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Short communication

Development of a headspace gas chromatographic test for the quantification of 1- and 2-bromopropane in human urine

C. B'Hymer*, K.L. Cheever

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Taft Laboratory, 4676 Columbia Parkway, Cincinnati, OH 45226, USA

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Abstract

A test procedure was developed for the detection and quantification of 1- and 2-bromopropane in human urine. 1-Bromopropane (1-BP) is a commonly used industrial solvent, and 2-bromopropane (2-BP) is often found as an impurity component in industrial grade 1-BP. Both compounds are a health concern for exposed workers due to their chronic toxicity. Bromopropanes have been associated with neurological disorders in both animals and humans. Sample preparation consisted of diluting urine with water and fortification with 1-bromobutane (1-BB), which was used as an internal standard; then each sample was sealed in a headspace vial. A static-headspace sampler (Teledyne-Tekmar Model 7000) was used to heat each sample at 75 °C for a 35-min equilibrium time. Quantification was by means of a gas chromatograph (GC) equipped with an electron capture detector (ECD) and a dimethylpolysiloxane (DB-1) capillary column. A recovery study using fortified urine samples at multiple concentrations (0.5–8 μg/ml) demonstrated full recovery; 104–121% recovery was obtained. Precision ranged from 5 to 17% for the 15–20 spiked samples used at each concentration, which were analyzed over multiple experimental trial days. The limit of detection (LOD) for this test procedure was approximately 2 ng/ml 1-BP and 7 ng/ml 2-BP in urine. A recovery study of 1- and 2-BP from fortified urine stored in vials appropriate for field collection was also completed. These results and other factors of the development and validation of this test procedure will be discussed.

Keywords: 1-Bromopropane; 2-Bromopropane

1. Introduction

1-Bromopropane (1-BP) is used extensively as a substitute for a number of chlorofluorocarbon solvents which have been withdrawn from use, because of their possible damaging effects to the earth's ozone layer [1]. 1-BP has numerous industrial applications including cleaning metal, optical instruments and electronics as well as being a component in spray adhesives. 1-BP has become a health concern and interest to this laboratory because of numerous toxicity studies in animals [2] and exposure for humans [3,4]. 2-Bromopropane (2-BP) is found as an impurity in some lots of industrial grade 1-BP and is also a health concern from occupational exposure [5–8].

The metabolism of 1-BP has been studied over several years and is complex [9,10]. Several analytical tests for possible biomarkers have been reported in the literature [11–13]; however, the simplicity of testing remaining unmetabolized 1- and 2-bromopropane in urine was desired for this current work. Urine testing offers a non-invasive approach for field sampling when studying exposed workers. Although dynamic headspace analysis of human urine for 1-BP has been reported in the literature [8], as well as a reported procedure of 1-BP testing on rat blood and urine [14], the current work reports a single test procedure for the determination of both 1- and 2-bromopropane. A description of the development and validation steps [15] of this test procedure using static headspace sampling is included. The two types of headspace

* Corresponding author. Tel.: +1 513 533 8148; fax: +1 513 533 8494.
E-mail address: cbhymer@cdc.gov (C. B'Hymer).

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sampling techniques, dynamic headspace analysis and static headspace analysis, have often been described and compared in the literature [16–20] and need not be repeated for this short communication. Static headspace sampling technique was used for this study of 1- and 2-bromopropane detection and quantification because of its inherent advantage of ease of use and eliminating the possibility of interfering artifacts from an adsorbent trap used in dynamic headspace sampling.

As mentioned previously, the objective of this reported work was to develop a simple and effective test to measure the levels of 1- and 2-BP in human urine samples. It was also an objective to have a validated [15] test procedure for use in monitoring dermally exposed workers in future field studies by this laboratory. The type of sample vials used for urine collection and storage conditions were also evaluated. The ECD was ultimately chosen for its greater sensitivity and specificity for halogenated compounds. 1-Bromobutane (1-BB) was chosen as the internal standard for this headspace/chromatographic procedure.

