Determine Blood Alcohol with Dual Column/Dual FID for Precision and Reproducibility

Application Note

Forensics and Toxicology

Authors

Haleigh Boswell and Frank Dorman
The Penn State University
University Park, PA, USA

Ken Lynam
Agilent Technologies, Inc.

Abstract

This application note highlights the use of Agilent J&W DB-ALC1 and DB-ALC2 columns for the analysis of blood alcohol concentration by static headspace GC/FID, using a Dual Channel Blood Alcohol Analyzer. The combination of a dual-column/dual-FID configuration delivers precision and reproducibility of the determined alcohol concentration within a complex blood matrix.

Introduction

Determining the ethanol content of blood from those charged with driving while intoxicated is one of the most common and widely used applications of headspace-gas chromatography [1]. With a universal threshold value of 0.08 g/dL, a robust and optimized method must be used to ensure the reported values are accurate. Although forensic laboratories perform this analysis routinely, minute errors can occur, ultimately altering the reported value.

Using an internal standard method for quantitative analysis helps compensate for matrix differences [1]. Other alcohols with similar characteristics to ethanol, such as *n*-propanol and *t*-butanol, are typically chosen as internal standards. This compensation occurs due to the internal standards undergoing the same matrix effects as the ethanol within the blood, due to their equivalent chemical polarity. Calibration using the internal standard method characteristically results in lower percent error when compared to the external standard method.

Agilent Technologies

Through the use of replicate samples at both the method detection limit and threshold value, a relative standard deviation can be calculated to demonstrate the precision of the method. Blood alcohol samples can be analyzed in a headspace vial up to a possible maximum of 0.30 g/dL with *n*-propanol and *t*-butanol as the internal standard.

Materials and Methods

An Agilent 7890B GC/FID equipped with a spit/splitless inlet, an Agilent 7697A Headspace Sampler with Headspace Control Software ChemStation Edition B.01.04, and Agilent OpenLab CDS ChemStation Edition for GC Systems C.01.05 software, were used for the GC/FID experiments.

GC conditions

Columns:	Agilent J&W DB-ALC1, 30 m × 0.32 mm, 1.8 µm (p/n 123-9134), DB-ALC2, 30 m × 0.32 mm, 1.2 µm (p/n 123-9234)
Carrier:	Helium
Oven:	40 °C (4.00 min)
Inlet:	Split mode, 250 °C, split ratio 20:1
Inlet liner:	Ultra Inert straight liner, 75 mm (p/n 5190-4048)
GC/FID:	Agilent 7890B GC equipped with dual FIDs
Sampler:	Agilent 7697A Headspace Sampler with 12-position tray

Flow path supplies

Vials:	Flat-bottom screw cap headspace vials, 20 mL (p/n 5188-2753)
Vial caps:	Screw caps and septa, PTFE/silicone, 18 mm (p/n 5188-2759, 100/pk)
Transfer line:	Deactivated tubing, 0.53-mm id (p/n 160-2535-5)
Tee fitting:	Capillary Flow Technology, 2-way unpurged splitter (p/n G3181B)
Septum:	Bleed and Temperature Optimized, BTO 11 mm septa (p/n 5183-4757, 50/pk)
Gold seals:	Ultra Inert Gold Seals (p/n 5190-6145, 10/pk)
CFT ferrules:	Flexible Metal ferrules (p/n G3188-27502 for 0.32-id column, 10/pk; p/n G3188-26503 for 0.53-mm id tubing, 10/pk), internal nut (p/n GB2855-20530)
Inlet/FID:	85:15 Vespel:graphite ferrules (p/n 5062-3514, 10/pk)

The general arrangement of the experimental setup is shown in Figure 1.

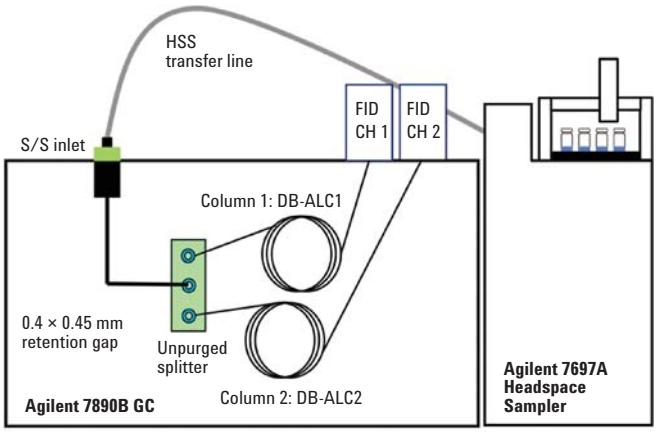

Figure 1. Experimental setup using Agilent dual-column/dual-FID for the detection of blood alcohol.

