Improved Separation of Blood Alcohols Using Zebron™ ZB-BAC1 and BAC2 GC Columns

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The Zebron ZB-BAC1 and ZB-BAC2 GC columns provide extremely reproducible and quantitative analysis of the blood alcohols. Adequate separation of ethanol and other blood alcohols is achieved in under 2 minutes with a ~1 – 3 % RSD. The Hta HT-200H autosampler provides consistent injection volumes.

Introduction

The determination of blood alcohol content (BAC) is one of the most common tests performed by forensic laboratories. A typical BAC analysis involves headspace-gas chromatography to prevent contamination at the head of the GC column from non-volatile components in the sample. Many places have recently begun using dual-column GC analyses to confirm the presence of ethanol. These two columns must have different selectivities to ensure that any analytes identified on the first column elute at a different retention time on the second column.

When performing dual column analysis, the major challenge is finding two dissimilar phases that provide adequate resolution of all analytes, while maintaining reasonable analysis times. In BAC analysis, baseline resolution for all analytes of interest and ultra fast analysis time (under five minutes) are critical because of the high throughput nature of most forensic labs.

The most critical blood alcohol compound is ethanol. However, there are other compounds that could be present in the blood stream that may interfere with the identification or quantitation of ethanol. First, a small amount of methanol is usually present in ethanol as a byproduct of the distillation process. Therefore, a small amount of methanol may also be present in blood as a result of ethanol consumption. Second, isopropanol (the main ingredient of rubbing alcohol) can also be present in the blood as a result of absorption through skin or from inhalation of rubbing alcohol vapor. Once in the blood stream, the consumed ethanol may also oxidize to acetaldehyde. Another metabolite to take into account when testing for BAC is acetone. This metabolite is particularly prominent in diabetics because acetone is produced as a result of ketoacidosis.

Since many of the mentioned compounds may co-elute with ethanol on a GC column, most BAC analyses need to take these possible interferences into account when determining blood alcohol content. To ensure proper identification and quantitation, it is important to use GC columns that can provide the best resolution of ethanol from all other components potential interferences.

Quantification of methanol, ethanol, isopropanol, acetaldehyde, and acetone is usually done using an internal standard method. Typical internal standards include *t*-butanol, *n*-propanol, and 2-butanol. Many forensic scientists will use any of these three internal standards depending on their specific needs. Another reason for using more than one internal standard is that n-propanol has been shown to be present in postmortem specimens, which would interfere with quantitative results.[1] Quantitative results must usually be precise to within ± 5 %.

The goal of this study is to demonstrate the capabilities of two new GC phases, Zebron™ ZB-BAC1 and ZB-BAC2, to accurately identify and quantify trace amounts of alcohols or possible alcohol interferents in blood with fast analysis times (from 2 to 4 minutes). All samples were analyzed by headspace-GC-FID. The blood alcohol analytes in each sample consisted of methanol, acetaldehyde, ethanol, acetone, and isopropanol diluted to 0.025, 0.050, 0.100, 0.200, and 0.400 % in water. This calibration range is in accordance with the typical 0.08 % blood alcohol limit for a DUI conviction in all 50 states in the US. Signal-to-noise data from the chromatograms have been used to determine a limit of detection (LOD) and a limit of quantitation (LOQ) for each of the five analytes.

The two new GC column phases provided enhanced resolution of ethanol from closely eluting compounds while also exhibiting the ability to quantify a blood alcohol concentration between 0.025 – 0.400 %. Relative standard deviations (RSD), absolute and relative to the internal standards, have been tabulated at the 0.025 and 0.100 % concentration levels to illustrate the precision of the application.

Experimental

All samples analyzed consisted of blood alcohol analytes diluted to 0.025, 0.050, 0.100, 0.200, and 0.400 % in 5.0 mL of water (total volume) inside a 20 mL headspace vial. Note that the concentration of internal standards in each sample was always at 0.100 %. Analysis of each sample was conducted on an HP6890 gas chromatograph (Agilent) equipped with an Overbrook Scientific (HT-200H) autosampler and two new capillary columns from Phenomenex. The Zebron ZB-BAC1 had dimensions of 30 m x 0.53 mm ID x 3.00 µm and the Zebron ZB-BAC2 had dimensions of 30 m x 0.53 mm ID x 2.00 µm. The columns were installed such that they would lead from the same injection port and guard column and split off into two separate flame ionization detectors. Additional parameters for the autosampler and GC method are listed in **Table 1**.