2. Experimental

2.1. Instrumental and chromatographic conditions

The headspace sampling was conducted using a Tekmar Model 7000 HT headspace sampler (Teledyne-Tekmar, Mason, Ohio, USA). The headspace sampling conditions are summarized in Table 1. The chromatographic analysis was carried out using an Agilent Technologies model 6890 gas chromatograph (Palo Alto, California, USA). The optimized chromatographic conditions used for this test procedure are also summarized in Table 1.

Table 1
Optimized headspace/GC conditions

Headspace conditions
Loop size: 1 ml
Sample temperature: 75 °C
Sample equilibrium time: 35 min
Vial size: 20 ml
Mixer: off
Vial pressurization time: 0.25 min
Vial pressurization equilibrium time: 0.05 min
Loop fill time: 0.25 min
Loop equilibrium time: 0.05 min
Sample loop temperature: 120 °C
Transfer line temperature: 140 °C
GC conditions
Injection type: split, 8:1 ratio
Injector temperature: 150 °C
Split flow: 8 ml/min
Column: Agilent J&W DB-1 (dimethylpolysiloxane) 60 m × 0.25 mm (i.d.), 1 μm film
Column program
Initial temperature 45 °C with a 10 min hold, then 12.5 °C/min to 170 °C and post run at 230 °C for 4 min
Flow rate: 1.0 ml/min helium
ECD temperature: 275 °C
ECD nitrogen flow: 60 ml/min

2.2. Chemicals and reagents

Commercial sources of the standard compounds, 1-bromopropane (Sigma-Aldrich, St. Louis, Missouri, USA), 2-bromopropane (Chem Service, West Chester, Pennsylvania, USA) and 1-bromobutane (Sigma-Aldrich), were used. Methanol used in the dilution of the spiking solutions was purge-and-trap grade (Burdick & Jackson, Muskegon, Michigan, USA). Doubly deionized water (Barnstead NANOpure, Dubuque, Iowa, USA) was used for urine dilution.

2.3. General urine sample preparation

Non-spiked urine samples or the 1- and 2-bromopropane-spiked urine samples were treated identically for headspace analysis. A 2.0 ml portion of the urine was place in a 20-ml headspace vial containing 8.0 ml of deionized water. A 0.25 ml aliquot of a 8 μg/ml solution of 1-bromobutane was added as the internal standard. A 0.25 ml portion of 1- and 2-BP spiking solution (prepared in purge-and-trap grade methanol) was added or 0.25 ml of purge-and-trap methanol for the non-spiked urine samples. The headspace vials were sealed with Teflon backed septa and crimp caps. The samples were then ready for placement into the headspace analyzer.

2.4. Standard sample preparation and recovery studies

1- and 2-BP standards for calibration were prepared at the 0.03, 0.1, 0.5, 1, 2, 5, 7.5, 10 and 12.5 μg/ml equivalent levels in urine plus a 0.01 μg/ml 1-BP and a blank 0 μg/ml level sample. All standard spiking solutions were prepared in trap-and-purge grade methanol. Urine was spiked at the 0.5, 2 and 8 μg/ml equivalent 1- and 2-BP level for each experimental day of the primary recovery study. A secondary recovery study consisted of collecting urine from 20 non-exposed volunteers (NIOSH employees who were not exposed to any known source of 1- or 2-BP). Urine samples containing no spikes (1-BP, 2-BP or internal standard were not added) and urine samples containing 5 μg/ml equivalent spikes of 1- and 2-BP with a 1 μg/ml equivalent level of 1-BB internal standard were prepared and analyzed by the described headspace/GC conditions. All spiked samples and the calibration standards contained the same quantity of urine; thus consisted of the same sample matrix in each headspace vial.

2.5. Vial and storage study

Glass serum vials (Wheaton Science Products, Millville, New Jersey, USA) were completely filled [minimum headspace] with 25 ml of urine from non-exposed humans

and spiked to 2 μg/ml equivalent levels of 1- and 2-BP. The vials were sealed with Teflon septa and crimp cap; vials were stored on their side to keep internal air space against the glass side, not the cap. After two and six week storage at 2–4 °C, aliquots were tested using the previously described procedure. Polypropylene tubes with spiked urine stored at −80 °C were also evaluated.