Sample preparation

Reference standards of ethanol were purchased from Restek, Bellefonte, PA, USA. These standards were subjected to the sample preparation process used for all samples by addition of 500 µL of each reference standard solution to 4.5 mL distilled water and 5 µL diluted internal standard.

The stock internal standard solution was prepared in a 1:10 dilution of either *n*-propanol or *t*-butanol (J. T. Baker) in distilled water, so that the final working concentration was nominally 0.08g/dL. Stock blood samples of known ethanol concentrations, 0.02 g/dL and 0.08 g/dL, were prepared with 20 mL of known sterilized sheep's blood with either 5 µL or 20 µL of ethanol (200 proof pure ethanol, KOPTEC USP), respectively.

Results and Discussion

Figures 2 and 3 show the chromatograms from DB-ALC1 (FID1) and DB-ALC2 (FID2) for the standard resolution mixture of eight separate compounds. Each standard was accurately matched with its corresponding individual standard by retention time, for qualitative identification. Despite the fact that the resolution mix had several coelutions on individual columns, retention times on DB-ALC1 and DB-ALC2 resolved into a discernible elution order for peak identification. This dual-column approach offers an advantage in that the elution order of ethanol and some other common metabolites differs on the two different stationary phases. This provides added confirmation and a potential reduction in interferences or coelutions with ethanol with a simple to use dual-column dual-FID approach.

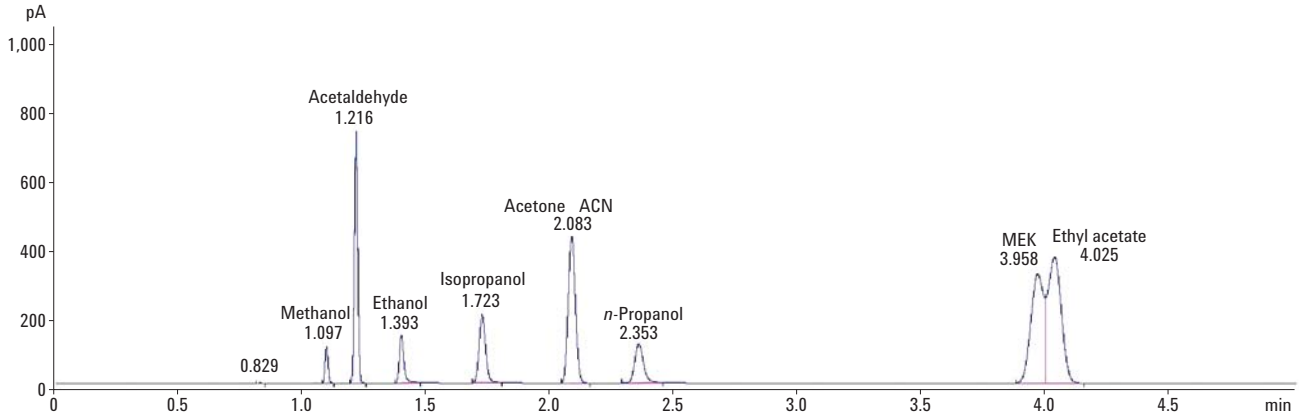

Figure 2. Resolution mix on an Agilent J&W DB-ALC1 GC column on FID 1.

