Improved Resolution and Peak Shape Performance for the Determination of Blood Alcohol Concentration Using Agilent J&W DB-BAC1 Ultra Inert and DB-BAC2 Ultra Inert Columns

Application Note

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Abstract

This application note highlights the use of Agilent J&W DB-BAC1 Ultra Inert and DB-BAC 2 Ultra Inert columns for the analysis of blood alcohol concentration by static headspace GC/FID using a Dual Channel Blood Alcohol Analyzer. The DB-BAC1 UI and DB-BAC2 UI showed good resolution for compounds encountered in the analysis of both ante- and post-mortem blood alcohol concentration.

Introduction

One of the most widely used applications of headspace gas chromatography is the determination of ethanol content in blood. This analysis was performed on samples from those charged with driving while intoxicated, with a universal threshold value of 0.08 g/dL [1]. Use of an internal standard for quantitative analysis, either *n*-propanol or *t*-butanol, assisted in compensating for matrix differences, due to their similarity to ethanol. Using the internal standard method for the calibration of ethanol resulted in lower percent errors when compared to the external standard method.

Analysis is often carried out using two columns with different stationary phases, for more reliable data. This study highlights the use of the Agilent J&W DB-BAC1 Ultra Inert and Agilent J&W DB-BAC2 Ultra Inert to improve the resolution of additional compounds, and inertness over time.

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Materials and Methods

An Agilent 7890B GC/dual FID equipped with a split/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 GC/FID experiments.

Experimental

Parameter	Value
GC Conditions	
Columns:	Agilent J&W DB-BAC1 UI, 30 m × 0.32 mm, 1.8 µm (p/n 123-9334UI) Agilent J&W DB-BAC2 UI, 30 m × 0.32 mm, 1.2 µm (p/n 123-9434UI)
Carrier:	Helium, constant flow, 6 mL/min
Oven:	40 °C (5.00 min)
Inlet:	Split Mode, 210 °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 a 108 position tray
FID Conditions	
Temperature:	250 °C
Hydrogen:	30 mL/min
Air:	350 mL/min
Col + Makeup:	35 mL/min
HS Conditions	
Oven:	70 °C (15 min equilibration)
Loop:	80 °C
Transfer line:	90 °C
Flow path supplies	
Vials:	Flat-bottom crimp cap headspace vials, 20 mL (p/n 5182-0837, 100/pk)
Vial caps:	Crip caps and septa, PTFE/silicone, 20 mm (p/n 5183-4478, 100/pk)
Transfer line:	Deactivated fused silica, 0.45 mm id (p/n 160-2455)
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 seal:	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;
Inlet/FID:	85:15 Vespel: graphite ferrules (p/n 5062-3514, 10/pk)

Standards

Calibration standards

Table 1. Ethanol calibration standards.

Part no.	Name	Description
5190-9756	Ethanol 20 mg/dL standard	Ethanol 20 mg/dL or 0.2 g/L, in water, (1 mL × 10)
5190-9757	Ethanol 50 mg/dL standard	Ethanol 50 mg/dL or 0.5 g/L, in water, (1 mL × 10)
5190-9758	Ethanol 80 mg/dL standard	Ethanol 80 mg/dL or 0.8 g/L, in water, (1 mL × 10)
5190-9759	Ethanol 100 mg/dL standard	Ethanol 100 mg/dL or 1.0 g/L, in water, (1 mL × 10)
5190-9760	Ethanol 150 mg/dL standard	Ethanol 150 mg/dL or 1.5 g/L, in water, (1 mL × 10)
5190-9761	Ethanol 200 mg/dL standard	Ethanol 200 mg/dL or 2.0 g/L, in water, (1 mL × 10)
5190-9762	Ethanol 300 mg/dL standard	Ethanol 300 mg/dL or 3.0 g/L, in water, (1 mL × 10)
5190-9763	Ethanol 400 mg/dL standard	Ethanol 400 mg/dL or 4.0 g/L, in water, (1 mL × 10)

Resolution mix

Agilent Blood Alcohol Checkout Mix (p/n 5190-9765)
50 mg/dL each in water, 1 mL.

