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BRIEF REPORT

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Quantification of bromide ion in biological samples using headspace gas chromatography–mass spectrometry

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Abstract

Objectives: In this study, we aimed to establish a method for quantifying bromide ions (Br^-) in blood and urine using gas chromatograph-mass spectrometer (GC-MS) equipped with a headspace sampler, for biological monitoring of workers exposed to methyl bromide.

Methods: Samples were mixed with dimethyl sulfate, and Br^- ions were detected using GC-MS with a headspace sampler. The validity of the proposed method was evaluated based on most of the US FDA guidance. The values obtained were compared with reference values by analysis using Seronorm™ Trace Elements Whole Blood L-1 RUO.

Results: The calibration curve showed good linearity in the Br^- concentration range of 0.1−20.0 mg/L, and the coefficient of determination R^2 value was >.999. Intraday and interday accuracy values were 99.3%−103.1% and 97.4%−101.8%, respectively. The measured and reference values of Seronorm were concordant. Herein, eight urine and serum samples of workers were analyzed; the samples' Br^- concentrations were known. The correlation coefficients of urine and serum samples were 0.97 and 0.96, respectively, and results were consistent.

Conclusions: This study established a simple and rapid method for the determination of Br^- concentration in biological samples using GC-MS with a headspace sampler. Moreover, it can be used for biological monitoring of occupational exposure to methyl bromide and for the determination of Br^- concentration in a wide range of biological samples.

KEYWORDS

biological monitoring, bromide ion, gas chromatography–mass spectrometry, methyl bromide

1 | INTRODUCTION

Methyl bromide is a colorless and odorless gas at normal temperature and pressure, and it can be liquefied under pressure and sealed in a container. It is used as a fumigant to kill insects and to disinfect cereals, fruits, and wood. However, owing to its low boiling point and high toxicity, fumigant workers who handle it often suffer from

serious poisoning accidents, and there have been many cases of unfortunate outcomes.[1] However, since methyl bromide was designated as an ozone-depleting substance by the Montreal Protocol in 1992, measures have been implemented to reduce its production, and the use of methyl bromide for soil fumigation has been banned since 2013. Its application is currently limited, for example, for phytosanitary treatments. Consequently, the volume of methyl bromide shipped for domestic use have also decreased over the years, from 4180 tons in 2004 to 506 tons in 2019, but the volume has remained constant at just over 500 tons for a while and remains considerably high for a pesticide. As workers are still involved in the production and use of methyl bromide, we have been conducting biological monitoring to determine the concentration of bromide ions (Br^-) in urine and serum as part of a special health examination.[2]

Quantification of Br^- ions in blood and urine is recommended for the differential diagnosis of industrial poisoning by methyl bromide and for estimating the extent of exposure among handlers.[3] There are several methods for determining Br^- concentration, including ion chromatography[4] and gas chromatography.[5] However, most of these methods require a large amount of samples, and as the methods are complicated and time-consuming, they are inconvenient as emergency diagnostic methods. Although a simple method for determining Br^- concentration in urine using headspace gas chromatography was developed,[6] which was suitable for the analysis of volatile substances and has been used for biological monitoring at industrial sites, it showed poor reproducibility and a low recovery rate when blood samples were tested directly. Yamano et al reported a simple method for determining Br^- concentration in the blood, with some modifications.[7]

We have also used a gas chromatograph-hydrogen flame ionization detector (GC-FID) to determine the Br^- concentration in biological samples. However, our GC-FID was nearing the end of its life, and thus, we decided to use introduce gas chromatography–mass spectrometry (GC-MS) equipped with headspace sampler. Therefore, we aimed to establish a simple method for the determination of Br^- concentrations using a headspace GC-MS.

2 | MATERIALS AND METHODS

2.1 | Materials

Dimethyl sulfate, potassium bromide, and fetal calf serum equivalent (EqualFETAL) were purchased from Wako Pure Chemicals, Kanto Chemical, and Atlas Biologicals, Inc, respectively. Purelab flex 3 (ELGA) was used as the ultrapure water system.

