide in biological materials has to be designed.

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[1]Guest researcher, professor, lecturer, and lecturer, respectively, Department of Legal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

[2]Forensic chemist, Forensic Science Laboratory, Fukuoka Prefectural Police Headquarters, Fukuoka, Japan.

While there are reports on the use of gas chromatography (GC) for analyzing hydrogen sulfide [*1,2*] and sulfide derivatives [*3–5*], these techniques are not always suitable for analysis of hydrogen sulfide in the blood, since this compound escapes from the blood and oxidization is rapid at room temperature [*6*].

The extraction methods reported by Funazo et al. [*5*] and Landini and Rolla [*7*] are convenient methods characterized by coupling extraction and derivatization, where the anion forms an ion pair and is transferred by a phase-transfer catalyst into an organic phase and derivatized. Their studies, however, were not related to biological samples.

We now report an improved technique for extractive alkylation, using PFBBr as the alkylating agent and TDMBA as the phase-transfer catalyst, in an alkaline medium. Sulfide in the blood was stabilized and qualitative and quantitative analyses of alkylated sulfide were made, using GC and gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Reagents

Oxygen-free water was prepared by bubbling nitrogen into distilled water for 15 min.

A solution of internal standard (IS) was prepared by dissolving 1,3,5-tribromobenzene (TBB) in ethyl acetate to give a concentration of 1 μM. TBB of analytical grade was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

A standard solution of sulfide was prepared by dissolving sodium sulfide ($Na_2S \cdot 9H_2O$) in oxygen-free water saturated in advance with sodium tetraborate, to give a concentration of about 10 μg/g. The concentration of sulfide was determined spectrophotometrically by the methylene blue method [*8*].

The alkylating agent, PFBBr, was dissolved in toluene. As the phase-transfer catalyst, tetradecyldimethylbenzylammonium chloride (TDMBA) was selected after a comparison with six other reagents: trimethyl-β-hydroxyethylammonium chloride, trimethylphenylammonium bromide, trimethylbenzylammonium chloride, dodecyltrimethylammonium chloride, tetra-*n*-butylammonium bromide, and *n*-hexadecyltrimethylammonium bromide. The reagent of analytical grade was purchased from Tokyo Kasei Kogyo Co., Tokyo, Japan and was dissolved in oxygen-free water.

Preparation of Water and Blood Samples

The samples were prepared by adding the standard solution of sulfide to the distilled water, or to the mixed blood of five healthy persons.

Basic Procedure Used for Extractive Alkylation

The basic factors for the analysis were as follows: ethyl acetate as the solvent for extraction, pH 9.3 for the alkylating reaction, 1-min reaction time, 20mM of PFBBr, and 5mM of TDMBA.

A 1/2 mL of 20mM PFBBr solution and 2.0 mL of ethyl acetate containing 1 μM of IS (TBB) were put into a 10-mL volume glass-stoppered test tube with 0.8 mL of 5mM TDMBA solution in the oxygen-free water saturated with sodium tetraborate. To the mixture was added 0.2 g of the sample solution and the preparation was stirred vigorously for 1 min at room temperature. After centrifugation at 2500 rpm for 10 min, the organic phase was transferred to a different test tube and a 0.1-μL aliquot of the extract was injected onto a GC or GC/MS apparatus.

Apparatus

The apparatus used was a Shimadzu GC-3BE model gas chromatograph equipped with a Ni^{63} electron capture detector connected with a computerized recorder of Shimadzu C-R3A model chromatopac and a Shimadzu QP-1000 model gas chromatograph-mass spectrometer of quadrupole type.

GC Conditions

The column was a glass tube of 2.1-m by 3-mm inside diameter (id) packed with 5% Apiezon grease L on Chromosorb W (acid-washed-dimethyldichlorosilane [AW-DMCS]), 60–80 mesh. The temperature of the column was kept at 200°C. Nitrogen was used as the carrier gas at a flow pressure of 0.3 kg/cm^2.

