3-BROMOPROPIONIC ACID in URINE

metabolite of 1-bromopropane

8324

FORMULA: $C_3H_5BrO_2$ MW: 152.97 CAS: 590-92-1 RTECS: UE7875000

METHOD: 8324, Issue 1 **EVALUATION: FULL** **Issue 1: 12 September 2014**

Exposure limits and guidelines:
OSHA: None
NIOSH: None
Other OELs: Because data on exposure limits and guidelines may change over time, NIOSH recommends referring to the following sources for updated limits [1 - 4].

PROPERTIES: Solid; d 1.48 g/mL at 25 °C; MP 58-62 °C

BIOLOGICAL INDICATOR OF: Exposure to 1-bromopropane

SYNONYMS: β-Bromopropionic acid; 2-carboxyethylbromide.

SAMPLING

SPECIMEN: Urine

VOLUME: At least 15 mL

PRESERVATIVE: None added
Refrigerate or freeze upon collection

SHIPMENT: Ship cold or frozen with ice or dry ice
Freeze upon receipt at the laboratory

SAMPLE STABILITY: Stable in frozen urine for at least 30 days

CONTROLS: Urine specimens obtained from non-exposed individuals

ACCURACY

RANGE STUDIED: Table 1

BIAS: None established

PRECISION: Table 1

RECOVERY ACCURACY: Full recovery (95% overall) was established by a spiked urine recovery, overall RSD was ±3.1% (Table 1).

The definitions of precision and accuracy in this method are those utilized by the US Food and Drug Administration [5].

MEASUREMENT

TECHNIQUE: GAS CHROMATOGRAPHY, MASS SPECTROMETRY with SELECTED ION MONITORING (GC/MS-SIM)

ANALYTE: tert-butyldimethylsilane derivative of 3-bromopropionic acid

EXTRACTION: Liquid-liquid extraction (LLE)

INJECTION VOLUME: 0.5 µL, splitless

TEMPERATURES
- **INJECTION:** 200 °C
- **COLUMN:** 60 °C initial, 4 °C/min to 180 °C,15 °C/min to 255 °C, post-run at 270 °C for 5 min
- **DETECTOR:** Source 230 °C, Quadrupole 150 °C; Solvent delay, 15 min

RUN TIME: Approximately 50 minute cycle time

CARRIER GAS: Helium, 0.8 mL/min constant flow

COLUMN: Capillary, fused silica, 100% dimethyl-polysiloxane, 50 m X 0.20 mm (ID), 0.33 µm film thickness

CALIBRATION: Analyte in control urine; with internal standard

QUALITY CONTROL: At least one level of spiked urine sample prepared from a separately weighed stock solution

RANGE: 2.0 to 100 µg/mL

ESTIMATED LOD: Approximately 0.01 µg/mL (IUPAC) [6]. 0.1 µg/mL is the lowest calibration standard.

APPLICABILITY: 3-Bromopropionic acid (3-BPA) has been reported to be a rat metabolite [7] and is a potential human biomarker for exposure to 1-bromopropane. This method measures the quantity of free 3-BPA in urine. 1-Bromopropane is used as an industrial solvent. In one limited study, 3-BPA was not detected in individuals exposed to low levels of 1-bromopropane [8].

INTERFERENCES: None found or identified.

OTHER METHODS: This method is from the one described by B'Hymer and Cheever [9] and further investigated by Mathias, et al. [8].

REAGENTS:

1. 3-Bromopropionic acid (3-BPA) reference standard as a 1.0 mg/mL stock solution in deionized water. Store in a refrigerator.
2. 3-Chloropropionic acid (3-CPA) reference standard, 20 µg/mL in deionized water, internal standard solution. Store in a refrigerator.
3. Ethyl acetate, HPLC grade or better*
4. N-Methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylchlorosilane (TBDMCS)
5. Magnesium sulfate, anhydrous, powdered, ACS reagent grade
6. Hydrochloric acid, concentrated, ACS reagent grade
7. Water, deionized (ASTM type II)
8. Nitrogen, prepurified grade or better
9. Urine, non-exposed*

