

Increasing Accuracy of Blood-Alcohol Analysis Using Automated Headspace-Gas Chromatography



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Abstract

Accuracy and precision are critical in blood-alcohol analysis because the toxicologist not only has to be confident in his or her results, but also must be prepared to withstand tough cross examination by defense attorneys. In addition, crime laboratories must comply with state regulations regarding blood-alcohol testing, including proficiencies which

require the result to be within $\pm 10\%$. The Phoenix Crime Laboratory uses the pressure-balanced approach to headspace-gas chromatography (HS-GC) that introduces the sample into the column in the form of a slug rather than a continuous flow. By removing much of the variability of conventional HS-GC methods, this approach achieved an RSD of 1.2% over an 18-month study.

Headspace time plus run time is typically in the area of 6 minutes. Headspace time plus run time was reduced to 2.5 minutes by increasing the column head pressure from 20 psi to 30 psi.

The purpose of this paper is to demonstrate the improved precision possible using pressure-balanced headspace-GC technology. The laboratory's time savings will also be considered.

Introduction

Blood-alcohol analysis is typically performed in driving under the influence (DUI) and driving while intoxicated (DWI) investigations and in traffic accidents where people have been critically injured or killed. Most states have “Per Se” laws where it is illegal to drive while having a concentration of 0.08% or above. Secondary Per Se levels are not uncommon, i.e., extreme DUI above 0.15%. With these defined blood-alcohol concentrations, the accuracy of a system must be assessed. Alcohol analysis is used primarily to determine the concentration of ethanol and, to a lesser extent, determine if methanol, acetone, 2-propanol or toluene is present in blood or urine of “huffers”. HS-GC has become the nearly universal method of choice for measuring blood alcohol in forensic laboratories because it allows a relatively large number of samples to be analyzed quickly and with a minimal amount of manual handling.

Conventional HS-GC systems use a combination of pressure, temperature and agitation at up to 40 psi to drive solution components into the

headspace. Then, pressure is used to stream headspace gas continuously into the column, which tends to reduce the sharpness of the analytical results. Large numbers of crime laboratories that utilize this method have typically seen RSD levels upwards of 4%.

According to Henry’s law, at equilibrium, in a sealed vessel, volatile compounds in the liquid state will be present in the vapor state at a concentration proportional to the concentration in liquid. By sampling this vapor (the headspace) and delivering it to a gas chromatograph, the volatile compounds may be qualitatively identified and quantitatively measured. A single headspace injection is normally split into two capillary columns, each exiting to a flame ionization detector (FID). The columns have different polarity for unique separations of the volatiles of interest.

Several years ago, the Phoenix Crime Laboratory implemented a pressure-balanced, time-based HS-GC method that pressurizes the headspace and then releases the headspace gas instantaneously as a single slug into the column.

This approach is explained in Figure 1. With the injection system on standby, the carrier gas enters the vial through Valve 1 (V1) and is directed partly through the heated transfer line to the GC and partly to the heated needle, which is constantly flushed to avoid cross contamination. The needle flush gas exits through the needle vent valve.

Prior to injection, each vial is pressurized to a preset pressure with carrier gas to ensure that all injections are performed under the exact same conditions, regardless of different equilibrium pressures in different samples. During injection, the carrier gas supply and the needle purge are switched off. Sample flows to the gas chromatography system from the pressurized headspace vial. The injected volume is proportional to the injection time.

At the end of the injection, the carrier gas and needle purge are once more switched on and the injected sample volume is driven through the gas chromatograph. The needle is retracted and the system goes into standby mode.