2.2 | Instruments and analytical condition

GC-MS-QP2010 SE (Shimadzu) with an HS-20 autosampler (Shimadzu) was used for headspace GC-MS analysis. The capillary column was a 60 m × 0.25 mm ID ZB-624 with a 1.40 μm film thickness (Phenomenex). The carrier gas was helium, and its linear gas velocity was set at 25.7 cm/sec. At the autosampler, the temperatures of the oven, sample line and transfer line were set to 85, 150 and 150°C, respectively. The pressure of the gas for vial pressurization was set to 90 kPa. The column oven temperatures at GC were set to 100°C for 1 minute and then increased to 160°C at a rate of 20°C/min. The temperature of the ion source and interface were set to 180 and 200°C, respectively. The vials were incubated at 85°C for 10 minutes in HS-20, and then 1 mL of the gaseous phase was injected in the split mode (split ratio, 20:1). The mass spectrometer was operated in the electron impact mode, and data were obtained in the selected ion monitoring (SIM) scan mode. The ions selected for SIM were as follows: m/z 95.95 (quantifier ion) and 93.53 (qualifier ion).

2.3 | Sample preparation and method validation

Aliquots of ultrapure water (800 μL) and serum (200 μL) were placed in a TORAST HS vial (GL Sciences Inc), and 50 μL of dimethyl sulfate was added to the vial. For urine analysis, 500 μL of ultrapure water, 500 μL of urine, and 20 μL of dimethyl sulfate were used. Subsequently, the vial was capped with a TORAST HS cap with a septum (GL Sciences Inc). The proposed method was validated based on most of the US FDA guidance.[8] However, we used potassium bromide dissolved in ultrapure water as an external standard instead of serum containing potassium bromide. Biological samples originally contain Br^-, whose concentration differs between individuals. For calibration, samples of potassium bromide dissolved in ultrapure water at concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 mg/L were prepared in triplicate and analyzed by the method described above. Calibration curves were obtained by plotting the ratio of the peak area to the Br^- concentration. The reproducibility of the method was determined by analyzing ultrapure water containing 0.1, 0.25, and 2.5 mg/L of Br^- on the same day (five replicates: intraday reproducibility) and on different days (five replicates: interday reproducibility). Recovery was evaluated using EqualFETAL containing 0.4, 1.0, 3.0, and 15.0 mg/L Br^- ions. Finally, the accuracy of the method was confirmed using Seronorm™ Trace Elements Whole Blood L-1 RUO (Nycomed), which helps determine the approximate values of Br^-.

2.4 | Comparison with other methods

We measured Br^- concentrations in the biological samples of workers handling methyl bromide using GC-FID as part of a special health examination. As the objective of this study was to develop an alternative method to determine Br^- concentration, it was necessary to evaluate the consistency of the measured values. Therefore, we selected eight urine and serum samples from the worker samples analyzed at our laboratory, quantified the Br^- concentration by GC-MS, and compared the results with those that were obtained previously by GC-FID. This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Showa University (Approval No. 2497). Informed consent was obtained from the participants during medical examination.

2.5 | Statistical analysis

Pearson's correlation coefficient was calculated for the GC-MS and GC-FID results. R version 4.0.1 was used to perform statistical analysis.

3 | RESULTS

The calibration curve showed linearity in the 0.1-20.0 mg/L Br^- concentration range, and the coefficient of determination R^2 value was >.999. Accuracy is defined as a variation from the expected value, and precision is a value evaluated by the relative standard deviation (RSD). The intraday accuracy for samples spiked with ultrapure water was 99.3%-103.1%, and the precision was 1.8%-3.8%, whereas the interday accuracy for samples spiked with ultrapure water was 97.4%-101.8%, and the precision was 2.7%-4.8% (Table 1). The recoveries of samples spiked with EqualFETAL were 103.7-112.5%, within a range of 0.4−15.0 mg/L (Table 2). Analysis of Seronorm, with a reference value of 0.753 mg/L, revealed a mean ±standard deviation, RSD, and deviation from the reference value obtained from five analyses of 0.7527 ± 0.0123 mg/L, 1.6%, and 0.04%, respectively. The limit of quantification was 0.1 mg/L.