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Gas chromatograph with mass spectrometric detector, column, autosampler, and data collector (page 8324-1)
2. Bottles, polypropylene, 125-mL with caps
3. Analytical balance, to ±0.0001 g
4. Analytical evaporator with nitrogen gas sweep
5. Test-tube vortex mixer
6. Heating block or oven
7. Automatic pipettor with disposable tips
8. Repeating pipet dispenser, 1 Liter
9. Tubes, disposable screw-top culture (16 X 100 mm and 16 X 150 mm), with polytetrafluoroethylene (PFTE)-lined caps
10. Metal spatula
11. Flasks, volumetric; 10, 50 and 100-mL
12. Autosampler vials, 2 mL, silanized, with caps and septa
13. Glass funnels
14. Glass wool, silanized
15. Disposable glass pipets
16. Dry ice or bagged refrigerant

SPECIAL PRECAUTIONS: Standard precautions should always be used when handling bodily fluids and/or extracts of bodily fluids [10]. Handle urine samples and urine extracts using powder-free latex or nitrile gloves. Ethyl acetate and MTBSTFA are flammable; handle with care and use in a chemical fume hood. Handle all chemicals using the required safety precautions. Manufacturer expiration dates for reagents should be observed.

SAMPLING:

1. Collect at least 15 mL urine in an appropriate polypropylene tube or bottle and cap. Refrigerate or freeze immediately after collection. Collect at least two urine specimens for each worker: one before the work shift and one after.
2. Ship the specimens stored in either wet or dry ice in an insulated container. Store frozen upon arrival at the laboratory. A reminder: commercial shippers have special labeling requirements for packages containing dry ice.

SAMPLE PREPARATION:

3. Thaw the urine specimen to room temperature.
4. Mix thoroughly to ensure urine homogeneity.
5. Transfer 2.0 mL of urine into a 16 X 100 mm (or larger) screw-capped culture tube.
6. Acidify by adding 40 µL of concentrated hydrochloric acid.
7. Add 0.5 mL of deionized water.
8. Add 0.5 mL of the 20 µg/mL 3-CPA internal standard solution.
9. Ethyl acetate extraction: Dispense 4 mL of ethyl acetate into the culture tube, cap, and vortex (or vigorously shake) for 1 minute.
10. Allow layers to separate, collect the ethyl acetate (top) layer.

11. Repeat the extraction (steps 9 and 10) three more times. Collect and combine all ethyl acetate extracts using a glass pipet into a 16 X 150 mm culture tube.
12. Dry the ethyl acetate extract by adding approximately 100 to 200 mg of anhydrous magnesium sulfate and swirl for about 15 seconds.
13. Transfer the extract solution into a 16 X 150 mm culture tube by means of a glass funnel with silanized glass wool patch to remove the wet magnesium sulfate. The glass wool patch must be packed tightly enough to prevent particles of magnesium sulfate from passing through.
14. Rinse the tube and the funnel with ethyl acetate to ensure complete transfer.
15. Concentrate the combined ethyl acetate extract for each sample to 1 mL using a nitrogen sweep at room temperature and transfer the solution to a 2-mL GC autosampler vial.
16. tert-Butyldimethylsilane derivatization: Add 50 µL of MTBSTFA with 1% TBDMCS silanizing reagent to each autosampler vial and cap immediately.
17. Heat the solution for 1.5 hours at 70 °C in a heating block or oven.

CALIBRATION AND QUALITY CONTROL:

