# TRICHLOROACETIC ACID IN URINE 8322

FORMULA: $C_2HCl_3O_2$ MW: 163.39 CAS: 76-03-9 RTECS: AJ7875000

**METHOD: 8322, Issue 1** **EVALUATION: FULL** **Issue 1: 17 April 2015**

**EXPOSURE LIMITS:**
**OSHA & NIOSH:** N/A

Because data on exposure limits and guidelines may change over time, NIOSH recommends referring to the following sources for updated limits and guidelines concerning trichloroacetic acid as well as its use as a marker for other compounds [1-4].

**PROPERTIES:** White solid; mp 59.1 °C; bp 198.2 °C; d = 1.61 g/cm3 [5]

**BIOLOGICAL INDICATOR OF:** Exposure to trichloroethylene, trichloroacetic acid, methyl chloroform, tetrachloroethene, chloral hydrate

**SYNONYMS:** Trichloroethanoic acid, Aceto-caustin

## SAMPLING

**SPECIMEN:** Urine

**VOLUME:** At least 10 mL

**PRESERVATIVE:** None

**SHIPMENT:** Freeze urine; ship in dry ice in an insulated container.

**SAMPLE STABILITY:** Stable at least 30 days at -17 ºC [6]

**CONTROLS:** Urine specimens from matched population of non-exposed persons

## ACCURACY

**RANGE STUDIED:** 0.9 to 100 µg/mL (as trichloroacetic acid)

**BIAS:** 0.0113

**OVERALL PRECISION ($\hat{S}_{rT}$):** 0.03656

**ACCURACY:** 7.5%

## MEASUREMENT

**TECHNIQUE** GAS CHROMATOGRAPHY with ELECTRON CAPTURE DETECTOR (GC-ECD)

**ANALYTES:** Trichloroacetic acid (determined as the methyl ester)

**PROCEDURE:** Methylation of an aliquot with $BF_3$·methanol to form the methyl ester followed by extraction into toluene

**INJECTION:** 1 µL, splitless for 0.5 min

**TEMPERATURES**
**-INJECTION:** 250 ºC
**-DETECTOR:** 300 ºC
**-OVEN:** 80 ºC (hold for 0.5 min), 80 to 180 ºC at 20 ºC/min, hold for 7 min

**CARRIER GAS:** Helium, ~3.5 mL/min

**MAKEUP GAS:** Nitrogen, 40 mL/min

**COLUMN:** Capillary, fused silica, 6% cyanopropylphenyl-94% dimethylpolysiloxane, 75 m x 0.53 mm ID, 3 µm film

**CALIBRATION:** Trichloroacetic acid prepared in water to cover range and derivatized with the samples

**ESTIMATED LOD:** 0.08 µg/mL (as trichloroacetic acid) [6]

**APPLICABILITY:** This method can be used for the determination of trichloroacetic acid (TCAA) in urine specimens. TCAA is one of several metabolites detected after exposure to a variety of chlorinated compounds (representative compounds listed above) or from contaminated drinking water [7-11].

**INTERFERENCES:** None observed in the analytical method apart from some carryover issues (see Evaluation of Method section.) TCAA is a non-specific metabolite of several compounds. Urinary TCAA levels reflect exposure to any and all of these precursors. Background TCAA was detected in 76% of urine samples in a US general population sample, with a median concentration of 3.3 µg/L, approximately 300 times lower than the range of this method [12].

**OTHER METHODS:** There are numerous literature methods for the determination of TCAA in urine [13]. The National Center for Environmental Health/Centers for Disease Control and Prevention (NCEH/CDC) has a method that is more expensive but also significantly more sensitive [11]. This method is based on the procedure used by O'Donnell [7] with some modifications.

