Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 817 (2005) 335–339
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of bromide in whole blood and urine from humans using gas chromatography–mass spectrometry

Shigetoshi Kage[a], Keiko Kudo[b], Hideaki Ikeda[a,b], Akira Tsujita[a], Noriaki Ikeda[b,*]

[a] *Forensic Science Laboratory, Fukuoka Prefectural Police Headquarters, 7-7 Higashikoen, Hakata-ku, Fukuoka 812-8576, Japan*
[b] *Department of Forensic Pathology and Sciences, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

Received 2 March 2004; accepted 16 December 2004

Abstract

We devised a sensitive and simple method for determination of bromide in whole blood and urine from humans using gas chromatography–mass spectrometry. Bromide was alkylated with pentafluorobenzyl *p*-toluenesulphonate in the mixture of acetone and phosphate buffer (pH 6.8). The derivative obtained was analyzed using gas chromatography–mass spectrometry with the positive-ion EI mode. The lower limit of detection for the compound was 1 mg/l. The calibration curve for bromide was linear over the concentration range from 2 to 100 mg/l. With use of this method, levels of bromide in whole blood and urine were determined in cases of poisoning by inhaled brominated hydrocarbons.

Keywords: Bromide; Methyl bromide; Toxicology; Gas poisoning; Metabolite; Gas chromatography–mass spectrometry

1. Introduction

Bromide in whole blood and urine are occasionally analyzed in cases of poisoning by exposure to brominated hydrocarbons such as halothane [1,2] and methyl bromide [3–5], since this compound is a metabolite of brominated hydrocarbons [2,6,7].

Numerous methods have been reported for the determination of bromide in blood and/or urine using ion chromatography [8], gas chromatography (GC) [1,2,9–13], radio activation analysis [3], inductively coupled plasma mass spectrometry [14], X-ray spectrometry [15], cyclic voltammetry [16] and photometry [17].

Majority of these methods, however, identify bromide only by the retention time [1,2,8–13] or the absorbance [17], thus they lack specificity. Gas chromatography–mass spectrometry (GC–MS) is a popular and specific method for forensic toxicological examination. Funazo et al. [18,19] have attempted simultaneous determination of inorganic anions including bromide with pentafluorobenzyl *p*-toluenesulphonate (PFB-TsO), however, these reports are not available concerning determination of bromide in biological materials. We developed a simple procedure to determine formate and acetate [20] in whole blood and urine, using pentafluorobenzyl bromide (PFBBr) as the alkylating agent. We therefore tried to derivatize bromide in whole blood and urine based on this procedure.

2. Experimental

2.1. Reagents

A standard solution of bromide (1000 mg/l) was purchased from Wako Pure Chemical Industries (Osaka, Japan), then was further diluted with the distilled water to prepare a 1–200 mg/l solution. A solution of internal standard (I.S.)

* Corresponding author. Tel.: +81 92 642 6124; fax: +81 92 642 6126.
E-mail address: norii@forensic.med.kyushu-u.ac.jp (N. Ikeda).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.12.017

was prepared by dissolving 1,3,5-tribromobenzene (TBB) in *n*-hexane to yield a concentration of 0.1 mM. TBB was purchased from Wako Pure Chemical Industries (Osaka, Japan). An alkylating agent, PFB-TsO (Tokyo Kasei Kogyo, Co., LTD. Tokyo), was dissolved in acetone at a concentration of 100 mM. The other reagents used were of analytical grade.

2.2. Preparation of whole blood and urine samples

Samples to be tested were prepared by adding the standard solution of bromide to whole blood and urine, both of which were collected from a healthy volunteer. For analysis of bromide, 0.1 ml sample was directly used for the alkylation procedure without steps of deproteinization. When bromide concentration in whole blood and urine is over the quantitation limit of 100 mg/l, the sample was diluted.