Table 1.
Instrument parameters for blood alcohol analysis.

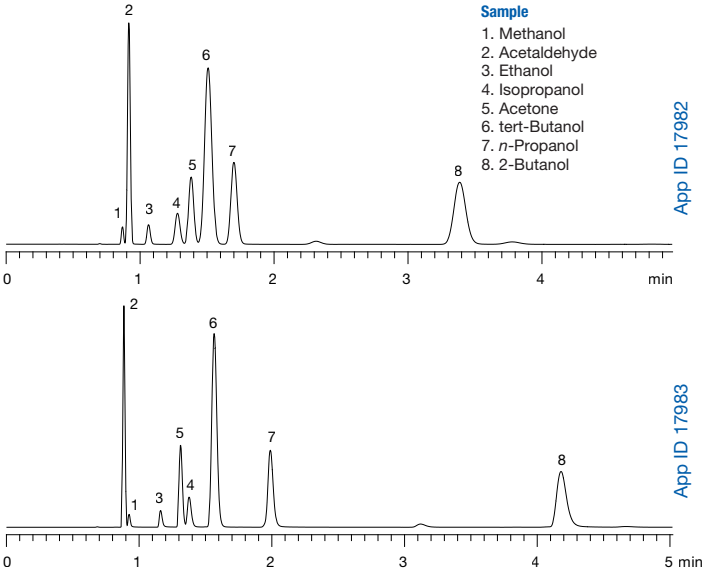
Column(s):	Zebron ZB-BAC1, 30 m x 0.53 mm x 3.00 µm Zebron ZB-BAC2, 30 m x 0.53 mm x 2.00 µm
Oven Program:	40 °C Isothermal for 5.0 min
Injection:	Headspace injection, 5.0 mL sample, 1.0 mL injection volume, 0.8:1 split at 150 °C constant, needle temperature constant at 70 °C, samples at 60 °C for 13.0 min, 40 mL/min injection speed, 30 mL/min fill speed
Detector:	FID @ 250 °C
Carrier Gas:	Helium @ 12.9 mL/min (constant flow)

Results and Discussion

A BAC analysis of five blood alcohol analytes at 0.025 % and three internal standards at 0.100 % was completed in less than 5 minutes (**Figure 1**). Under normal circumstances, labs would not typically use more than one internal standard. However, this analysis was performed this way to demonstrate that the ZB-BAC phases provide good resolution of all analytes irregardless of the internal standard used. It should be noted that if *n*-propanol was chosen as the internal standard, then the analysis time could be reduced to just 1.9 and 2.2 minutes on the Zebron ZB-BAC1 and the Zebron ZB-BAC2 phases, respectively. If a lab was able to use *t*-butanol as an internal standard then the analysis time could be further reduced to 1.7 minutes on both phases.

Both phases provided excellent resolution of ethanol from other common solvent interferences encountered in blood. It should be noted that the elution order of the eight solvents analyzed was significantly different between the two phases. This selectivity difference demonstrates that when used together, the pair provides positive confirmation of alcohol abuse.

Figure 1.
GC-FID chromatograms of a 0.025 % blood alcohol mix for Zebron ZB-BAC1 (top) and ZB-BAC2 (bottom).



Replicate samples were run for the 0.025 and 0.100 % concentrations. All other concentrations were run as a single injection. The peak areas calculated from the chromatograms for the 0.025 % blood alcohol samples were used to tabulate relative standard deviations (RSDs) relative to detector response (absolute) and also relative to the three internal standards. As shown in **Table 2**, the blood alcohol samples showed excellent reproducibility exhibiting absolute and relative RSDs ranging from approximately one to three percent for both the Zebron ZB-BAC1 and ZB-BAC2 phases. Such small deviations are a testament to the integrity of the phases to give consistent separation and to the Overbrook Scientific HT-200H autosampler for providing consistent injection volumes.