2.6. Calculations

Calculations were base on peak area ratios of 1- and 2-BP to 1-BB internal standard. Standard calibration curves were linear within the 0.01–12.5 μg/ml 1-BP range used; correlation coefficients were 0.98 or greater and *y*-intercepts approached zero for all curves generated with this procedure. Standard calibration curves for 2-BP were linear within the 0.03–12.5 μg/ml range used; correlation coefficients were 0.96 or greater and *y*-intercepts approached zero for all curves generated with this procedure. Full calibration curves were generated at the beginning and end of each set of experimental runs using this procedure. Calibration curve slope drift was generally minimal during a sample run.

The limit of detection (LOD) was calculated in the traditional way; three times [15] the standard deviation of the noise level divided by the slope of calibration curves generated from spiked solutions. The average baseline noise level was determined for several batch runs in chromatograms at the retention time windows for 1- and 2-BP from the 0 μg/ml level blank standard. The standard deviation from the baseline as height was determined using 100 data points. The slope from the calibration curve using peak heights of the standard solutions was used for this calculation.

3. Results and discussion

3.1. Chromatographic separation, detection and detection limits

The chromatographic conditions developed for this test procedure proved to be specific and had no apparent interferences. The unspiked urine sample chromatographs showed no interfering peaks for 1- and 2-BP; the blank samples from the 20 non-exposed volunteers showed no interfering peaks for the 1-BP, 2-BP and the 1-BB internal standard. These chromatograms were generated using the electron capture detector (ECD). Early development work using the same headspace and chromatographic conditions with a flame ionization detector (FID) did have problems with interfering peaks for 1-BP in a few individual volunteer samples. No interfering peaks were noticed when using the ECD, and this chromatographic test appears to be specific for testing urine for the presence of 1- and 2-BP. Typical chromatograms from urine samples of a non-exposed volunteer are shown in Fig. 1. A blank chromatogram generated from the preparation of an unspiked urine sample is shown in the lower portion of

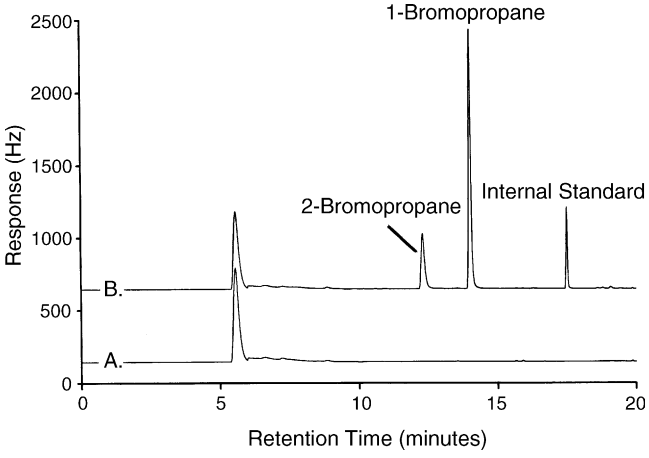

Fig. 1. A chromatogram of an (A) unspiked non-exposed volunteer urine sample analyzed by the described headspace/GC procedure; (B) 5 μg/ml spiked 1- and 2-bromopropane urine solution with 1 μg/ml equivalent level 1-bromobutane used as the internal standard.

Fig. 1(A) and a spiked sample showing the peaks for 2-BP, 1-BP and 1-BB is displayed in the upper chromatogram (B). Peak shape was good and there was no evidence of carryover between injections; analyte peaks did not appear in the chromatograms of unspiked urine samples injected immediately after the higher level standard samples.

Another advantage of the ECD used in the optimized chromatographic procedure was increased sensitivity and lower detection limits observed versus the FID. The limit of detection (LOD) for 1-BP was 2 ng/ml, and 7 ng/ml was the LOD for 2-BP. The FID used in the early development stage of this work gave a detection limit of roughly 20 ng/ml for both compounds using the same headspace and chromatographic conditions. All of these values are acceptable for a headspace testing procedure; Ichihara et al. [8] reported a detection limit of 0.5 ng/ml for 1-BP in urine using a dynamic (purge-and-trap) headspace procedure. Generally, dynamic headspace analysis has lower detection limits; thus, the results of this study are comparable to the other analysis procedures reported in the literature. The limit of detection for 2-BP was higher for the simple reason that detector response was lower for 2-BP using the ECD. It should also be noted that limits for this optimized headspace/GC procedure are based upon 2.0 ml urine sample volumes. Larger sample volumes would lower the effective detection limits of the test procedure if such volumes were available for testing. Initial field collection by this laboratory restricted the supply of urine for full biomarker/exposure testing; therefore, the method was developed using the smaller urine sample volumes.