The correlation coefficients (95% confidence intervals) of Br^- concentrations in the urine and serum of workers obtained by GC-FID and GC-MS were 0.97 (0.82-0.99) and 0.96 (0.79-0.99), respectively ($P = .001$).

4 | DISCUSSION

The method described in our study showed small intraday and interday differences, high recovery rates, and extremely small deviations from the reference values, suggesting its utility in quantifying the concentration of Br^- in biological samples. Further, we confirmed that there was no difference in the slopes of the calibration curves of water and serum. Methyl bromide is designated as a Class II substance of specified chemical substances under the Industrial Safety and Health Law.[9] Its occupational exposure limit and administrative control levels are 1 ppm (3.89 mg/m^3)[10] and 1 ppm,[11] respectively. Biological monitoring is not specified as a mandatory health examination, but previous studies have shown that the Br^- concentration in the blood of workers exposed to methyl bromide and controls was 6.9 ± 4.5 and 3.7 ± 1.5 mg/L, respectively.[7] We confirmed patients' work history and conducted a reexamination when the Br^- concentration in the serum or urine exceeded 10 and 20 mg/L, respectively. The method proposed in this study showed good quantification, in the range of 0.1−20.0 mg/L, which could be validated, suggesting that the method can be used for quantifying Br^- concentration in serum or urine.

Kawai et al reported a method for determining Br^- in biological samples by improving the method reported by Yamano et al[12] This method uses an electron capture detector instead of an FID, which can detect concentrations as low as 0.01 mg/L. However, at least 1 hour is required to complete the procedure from sample preparation to the end of analysis. Hori et al have also used a similar analytical method in animal experiments.[13] Their method is almost the same as our method; their method includes sample pretreatment and also employs GC-MS as the analytical tool. However, it is

TABLE 1 Intraday and interday coefficients of variation of the proposed method in spiked ultrapure water samples

	Intraday (n = 5)[a]			Interday (n = 15)[b]		
Spiked ultrapure water concentration (mg/L)	Mean ± SD (mg/L)	RSD (%)	Accuracy (%)	Mean ± SD (mg/L)	RSD (%)	Accuracy (%)
0.1	0.1031 ± 0.0039	3.8	103.1	0.1018 ± 0.0031	3.0	101.8
0.25	0.2547 ± 0.0048	1.9	101.9	0.2436 ± 0.0116	4.8	97.4
2.5	2.4827 ± 0.0454	1.8	99.3	2.4836 ± 0.0667	2.7	99.3

Abbreviation: RSD, relative standard deviation.

[a]Intraday reproducibility analysis was performed on a single day.

[b]Interday reproducibility analysis was performed over consecutive days in five replicates.

TABLE 2 Variation in the recoveries using the proposed method for spiked serum samples

Spiked serum concentration (mg/L)	n = 5 Mean ± SD (mg/L)	RSD (%)	Recovery (%)
0	4.4678 ± 0.0787	1.8	—
0.4	4.8861 ± 0.1431	2.9	104.6
1.0	5.1671 ± 0.0866	1.7	112.5
3.0	7.2169 ± 0.2495	3.5	105.8
15.0	19.5951 ± 0.5751	2.9	103.7

Abbreviation: RSD, relative standard deviation.