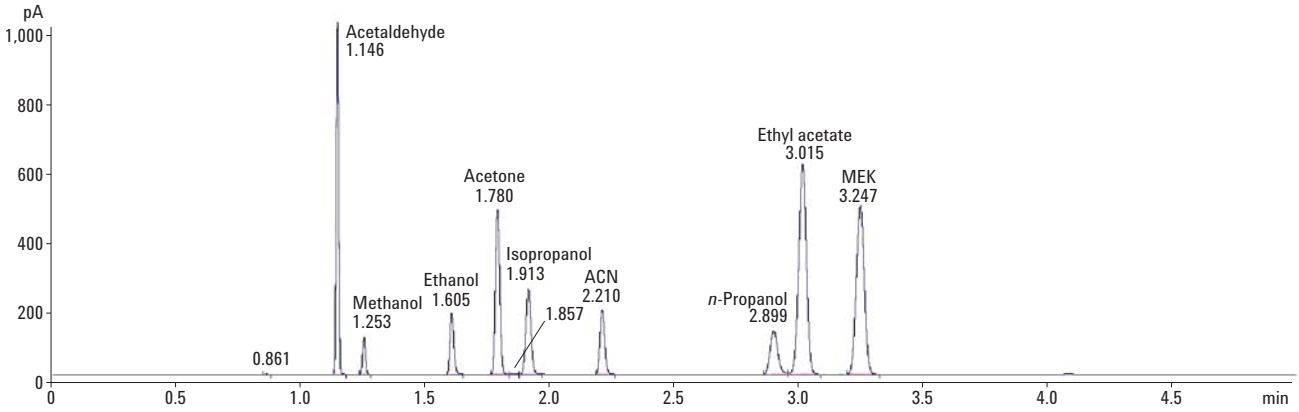

Figure 3. Resolution mix on an Agilent J&W DB-ALC2 GC column on FID 2.

Table 1 summarizes the results obtained for 10 replicate samples run at nominal concentrations of 0.02% and 0.08% ethanol using both *t*-butanol and *n*-propanol internal standard approaches. In general, the variability observed with the *t*-butanol approach was higher than the n-propanol approach. The % RSD values for the 0.02% and 0.08% ethanol replicates ranged as high as 17.08% using *t*-butanol internal standard and to 4.53% using *n*-propanol as an internal standard. Consistent % RSD values below 5% for the *n*-propanol internal standard at both the 0.02 and 0.08% ethanol level speak well for this approach.

Figures 4 and 5 are calibration curves for the two internal standard approaches. It is evident that *n*-propanol is the better choice, given that it produced results closer to known values. The higher relative response factor and higher regression line slope are arguments for using *n*-propanol as an internal standard over *t*-butanol.

t-Butanol, being a low melting solid (melting point 25 to 26 °C) can be problematic to handle in laboratories where temperature control is less than ideal, particularly in the winter months. *n*-Isopropanol has a melting point of –89 °C and remains liquid well below the freezing point of water.

Table 1.Mean relative response factor and calculated ethanol mean, standard deviation, variance, and percent RSD values for *t*-butanol and *n*-propanol internal standards.

	***t*-Butanol**				***n*-Propanol**			
	0.02 reps		**0.08 reps**		**0.02 reps**		**0.08 reps**	
	FID 1	**FID2**	**FID 1**	**FID 2**	**FID 1**	**FID 2**	**FID 1**	**FID 2**
Mean	0.20586	0.01562	0.08132	0.08156	0.01986	0.01938	0.08916	0.08823
St Deviation	0.03516	0.00067	0.00755	0.00749	0.00087	0.00088	0.00398	0.00400
Variance	1.24E-03	4.43E-07	5.71E-05	5.61E-05	7.57E-07	7.71E-07	1.59E-05	1.60E-05
% RSD	17.08%	4.26%	9.29%	9.18%	4.38%	4.53%	4.47%	4.53

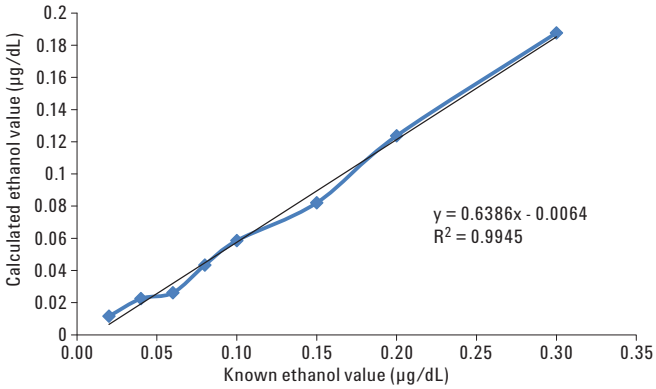

Figure 4A. Calibration curve for *n*-propanol internal standard on FID 1

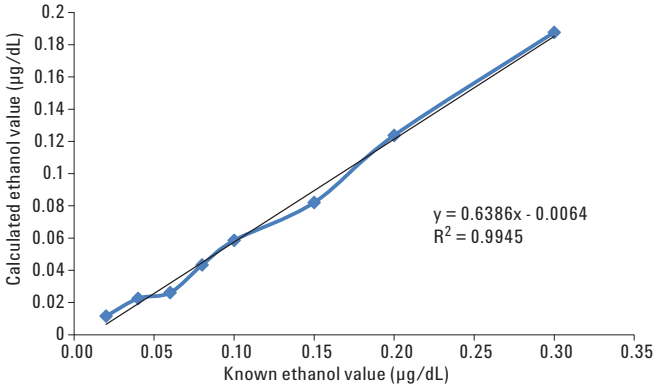

Figure 4B. Calibration curve for *n*-propanol internal standard on FID 2.