2.3. Alkylation procedure

A 0.5 ml volume of 100 mM PFB-TsO solution in acetone was put into a 10 ml volume glass-stoppered test tube with 0.1 ml of 0.5 M phosphate buffer (pH 6.8). 0.1 ml of the sample solution was added to the mixture and the preparation was vortexed for 1 min at room temperature, maintained at 60 °C in a water bath for 30 min. 1.0 ml of 0.1 mM TBB solution in *n*-hexane was added to the preparation. The preparation was vortexed for 1 min at room temperature then centrifuged at 1400 × *g* for 15 min. The organic phase was placed in another test tube and 1.0 μl aliquot of the solution was injected onto the GC–MS apparatus. Fig. 1 shows the outline of our procedure.

2.4. GC–MS conditions

Gas chromatography–mass spectrometry (GC–MS) was done using a Hewlett-Packard HP 5790A gas chromatograph (Palo Alto, CA, USA) interfaced to a JEOL AX505A mass spectrometer (Tokyo, Japan). The column was a J&W fused-silica capillary tube of DB-225 (30 m × 0.32 mm i.d., 0.25 μm film thickness). A splitless injection mode was selected with a valve off-time of 1.5 min. The initial temperature of the column was held at 50 °C for 3 min, and then programmed to rise to 220 °C at 20 °C/min. The injection port, separator and ion source were kept at 220, 200 and 220 °C, respectively. Helium was used as the carrier gas at a flow-rate of 2 ml/min. The ionization energy of the positive-ion electron ionization (EI) condition was 70 eV. The ionization energy and reagent gas of the negative-ion chemical ionization (CI) conditions were 200 eV and isobutane, respectively.

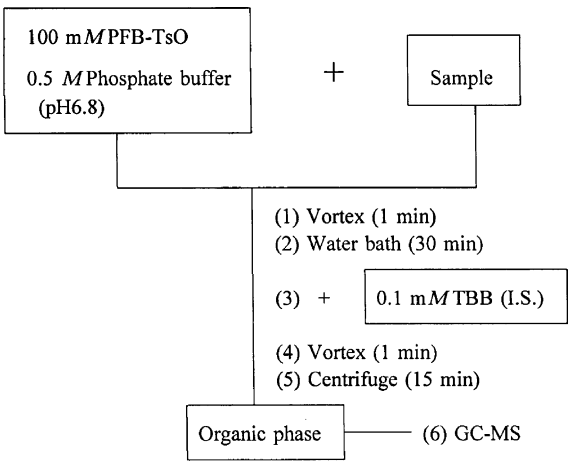

Fig. 1. Scheme of analytical procedures for bromide analysis.

2.5. Preparation of calibration graphs

Fresh whole blood and urine samples were prepared to contain bromide at concentrations of 1, 2, 5, 10, 20, 50, 75 and 100 mg/l. These samples were extracted and derivatized in the same manner as described above. Calibration graphs were obtained by plotting the peak-area ratio of the molecular ion peak, *m/z* $[260]^+$ of the derivative of bromide to the base ion peak, *m/z* $[314]^+$ of TBB (I.S.) against the concentration of bromide, using mass chromatography in positive-ion EI mode.

3. Results

3.1. Analysis by GC–MS

Mass spectra of the derivative of bromide using positive-ion EI and negative-ion CI modes, are shown in Fig. 2. The molecular ion peak of the derivative of bromide was observed at *m/z* 260 when using the positive-ion EI mode, and the

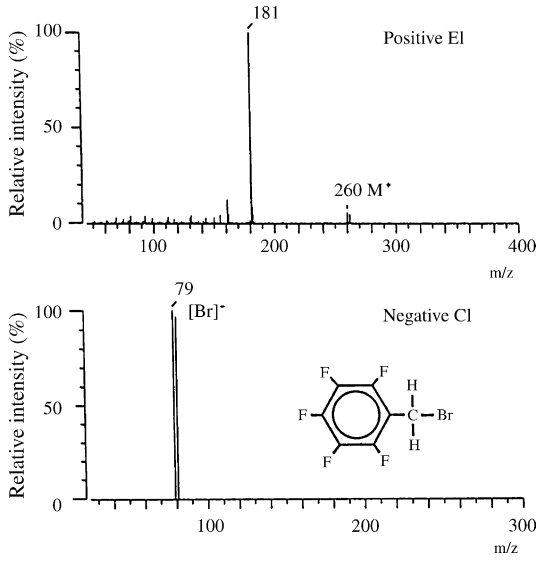

Fig. 2. Mass spectra of the derivative of bromide, using positive-ion EI and negative-ion CI modes of GC–MS.