18. 3-Bromopropionic acid (3-BPA) standards are prepared in blank, non-exposed urine. The 1 mg/mL stock 3-BPA solution is diluted in deionized water to make 0.4, 1, 2, 4, 8, 20, 80, 200, 400, 600, and 800 µg/mL 3-BPA solutions for spiking.
19. Transfer 2.0 mL of non-exposed urine into a 16 X 100 mm (or larger) screw-capped culture tube.
20. Acidify by adding 40 µL of concentrated hydrochloric acid.
21. Add 0.5 mL of the 20 µg/mL 3-CPA internal standard solution.
22. Add 0.5 mL of the appropriate 3-BPA spiking solution described in step 18 to make urine samples equivalent to 0.1, 0.25, 0.5, 1, 2, 5, 20, 50,100, 150 and 200 µg/mL of 3-BPA in the original 2.0 mL volume of urine.
23. Prepare at least one blank urine without a 3-BPA spike to verify the source of blank urine contains no detectable quantity of 3-BPA.
24. Prepare at least two levels of quality control (QC) standard of 3-BPA fortified urine using a separately weighed and prepared 3-BPA stock solution. One level should be within the lower 25% of the calibration curve and one level within the upper 25% of the calibration curve. More than two QC levels can be used. QC samples should be analyzed with every batch such that they constitute at least 5% of the sample batch.
25. QC values should be within ±20% of the spiked values. If not, the batch is considered out of control, the batch data discarded, and corrective actions should be taken before more samples are analyzed.
26. Ethyl acetate extraction: Prepare the spiked standard urine samples, the blank urine sample(s), and the QC standards the same as described in the preceding Sample Preparation section using Steps 9 through 17.

MEASUREMENT:

27. Set the gas chromatograph according to the manufacturer's recommendations and to the conditions listed on page 8324-1.
28. Set the mass selective detector to selected ion monitoring mode for ions *m/z* 211 (derivative of 3-BPA) and 165 (derivative of 3-CPA).
    NOTE: The use/non-use of qualifier ions for this method is discussed in the literature [9].
29. Inject 0.5 µL of each sample, standard, blank, and QC standard extract from Steps 17 and 26.
30. Measure the peak areas of the tert-butyldimethylsilane derivatives of 3-BPA and 3-CPA in the chromatograms of the standards.
31. Divide the peak area of the derivative of 3-BPA by the peak area from the derivative of 3-CPA in the same chromatogram.

32. Prepare a linear calibration curve of the peak Area Std./Area Int. Std. versus the concentration of the standard for 3-BPA.
33. Measure the peak areas of the derivatives of 3-BPA and 3-CPA in the remainder of the chromatograms.
34. Divide the peak area of the derivative of 3-BPA by the peak area from the derivative of 3-CPA in the same chromatogram.

CALCULATIONS:

35. Determine the concentration of 3-BPA in the extracts from the original urine (2.0 mL specimen) from the curve obtained in step 32. The results are expressed as µg/mL of 3-BPA in urine.

EVALUATION OF METHOD:

This method was evaluated and described by B'Hymer [6] and in detail by B'Hymer and Cheever [9]. A general summary of this published information is given below:

Accuracy and Precision: Two recovery studies using multiple GC columns over several days demonstrated the accuracy and precision of this test method. The first recovery study was performed over three separate experimental batch runs, and these data are presented in Table 1A. Average recovery was between 93 and 98% for the three 3-BPA spiked level urine samples investigated. For each batch run, the experimental trial consisted of three samples at three different concentration levels. The recovery for each level (n=9 samples) is displayed in Table 1A. The second recovery study (data shown in Table 1B) used spiked urine samples from 20 non-exposed volunteers and demonstrated that the procedure was accurate (95% average recovery) and precise (2.5% relative standard deviation.) No interferences were detected in the unspiked urine from the 20 volunteer specimens. Both recovery studies generated a total of 47 spiked urine samples at 2, 10, 20 and 50 µg/mL 3-BPA levels. Precision expressed as percent relative deviation (% RSD) was as high as 5.7% on the 2 µg/mL recovery samples (n = 9). Overall recovery was 95% and overall RSD was 3.1% (n = 47).

Linearity: All calibration curves used during the development of this method were linear and had correlation coefficients of 0.98 and greater. The concentration range was 0.1 to 200 µg/mL 3-BPA in urine with 2.0 mL urine sample size. Calibration curves were run at the beginning and end of all sample batch runs; calibration curve slope drift was found to be acceptable.