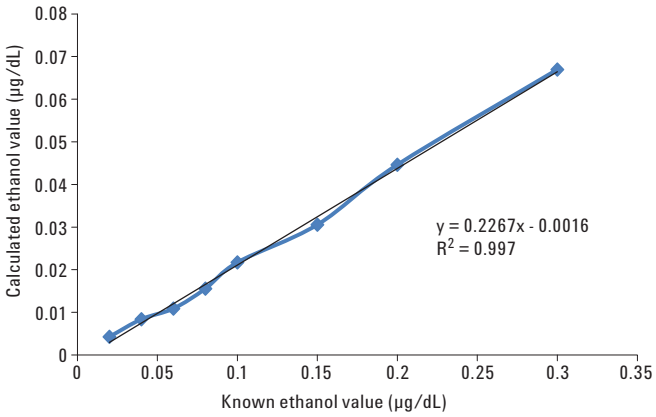

Figure 5A. Calibration curve for *t*-butanol internal standard on FID 1.

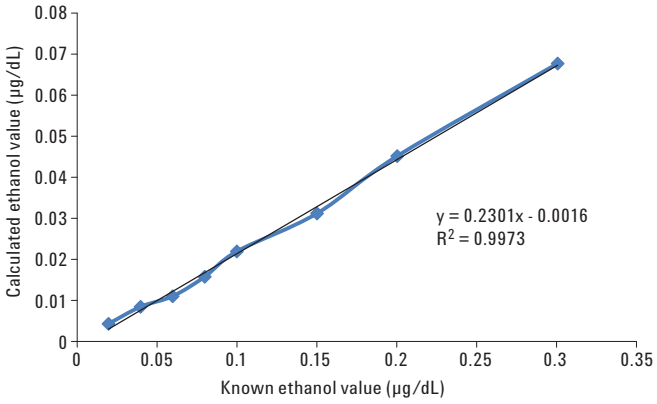

Figure 5B. Calibration curve for *t*-butanol internal standard on FID 2.

Figure 6 is an overlay showing the early system peak and the peaks of interest on both columns. It is evident that the system peak does not interfere with the peaks of interest.

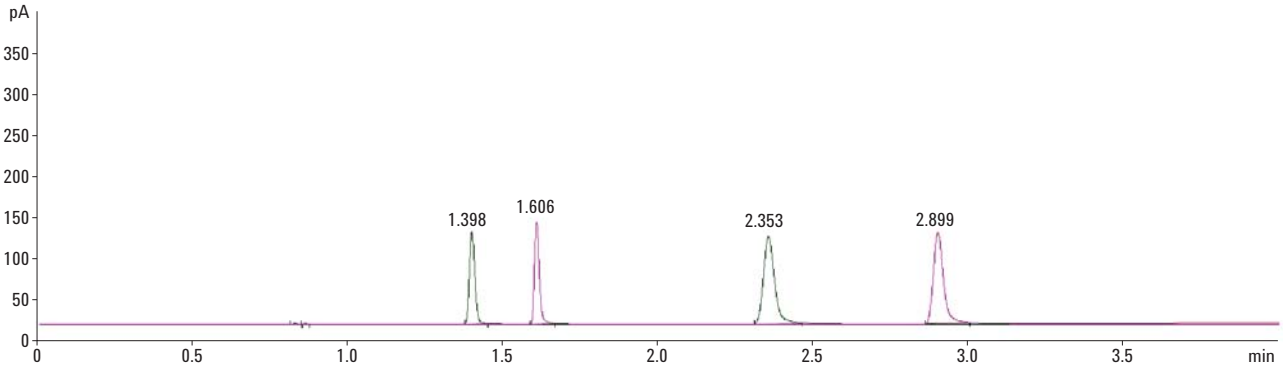

Figure 6. Overlay chromatogram showing early elution of a system peak that is well separated from ethanol and internal standards.

Figures 7 and 8 are overlays of the first and last replicate of both ethanol concentrations. There is some variation in peak areas but baseline resolution is still achieved. It is noteworthy that variation was apparent even though all the samples were prepared by the same analyst at the same time.

