Improved Blood Alcohol Analysis by Resolution of Propanal from Ethanol Using Two Unique GC Column Phases, Zebron™ ZB-BAC1 and ZB-BAC2

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Though the determination of Blood Alcohol Content (BAC) using Gas Chromatography (GC) is routinely used, the analytical procedure is not infallible. This work provides a method for resolving propanal from ethanol for accurate BAC analysis via headspace GC using two novel GC phases, Zebron ZB-BAC1 and ZB-BAC2. This allows laboratories to ensure the utmost accuracy when doing BAC anaylsis.

Introduction

Today, GC has become the standard analytical method for testing BAC in both confirmatory testing as well as post-mortem investigations. To improve the accuracy of this test, many labs are moving towards dual column approaches. Though n-propanol is commonly used as the internal standard, it can be problematic when analyzing post-mortem samples because it can also be formed in the body's natural decay process.[1]

Another possible source of error comes from propanal. A recent study suggests that propanal occurs naturally in the body through two different metabolic pathways in the liver.[2] Propanal may be undetected via GC analysis because not only does it typically occur in minute amounts, but it frequently co-elutes with ethanol when using standard GC columns.

The objective of this work was to adequately separate propanal from all key components in a blood alcohol sample using two unique GC columns, the Zebron ZB-BAC1 and ZB-BAC2. We also aimed to demonstrate how these columns' unique selectivities can resolve other compounds, such as t-butanol, n-propanol, and 2-butanol. This will allow laboratories to use another internal standard besides n-propanol for more accurate blood alcohol analyses.

Methods

Sample Preparation

All samples 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. The concentration of internal standards in each sample was always at 0.100 %.

Gas Chromatography Analysis

The analysis of each sample was conducted on an HP 6890 Gas Chromatograph (Agilent®) equipped with an Overbrook Scientific® Inc. (HT-200H) autosampler and two capillary columns from various manufacturers. 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. All parameters for the GC methods are listed with each corresponding chromatogram. Linearity, LOD, LOQ, and reproducibility were evaluated for the Zebron BAC1 and BAC2 columns.

Results and Discussion

Two possible sources of error can occur when doing blood alcohol analysis via GC. First, n-propanol may be present in post-mortem samples and therefore its use as an internal standard should be avoided. Second, propanal can occur naturally in some blood samples and may co-elute with ethanol and interfere with quantitation.

Previous work has shown that the Zebron ZB-BAC1 and ZB-BAC2 columns can adequately resolve alternative internal standards, such as t-butanol and 2-butanol, while maintaining fast analysis time.[3] This allows laboratories to more accurately determine BAC levels. In contrast, traditional BAC column pairs cannot fully resolve t-butanol from acetone on both columns. Using 2-butanol as an internal standard will double the run time on these columns.