Table 2. Target compounds contained in Agilent Blood Alcohol Checkout Mix (p/n 5190-9765).

No	Compound	No	Compound
1	Methanol (MeOH)	7	*n*-Propanol (*n*-C_3OH)
2	Acetaldehyde	8	Acetone
3	Ethanol (EtOH)	9	Acetonitrile (ACN)
4	Isopropanol (IPA)	10	2-Butanol (2-BuOH)
5	*t*-Butanol (*t*-BuOH)	11	Ethyl acetate (EtAc)
6	Propanal	12	2-Butanone

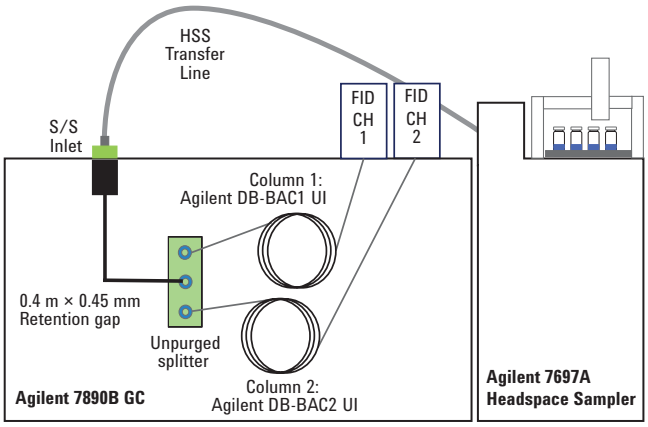

Figure 1. Experimental setup using Agilent dual-column/FID for the detection of blood alcohol.

Sample preparation

Agilent reference standards were used for the experiments. These standards were subjected to the sample preparation process used for all samples, that is, the addition of 500 µL of each reference standard solution to 4.5 mL deionized water and 5 mL diluted internal standard.

Stock internal standard solution of 1.5 g/dL of *t*-butanol or *n*-propanol (Fluka) was prepared by dilution in deionized water. From this stock, a working solution was prepared by dilution in deionized water to a final working concentration of 0.150 g/dL in vial. Known ethanol calibration standards and resolution mix were prepared according to the sample preparation procedure.

Results and Discussion

Figures 2 and 3 show the chromatograms from DB-BAC1 UI (FID1) and DB-BAC2 UI (FID 2) for the Agilent blood alcohol checkout mix containing 12 separate compounds. Each standard was accurately matched with its corresponding standard retention time, for qualitative identification. All 12 compounds were resolved completely on both the DB-BAC1 UI and the DB-BAC2 UI, and were in a discernible elution order for peak identification. The dual-column approach offers an advantage in that the elution order of ethanol and some other compounds differs on the two stationary phases. This approach provides added confirmation, and further reduces interferences and coelutions with ethanol.

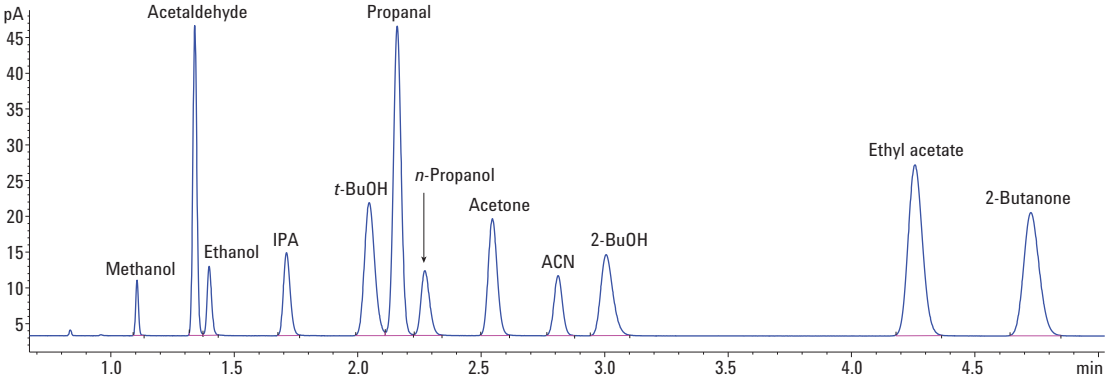

Figure 2. Resolution Mix on an Agilent J&W DB-BAC1 Ultra Inert GC column on FID1.