GC/MS Conditions

The column was a glass tube of 2.1-m by 3-mm id packed with 1.5% SE-30 silicone on Chromosorb W (AW-DMCS), 60–80 mesh. The temperature settings were: 170°C at column, 230°C at injection port, and 250°C at separator and ion source. Helium was used as the carrier gas at a flow rate of 20 mL/min. Ionization energy was 70 eV.

Results

Stabilization of Sulfide in the Blood

When the extract from a two- or three-day-old blood sample was stored for a few days before analysis, there was an increase in the sulfide derivative, presumably originating from the sulfur containing organic materials such as degraded peptide, glutathione, or cysteine transferred into the ethyl acetate layer in the alkaline medium.

To prevent sulfide formation, potassium dihydrogenphosphate (KH_2PO_4) was added as a buffer to the sample before the step of centrifugation in the basic procedure, and the preparation was mixed for 10 s at room temperature. The formation of sulfide derivative in the blood sample as well as in the cysteine solution was suppressed.

The established procedure of extractive alkylation is summarized in the scheme (Fig. 1).

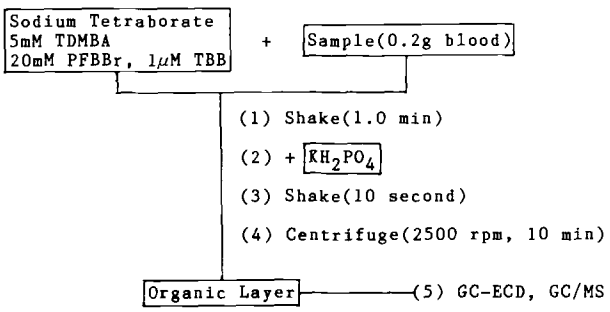

FIG. 1—*Procedure for extractive alkylation.*

Analysis by GC and Calibration

A gas chromatogram of the sulfide derivative obtained from the blood sample and that of IS, TBB, are shown in Fig. 2. Both gave sharp and symmetrical peaks with a retention time of 4.2 and 4.8 min, respectively. There were no interfering peaks.

The calibration curves were obtained by plotting the peak area ratios of the sulfide derivative to IS on the y axis and the concentrations of sulfide from 0 to 0.8 μg/g on the x axis using GC. The curve of the water sample presented a straight line through zero with a regression equation of $y = 2.67x$ and a correlation coefficient of 0.997. In case of the blood sample, a linear relationship was also observed with a regression equation of $y = 1.06x + 0.18$ and the correlation coefficient was 0.996. The lowest limit of detection was about 0.01 μg/g as the sulfide in both samples.

Analysis by GC/MS

The peaks of BPFBS and TBB on GC were analyzed by GC/MS. Their mass spectra are shown in Fig. 3. The molecular ion of BPFBS was observed at m/z 394 (M^+), the base peak at m/z 181 ($M-C_6F_5CH_2S$), and a small fragment ion at m/z 213 ($M-C_6F_5CH_2$). The mass spectral pattern indicates that the derivative is bis(pentafluorobenzyl) sulfide (BPFBS). The molecular ion of TBB was observed at m/z 314 and 316, and the fragment ion at m/z 235 (M−Br).

As no mass spectra could be obtained from a small amount of sulfide derivatives in the ppb order, mass fragmentography was used to confirm the GC peaks. Mass fragmentograms monitored with three ions of BPFBS, m/z 394, 213 and 181, and with two ions of IS, m/z 314 and 235, were obtained. All peaks were clearly separated and there was no interference. Quantitative determination was attempted to obtain calibration curves in case of the blood

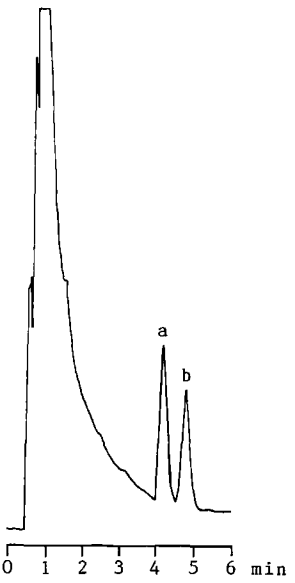

FIG. 2—*Gas chromatogram of the sulfide derivative, BPFBS, and the internal standard, TBB, in the blood sample.* (a) *BPFBS with the concentration of 1.0 μg/g as sulfide.* (b) *TBB contained with the concentration of 1 μM.*

