

## Analysis of Blood Plasma for Ethanol by Gas Chromatography

### References

- Saferstein, Richard. "Chapter 10: Forensic Toxicology." *Criminalistics: An Introduction to Forensic Science*. Prentice Hall: Englewood Cliffs, 1995, pp. 278-313.
- Logan, Barry K. "Analysis of alcohol and other volatiles." Ian Tebbett, ed. *Gas Chromatography in Forensic Science*. Ellis Horwood: New York, 1992, pp. 87-108.
- Tietz, Norbert W., ed. "Determination of Alcohols by Gas Chromatography." *Fundamentals of Clinical Chemistry*. Saunders Company: Philadelphia, 1976, pp. 1110-1111.

### Goal

To determine the concentration of alcohol in blood.

### Suggested Method of Analysis

Gas chromatography; aqueous injection

### Preparation of standards and samples

Prepare aqueous standard solutions of ethanol and internal standard (n-propanol). The ethanol concentrations should cover the range expected for sober and impaired drivers (0 to 0.25 g ethanol per 100 mL aqueous solution; w/v%). The internal standard concentration should be approximately equal to the average standard ethanol concentration (~0.10 w/v%) and should be identical in each ethanol standard and in each sample. Prepare first an aqueous solution of n-propanol by dissolving 1.00 g n-propanol in 1.00 L of water. Use this internal standard solution as the blank and as the diluent for the standard solutions. Prepare the standard solutions by weighing ethanol into a 100-mL volumetric flask and diluting to the mark with the internal standard solution.

Sample test solutions are prepared by mixing 10.0 mL of existing sample with 1.00 mL of an n-propanol solution that is exactly 11 times the concentration of the diluent solution prepared above. The factor of 11 is the reciprocal of the dilution of the internal standard upon mixing ( $1.00 \text{ mL} / \{1.00 \text{ mL} + 10.00 \text{ mL}\}$ ). Remember to account for the dilution of the original sample in your final calculations.

Store aliquots of the standard and sample solutions in glass vials fitted with open screw caps and teflon-lined septa. To preserve the solutions for up to one week, tightly seal the vials and store the solutions in a refrigerator.

### Gas chromatographic analysis

Follow instructions to set up the gas chromatograph for split injection of the sample (but use a splitless injection liner, an open tube) and isothermal separation in a polar column (e.g. thick-film Carbowax column; AT-WAX;  $15 \text{ m} \times 0.25 \text{ mm} \times 0.50 \mu\text{m}$ ). By experiment determine the highest oven temperature that gives baseline separation of the peaks; this usually translates into an oven temperature of 60 – 80 degrees C. Note: If a more common, 0.25  $\mu\text{m}$ -thick stationary phase is used instead, the oven temperature will need to be less than 40 °C to just resolve the two components.

Perform the analysis in the following manner. Push the syringe needle through the vial's septum. Slowly fill and rapidly empty the syringe several times to remove air. Fill the syringe

with aqueous solution and set the plunger to the exactly 1  $\mu\text{L}$ . Remove the syringe/needle from the vial, and then pull  $\sim 1 \mu\text{L}$  of air into the syringe. Immediately insert the syringe/needle into the GC injection port and inject the sample. Start the instrument and data acquisition. Record the peak areas for ethanol (first to elute) and n-propanol. Repeat the process for each vial.

Use the peak areas and ethanol concentrations to construct an internal standard calibration line, and then calculate the concentrations of the SRM and samples. The result for the SRM should be compared (t-test) to the listed concentration to determine the accuracy of the method. Calculate uncertainties for all final quantitative values.