3.2. Headspace conditions and choice of the internal standard

The headspace analysis conditions were optimized for the analysis of 1- and 2-BP. The activity coefficient or partitioning of the analyte into the gas phase is relatively high for both

1- and 2-BP. Both are bromoalkanes and have relatively low boiling points; 1-BP has a boiling point of 71 °C, and 2-BP has a boiling point of 59 °C. Sample heating temperatures of 70, 80 and 90 °C were investigated, and little increase in headspace concentration was noticed at the higher temperatures. Therefore, 75 °C proved adequate, being above the boiling point of both analytes. The 35-min equilibrium time was typical of static headspace procedures with an aqueous sample matrix [20,21].

In general, sensitivity of static headspace analysis can be increased by use of an inorganic salt to "salt-out" non-polar compounds [22]. Sodium sulfate was investigated during this study using 2% (w/v) concentrations in the final headspace sample matrix. A significant increase in the response of the bromopropanes was not found, but increases in background peaks attributed to the source of methanol used to prepare the spike solutions was noticed. Because the headspace equilibrium temperature was set above the boiling point of the analytes, improvement in response or "enrichment" of the headspace was not achieved by using the inorganic salt. One general problem cited with static headspace analysis is the purity of the dissolution solvent [20]. Purge-and-trap grade methanol available from Burdick and Jackson (Muskegon, Michigan, USA) was found to have the lowest level of background peaks of all methanol brands and grades investigated during the development of this procedure, and was used for the preparation of the spiking solutions.

The choice of the internal standard was based on having a compound with a similar headspace activity and similar response using the electron capture detector. 1-Bromobutane was ultimately chosen for this test procedure because of its better response with the ECD, and it similar headspace activity. 1-Chloropropane had a similar boiling point to the analytes, but had a much lower detector response and was subsequently dropped from consideration as a possible internal standard. 2-Bromobutane was also evaluated as a possible internal standard; it demonstrated no advantage during recovery studies and it had a slightly lower detection response than 1-BB.

3.3. Analyte recovery studies

A primary recovery study of blank urine spiked with 1- and 2-BP was performed over three separate experimental runs to demonstrate accuracy and precision of the test procedure. These results are presented in Table 2; average recovery was between 104 and 120% for 1-BP and from 107 to 121% for 2-BP for the three concentration levels investigated. The 0.5 μg/ml level had the highest average recovery (120% for 1-BP and 121% for 2-BP) which might indicate some high bias at the lower concentrations for this test procedure; however, the percent relative standard deviation (%R.S.D.) was also high (11%) for both analytes for the low concentration level recovery. This positive bias can be attributed to a slight shift in the slope of the calibration curve at this lower level. The 2 and 8 μg/ml level recovery results were more accurate; 104–114% recovery was obtained for 1- and 2-BP as is shown in Table 2. The second recovery study used urine samples from 20 non-exposed volunteers and demonstrated that the procedure was relatively accurate (Table 3). The 5 μg/ml spiked samples showed an average recovery of 105% for 1-BP and 113% for 2-BP. The percent relative deviations for the recovery were both approximately 13%. This is reasonable for a headspace analysis of volatiles from a biological matrix; headspace anaylsis tends to have less reproducibility than direct measurement procedures [21]. Also, the polarity differences of 1- and 2-BP in the aqueous matrix would be expected to cause less precision in headspace analysis; therefore, the recovery data showing 5–17% R.S.D. is not inconsistent with the headspace analysis.