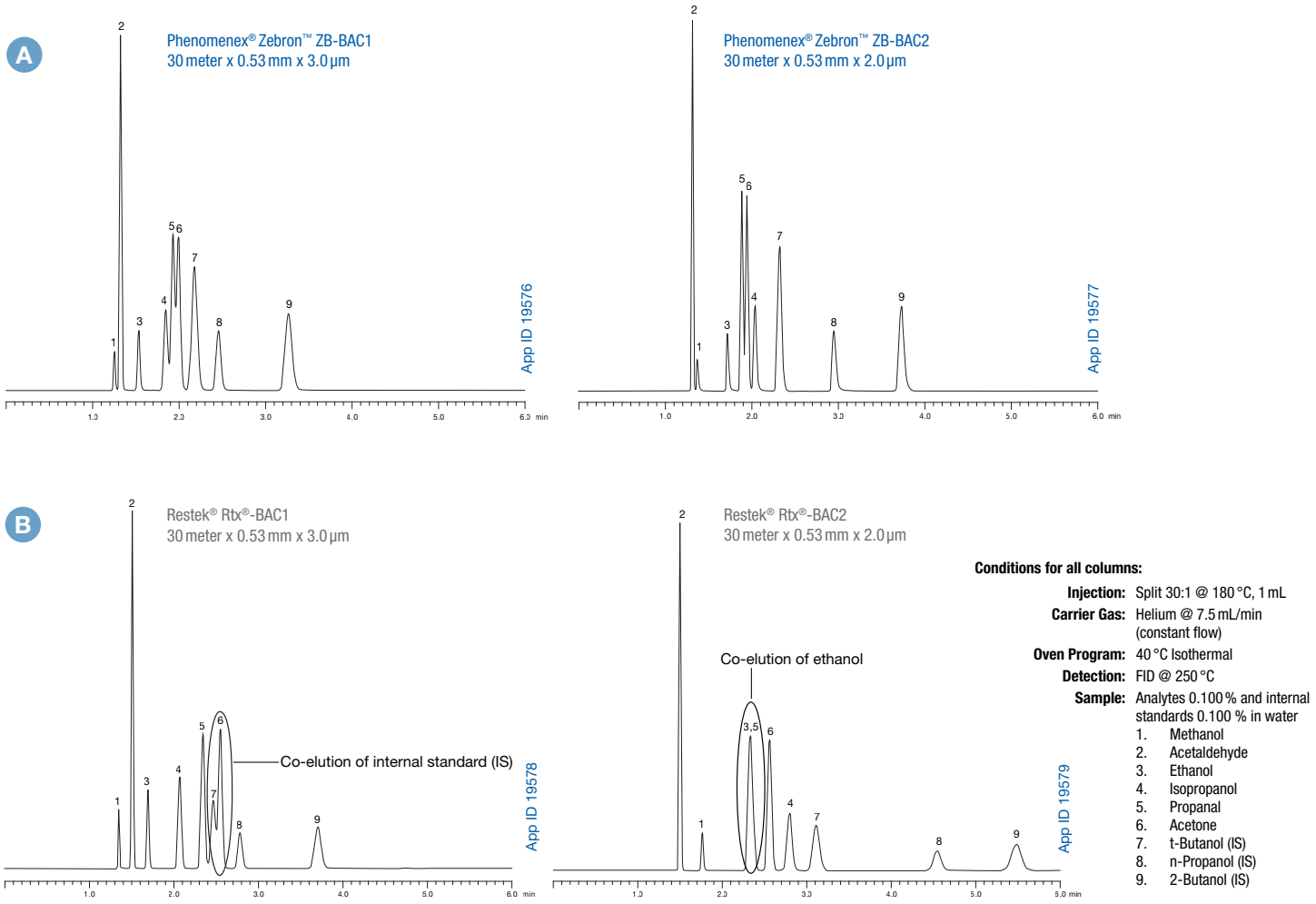
The unique selectivity of the Zebron ZB-BAC1 and ZB-BAC2 columns also allows them to resolve propanal from all other key components in a dual column GC analysis. Using a simple 40 °C isothermal program and a flow of 7.5 mL/min, propanal was separated from ethanol **(Figure 1A)**. In contrast, propanal completely co-elutes with ethanol on the Restek® Rtx®-BAC2 column, making it essentially a single column analysis. This eliminates any benefit of being able to reliably confirm a peak's identification **(Figure 1B)**.

Though the Restek Rtx-BAC1 column can resolve propanal, it comes at the sacrifice of not being able to use t-butanol as an internal standard. t-Butanol co-elutes with acetone on the Restek Rtx-BAC1 column **(Figure 1B)**. Since n-propanol can occur naturally in certain samples, this forces analysts to use 2-butanol with the Restek BAC column pair, nearly doubling the run time.

A further benefit of the Zebron BAC column pair is the increased resolution and high reproducibility of ethanol from other close eluting components, thus improving analytical precision. Using Zebron columns, the calibration curve for all compounds was found to be linear with correlation coefficients (R^2) within a range of 0.9961 to 0.9998 **(Figure 2)**. The agreement in quantitative results between each of the three possible internal standards was very good, indicating that either t-butanol or 2-butanol would be suitable alternatives to n-propanol for method quantitation **(Table 1)**. Method LOD and LOQ were determined to be less than 0.0001 % and 0.001 % respectively for all compounds **(Table 2)**. These levels are significantly below the required detection limits of the method indicating that sensitivity requirements can easily be met.