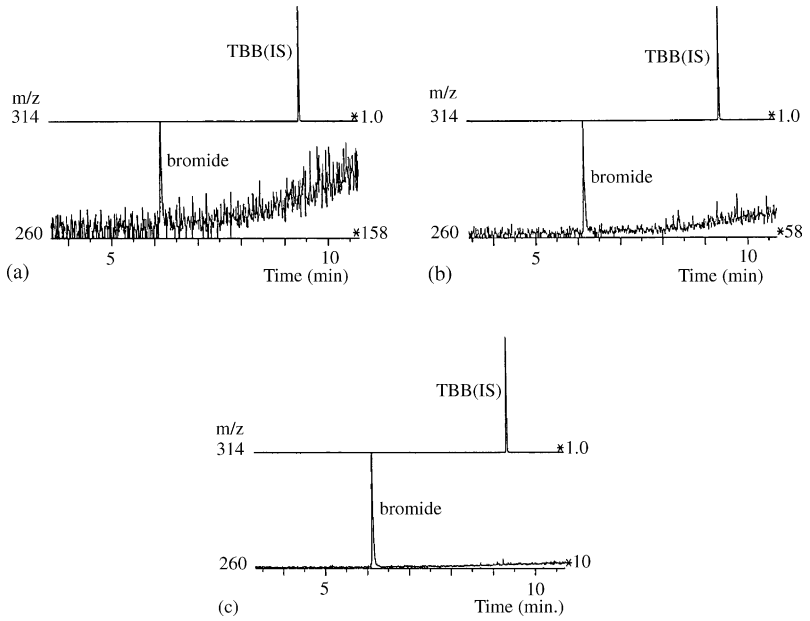

Fig. 3. Mass chromatograms of the derivatized extract obtained from (a) blank whole blood (unspiked), (b) whole blood spiked with 20 mg/l of bromide, and (c) whole blood of the victim (A), using positive-ion EI mode of GC–MS. The numbers to the right of the chromatograms indicate the degree of enlargement used to obtain similar-sized peaks in the chromatograms.

base ion peak was observed at *m*/*z* 181 $[M-\mathrm{Br}]^+$. Using the negative-ion CI mode, the base ion peak of the derivative of bromide was $[\mathrm{Br}]^-$ at *m*/*z* 79. The mass spectral pattern indicated that the derivative obtained was PFB-Br. The most abundant ion of TBB was observed at *m*/*z* 314 $[M+2]^+$, using the positive-ion EI mode. Using the negative-ion CI mode, the base ion peak of TBB was observed at *m*/*z* 79 $[\mathrm{Br}]^-$.

3.2. Determination of bromide in whole blood and urine

Mass chromatograms of the derivatized extracts from (a) blank whole blood (unspiked) and (b) whole blood spiked with 20 mg/l of bromide, using the positive-ion EI mode, are shown in Fig. 3. Mass chromatograms of the derivatized extracts from (a) blank urine (unspiked) and (b) urine spiked with 20 mg/l of bromide, using the positive-ion EI mode,