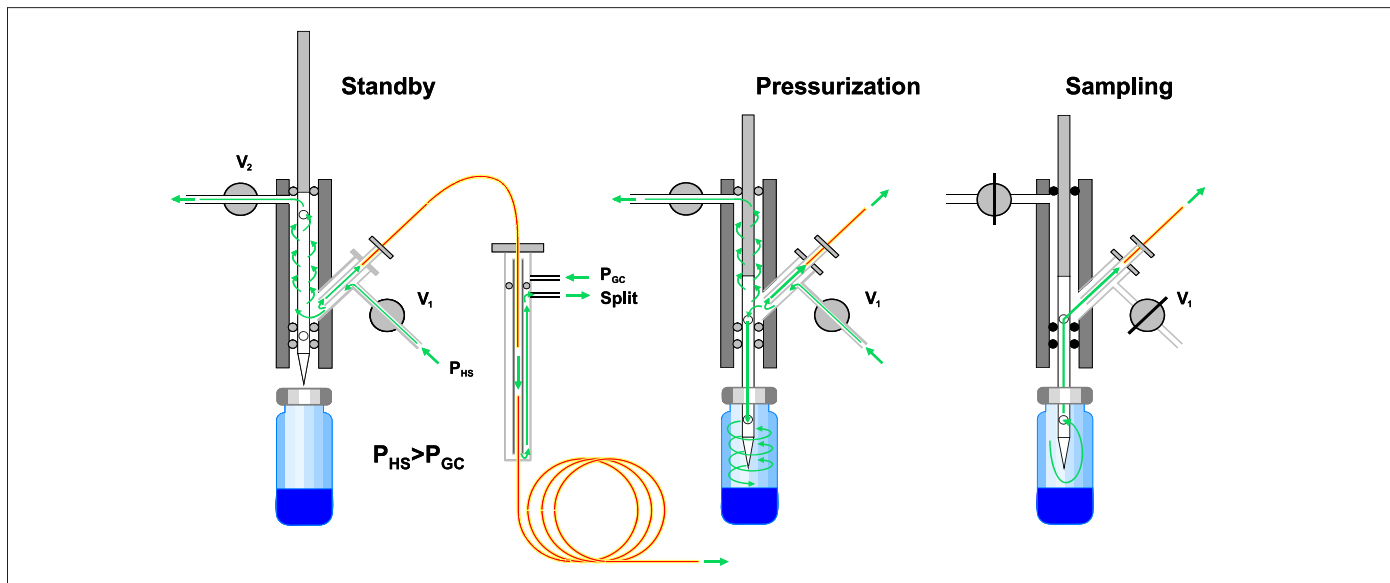


Figure 1. Headspace operation.

Experimental

Equipment

A PerkinElmer® AutoSystem™ XL GC equipped with a TurboMatrix™ HS 110 headspace sampler, dual FIDs and TotalChrom® software was used (PerkinElmer part no. N5150511). The GC was equipped with dual capillary columns from Restek Corp. The BAC 1-WCOT is a 30-m long fused-silica column with a 0.32-mm i.d. stationary phase and 1.8- μ m film thickness and the BAC 2-WCOT is a 30-m long fused-silica column with a 0.32-mm i.d. stationary phase and 1.2- μ m film thickness.

Materials

Internal standard solutions were prepared including:

1. A concentration of 0.015% v/v n-propanol and 0.5 M ammonium sulfate in deionized water.
2. A qualitative mixed-volatiles standard was prepared by adding 20 μ L acetaldehyde, 80 μ L methanol, 20 μ L acetone, 80 μ L ethanol and 50 μ L isopropyl alcohol to 100 mL deionized water.

Ethanol calibration solutions with concentrations of 0.025%, 0.050%, 0.100%, 0.200% and 0.400% were procured from an outside vendor. Positive ethanol controls from a separate vendor at 0.100% aqueous and 0.190% whole blood were also procured. A negative control was prepared using deionized water and the internal standard.

Methods

All calibration solutions, controls and samples were allowed to come to room temperature before starting. Whole blood samples were mixed thoroughly before pipetting. Serum samples required no preparation. Clotted samples were thoroughly homogenized before pipetting.

A Hamilton Microlab® 500A Series dispenser diluter was primed with an internal standard solution for at least three cycles. The left syringe was set to a speed of 4 and used to deliver 1000 μ L of internal standard. The right syringe was set to a speed of 2 and used to deliver 100 μ L of sample.

The dispenser diluter was then used to prepare each sample, control and calibration solution in duplicate. Each vial was then sealed and loaded onto the headspace sampler in the designated sequence. Diluter precision was determined to be 0.6%.

Samples and calibrators were equilibrated for 13 minutes at 60 °C and pressurized with helium at 36.5 psig. The sample was injected into the column in a 0.02-minute period through a 1.3-m x 0.32-mm fused-silica transfer line at 100 °C. The cycle time was 3.2 minutes. The needle temperature was maintained at 70 °C and the withdrawal time was 0.2 minutes.

The GC run time was 2.5 minutes and the sampling rate was set at 12.5 points per second. Oven temperature was isothermal 45 °C and pressure was isobaric 30 psi. Helium was used as the carrier gas. FIDs were used on each column with a temperature of 220 °C. Gas flows through each detector were 450 mL/min of air and 45.0 mL/min of hydrogen.

Elutions on the Restek Rtx® BAC I are as follows:

- Methanol eluted as a single-peak component at 0.716 min
- Acetaldehyde eluted as a single-peak component at 0.790 min
- Ethanol eluted as a single-peak component at 0.877 min

- Isopropanol eluted as a single-peak component at 1.050 min
- Acetone eluted as a single-peak component at 1.269 min
- N-propanol eluted as a single-peak component at 1.374 min
- Toluene eluted as a single-peak component at 5.32 min

With the exception of toluene, all analytes elute prior to 2.5 minutes on both columns.