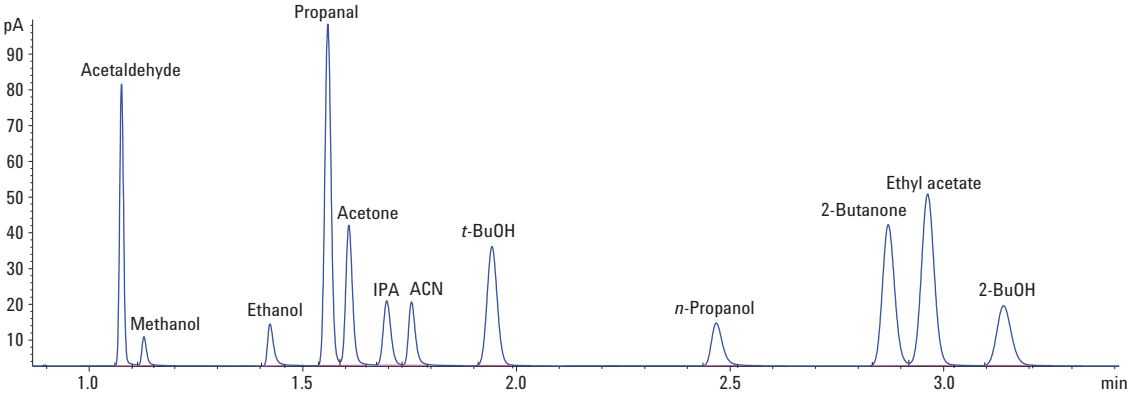

Figure 3. Resolution Mix on an Agilent J&W DB-BAC2 Ultra Inert GC column on FID2.

Reproducibility and Resolution Study

Figure 4 shows the offset expanded view of the resolution between acetaldehyde and ethanol from the first and last injection of a blood alcohol checkout mix of more than 175 injections. Over this period, the resolution and peak shape remained consistent, demonstrating the extended longevity and robustness of these columns.

Ten replicates of a 12-component resolution mix were run on both DB-BAC1 Ultra Inert (Table 3) and DB-BAC2 Ultra Inert (Table 4) columns, over 100 injections. All of the compounds had a %RSD less than 0.1%, with most less than 0.05%, illustrating the reproducibility and stability of the DB-BAC1 Ultra Inert and DB-BAC2 Ultra Inert columns over an extended period of time.

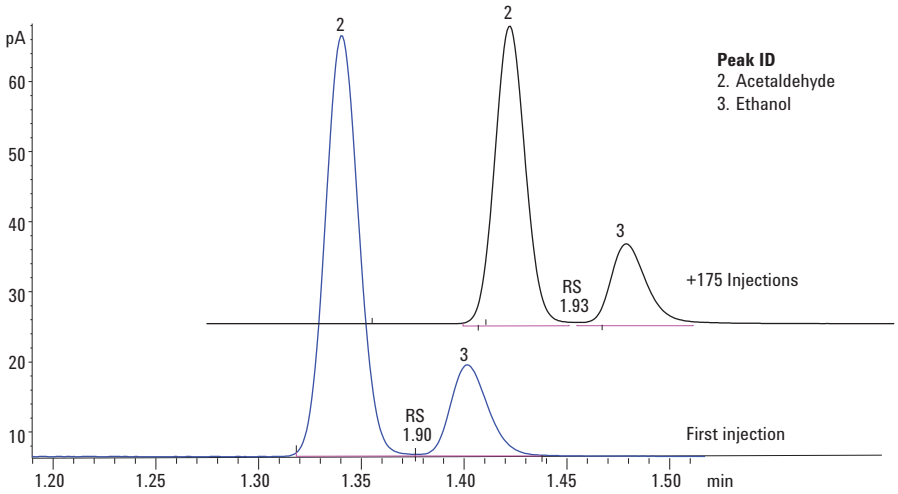

Figure 4. Expanded view of the resolution between acetaldehyde and ethanol, offset of the first and 175th+ injection of a 12-component resolution mix on an Agilent J&W DB-BAC1 Ultra Inert GC column on FID1.

