

Houston Police Department Crime Laboratory

Toxicology: Ethanol Quantitation Procedure

Purpose

This Standard Operating Procedure delineates the process by which samples submitted to the Toxicology Section are to be analyzed for the presence of ethyl alcohol.

Equipment and Reagents

- 1) Instrumentation
 - a) A Perkin-Elmer gas chromatograph equipped with dual columns.
 - i) BAC1, 30 M, 0.32 mm i.d. stationary phase, 0.32 mm i.d. stationary phase, 1.8 μm film thickness, or equivalent
 - ii) BAC2, 30 M, 0.32 mm i.d. stationary phase, 0.32 mm i.d. stationary phase, 1.2 μm film thickness, or equivalent
 - b) TurboMatrix 110 Headspace Autosampler
- 2) Solutions and Reagents
 - a) Internal Standard Solution – 0.5M Ammonium Sulfate
 - i) Pipette 300 μl of n-propanol into a 2000 ml volumetric flask containing approximately 1000 ml of deionized water. Add 132g $(\text{NH}_4)_2\text{SO}_4$. Fill with deionized water to the mark and mix well.
 - ii) Label the container to be used with the preparers initials and the date of preparation.
 - iii) This solution may be stored for up to one year at room temperature.
 - iv) Record in the reagent and component lot numbers in the reagent log book.
 - b) Volatile mixture standard solution
 - i) Add the following to a 100 ml volumetric flask and dilute with deionized water. (Note: acetaldehyde boils at 20.8°C, and must be kept cold.)
 - (1) 64 μl of acetone
 - (2) 127 μl of isopropanol
 - (3) 224 μl of methanol
 - (4) 200 μl of acetaldehyde
 - (5) 133 μl of ethanol
 - ii) Label the container to be used with the preparer's initials and the date of the preparation.
 - iii) This solution may be stored for up to one year, refrigerated.
 - iv) Record the reagent and component lot numbers in the reagent log book.
 - c) Calibrators should be procured from an outside vendor.
 - i) The current calibration levels available are 0.010 g/100ml, 0.020 g/100ml, 0.050 g/100ml, 0.080 g/100ml, 0.100 g/100ml, 0.200 g/100ml, 0.300 g/100ml and 0.400 g/100ml.
 - ii) A minimum of three calibration levels will be used to establish a calibration curve.
 - iii) It is not necessary to establish a new curve with each batch of samples. However, the controls associated with the run must be within the specified range that has been previously established for the lot number.

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- iv) Controls should be procured from a separate outside vendor that the calibrators. These may be comprised of an aqueous control and/or whole blood controls.
- v) All quantitative values reported must be bracketed by the calibrators used to calibrate the instrument.
- 3) Priming of the Hamilton Microlab 600 Series Dispenser Diluter
 - a) Verify that the pipettor is set to deliver 1000 μ l of the n-propanol internal standard and 100 μ l of sample.
 - b) Prime the pipette with internal standard solution for at least three cycles.
 - c) Ensure there are no air bubbles trapped in the system.
 - d) Small air bubbles in the lines may be removed by flicking the line with your finger while the pump is priming.
- 4) Sample Preparation
 - a) All solutions, calibrators, controls and specimens must be at room temperature.
 - b) All samples should be mixed thoroughly before analysis.
 - c) If a specimen is clotted, it must be thoroughly homogenized before analysis.
 - i) If it is not possible to homogenize the sample, it may be centrifuged and the liquid portion analyzed. In this case, the sample will be reported as plasma.
- 5) Pipette Samples, Volatile Mix, Calibrators and Controls
 - a) Insert the probe tip into the sample vial and push the button on the probe tip one time to remove an aliquot.
 - b) Carefully wipe the tip in a downward motion to remove any excess sample from the outside of the pipette tip
 - c) Insert the tip into a labeled glass vial and again press the button one time to dispense the aliquot along with the internal standard solution.
 - d) Rinse the tip thoroughly with deionized water between samples.
 - e) Cap the vial(s) tightly before moving to the next specimen.
 - f) Repeat this procedure for each sample, control and calibrator to be analyzed.
 - g) Each five samples must be bracketed by controls.
 - h) Each sample must be run in duplicate.
- 6) Preparing non-biological specimens
 - a) Beer should be diluted 1:20 with deionized water.
 - b) Wine should be diluted 1:50 with deionized water.
 - c) Spirits and other specimens should be diluted 1:100 or other appropriate dilution with deionized water.
 - d) If the type is unknown, start with a 1:100 dilution and move to a higher concentration if necessary.
- 7) Analysis
 - a) Create the instrument sequence table for the specimens to be analyzed.
 - b) Following the sequence list, load the auto sampler accordingly.
 - i) It is recommended that two people load the instrument. One person should call out the information recorded on the vial and the position on the magazine while the other checks this information against the sequence table.
 - c) Ensure the lot numbers for all reagents utilized are recorded.
- 8) Check the analytic results
 - a) Check the linearity for each column. Each shall have $R^2 \geq 0.99$.

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- b) Check standards and controls for accuracy and precision. Standards and controls must be within $\pm 5\%$ or ± 0.005 g/dl, whichever is greater relative to the target value.
 - i) Each lot of control will be run at least ten times to determine the target value prior to being used in casework. The mean will be used as the target value and the mean must be within $\pm 10\%$ of the manufacturers stated mean if purchased from an outside source.
- c) Any compound detected from the mixed volatile solution must be identified on both the A and B columns for both aliquots to be reported.
 - i) Compounds other than ethanol will be reports as 'detected'.
- d) Duplicate samples must be within $\pm 5\%$ or ± 0.005 g/dl, whichever is greater relative to the lower value.
- e) Verify that the instrument is able to resolve each of the peaks in the mixed volatile standard.
- f) Verify that the blank sample(s) is negative.
- g) Any specimen of 0.005 g/dl or less will be reported as negative.
- h) Any specimen that is >0.005 g/dl and <0.010 g/dl will be reported as ethanol detected.
- i) For liquors, convert w/v to v/v. ($v/v = (w/v)/0.789 * \text{dilution}$)
- j) Urine specimens will be reported as grams per 67 ml. Multiply the alcohol concentration by 0.67 or divide by 1.49 to obtain grams of alcohol per 67 milliliters of urine.
- k) Serum and Plasma specimens are typically 15% higher than the whole blood concentration. If serum or serum plasma are analyzed, the specimen type and original value will be included in the final report along with the calculated whole blood concentration.
- l) Always report the lowest valid result.