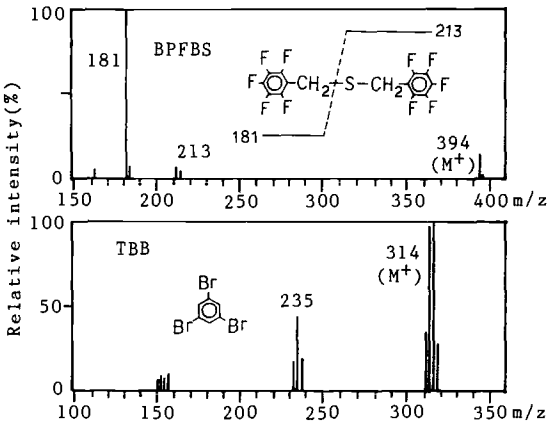

FIG. 3—*Mass spectra of the sulfide derivative, BPFBS, and internal standard, TBB.*

samples. The peak area ratios of the parent ion of sulfide derivative, *m/z* 394, to that of IS, *m/z* 314, were calculated. The lowest limit of detection of BPFBS was about 0.01 μg/g as the sulfide, much the same as that on GC. Although the calibration curve gave a straight line, the equations varied slightly with each examination. Thus, the calibration curve of GC was used.

Testing of Blood

Five male Wistar rats weighing 200 to 250 g were exposed to hydrogen sulfide (H_2S) gas controlled from 500 to 600 ppm until they died. The blood samples collected after 8 to 27 min of exposure contained levels of sulfide varying from 0.19 to 0.61 μg/g. Concentrations in the controls were less than 0.01 μg/g.

Discussion

Wu et al. alkylated sulfide in spring water using PFBBr without the phase-transfer catalyst by stirring for 60 min [*3*]. This method is of little use for biological materials as a 1-h reaction in an alkaline medium may lead to production of sulfide, derived from a decomposition of the organic substances.

Landini and Rolla converted sulfide into dibenzyl sulfide, using a phase-transfer catalyst, and within 10 min, under vigorous stirring at 70°C [*7*]. Their study was focussed on the synthesis and attention was not directed to the sensitivity or to the loss of sulfide which readily escapes at high temperatures.

Funazo et al. used gas chromatography-flame ionization detection (GC-FID) to analyze derivatives of bromide, iodide, cyanide, thiocyanate, nitrite, nitrate, and sulfide alkylated with pentafluorobenzyl *p*-toluenesulfonate (TsO-PFB) [*5*]. The sensitivity of their method is limited to over 5 μg/mL, hence it is not practical in cases of a small amount of sulfide.

Using gas chromatography-electron capture detection (GC-ECD), they detected derivatives of bromide, iodide, thiocyanate, and nitrite at a concentration of less than 5 μg/mL. However, peaks of PFB derivatives of cyanide, nitrate, and sulfide were not obtained on their gas chromatogram [*9*].

The problems related to stability, sensitivity, and applicability to biological materials were overcome using our extractive alkylation technique combined with GC and GC/MS.

Summary

Analyses of a small amount of sulfide in the blood were made feasible using an extractive alkylation technique. PFBBr as the alkylating reagent together with the phase-transfer catalyst, TDMBA, stabilized sulfide as BPFBS, and potassium dihydrogenphosphate as the buffer suppressed the formation of sulfide. The production of BPFBS was identified by mass fragmentography, and was determined quantitatively by GC-ECD, in the ppb order.

Our method could be used to analyze the blood of experimental animals, where the acute fatal blood level of sulfide was estimated as 0.2 to 0.6 μg/g.

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Address requests for reprints or additional information to
Takeaki Nagata, M.D.
Department of Legal Medicine
Kyushu University
3-1-1, Maidashi, Higashi
Fukuoka 812, Japan