APPLICATIONS

Figure 1.
Propanal separation on the Zebron™ and Restek® BAC column pair

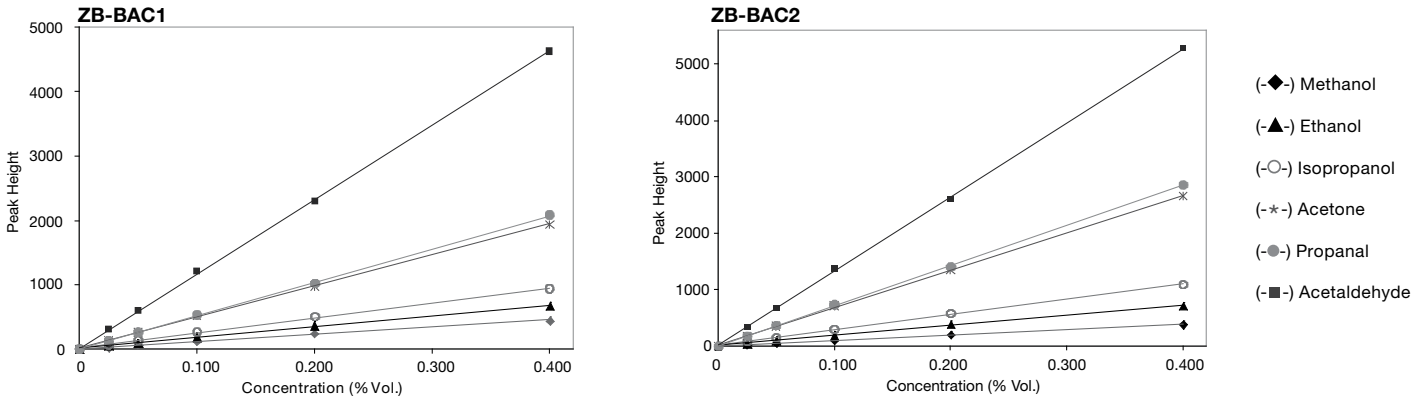


Conditions for all columns:

Injection: Split 30:1 @ 180 °C, 1 mL
Carrier Gas: Helium @ 7.5 mL/min (constant flow)
Oven Program: 40 °C Isothermal
Detection: FID @ 250 °C
Sample: Analytes 0.100 % and internal standards 0.100 % in water

1. Methanol
2. Acetaldehyde
3. Ethanol
4. Isopropanol
5. Propanal
6. Acetone
7. t-Butanol (IS)
8. n-Propanol (IS)
9. 2-Butanol (IS)

Figure 2.
Calibration curves on Zebron ZB-BAC1 and BAC2 phases



Comparative separations may not be representative of all applications.

Table 1.
Absolute and relative reproducibilities on Zebron™ ZB-BAC1 and ZB-BAC2 for blood alcohols and related analytes

Zebron ZB-BAC1

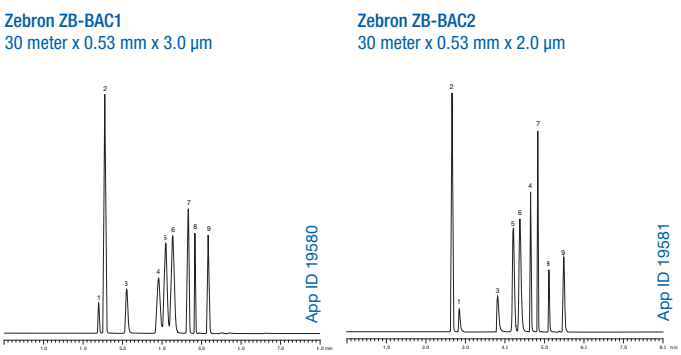
Compound	Peak Area % RSD (Absolute)	Peak Area % RSD (Relative to t-Butanol)	Peak Area % RSD (Relative to n-Propanol)	Peak Area % RSD (Relative to 2-Butanol)
Methanol	1.7	3.8	3.7	3.9
Acetaldehyde	1.3	3.4	3.3	3.5
Ethanol	1.7	3.8	3.7	3.8
Isopropanol	1.4	3.5	3.4	3.5
Propanal	1.1	3.2	3.1	3.3
Acetone	1.2	3.4	3.3	3.4

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	1.3	3.4	3.4	3.5
Acetaldehyde	1.9	4.0	3.9	4.0
Ethanol	1.8	3.9	3.8	4.0
Isopropanol	1.1	3.2	3.2	3.3
Propanal	1.3	3.4	3.4	3.5
Acetone	1.4	3.5	3.4	3.6

Note: The LODs and LOQs are also displayed in units of ppm in addition to % blood alcohol content.