Table 2
Multilevel recovery study of 1- and 2-bromopropane

Spike level (μg/ml)	Mean recovery (n = 15) (μg/ml)	Average percent recovery	S.D. (μg/ml)	%R.S.D.
1-Bromopropane				
0.5	0.60	120	0.063	11
2	2.27	114	0.21	9.2
8	8.31	104	1.1	14
2-Bromopropane				
0.5	0.61	121	0.066	11
2	2.25	112	0.11	4.9
8	8.57	107	1.4	17

Notes: Five different spiked samples were prepared at each level and chromatographed on three separate experimental trial runs (a total of 15 samples at each spike level were analyzed). The same DB-1 GC column was used for experimental batch trials 1 and 2; a second DB-1 column was used on trial run 3. S.D., standard deviation; %R.S.D., percent relative standard deviation.

3.4. Vial and storage evaluation

The Teflon septa sealed glass serum vials were found to be the best storage system. After two weeks of storage at 2–4 °C, average recovery for 10 of the 2 ng/ml fortified urine vials was 116% for 1-BP and 108% for 2-BP. At six weeks, average recovery was 113% for 1-BP and 103% for 2-BP (n = 10). This slightly high recovery is consistent with the

Table 3
Recovery of 5 μg/ml 1- and 2-bromopropane spikes from urine samples of 20 non-exposed volunteers

Mean recovery (μg/ml)	Average percent recovery	Lowest value (μg/ml)	Highest value (μg/ml)	%R.S.D.
1-Bromopropane				
5.27	105	4.2	6.0	13
2-Bromopropane				
5.63	113	4.4	6.4	13

Notes: All non-spiked samples did not show any peaks in the retention time region of 1- or 2-bromopropane. Each individual volunteer sample was prepared in duplicate and the result was averaged for the two determinations. %R.S.D., percent relative standard deviation.

previous recovery studies; some high bias for this method has been demonstrated. This would seem to indicate statistically that full recovery of the bromopropane was obtained. Frozen urine (−80 °C) in polypropylene tubes showed loss of the volatile analytes after two weeks. Average recovery for 1-BP measured 93% and 2-BP measured 92% ($n = 10$). At six weeks, bromopropane loss from the plastic tubes was more significant; recovery of 68% for 1-BP and 77% for 2-BP was measured ($n = 10$). Plastic tube collection and frozen storage for urine samples is a common technique for this laboratory and was found not to be appropriate for bromopropane analysis. Loss of the bromopropanes was minimal with the glass vials, and more significant for the plastic tubes. The loss was likely due to freezing and thawing times for the tubes, but this study was conducted under ideal laboratory conditions. Field urine sampling would probably incur greater loss of the volatile bromopropanes.

3.5. Method reproducibility and future work

Two different DB-1 capillary columns of different manufacturing lots were used during the recovery studies of this test procedure's development and the results were not significantly different between the two columns. It, therefore, should be expected that this procedure should give consistent and reproducible results using DB-1 columns of different manufacturing lots. This test procedure should be considered accurate for the quantification of remaining 1- and 2-BP in human urine within the standard calibration curve range. Although the higher levels of 1-BP and certainly 2-BP are not expected in the field, this method has demonstrated recovery data for 1- and 2-BP up to 8 μg/ml concentration levels.

Collection and testing of field samples are currently under consideration by this laboratory, and is part of planned future work. This larger comprehensive study, which includes the analysis of other biomarkers for 1-BP exposure, is beyond the scope of this manuscript and will be reported in detail elsewhere.

4. Conclusions

An accurate procedure to monitor the level of 1- and 2-bromopropane in human urine has been developed and validated. A static-headspace sampler and gas chromatograph equipped with an electron capture detector were used in the test procedure. Average recovery of known 1- and 2-BP fortified blank urine samples was 104–121% with relative standard deviations as high as 17% using samples with 1- and 2-BP concentrations of 0.5, 2, 5 and 8 μg/ml. The limit of detection was found to be approximately 2 ng/ml for 1-BP and 7 ng/ml for 2-BP. The procedure has been demonstrated to be useable for the quantification of 1- and 2-BP in human urine. Urine samples stored in sealed glass serum vials at 2–4 °C were demonstrated to have insignificant analyte loss for at least six weeks.

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