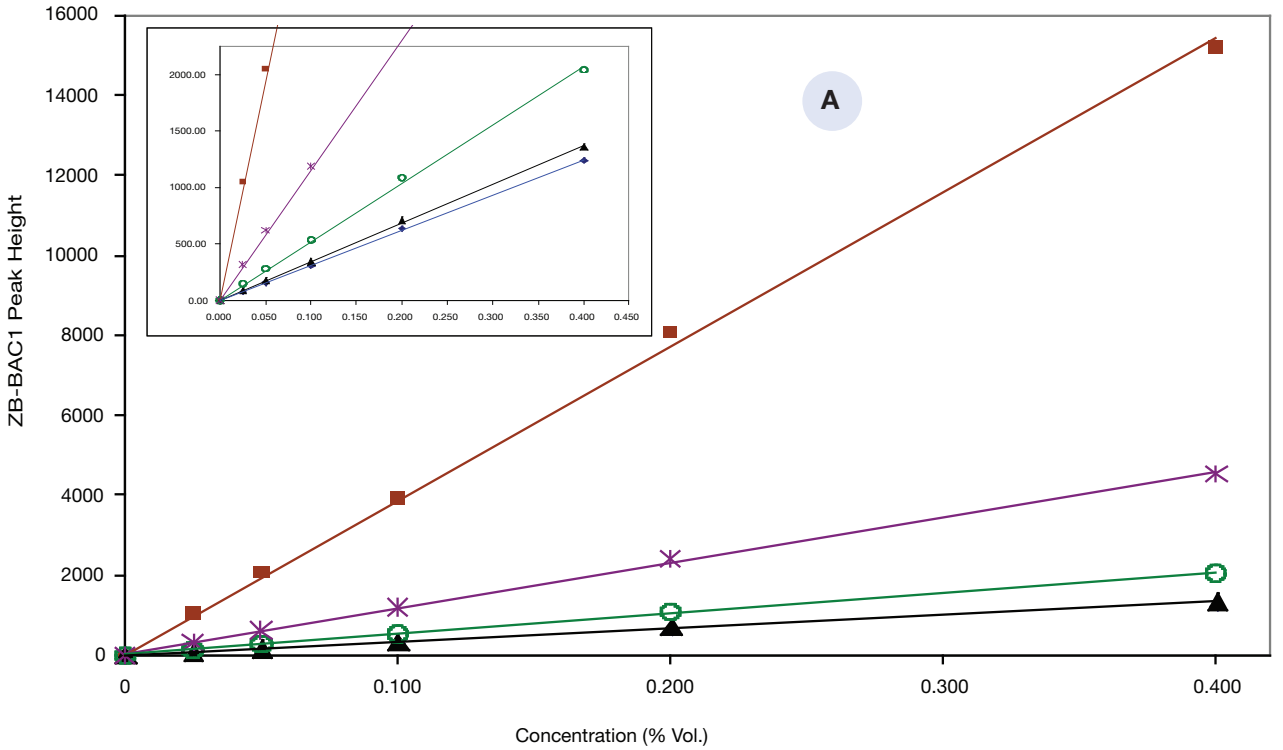
Table 2.
Reproducibility on Zebron ZB-BAC1 and ZB-BAC2 phases.

Zebron ZB-BAC1				
Compound	Peak Area % RSD (Absolute)	Peak Area % RSD (Relative to *t*-Butanol)	Peak Area % RSD (Relative to *n*-Propanol)	Peak Area % RSD (Relative to 2-Butanol)
Methanol	3.1	2.8	2.7	2.6
Acetaldehyde	2.1	1.6	1.5	1.4
Ethanol	3.4	2.8	2.7	2.6
Isopropanol	3.2	2.6	2.5	2.4
Acetone	2.2	1.6	1.5	1.4