Specificity: The optimized chromatographic conditions developed for this procedure proved to be specific and have no major interferences. The mass spectrometric detector was useful in adding additional specificity to the method. The ion *m/z* 211 was chosen for monitoring the calibration curve used in the calculations because of its greater abundance, and it was a characteristic fragment for the tert-butyldimethylsilane (TBDMS) derivative of 3-BPA. This is the molecular ion less the tert-butyl group, *m/z* 57 (Figure 1). Ion *m/z* 165 was used to monitor the TBDMS derivative of 3-CPA, the internal standard, for the same reasons. Full-scan mass spectra of the TBDMS derivatives are presented in Figure 2. An example chromatogram for blank urine and urine spiked with 3-BPA and 3-CPA is shown in Figure 3.

Robustness: Multiple HP-1 (100% dimethylpolysiloxane) columns of different manufacturing lots were used during the recovery studies. Accuracy and precision did not appear to be affected; therefore, the method appears to be reproducible with any normal functioning HP-1 capillary column. Recovery results from individual urine samples spiked with 3-BPA indicate that the method is accurate and not significantly affected by individual urine sample matrix differences during analyte extraction.

Stability: While sample stability was not exhaustively evaluated, an aqueous stock standard solution of 3-BPA stored for two weeks at 4 °C gave full recovery assay values when compared to a freshly prepared 3-BPA standard. The 3-BPA in the urine specimens appears to be stable in frozen urine for a much

longer time frame of two months or more. Derivatized sample extracts appeared to be stable during a one week time frame.

Range: This method should be considered accurate for the estimation of 3-BPA in human urine within the 2.0 to 100 µg/mL method validation range.

REFERENCES:

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METHOD WRITTEN BY:

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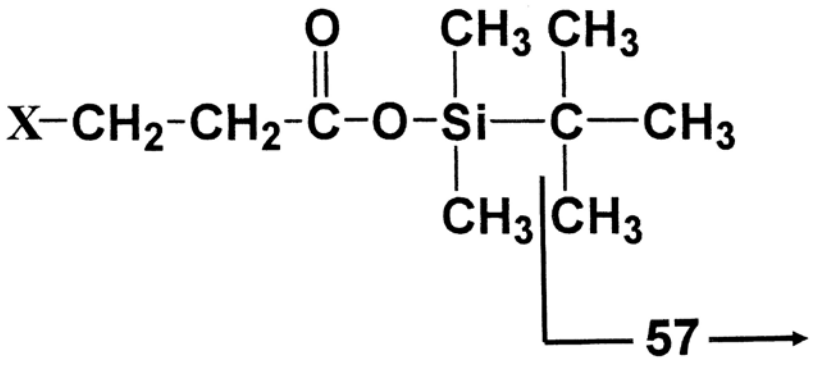

X = ^{35}Cl, m/z = 165 or for ^{37}Cl, m/z = 167.

X = ^{79}Br, m/z = 209 or for ^{81}Br, m/z = 211.

Figure 1. The major ions monitored for the tert-butyldimethylsilane derivatives were the molecular ions less the tert-butyl group (*m/z* = 57). The ions used for quantitation are *m/z (mass to charge)* 165 for the internal standard derivative and *m/z* 211 for the 3-bromopropionic acid derivative.