Results

In order to evaluate the linear range of ethanol quantitation using this method, a set of 0.025, 0.050, 0.100, 0.200 and 0.400% ethanol calibration solutions were prepared and run according to protocol. Spiked samples below and above the calibration solutions were run at the following concentrations: 0.005, 0.010, 0.015, 0.020, 0.600, 0.700, 0.800, 0.900 and 1.000. The sample was determined to be in the linear range, if the quantitation fell within +10%. The method was linear from 0.010-1.0%.

The precision, accuracy, limits of detection (LODs) and limits of quantitation (LOQs) were determined by the following method: ten replicates of ethanol at concentrations of 0.005, 0.01, 0.100, 0.188 (whole blood control) and 0.400% were run according to the method described above; accuracy was defined as the deviations from the actual concentration; precision was defined as the coefficient of variation (CV) for each of the replicate concentrations. Precision and accuracy at different concentration levels are shown in Table 1 (Page 4). The LOD was determined to be 0.005% and the LOQ was 0.010%.

The Phoenix Crime Laboratory analyzes approximately 1000 blood samples per year for alcohol concentration. Figure 2 shows the run-to-run precision utilizing this method over a period of 18 months. For the target value of 0.1900% ethanol, the average result was 0.18911 (n=537) with a standard deviation of 0.0023. This yields an RSD of 1.23%, an upper control limit at three standard deviations of 0.19697 and a lower control limit at three standard deviations of 0.18303.

A study was run to evaluate the interferences of other volatile substances, which may be found in biological fluids either in the presence or absence of ethanol. Positive interferences were provided by the mixed-volatiles standard described above. The peaks were resolved with a resolution better than 1 and a peak-to-valley ratio better than 90%. This study showed that acetaldehyde, methanol, acetone, isopropanol, toluene and methyl ethyl ketone do not interfere with the identification or quantification of ethanol by HS-GC.

Conclusions

An analytical method was developed to quantify ethanol in blood alcohol using HS-GC. The method appears to be extremely robust and reliable and achieved an RSD value of 1.23%, which is well below those achieved by other methods. RSD values of this level make it possible for toxicologists to testify with confidence that their results are accurate within $\pm 5\%$. The result is that DUI and DWI defendants in the Phoenix area normally plead guilty to driving under the influence or driving while impaired, saving the toxicologists the time they would otherwise have to spend testifying and saving the local government the cost of a trial.

Table 1. Precision and Accuracy at Different Concentrations.

Concentration (%)	0.005	0.010	0.100	0.188	0.400
Accuracy	+13.0%	+8.5%	-1.8%	+0.6%	-1.6%
Precision	1.8%	0.4%	0.2%	0.5%	0.2%