Zebron ZB-BAC2				
Compound	Peak Area % RSD (Absolute)	Peak Area % RSD (Relative to *t*-Butanol)	Peak Area % RSD (Relative to *n*-Propanol)	Peak Area % RSD (Relative to 2-Butanol)
Methanol	2.1	1.6	1.4	1.3
Acetaldehyde	3.1	2.7	2.5	2.4
Ethanol	3.2	2.7	2.5	2.4
Isopropanol	2.2	1.6	1.4	1.3
Acetone	3.2	2.7	2.5	2.4

The LOD and LOQ for each analyte on each column were obtained from a calibration curve of average peak height vs. concentration (**Figure 2**). All calibration curves showed good linearity as all correlation coefficients (R^2) fell within a range of 0.9980 – 0.9998. The LOD and LOQ are defined as the height with a value that is three or ten times the signal-to-noise ratio, respectively, of an adjacent portion of baseline devoid of peaks. Therefore, plugging in a peak height value that is three and ten times the noise (N) into the best-fit equation from a linear regression would yield a percent concentration for the LOD and LOQ. Consequently, it was necessary to tabulate the average noise from all of the chromatograms for this purpose. Noise levels of $3N_{avg}$ and $10N_{avg}$ were then plugged into the best-fit linear equation for each analyte (as a y-value) to obtain the corresponding x-values, LOD and LOQ, for each analyte (**Table 3**). Note that the LODs and LOQs are also displayed in units of ppm (in parentheses) in addition to % blood alcohol content. It should be noted that the 0.08 % (800 ppm) blood alcohol limit for a DUI conviction is nearly 80 times greater than the highest LOD's and LOQ's estimated in this work. Therefore, it is quite easy to detect and quantify blood alcohol concentrations between 0.025 and 0.400 % when using the Zebron ZB-BAC1 and ZB-BAC2 phases for the separation.

Figure 2.
Calibration curves on (a) Zebron ZB-BAC1 and (b) Zebron ZB-BAC2 phases. Each analyte is designated by the following: Methanol (-♦-), Ethanol (-▲-), Isopropanol (-○-), Acetone (-✱-), and Acetaldehyde (-■-). Insets show a magnified view to decipher the methanol and ethanol calibration curves.