**REAGENTS:**

1. Sodium trichloroacetate [CAS #650-51-1], 97% purity or greater*
2. Boron trifluoride-methanol solution, 14%*
3. Toluene, ACS reagent grade or better*
4. Sodium sulfate, anhydrous, granular; reagent grade or better
5. Acetic acid, glacial; reagent grade or better*
6. Acetone, reagent grade or better*
7. Methanol, reagent grade or better*
8. Helium, purified
9. Nitrogen, Ultra High Purity or P5
10. Water, ASTM Type II [14]

**SOLUTIONS:**

1. 1:3 glacial acetic acid:deionized water
2. 1:1 acetone:methanol

*** See SPECIAL PRECAUTIONS**

**EQUIPMENT:**

1. Centrifuge tubes, polypropylene, ~15-mL, with screw caps, or other suitable container for specimen collection and storage
2. Gas chromatograph with electron capture detector, autosampler, data collection system and column (page 8322-1)
3. Microliter syringes, various sizes
4. Volumetric flasks, glass, various sizes
5. Adjustable pipettor with disposable plastic tips, 0.1 to 1-mL
6. Disposable Pasteur transfer pipettes, 15 and 23 cm
7. Culture tubes, 13 mm x 100 mm (~8 mL), with PTFE-lined caps
8. Vortex mixer
9. Glass wool
10. Vials, autosampler, glass, 2-mL with caps
11. Oven, capable of maintaining 60 °C

**SPECIAL PRECAUTIONS:** Standard precautions should always be used when handling bodily fluids and/or extracts of bodily fluids [15]. Handle urine specimens and urine extracts using powder-free latex or nitrile gloves. Acetic acid, acetone, toluene, and methanol are flammable; handle with care and use in a chemical fume hood. Handle all chemicals using the required safety precautions. Reagents with manufacturer expiration dates should be observed. The Environmental Protection Agency (EPA) has designated TCAA as a known mouse carcinogen and a possible human carcinogen [16].

**SAMPLING:**

1. Collect at least 10 mL of urine in ~15-mL polypropylene tubes or other suitable container.
   NOTE: Because of the relatively lengthy half-life values of TCAA, ACGIH recommends sampling at the end of shift at the end of workweek [1].
2. Freeze the urine and ship in dry ice in an insulated container.
   Reminder: commercial shippers have special labeling requirements for packages containing biological samples and dry ice.

**SAMPLE PREPARATION:**

3. Thaw urine specimens, bring to room temperature, and mix thoroughly.
4. Place 200 µL of urine specimen in an 8-mL glass culture tube with a PTFE-lined cap.
5. Add 0.5 mL 14% boron trifluoride in methanol; cap and mix.
6. Heat in oven at 60 °C for a minimum of 1.5 hr (maximum 2.5 hr).
7. Cool to room temperature and then add 2.0 mL toluene.
8. Vortex or shake vigorously for 1 min.
9. After the layers separate, transfer the upper toluene layer to a drying column containing anhydrous sodium sulfate. The drying columns are prepared in 15-cm Pasteur pipettes with a glass wool plug and about 200 to 300 mg anhydrous sodium sulfate, sufficient to form a bed depth of ~1 cm.
10. Collect the eluate in a 2-mL GC vial. Cap vial.

**CALIBRATION AND QUALITY CONTROL:**

11. Prepare a stock solution by accurately weighing a known quantity of sodium trichloroacetate into a volumetric flask. Add a known volume of deionized water and mix. Convert the weight of the sodium trichloroacetate to TCAA by multiplying by 0.8814 (MW TCAA divided by MW sodium trichloroacetate = 0.8814). As an example, 34 mg of sodium trichloroacetate into a 10-mL flask makes a 3 mg/mL stock solution to be used in preparing the calibration standards.
    NOTE: Sodium trichloroacetate was used instead of trichloroacetic acid for all phases of this method development as well as in the preparation of standards. Trichloroacetic acid is very hygroscopic; the salt is much less so.
12. Prepare working (calibration) standards by serial dilution to cover the analytical range. A suggested working standard concentration range is 0.08 to 300 µg/mL. Withdraw 200 µL of each calibration standard and follow steps 4 through 10.
13. Determine the retention time for the analyte of interest.
14. Prepare at least one blank urine specimen without an analyte spike to verify whether the source (of blank urine) contained no detectable quantity of TCAA.
15. Prepare at least two levels of quality control spikes of TCAA, sodium salt to be analyzed with each analysis batch. These levels should be at ~10 X the limit of quantitation (LOQ) and 200 X LOQ, but can be adjusted to better suit the anticipated levels of the set of specimens. QC samples must be analyzed with every batch such that they constitute 10% of the sample batch.
16. QC values must be within ±20% of the spiked values. If not, the batch is considered out of control, the batch data discarded, and corrective actions taken before more samples are analyzed.
17. Calibrate daily with at least six liquid working standards covering the expected concentration range of the samples.