Table 3. Ten replicates of a 12-component mix run over 100 injections on an Agilent J&W DB-BAC1 Ultra Inert Column on FID1.

	Replicate number											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Average**	**%RSD**
Methanol	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	1.107	0.000
Acetaldehyde	1.340	1.340	1.341	1.340	1.341	1.341	1.341	1.340	1.341	1.340	1.341	0.039
EtOH	1.402	1.402	1.403	1.402	1.403	1.403	1.403	1.403	1.403	1.403	1.403	0.034
IPA	1.717	1.718	1.718	1.718	1.719	1.719	1.719	1.719	1.719	1.718	1.718	0.041
t-BuOH	2.054	2.055	2.055	2.055	2.056	2.055	2.056	2.055	2.056	2.055	2.055	0.031
Propanal	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	2.162	0.000
n-C_3OH	2.281	2.282	2.283	2.283	2.284	2.284	2.285	2.284	2.285	2.284	2.284	0.056
Acetone	2.551	2.552	2.552	2.552	2.552	2.552	2.552	2.552	2.552	2.551	2.552	0.017
ACN	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.815	2.814	2.815	0.011
2-BuOH	3.019	3.020	3.021	3.021	3.023	3.023	3.024	3.023	3.024	3.023	3.022	0.057
EtAc	4.267	4.267	4.267	4.267	4.268	4.268	4.268	4.268	4.268	4.267	4.268	0.012
2-Butanone	4.739	4.739	4.740	4.740	4.740	4.740	4.740	4.739	4.740	4.739	4.740	0.011

Table 4. Ten replicates of a 12-component mix run over 100 injections on an Agilent J&W DB-BAC2 Ultra Inert Column on FID2.

	Replicate number											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Average**	**%RSD**
Acetaldehyde	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	1.075	0.000
Methanol	1.129	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	1.130	0.028
EtOH	1.426	1.427	1.427	1.426	1.428	1.427	1.428	1.427	1.428	1.426	1.427	0.057
Propanal	1.559	1.559	1.559	1.559	1.559	1.559	1.559	1.558	1.559	1.558	1.559	0.027
Acetone	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	1.609	0.000
IPA	1.700	1.701	1.701	1.701	1.702	1.701	1.702	1.701	1.702	1.700	1.701	0.043
ACN	1.755	1.755	1.755	1.755	1.756	1.755	1.756	1.755	1.756	1.755	1.755	0.028
t-BuOH	1.948	1.948	1.948	1.948	1.949	1.948	1.949	1.948	1.949	1.948	1.948	0.025
n-C_3OH	2.475	2.475	2.476	2.475	2.476	2.476	2.476	2.475	2.477	2.475	2.476	0.028
MEK	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.873	2.872	2.873	0.011
EtAc	2.965	2.965	2.965	2.965	2.965	2.965	2.965	2.964	2.965	2.964	2.965	0.014
2-BuOH	3.150	3.150	3.150	3.150	3.151	3.150	3.151	3.150	3.151	3.149	3.150	0.020

Calibration and internal standard selection

Figures 5 and 6 show the calibration curves for the two internal standard approaches. Figures 5A and 5B demonstrate the linearity of the calibration standards, when used with *t*-butanol as an internal standard.