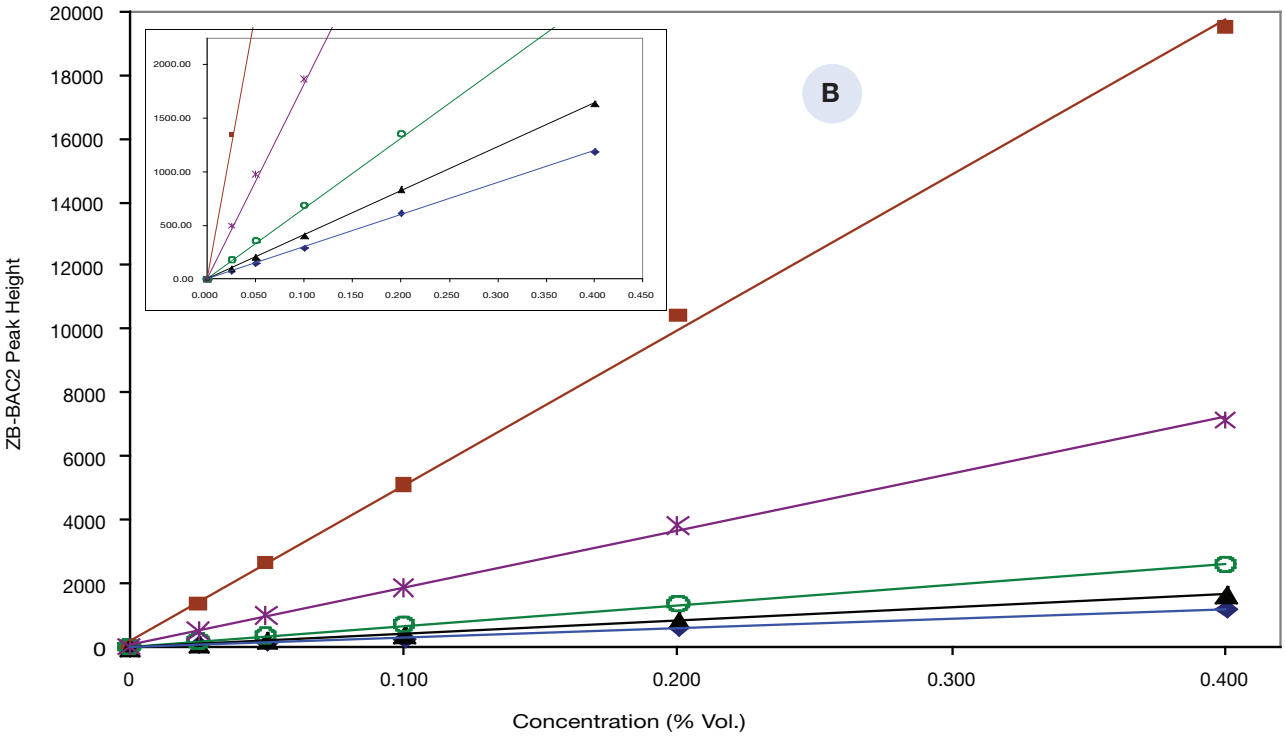


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Table 3.
Estimated limits of detection and quantitation on Zebron ZB-BAC1 and ZB-BAC2 phases.

Compound	Zebron ZB-BAC1		Zebron ZB-BAC2	
	LOD	LOQ	LOD	LOQ
Methanol	0.0003 % (2.9 ppm)	0.001 % (9.7 ppm)	0.0003 % (3.0 ppm)	0.001 % (10.1 ppm)
Acetaldehyde	0.00002 % (0.2 ppm)	0.00008 % (0.8 ppm)	0.00002 % (0.2 ppm)	0.00006 % (0.6 ppm)
Ethanol	0.0003 % (2.6 ppm)	0.0009 % (8.8 ppm)	0.0002 % (2.2 ppm)	0.0007 % (7.4 ppm)
Isopropanol	0.0002 % (1.8 ppm)	0.0006 % (5.8 ppm)	0.0001 % (1.4 ppm)	0.0005 % (4.6 ppm)
Acetone	0.00008 % (0.8 ppm)	0.0003 % (2.6 ppm)	0.00005 % (0.5 ppm)	0.0002 % (1.7 ppm)

Conclusions

Two new capillary phases, Zebron ZB-BAC1 and ZB-BAC2, have been designed to successfully identify and quantify ethanol and other blood alcohols by headspace-gas chromatography. When used together, their complementary selectivities provides positive identification of all blood alcohol related compounds. Successful separation of the blood alcohols and internal standards can take place in less than 5 minutes and as quickly as 1.7 minutes depending on which internal standard is used. The ~1 – 3 % RSDs achieved with this method show that these newly developed phases provide extremely reproducible and quantitative analysis of the blood alcohols. It also demonstrates the ability of the Overbrook Scientific HT-200H autosampler to provide consistent injection volumes. The low ppm limits of detection and quantitation achieved in this work also demonstrate that a 0.08 % blood alcohol concentration for a DUI conviction is easily detectable and quantifiable on these phases.

References

1. Nanikawa, R.; Ameno, K.; Hashimoto, Y.; Hamada, K. Medicolegal Studies on Alcohol Detected in Dead Bodies – Alcohol Levels in Skeletal Muscle. *Forensic Sci. Int.*, 20, 133-140.

For more information on the Overbrook Scientific HT-200 autosampler, go to: http://www.overbrookscientific.com/

guarantee

If Zebron does not provide you with equivalent separations as compared to any other GC column of the same phase, and comparable dimensions, return the column with your comparative data within 45 days for a FULL REFUND.

Ordering Information

Zebron ZB-BAC1 GC Columns			
ID(mm)	**df(µm)**	**Temp. Limits °C**	**Part No.**
30-Meter			
0.32	1.80	-20 to 260/280 °C	7HM-G021-31
0.53	3.00	-20 to 260/280 °C	7HK-G021-36

Zebron ZB-BAC2 GC Columns			
ID(mm)	**df(µm)**	**Temp. Limits °C**	**Part No.**
30-Meter			
0.32	1.20	-20 to 260/280 °C	7HM-G022-25
0.53	2.00	-20 to 260/280 °C	7HK-G022-32

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