**MEASUREMENT:**

PRECAUTION: SYRINGE-RINSE SOLUTIONS: Toluene will extract material from some urine specimens that may eventually clog the syringe and cause injection errors unless the syringe is rinsed with the following solutions following each injection.
First rinse solution: 1:3 glacial acetic acid:deionized water
Second rinse solution: 1:1 acetone:methanol

18. Set the gas chromatograph according to manufacturer's recommendations and to conditions given on page 8322-1. With the chromatographic conditions listed, the retention time of the methyl ester of TCAA was 8.87 min [6].
19. Inject each of the samples, standards, blanks, and quality control samples.
20. Measure peak area or peak height; peak area is recommended.
    NOTE: If the sample peak area or height is greater than that of the highest calibration standard, dilute with toluene and reanalyze. Apply the appropriate dilution factor in the calculations.
21. Prepare a calibration curve by plotting instrument responses (usually peak area) for the standards vs. concentration. The simplest model that adequately describes the data should be used, but either a linear (mostly likely 1/x weighted because of the range of the calibration curve) or a quadratic model may be utilized in processing the analytical results. The standard curve must have a coefficient of determination ($r^2$) of equal to or greater than 0.98 to be acceptable for use. Furthermore, when each standard is plugged back into the calibration equation, the measured value must be within ±20% of the expected value.

**CALCULATION OF ANALYTE PER SAMPLE:**

22. Determine the concentration of TCAA in µg/mL (mg/L) using the response of each sample and the calibration curve prepared in step 21. Apply any dilution factor if applicable.

NOTE: If the creatinine value is available, the concentration may be reported as µg/g creatinine if desired.

**EVALUATION OF METHOD:**

This method was evaluated over a range of 0.9-30 µg/mL. This range covers 3x, 10x, 30x, and 100x of the estimated LOQ. Six replicates were prepared and analyzed at each concentration level. The average recoveries for each of the concentration levels were 94.8% (3 x LOQ), 102.3% (10 x LOQ), 110.4% (30 x LOQ), and 97.1% (100 x LOQ). Recoveries were determined by comparison against spiked and derivatized liquid standards (standards prepared in deionized water). The upper concentration range was extended to 100 µg/mL during testing by an independent laboratory. Five samples were analyzed at this concentration and the average recovery was 105.0%. Overall accuracy was calculated to be 7.5%; bias was 0.0113, and overall precision was 0.0366 [6]. The limit of detection (LOD) and LOQ were determined by analyzing a series of derivatized spiked standards, with the data fitted to a quadratic curve, then estimated according to the Burkart method [17]. A long-term storage stability study was carried out at the 10x, 30x, and 100x LOQ levels. Urine samples were spiked with trichloroacetate and stored at -17 ºC for 7, 14, 21, 30, and 46 days and then analyzed. Recoveries at 30 and 46 days were all greater than 90% [6].

During the testing performed by the independent laboratory ("User Check"), broad, interfering carryover peaks from the urine matrix were noticed. The lab found that raising the final temperature of the GC program to 240 ºC (instead of 180 ºC) and adding a longer hold time 10 min (instead of 7 min) reduced the carryover problem, allowing more precise and accurate measurement of the peak of interest. This adjusted GC program would now be: 80 ºC for 0.5 min, heat to 240 ºC at 20 ºC/min, and hold for 10 min. Either set of conditions may be used.

NOTE: While the overall accuracy and precision for the User-Check samples were within acceptable limits [6], there were spurious results in 10% of the samples (2 out of 20.) No reason is known for these outliers, nor were the samples able to be re-injected, re-extracted, or re-analyzed. To improve user confidence in the results obtained by this method, it is suggested to randomly run duplicate analyses of 10-20% of the samples and to randomly re-inject 10-20% of the samples. If the method is used in an on-going manner and no problems or spurious results are noted, this recommendation could be lowered or eliminated.

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