# BUTOXYACETIC ACID IN URINE 8316

$C_6H_{12}O_3$ MW: 132.16 CAS: 2516-93-0 RTECS: None

| METHOD: 8316, Issue 1 | EVALUATION: PARTIAL | Issue 1: 15 March 2003 |
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**BIOLOGICAL INDICATOR OF:** Exposure to 2-butoxyethanol (CAS # 111-76-2, RTECS # KJ8575000)
2-butoxyethyl acetate (CAS # 112-07-2, RTECS # KJ8925000).

**SYNONYMS:**

2-butoxyethanol: Ethylene glycol monobutyl ether, Monobutyl glycol ether, Butyl Cellosolve®, Butyl oxitol, Dowanol® EB, EGBE, Ektasolve EB®, Jeffersol EB

2-butoxyethyl acetate: Ethylene glycol monobutyl ether acetate, Butyl Cellosolve® acetate, Butyl glycol acetate, EGBEA, Ektasolve EB® acetate,

## SAMPLING

**SPECIMEN:** Urine

**VOLUME:** 20 mL of sample

**PRESERVATIVE:** None

**SHIPMENT:** Frozen; on dry ice

**SAMPLE STABILITY:** At least 9 months at -70 °C

**CONTROLS:** Urine specimens from non-exposed persons, number determined by design of study.

## MEASUREMENT

**TECHNIQUE:** GAS CHROMATOGRAPHY, ECD Ni-63

**ANALYTE:** Pentafluorobenzyl butoxyacetate, PFB-BAA

**INJECTION VOLUME:** 5 µL

**TEMPERATURE**
**-INJECTION:** 150 °C
**-DETECTOR:** 177 °C
**-COLUMN:** 70 °C for 2 min, 50 °C/min to 120 °C, 2 °C/min to 170 °C

**CARRIER GAS:** Helium, 10 mL/min

**COLUMN:** Capillary, fused silica, 5 m x 0.53-mm ID, deactivated and uncoated, followed by 30 m x 0.53-mm ID fused silica capillary with a 2.65-µm film of polydimethyl siloxane, HP-1 or equivalent.

**CALIBRATION:** Standard solutions of PFB-BAA in toluene/2-propanol

**QUALITY CONTROL:** Standard solutions of butoxyacetic acid (BAA) in urine

**RANGE:** 10 to 450 µmol/L in urine

**ESTIMATED LOD:** 10 µmol/L in urine

**PRECISION ($\bar{S}_r$):** 0.13

**ACCURACY:** Not determined

**APPLICABILITY:** Urinary butoxyacetic acid (BAA) is a biomarker of exposure to 2-butoxyethanol and 2-butoxyethyl acetate. Both 2-butoxyethanol and 2-butoxyethyl acetate are metabolized to butoxyacetic acid (BAA) and *N*-butoxyacetylglutamine, which are excreted in urine [1]. Since BAA produces the adverse hematogic effects attributed to exposures to 2-butoxyethanol and 2-butoxyethyl acetate, urinary BAA serves also as a biomarker to these particular exposure-related adverse health effects [2].

**INTERFERENCES:** No analytical interferences found. Consumption of ethanol is predicted to inhibit metabolism of 2-butoxyethanol to BAA [2], and thus may effect the accuracy of biomonitoring.

**OTHER METHODS:** This method is based on those of Smallwood *et al.* [3] and Johanson [4]. Grosenken *et al.* [5] published a method using lyophilization, derivatization with pentafluorobenzyl (PFB) bromide, then GC. The method of Rettenmeier *et al.* [1] determined both free BAA and its conjugate with glutamine using extraction and derivatization with 4-nitrobenzyl bromide, then HPLC. Sakai *et al.* [6] used acid hydrolysis, extraction, derivatization with trimethylsilyldiazomethane, then GC to determine free plus conjugated BAA.