difficult to compare the results since the GC method that was developed on the instrument is not described. Kage et al also reported a method for alkylation of Br^- with pentafluorobenzyl *p*-toluenesulfonate and detection by GC-MS.[14] Although the lower limit of detection and lower limit of quantification are slightly higher at 1 mg/L and 2 mg/L, respectively, the calibration curve is linear in the range of 2-100 mg/L, which is a great advantage. However, this procedure also requires nearly 1 hour to complete from sample preparation to the end of the analysis. In contrast, in our procedure, it takes less than 20 minutes. We believe that our method is particularly useful for rapid diagnosis of acute poisoning. In addition, an empirical lower limit of quantification was adopted in this study. Our analyses results suggest that the Br^- concentration in serum was never less than 1 mg/L, irrespective of exposure; therefore, we set the lower limit of quantification as 0.1 mg/L, which is one tenth of 1 mg/L. However, when the limits of detection and quantification were defined as the amount of Br^- in water corresponding to three and ten times the baseline noise, respectively, the lower limits of detection and quantification obtained via our method were 0.0053 and 0.015 mg/L, respectively. Therefore, our method may be more sensitive than the previously reported methods. The actual concentration in the samples will not be as low as the lower limits of quantification or detection. However, when analyzing specimens from workers exposed to high concentrations of methyl bromide, a sensitive method may be less affected by dilution factor and may allow for more efficient analysis.

The results of our method are consistent with those of previous methods. For serum, the concentration determined by GC-MS was higher than that determined by GC-FID, and only one sample had a lower concentration than that obtained on GC-FID. The FID, in general detects higher concentrations of foreign substances and thus there may be some bias in GC-MS analysis. As the samples were stored at −20°C for >5 years, evaporation of water might have led to concentration. Further, default quantification programs were used for each instrument, but the peak widths determined by GC-MS were <25% of those determined by GC-FID and thus there could be differences in the quantification stage. However, there are only minor differences in the mean value and standard deviation between the two (1.3 ± 1.6 mg/L), and the concentration may not affect the utility of the method. In contrast, in the case of urine, the concentration determined by GC-MS is generally higher than that determined by a GC-FID in the range >10 mg/L, but it is lower than that determined by a GC-FID below this level. This may be mainly associated with the range of the calibration curve in routine testing. As calibration curves are frequently prepared in the range of 20-100 mg/L for urine analysis, measurements beyond this range increase the uncertainty; therefore, the values determined by GC-MS may be lesser than those determined by a GC-FID at levels below <10 mg/L.

One limitation of our study was that we could not conduct reanalysis by a GC-FID because the GC-FID we were using was broken and beyond repair. Therefore, it is no longer possible to verify the reported differences between the GC-FID and GC-MS results. However, since the differences were small, they are not expected to have a significant impact on the findings of this study. Further, we could not find any certified substances with certified values and confidence intervals, which is also a limitation. The Seronorm™ Trace Elements Whole Blood L-1 RUO only had a reference value, without any confidence interval. Therefore, complete validation was not obtained.

The method described in this study can be used for determining Br^- concentrations in biological samples. It shows a high correlation with the values obtained by the conventional GC-FID method. Further, by setting the qualifier ion in SIM mode, it is possible to reduce the influence of other interfering components and perform highly accurate analysis. Additionally, this method does not require complicated pretreatment, and the linearity of the calibration curve is high over a wide range, making it suitable for routine analysis. The number of workers exposed to methyl bromide may not increase in the future, but it is important to establish a simple method for evaluating the concentration of Br^- in biological samples.

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DISCLOSURE

Approval of the research protocol: This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Showa University (Approval No. 2497). *Informed consent*: Informed consent was obtained from participants at medical examination. *Registry and the registration no. of the study/trial*: N/A. *Animal Studies*: N/A. *Conflict of Interest*: The Authors declare no Conflict of Interests for this article.

AUTHOR CONTRIBUTIONS

TY, AK, and YY conceived the study; TY, DN and SO analyzed samples; TY statistically analyzed the data; and TY led the writing.

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