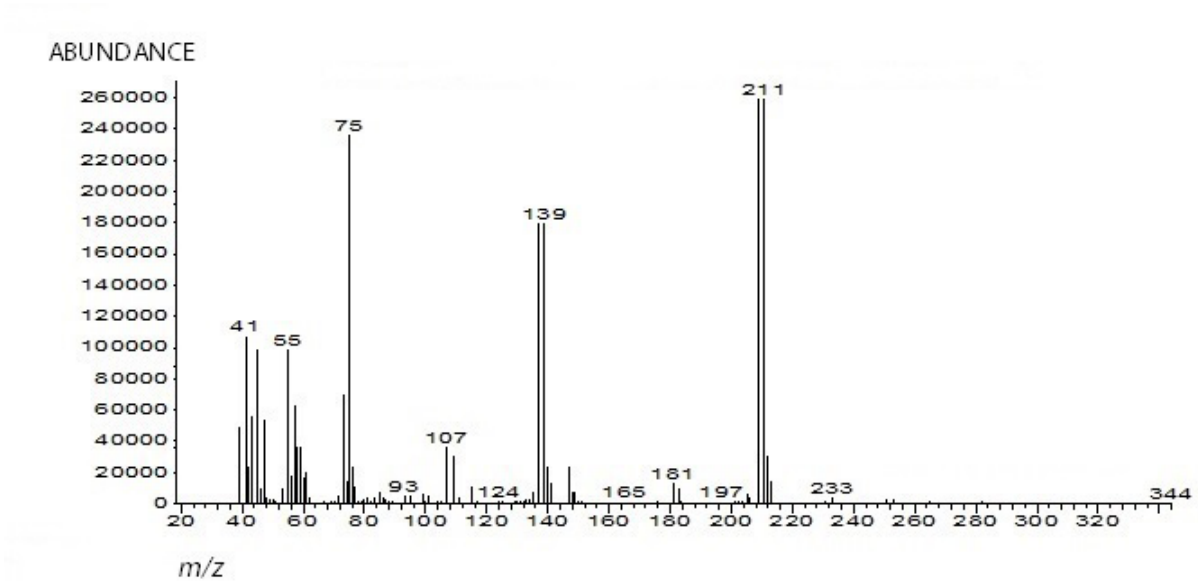

Figure 2a. **Mass spectrum for 3-Bromopropionic acid, t-butyldimethylsilyl ester**

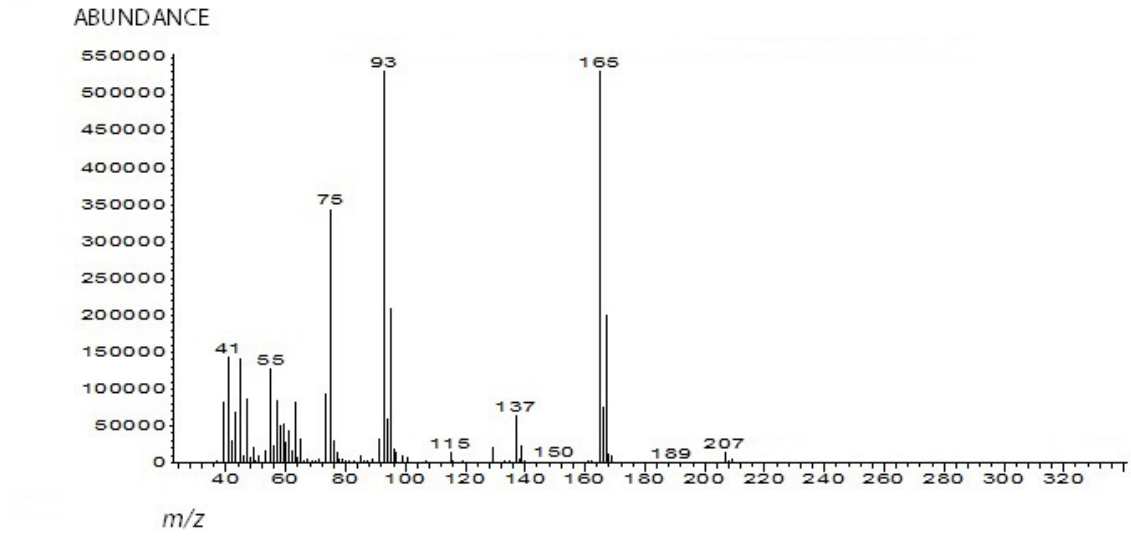

Figure 2b. **Mass spectrum for 3-Chloropropionic acid, t-butyldimethylsilyl ester**

