

# Blood Alcohol Analysis Utilizing Headspace Autosampling and Dual-column GC Confirmation

## Introduction

Testing for the presence and subsequent quantitation of ethanol in blood, breath, and urine are the highest volume tests performed in forensic laboratories. In addition to ethanol, the detection of several other significant alcohols and their metabolites is necessary. Gas chromatographic (GC) assays provide the greatest amount of flexibility and specificity in analyzing for these volatile compounds. Direct injection of biological samples into GC columns has been used in the past as the method of sample introduction. This typically leads to column contamination and decreased performance. The incorporation of headspace sampling into the method prevents the buildup of non-volatile contamination at the head of the column and helps to maintain consistent performance and extend column lifetime.

Analysis time and resolution are two critical factors when developing a GC assay for ethanol. Analysis time for each sample should be as short as possible, while

still maintaining baseline resolution for all analytes. Isothermal operation is the preferred method of analysis because it eliminates the cool-down period between temperature programmed runs. Overall analysis time can be reduced in isothermal analysis by raising the temperature of the analysis or by increasing carrier gas flow rate. However, in attempting to shorten the analysis time either by increasing the flow rate or by raising the temperature, many traditional capillary column stationary phases fail to provide adequate resolution of all of the components commonly tested during blood alcohol analysis. PerkinElmer supplies the Elite BAC-1 and BAC-2, two novel capillary column stationary phases to meet all of these requirements.

## Experimental

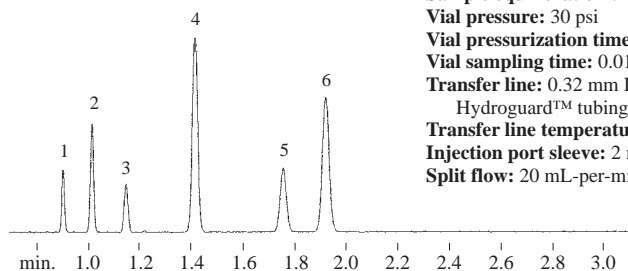
The goal of the method developed here was to use headspace sampling and dual column GC analysis to provide blood alcohol confirmation in less than 3 minutes.

**Figure 1**

Achieve baseline resolution of all blood alcohol components in less than 3 minutes using the PerkinElmer headspace autosampler, gas chromatograph and Elite BAC-1 and Elite BAC-2 columns.

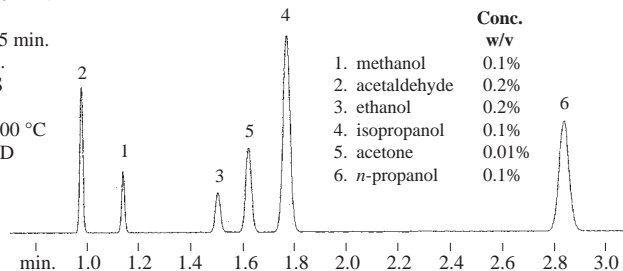
**30 m, 0.32 mm ID, 1.8  $\mu$ m  
Elite BAC-1  
(part # N9316579)**

Dual column analysis using a two-hole ferrule.  
1.0 mL headspace sample of a blood alcohol mix.  
**Oven temperature:** 40 °C isothermal  
**Inj. temperature:** 200 °C  
**Carrier gas:** He  
**Sample equilibration temperature:** 70 °C  
**Sample equilibration time:** 15 min.  
**Vial pressure:** 30 psi  
**Vial pressurization time:** 0.15 min.  
**Vial sampling time:** 0.01 min.  
**Transfer line:** 0.32 mm ID FS  
Hydroguard™ tubing  
**Transfer line temperature:** 200 °C  
**Injection port sleeve:** 2 mm ID  
**Split flow:** 20 mL-per-minute



**30 m, 0.32 mm ID, 1.2  $\mu$ m  
Elite BAC-2  
(part # N9316577)**

	Conc. w/v
1. methanol	0.1%
2. acetaldehyde	0.2%
3. ethanol	0.2%
4. isopropanol	0.1%
5. acetone	0.01%
6. n-propanol	0.1%



The instrumentation requirements for this type of analysis can be satisfied by selecting from a wide variety of PerkinElmer products. Any current TurboMatrix™ headspace autosampler (as well as previous models such as the HS40 or HS110) can be used for sample introduction. Similarly, the GC requirements can be fulfilled using the Clarus™ 500 as well as previous models such as the AutoSystem XL or AutoSystem (as long as the system incorporates dual FID detectors). Data acquisition and data processing requirements can easily be addressed using SoftLINK™ and TotalChrom™ software (or Series 600 link box and TurboChrom® software).