**REAGENTS:**

1. Tetrabutylammonium hydrogen sulfate ($C_{16}H_{36}N{\cdot}HSO_4$).
2. Potassium dihydrogen phosphate.
3. Potassium hydroxide.
4. Phosphoric acid, 85%.*
5. Water, deionized.
6. $C_{16}H_{36}N{\cdot}HSO_4$ in phosphate buffer: 0.2 M $KH_2PO_4$ and 0.1 M $C_{16}H_{36}N{\cdot}HSO_4$ in deionized water, made pH 6 with 85% $H_3PO_4$ or 10 M KOH.
7. Methylene chloride.*
8. PFB bromide, 2,3,4,5,6-pentafluorobenzyl bromide.*
9. 2-Propanol.*
10. Toluene.*
11. Isopropanol-toluene mixture, 1:1 (v/v).
12. PFB-BAA, pentafluorobenzyl ester of butoxyacetic acid, MW 312.24 amu. Synthesized according to Groeseneken [5], purified by RP-HPLC, and analyzed to one major peak by GC/MS. Density 1.359 g/mL GC-MS (EI) fragmentation pattern; 240, 181, 131, 87, 73, 51amu.
13. PFB-BAA stock standard solution, 680 µmol/L. Dissolve 21 ± 0.1 mg of PFB-BAA in 100 mL of 1:1 isopropanol-toluene using an amber 100-mL volumetric flask. Store at 0 to 5 ˚C.
14. BAA, Butoxyacetic acid, >99%.
15 Urine from unexposed volunteers, stored at -20 to -15 $^{o}$C for a maximum of 2 weeks.
16. BAA-in-urine stock solution, 4000 µmol/L. Dissolve 53 ± 1 mg of BAA in 100 mL of urine. Dilute with urine from unexposed volunteers for preparation of the quality control samples.
17 Argon with 5% methane.
18 Helium.

* See SPECIAL PRECAUTIONS

**EQUIPMENT:**

1. Gas chromatograph with $^{63}$Ni electron capture detector, temperature programming, splitless injection port with purge, and autoinjector. The detector makeup gas was 5% methane in argon at 60 mL/min.
2. Polypropylene bottles, 30- and 250-mL with screw caps.
3. Graduated cylinders, 100- and 250-mL.
4. Shipping container, polystyrene foam, with dry ice.
5. Culture tubes, 16-mm x 100-mm, with teflon-lined screw caps, disposable.
6. Disposable serological pipettes, 0.2-mL in 0.001-mL increments, and 2-mL in 0.1-mL increments. Micropipette, 10-µL.
7. Aluminum foil.
8. Tube rotator.
9. Centrifuge, 3000 rpm.
10. Evaporator, nitrogen purge-type, with 30 ˚C heating bath.
11. Vortex mixer.
12. Ultrasonic water bath, room temperature.
13. Autoinjector vials, amber with crimp caps.
14. Balance, five-decimal-place analytical.
15. Volumetric flasks, amber, 10- and 100-mL.

**SPECIAL PRECAUTIONS:** See material safety data sheets. Urine samples may contain a number of bacterial and viral agents, including hepatitis B virus, and should be handled using Biosafety Level 2 practices, containment equipment and facilities [CDC & NIH, *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed., HHS Publication No. (CDC) 93-8395 (1993)]. Pentafluorobenzyl bromide is volatile compound, a powerful lacrimator, and a suspected carcinogen. Methylene chloride, isopropanol, and toluene are NFPA level 3 fire hazards. Phosphoric acid is highly corrosive.

**SAMPLING:**

1. Collect a spot urine sample in one or several 250-mL polypropylene bottles. Measure and record the volume of the whole voiding and transfer approximately 20 mL to a 30-mL polypropylene bottle. Label the bottle with the code unique to that specimen. Freeze immediately with dry ice.
2. Ship in a polystyrene-foam shipping container kept frozen with dry ice.
3. Store the samples at -76 °C until time of analysis.

**SAMPLE PREPARATION:**

4. Thaw the urine sample to room temperature. Remove a portion for creatinine analysis [8].
5. Transfer 0.200 mL of the sample to a culture tube.
6. Add 1.80 mL of tetrabutylammonium hydrogen sulfate in phosphate buffer, 2.00 mL of methylene chloride, and then 10 µL of pentafluorobenzyl bromide. Cap the tube.
7. Wrap the tube with aluminum foil to prevent exposure to light, and tumble with the rotator for 20 hr at 30 rev/min. Timing is critical, because analyte recovery reaches a maximum at 20 hr then decreases.
8. At 20 hours, centrifuge culture tubes for 5 min at 3000 rpm.
9. Transfer 1.5 mL of the methylene chloride (lower layer)to a clean culture tube. Evaporate to dryness under nitrogen at 30 °C.
10. Add 1.00 mL of 1:1 isopropanol-toluene to the residue, vortex for 1 min, then sonicate for 1 min.
11. Transfer the solution to an autoinjector vial, cap, and seal.