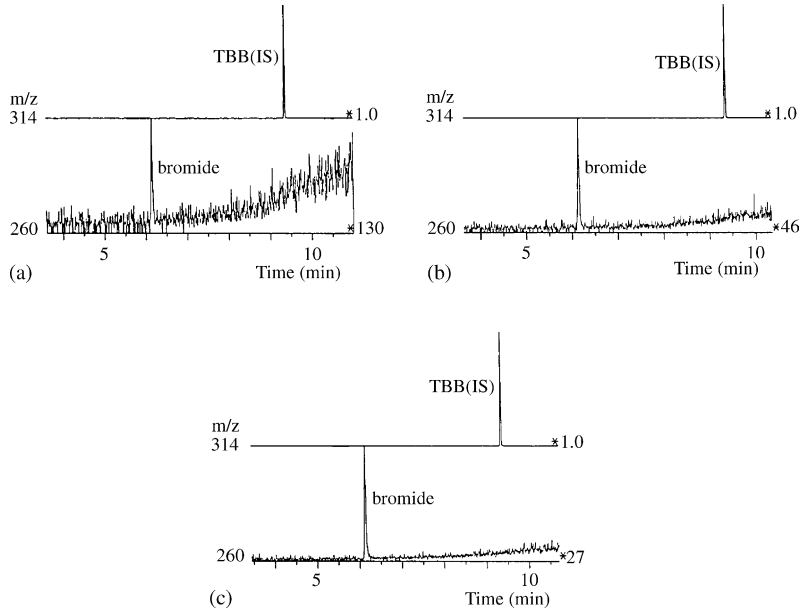

Fig. 4. Mass chromatograms of the derivatized extract obtained from (a) blank urine (unspiked), (b) urine spiked with 20 mg/l of bromide, and (c) urine of the victim (A), using positive-ion EI mode of GC–MS. The numbers to the right of the chromatograms indicate the degree of enlargement used to obtain similar-sized peaks in the chromatograms.

Table 1
Accuracy and precision of bromide determination in human whole blood and urine

Sample	Added (mg/l)	n	Detected (mg/l)		R.S.D. (%)	Accuracy (%)
			Mean	S.D.		
Blood						
Intra-day	0	5	5.7	0.52	9.1	–
	20	5	25.4	1.36	5.3	98.4
	50	5	55.2	2.14	3.9	99.0
Inter-day	0	5	6.9	0.61	8.8	–
	20	5	26.2	2.14	8.2	96.4
	50	5	56.2	2.79	5.0	98.6
Urine						
Intra-day	0	5	6.8	0.49	7.1	–
	20	5	25.8	1.17	4.5	95.0
	50	5	56.8	2.14	3.8	100.0
Inter-day	0	5	7.7	0.66	8.6	–
	20	5	26.6	2.42	9.1	94.5
	50	5	57.1	3.61	6.3	98.8

S.D.: standard deviation; R.S.D.: relative standard deviation.

are shown in Fig. 4. Sharp and symmetrical peaks of the derivative from bromide and TBB (I.S.) were observed, with retention times of 6.1 and 9.3 min, respectively. Bromide was detected in blank whole blood and urine, the bromide level was below 10 mg/l. The calibration curves were linear within the concentration range from 2 to 100 mg/l for bromide in whole blood and urine. The equations and *r*-values for curves were $y = 0.001x + 0.0063$ ($r = 0.995$) in whole blood and $y = 0.0011x + 0.0092$ ($r = 0.996$) in urine. Relative recoveries of bromide in the whole blood and urine at three different concentrations, 20, 50 and 100 mg/l, were determined by comparing the peak area ratios of *m*/*z* 260 of bromide derivative to *m*/*z* 314 of TBB in samples with that in the water sample, using mass chromatography. The gross recoveries of bromide in the whole blood and urine were 85 and 95%, respectively.

The lower limit of detection for bromide in whole blood and urine, based on a concentration giving a signal three times stronger than the average noise intensity, was 1 mg/l, the same level as in reported methods. The lower limit of quantitation for bromide in whole blood and urine, based on a concentration giving a signal 10 times stronger than the average noise intensity, was 2 mg/l, that was estimated with intra- and inter-day precision less than 20%, and accuracy between 80 and 120% of the theoretical value. Intra- and inter-day precisions were obtained using two different concentrations (20 and 50 mg/l) by adding bromide to blank whole blood and urine. The precision and the accuracy for whole blood and urine ranged from 3.8 to 9.1% and from 94.5 to 100%, respectively (Table 1).