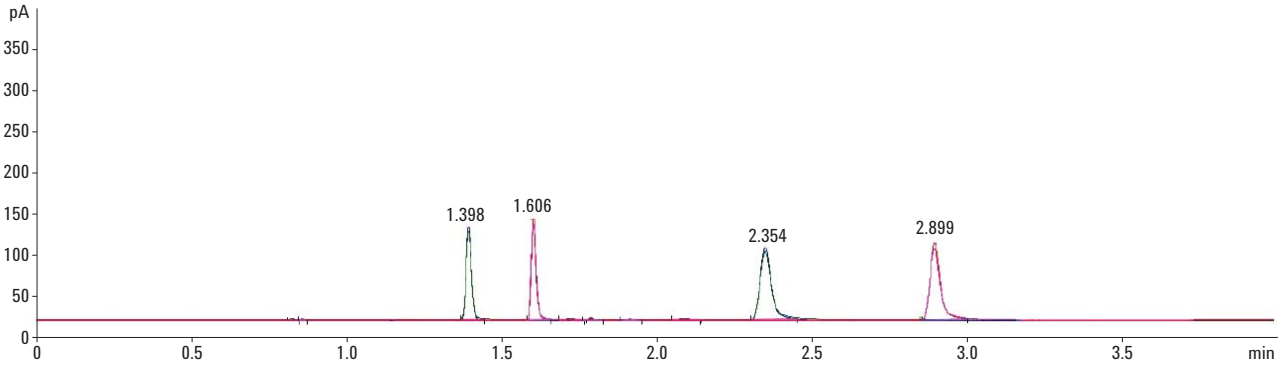

Figure 7. Overlay of the first and tenth replicates at 0.08% level.

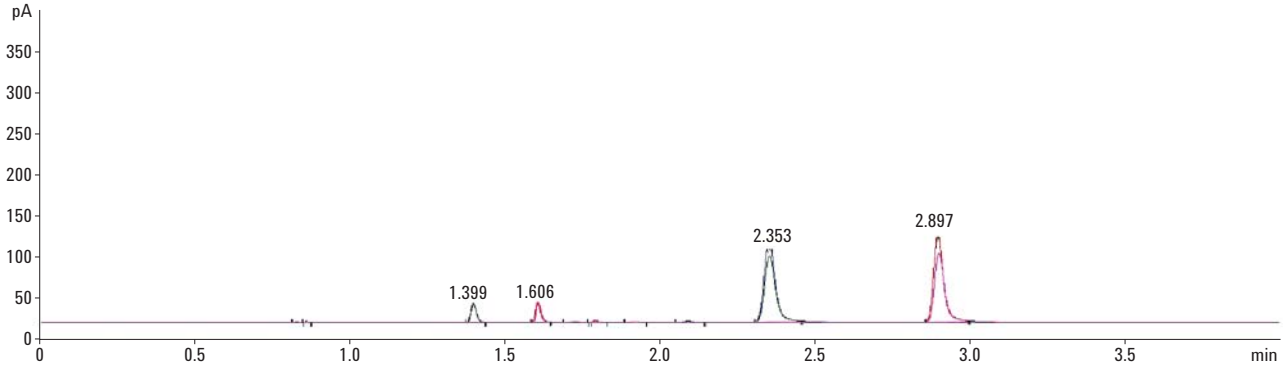

Figure 8. Overlay of the first and tenth replicates at 0.02% level.

Conclusions

The Agilent J&W DB-ALC1, 30 m × 0.32 mm, 1.8 µm and DB-ALC2, 30 m × 0.32 mm, 1.2 µm GC columns show excellent precision and reproducibility for the determination of blood alcohol concentration from a complex blood matrix. There was a significant difference between the internal standards, *n*-propanol, and *t*-butanol. Overall, *n*-propanol reproduced more precise results over replicate samples. Both columns showed 5% relative standard deviation or less with the 0.02 and 0.08 g/dL replicate samples, with *n*-propanol as the internal standard.

When the internal standards' method was applied to DB-ALC1 and DB-ALC2 using *n*-propanol, both columns produced less than 6% error on the continuing calibration verification. An error of about 10% was seen when using *t*-butanol internal standards continuing calibration verification.

The internal standards' method using *n*-propanol is the definitive choice for performing blood alcohol concentration analysis by static headspace GC/FID with a dual-column, DB-ALC1 and DB-ALC2, dual-FID configuration.

The fully automated Agilent GC headspace system for high-throughput blood alcohol analysis has a 3-minute cycle time, and provides excellent separation for ethanol, ISTD, and four potential interferences using dual column confirmation from split injection with CFT splitter, Flexible Metal ferrules, and Agilent J&W DB-ALC1 and DB-ALC2 columns.

Reference

1. B. Kolb, L. B. Ettre. *Static Headspace-Gas Chromatography: Theory and Practice* (2nd ed), John Wiley & Sons, Inc., Hoboken, NJ, USA (2006).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
November 27, 2013
5991-3671EN

Agilent Technologies