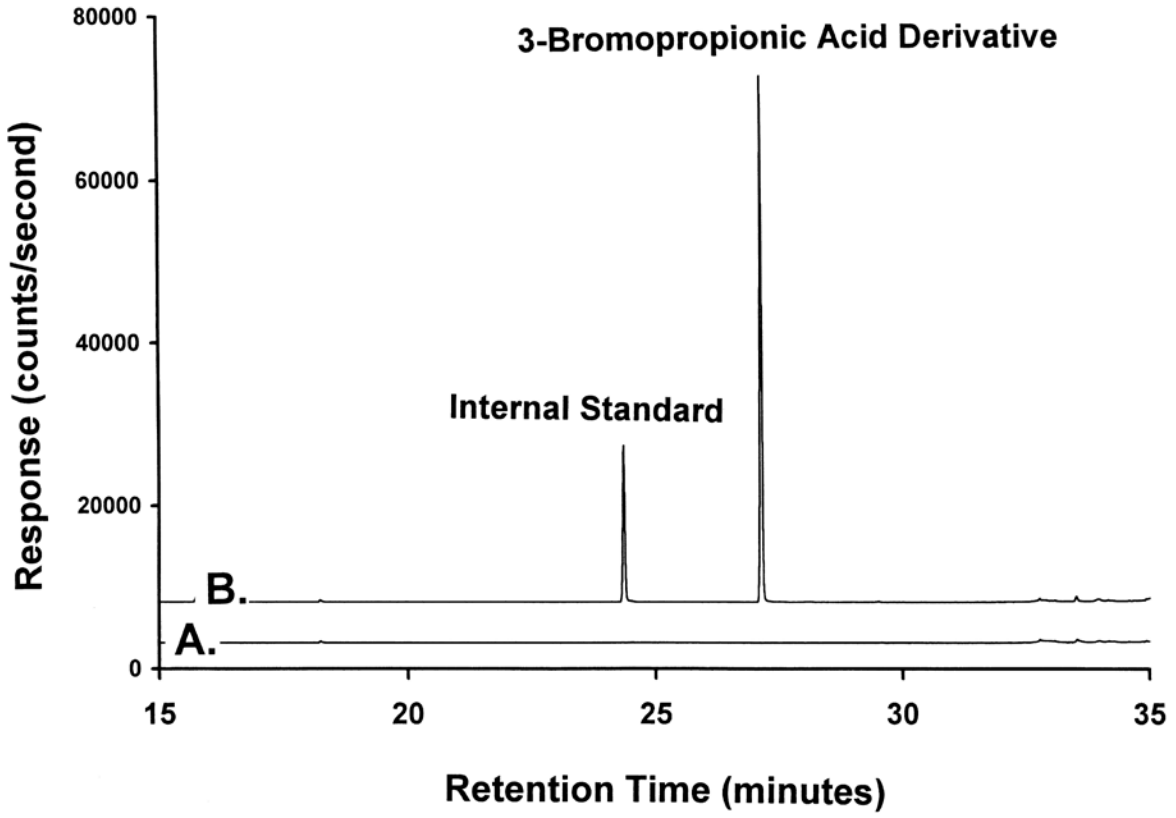

Figure 3. A total ion chromatogram of a (A) blank non-exposed volunteer urine specimen and (B) 20 µg/mL spiked 3-BPA urine solution with 5 µg/mL equivalent 3-CPA as the internal standard. No interfering peaks were evident in any of the group of 20 non-exposed volunteer specimens.

Table 1. Recovery studies of 3-bromopropionic acid:
(A) multilevel recovery study of 3-BPA from spiked urine samples1,
(B) recovery of 20 µg/mL 3-BPA spikes from individual urines of 20 non-exposed volunteers2
Note: Overall recovery of all samples was 95% and overall RSD was 3.1% (n = 47.)

(A)

Spike level (µg/mL)	Mean 3-BPA recovered, (n = 9, µg/mL)	Average % Recovery	Standard Deviation (µg/mL)	% Relative Standard Deviation
2	1.91	96	0.11	5.7
10	9.32	93	0.13	1.4
50	48.9	98	0.36	0.7

(B)

Volunteer urine Spike level (µg/mL)	Mean 3-BPA recovered (n = 20, µg/mL)	Average % Recovery	Standard Deviation (µg/mL)	% Relative Standard Deviation
20	19.0	95	0.48	2.5

[1] Three different spiked urine samples were prepared at each level and chromatographed on three separate experimental trial runs (a total of nine samples at each spike level were analyzed.)

[2] All non-spiked specimens showed no 3-BPA derivative peak in the chromatograms.