4. Discussion

Derivatization of bromide followed by GC–MS analysis is a superior and convenient technique, which can be used to identify this compound. The pentafluorobenzylation of bromide has been carried out by extractive alkylation in the presence of counter-ion, tetra-*n*-amylammonium chloride [18,19]. Funazo et al. [18] attempted to determine inorganic anions, including bromide in water samples as the pentafluorobenzyl ester, using GC with flame ionization detection (FID). The calibration curve was linear within the concentration range from 30 to 300 mg/l for bromide, which is not sensitive enough for analysis of biological samples. They also investigated the method to determine trace amounts of four inorganic anions (bromide, iodide, nitrite and thiocyanate) using GC with electron capture detection (ECD) [19]. The water samples containing bromide were determined over the concentration range 0.16–1.6 mg/l, however, derivatization was interfered within the presence of 50 mg/l carbonate. Since human whole blood contains over 1000 mg/l carbonate, further investigation was made based on conditions used for derivatization [20]. We derivatized bromide in whole blood and urine using PFB-TsO instead of PFBBr as the alkylating agent. We increased the amount of PFB-TsO 20 times compared with the method of Funazo et al. [19], and we derivatized bromide without adding a counter-ion.

We also derivatized the other anions (chloride, azide, iodide, nitrite and thiocyanate) using this same condition, and derivatives of these anions showed sharp peaks at retention times of 5.3, 6.5, 7.1, 8.5 and 10.0 min, respectively. The molecular ion peaks of chloride, azide, iodide and thiocyanate were observed at *m*/*z* 216, 223, 308 and 239, respectively, using positive-ion EI mode, but that of nitrite was not observed. All anions showed the same base ion peak at *m*/*z* 181 $[pentafluorobenzyl]^+$. The derivatives of these compounds were at least stable for 1 month at room temperature. The lower limits of detection for these compounds were below 1 mg/l using positive-ion EI mode. In the negative-ion CI mode, the base ion peaks of chloride, azide, iodide, nitrite and thiocyanate were observed at *m*/*z* 35, 42, 127, 46 and 58, respectively. Since the base peak of each anion was compound specific in the negative-ion CI mode and the sensitivity was 10 times higher in the negative-ion CI mode than in the positive-ion EI mode, simultaneous detection of chloride, bromide, azide, iodide, nitrite and thiocyanate can be effectively carried out using negative-ion CI mode. However, the calibration curves for bromide using negative-ion CI mode were linear within narrower range than those using the positive-ion EI mode. Therefore, bromide in whole blood and urine for forensic toxicological examination was analyzed using the positive-ion EI mode.

5. Practical applications

We examined blood and urine samples collected from a 29-year-old man (A), a 31-year-old man (B) and a 46-year-old man (C) who had inhaled methyl bromide. They had been fumigating closets of exhibition in a national museum using methyl bromide from 2 day before. They stayed

at the museum for 2 days, and inhaled the gas leaked from the closets through an air conditioner. They were sent to a hospital by ambulance, two men (A and C) were in serious condition and condition of the other man (B) was not so serious.