Figure 3.
Optimized propanal separation on the Zebron BAC column pair



Conditions for both columns:

Injection: Headspace 30:1 @ 180 °C, 1 mL
Carrier Gas: Helium @ 4 mL/min for 4.5 min to 40 mL/min @ 80 mL/min² for rest of run
Oven Program: 30 °C Isothermal
Detection: FID @ 250 °C
Sample: Analytes 0.100 % and internal standards 0.100 % in water

1. Methanol
2. Acetaldehyde
3. Ethanol
4. Isopropanol
5. Propanal
6. Acetone
7. t-Butanol (IS)
8. n-Propanol (IS)
9. 2-Butanol (IS)

Table 2.
Limits of Detection (LOD) and Quantitation (LOQ) on Zebron ZB-BAC1 and ZB-BAC2 phases

	Zebron ZB-BAC1		Zebron ZB-BAC2	
Compound	LOD	LOQ	LOD	LOQ
Methanol	0.00006 % (0.6 ppm)	0.0002% (1.9 ppm)	0.00006 % (0.6 ppm)	0.0002 % (1.9 ppm)
Acetaldehyde	0.00001 % (0.1 ppm)	0.00002% (0.2 ppm)	0.000004 % (0.04 ppm)	0.00001 % (0.1 ppm)
Ethanol	0.00004 % (0.4 ppm)	0.0001% (1.2 ppm)	0.00003 % (0.3 ppm)	0.0001 % (1.0 ppm)
Isopropanol	0.00003 % (0.3 ppm)	0.00009% (0.9 ppm)	0.00002 % (0.2 ppm)	0.00007 % (0.7 ppm)
Acetone	0.00001 % (0.1 ppm)	0.00004% (0.4 ppm)	0.00001 % (0.1 ppm)	0.00003 % (0.3 ppm)
Propanal	0.00001 % (0.1 ppm)	0.00004% (0.4 ppm)	0.00001 % (0.1 ppm)	0.00003 % (0.3 ppm)

All RSD values for replicate injections (n = 5) were taken at the lowest concentration in the calibration curve of 0.025 %.

To further explore the unique selectivity advantage that the Zebron BAC column pair provides, we optimized the propanal separation on these columns. Utilizing an oven program of 30 °C isothermal and a flow of 4 mL/min, propanal had near baseline resolution on both columns **(Figure 3)**. This was accomplished while maintaining baseline resolution for all commonly used internal standards, t-butanol, n-propanol, and 2-butanol.

Conclusion

Chromatographic BAC analysis is conducted to either confirm alcohol use/abuse or to identify its contribution in post-mortem cases. Many analytical methods today utilize dual column headspace GC to confirm the presence of alcohol in a blood sample. Though this technique is fairly accurate, it is not infallible.

In this work, we looked at two possible sources of error that can occur when doing blood alcohol analysis via GC. First, n-propanol has been shown to be present in some post-mortem samples and therefore should be avoided as an internal standard. Second, propanal can occur naturally in some blood samples. Because propanal co-elutes with key components, such as ethanol, its presence may go undetected and result in inaccurate analysis.

To help improve method accuracy, we performed dual column GC analyses using two unique GC columns, the Zebron ZB-BAC1 and ZB-BAC2. These columns were able to fully resolve all commonly used internal standards, such as t-butanol, n-propanol, and 2-butanol, from all other analytes. This allows laboratories to utilize either t-butanol or 2-butanol as an internal standard, instead of risking inaccurate analysis by using n-propanol.

The Zebron BAC column pair was also able to resolve propanal from all key components in a blood alcohol sample utilizing a simple 40 °C isothermal program and a flow of 7.5 mL/min. In contrast, the Restek® Rtx®-BAC column pair did not adequately separate propanal. On the Restek Rtx-BAC2 GC column, propanal co-elutes with ethanol and therefore will go undetected and falsely inflate ethanol levels. This negates the added benefit of

improved accuracy from a dual column analysis. On its complementary column, the Restek® Rtx®-BAC1, propanal can only be separated if analysts are willing to forgo using t-butanol as an internal standard.

Given that accuracy is very critical for confirming alcohol use and/or abuse, it is best to select a GC column pair that will provide the most reliable results. The Zebron™ ZB-BAC1 and ZB-BAC2 columns are able to address two possible sources of errors in BAC analysis. Not only do these columns allow you to utilize an alternative internal standard besides the problematic n-propanol, they also provide adequate separation for the detection of propanal.

References

1. Nanikawa, R.; Ameno, K.; Hashimoto, Y.; Hamada, K. Medicolegal Studies on Alcohol Detected in Dead Bodies – Alcohol Levels in Skeletal Muscle.
2. Bessonneau, V.; Clement, M.; Thomas, O. Can Intensive Use of Alcohol-Based Hand Rubs Lead to Passive Alcoholism? Int. J. Environ. Res. Public Health, Pages 3038-3050, 7, 2010.
3. Countryman, S.; Kelly K.; Fernandez, C. Critical Factors in Selecting an Internal Standard for Accurate Determination of Blood Alcohols in Post-Mortem Samples, 2010.

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

guarantee

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

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