**CALIBRATION AND QUALITY CONTROL:**

12. Calibrate the GC for each batch of samples.
    a. Prepare 9 working standards by diluting the PFB-BAA stock solution with 1:1 toluene/propanol to 9 equally-spaced concentrations between 0.8 and 68 µmol/L (equivalent to urinary levels of 5.3 to 453 µmol/L).
    b. Analyze these standards with the unknowns in step 17 in random order but after every third unknown in the batch.
    c. Prepare a calibration graph of peak area versus analyte concentration, using, if necessary, quadratic regression to fit the data.
13. Prepare and analyze a minimum of 4 BAA-in-urine quality-control samples per batch.
    a. Prepare control samples at 0, 20, 90, and 400 µmol/L by diluting aliquots of BAA-in-urine stock solution with urine from unexposed individuals.
    b. Analyze quality control samples and five samples of the unspiked urine with the unknowns.
    c. Correct the nominal values for the control samples for the background level of BAA in the blank urine.
    d. Calculate the recoveries and plot them on a control chart for the method.
14. Re-analyze two field samples from the previous batch with each batch.
    a. If possible, select field samples with levels at both ends of the analytical range, but above the detection limit.
    b. Calculate the percent difference for each duplicate and plot it on a control chart for the method.
15. Analyze at least one sample of pure water with each batch as a check for background interferences.

**MEASUREMENT:**

16. Set the gas chromatograph system according to the manufacturer's recommendations and the conditions given on page 8316-1.
17. Inject 5 µL of sample from step 11 or standard from step 12.
    Note: With the chromatographic system used for method development, the PFB-BAA peak had an efficiency of 124,000 plates, an asymmetry factor of 1.4, and a retention time of 31.7 min. The intensity and precision of the detector's response to PFB-BAA were sensitive to temperature, with an optimal precision obtained at 177 °C.
18. Measure peak area.

**CALCULATIONS:**

19. Calculate from the calibration curve the concentration of PFB-BAA ($C_c$, µmol/L) in the final solution.
20. Calculate the concentration of BAA ($C_u$, µmol/L) in the urine sample from the equation below.

$$Cu = 6.67 \cdot Cc, \mu mol / L$$

where 6.67 is the dilution for the sample preparation steps.

21. Adjust the result for the creatinine level and report in micromoles per mole creatinine or in milligrams per gram creatinine, using the conversion 1 µmol/mol equals 1.17 mg/g.

**GUIDES FOR INTERPRETATION:**

A urinary BAA level of 51 mmol/mol creatinine (60 mg/g creatinine) corresponds to the NIOSH recommended exposure level for a 10-hr work shift of 5-ppm TWA for 2-butoxyethanol and 2-butoxyethyl acetate, assuming no dermal exposure to the liquid. Biological monitoring for exposure is recommended, because 1) dermal absorption may be a major route of exposure, 2) workload can significantly influence inhalation exposure, and 3) butoxyacetic acid itself exerts hematologic toxicity [2].

BAA levels up to 282 µmol/mol creatinine (330 mg/g creatinine) have been measured in occupationally exposed persons [7]. Levels as high as 0.5 µmol/mol creatinine (0.6 mg/g creatinine) have been found in urine of people not occupationally exposed to 2-butoxyethanol or 2-butoxyethyl acetate [6]. 2-Butoxyethanol is commonly found in household products, such as paints and cleaners.

**EVALUATION OF METHOD:**

A detection limit of 10 µmol/L was estimated using results from 11 urine samples spiked with BAA over the range 0.27 to 32 µmol/L. Recovery averaged 93% (range 53% to 146%) for 20 quality-control samples containing BAA at concentrations 12 to 3000 µmol/L. Precision was estimated at 13% relative standard deviation from duplicate analysis of 12 field samples from workers exposed to 2-butoxyethanol.

**REFERENCES:**

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**METHOD DEVELOPED BY:**

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