Since the elimination rate of bromide was very slow with a half-life of about 5 days [7], compared to methyl bromide with a half-life of 30 min, bromide, a metabolite of methyl bromide, was determined using our method. Mass chromatograms of the derivatized extract from whole blood obtained from the victim (A), using the positive-ion EI mode, are shown in Fig. 3. The blood levels of bromide in the victims (A–C) were 145, 66 and 148 mg/l, respectively. Rathus and Landy [21] correlated the following blood bromide levels with the degree of intoxication observed in their cases: 400 mg/l, gross disability; 250 mg/l, convulsive seizures; 176 mg/l, slight residual ataxia; 135 mg/l, moderate disability; and 100 mg/l and less, recovery. Hine [22] reported blood bromide at 400 and 250 ppm, respectively, in two fatal cases, and patients with severe, moderate and light symptoms had blood concentrations of 220, 180 and 120 ppm, respectively. Mass chromatograms of the derivatized extract from urine obtained from the victim (A), using the positive-ion EI mode, are shown in Fig. 4. The urine levels of bromide in the victims (A–C) were 50, 56, and 190 mg/l, respectively. Bromide concentrations in whole blood and urine in normal healthy subjects are reported to be below 10 mg/l, when various methods were used [23]. Therefore, all three victims were diagnosed as cases of methyl bromide poisoning.

Acknowledgment

We thank M. Ohara (Fukuoka, Japan) for helpful comments.

References

[1] D.L. Corina, K.E. Ballard, J. Chromatogr. 162 (1979) 382.
[2] R.M. Maiorino, A.J. Gandolfi, I.G. Sipes, J. Anal. Toxicol. 4 (1980) 250.
[3] S. Ohmori, M. Hirata, Jpn. J. Ind. Health 24 (1982) 119.
[4] S. Ishizu, N. Kato, S. Morinobu, N. Nagao, Y. Yamano, I. Ito, Jpn. J. Ind. Health 30 (1988) 54.
[5] S. Langard, T. Rognum, O. Fløtterød, V. Skaug, J. Appl. Toxicol. 16 (1996) 445.
[6] M.L. Gargas, M.E. Andersen, Toxicol. Appl. Pharmalcol. 66 (1982) 55.
[7] T. Honma, M. Miyagawa, M. Sato, H. Hasegawa, Toxicol. Appl. Pharmacol. 81 (1985) 183.
[8] Y. Michigami, Y. Yamamoto, K. Ueda, Analyst 114 (1989) 1201.
[9] A.W. Archer, Analyst 97 (1972) 428.
[10] N. Sugiyama, K. Saito, A. Nomoto, T. Hanabusa, H. Sato, M. Kawai, Bunseki Kagaku 34 (1985) 335.
[11] Y. Yamano, I. Ito, N. Nagao, S. Ishizu, Jpn. J. Ind. Health 29 (1987) 196.
[12] I. Maros, M. Káldy, S. Igaz, Anal. Chem. 61 (1989) 733.
[13] T. Kawai, Z.-W. Zhang, C.-S. Moon, S. Shimbo, T. Watanabe, N. Matsuda-Inoguchi, K. Higashikawa, M. Ikeda, Toxicol. Lett. 134 (2002) 285.
[14] P. Allain, Y. Mauras, C. Dougé, L. Jaunault, T. Delaporte, C. Beaugrand, Analyst 115 (1990) 813.
[15] J.A. Hurst, C.E. Tonks, R. Geyer, J. Anal. Toxicol. 18 (1994) 147.
[16] K. Arai, F. Kusu, N. Noguchi, K. Takamura, H. Osawa, Anal. Biochem. 240 (1996) 109.
[17] M. Müller, P. Reinhold, M. Lange, M. Zeise, U. Jürgens, E. Hallier, Toxcol. Lett. 107 (1999) 155.
[18] K. Funazo, M. Tanaka, K. Morita, M. Kamino, T. Shono, H.-L. Wu, J. Chromatogr. 346 (1985) 215.
[19] K. Funazo, M. Tanaka, K. Morita, M. Kamino, T. Shono, J. Chromatogr. 354 (1986) 259.
[20] S. Kage, K. Kudo, H. Ikeda, N. Ikeda, J. Chromatogr. B 805 (2004) 113.
[21] E.M. Rathus, P.J. Landy, Brit. J. Ind. Med. 18 (1961) 53.
[22] C.H. Hine, J. Occup. Med. 11 (1969) 1.
[23] H.A. Olszowy, J. Rossiter, J. Hegarty, P. Geoghegan, J. Anal. Toxicol. 22 (1998) 225.