### Instrument parameters

Dual column analysis using a two-hole ferrule. 1.0 mL headspace sample of a blood alcohol mix.

<b>Oven temperature:</b>	40 °C isothermal
<b>Inj. temperature:</b>	200 °C
<b>Carrier gas:</b>	He
<b>Sample equilibration temperature:</b>	70 °C
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<b>Vial pressurization time:</b>	0.15 min.
<b>Vial sampling time:</b>	0.01 min.
<b>Transfer line:</b>	0.32 mm ID FS Hydroguard™ tubing
<b>Transfer line temperature:</b>	200 °C
<b>Injection port sleeve:</b>	2 mm ID
<b>Split flow:</b>	20 mL-per-minute

The TurboMatrix headspace products use a balanced pressure sampling procedure to transport the sample to the GC. This type of sampling works better with columns that require higher head pressure, so 0.32 mm ID analytical columns were chosen for this application because of their higher operating pressure. Optimal performance of these columns during headspace analysis depends on GC/headspace system set up. Band broadening can occur if there is excess dead volume in the sample flow path between the sample valve and the head of the column. Low volume inlet liners or interfaces in the injection port should be used to reduce the amount of excess volume at the exit end of the transfer line. A 2 mm ID zero dilution liner was used in this analysis to reduce dead volume and maintain narrow

peak widths. High carrier gas flow rates through the transfer line can also be used to maintain narrow sample bandwidths and speed up sample transfer to the head of the column. A flow of 40 mL-per-minute was used to optimize the analysis on the headspace autosampler.

Simulated blood alcohol samples were prepared and analyzed using a modification of a procedure published by Christmore et al.<sup>1</sup> *n*-Propanol was used as the internal standard and was prepared at a concentration of 0.03 g/dL in 1.0 M ammonium sulfate as a diluent. Five milliliters of diluent was added to 1 mL of sample in a 20 mL headspace vial.

### Results

We demonstrated here that a PerkinElmer headspace autosampler coupled with dual-column GC confirmational analysis provided excellent accuracy and precision in the analysis of blood alcohol. Calibration curves were constructed using concentrations ranging from 0.01% to 0.5% ethanol. Correlation coefficients above 0.999 were easily obtained for all compounds. Response factor repeatability was less than +1% standard deviation while analyzing six samples at a concentration of 0.2% ethanol. Based on our experimentation, a system detection limit of approximately 0.001% ethanol should be achievable while maintaining a minimum signal-to-noise ratio of 10.

### Conclusion

Successful blood alcohol analysis can be performed in less than 3 minutes per sample using PerkinElmer headspace autosampler and gas chromatograph equipped with Elite BAC-1 and BAC-2 GC columns and dual FID detectors.

### References

1. D. S. Christmore, R. C. Kelly, and L. A. Doshier, *Journal of Forensic Science*, Volume 29, No. 4, October 1984, pp.1038-1044.

## Product listing

### Elite BAC-1 GC Columns

Length (m)	ID (mm)	df (µm)	Part No.
30	0.53	3.0	N9316578
30	0.32	1.8	N9316579

### Deactivated Fused Silica Transfer Lines

Length (m)	ID (mm)	OD (mm)	Part No.
5	0.18	0.34	N9301354
5	0.25	0.37	N9301356
5	0.32	0.45	N9301357
5	0.53	0.69	N9301358

### Dual-Column Analysis Replacement Ferrules – Two-hole

Ferrule i.d. (mm)	Nut Size (in.)	Column i.d. (mm)	Graphite	Graphite/Vespel®
0.4	1/16	0.18-0.25	N/A	04972392 (1-pk)
0.5	1/16	0.32	N9306001 (10-pk)	N9306000 (10-pk)
0.5	1/8	0.32	09903395 (10-pk)	N/A

### Elite BAC-2 GC Columns

Length (m)	ID (mm)	df (µm)	Part No.
30	0.53	2.0	N9316576
30	0.32	1.2	N9316577

### Splitless Liners

Description	Part No.
Quartz Liner (2 mm)	N6121002
Glass Liner (2 mm)	N6101372
Deactivated Liner (2 mm, packed with wool)	N6121021

### Headspace Autosampler Vials & Seals

Description	Part No.
22 mL clear vial, rounded bottom (100-pk)	B0104236
Headspace vial cap w/PTFE/Silicone septa (1000-pk)	B0104242

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