Within-run precision data

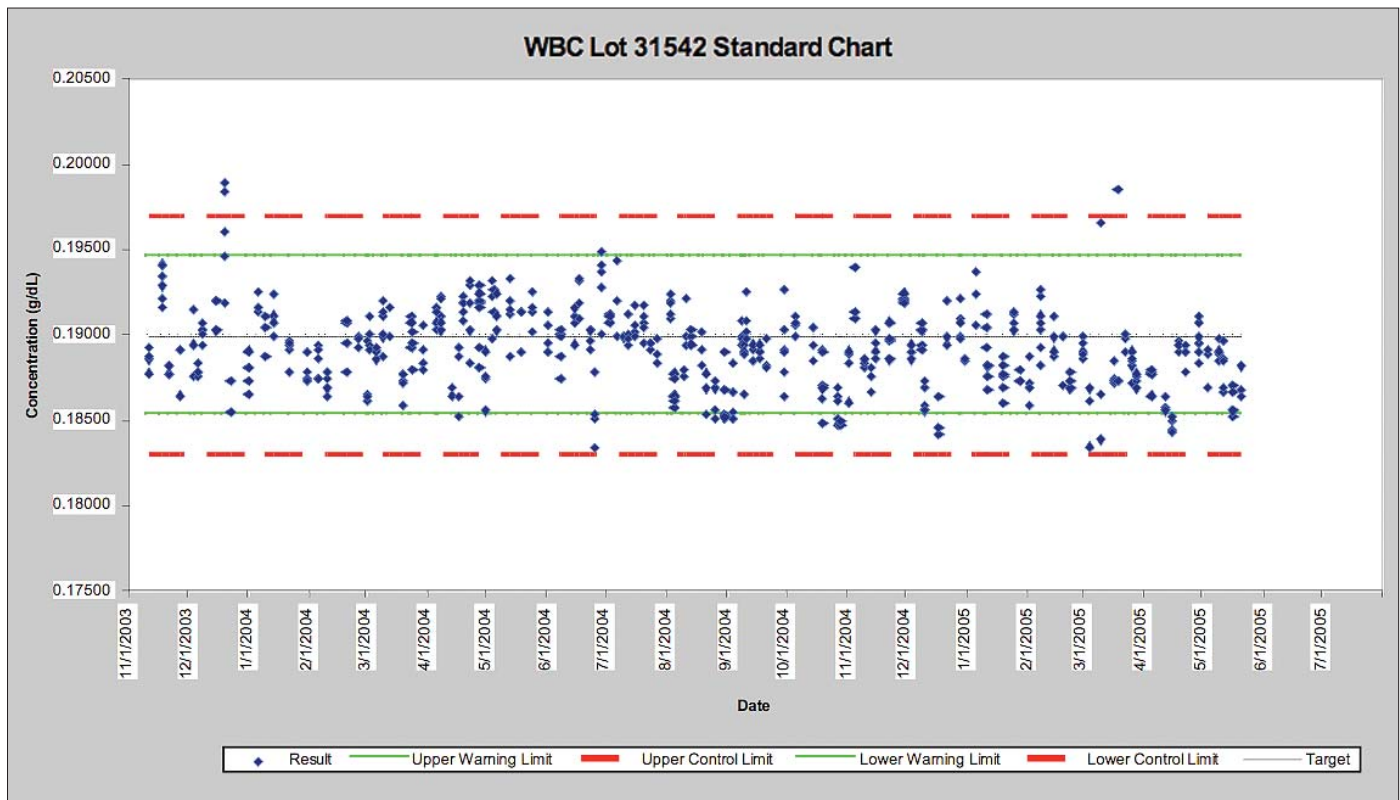


Figure 2. Run-to-run precision over 1.5 years.

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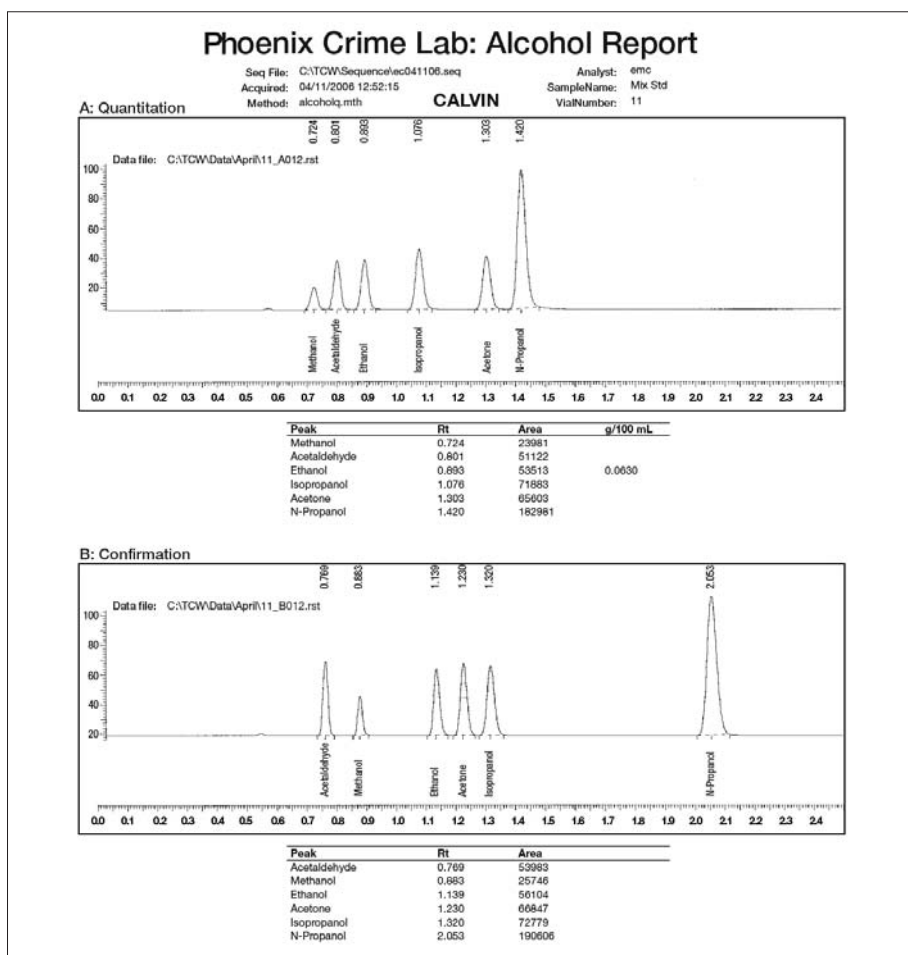


Figure 3. Quantitation column and confirmation column chromatographs.

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