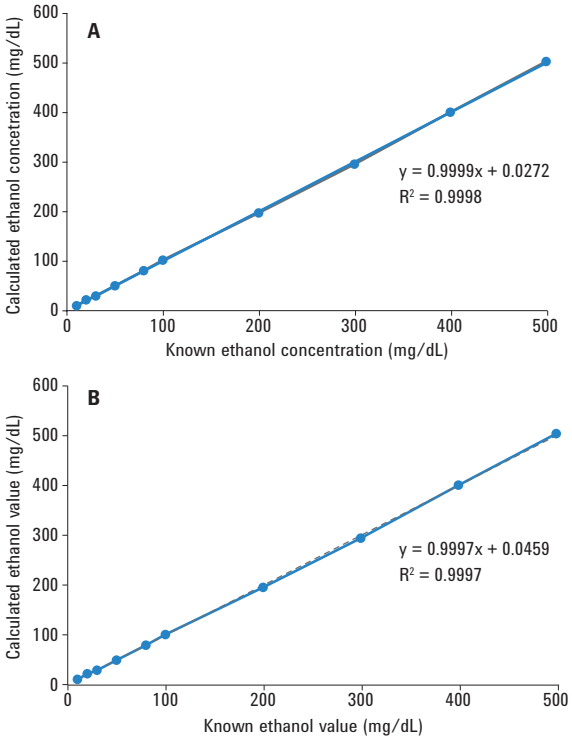

Figure 5. Calibration curve with *t*-butanol as the internal standard on FID1 (A) and FID 2 (B).

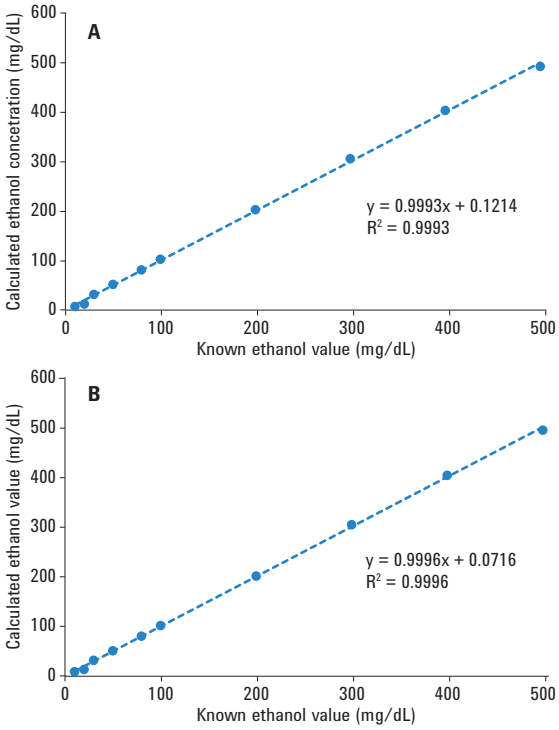

Figure 6. Calibration curve with *n*-propanol as the internal standard on FID1 (A) and FID 2 (B).

Due to its low-melting point (25 °C to 26 °C), *t*-Butanol can be difficult to work with in laboratories where temperature control is less than ideal. Working with a higher concentration stock solution (1.5 g/dL) helps keep *t*-butanol dissolved and in solution, making it easier to accurately dilute, and use as a working internal standard solution.

While *n*-propanol still provides a higher response factor than *t*-butanol, both internal standard approaches display great linearity in conjunction with the ethanol calibration standards.

Conclusions

Agilent J&W DB-BAC1 Ultra Inert, 30 m × 0.32 mm, 1.8 µm and Agilent J&W DB-BAC2 Ultra Inert, 30 m × 0.32 mm, 1.2 µm columns were evaluated for the analysis of blood alcohol concentration from a water matrix, with the addition of a 12-component checkout mix. Two internal standards, *n*-propanol and *t*-butanol, were used, and no significant differences were found in their use for the calibration of ethanol.

The internal standard method can be performed using *n*-propanol or *t*-butanol as the internal standard for the analysis of blood alcohol concentration analysis by static headspace GC/FID with a dual-column, DB-BAC1 UI and DB-BAC2 UI, dual FID configuration.

The improved inertness of the of the DB-BAC1 UI and DB-BAC2 UI columns provides better peak shape and resolution for 11 additional compounds that have been associated with potential interference using dual-column confirmation.

Reference

1. K. Lynam, F. Droman, H. Boswell, *Determine Blood Alcohol with Dual Column/Dual FID Precision and Reproducibility*; Application note, Agilent Technologies, Inc. Publication number 5991-3671EN, **2013**.

For More Information

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