

Forensic Analysis of Blood Alcohol Concentration

Using the Agilent 8860 GC with Agilent J&W DB-BAC1 UI and Agilent J&W DB-BAC2 UI columns and the Agilent 7697A headspace sampler

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Abstract

This Application Note highlights the determination of blood alcohol concentration (BAC) using the Agilent 8860 GC configured with dual flame ionization detectors (FIDs) and an Agilent 7697A headspace sampler. The system demonstrates exceptional statistical performance, with linearity across an expected range of concentrations and repeatability through many injections. Productivity is greatly increased when implementing a splitter to simultaneously resolve the sample components on two columns: the Agilent J&W DB-BAC1 UI and the Agilent J&W DB-BAC2 UI.

Introduction

BAC corresponds to the percentage of ethanol (ethyl alcohol) in the blood stream, and can be measured by blood, urine, or breath tests. BAC analysis is routine in many forensic labs, with a standard workflow comprising headspace sampling and FID¹. The 8860 GC system is well suited for routine analyses such as BAC. Simple hardware configurations such as HSS-dual FID are easily accomplished with the 8860 GC interfaced to OpenLab CDS version 2.2, and will meet most method requirements. This Application Note describes a method for determining BAC with the 8860 GC, dual FIDs, and the 7697A headspace sampler.

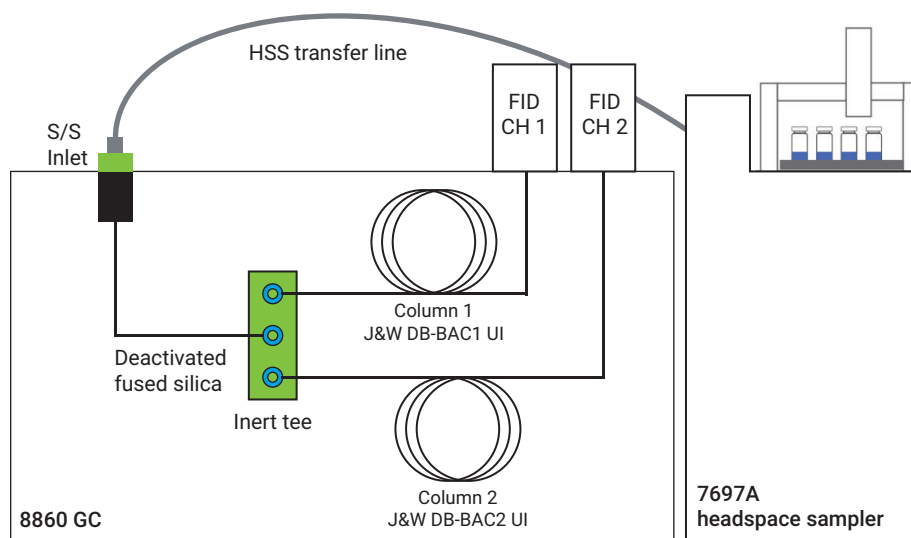


Figure 1. Experimental setup using dual-column/FID for the analysis of blood alcohol concentration.

Experimental

Equipment

An 8860 GC was configured with a split/splitless inlet (SSL) and dual FIDs, and sampling was performed using a 7697A headspace sampler.

The 8860 GC can use unpurged flow devices. In this case, an inert tee was installed to run the analysis in the dual-column, dual-FID configuration. Figure 1 displays a diagram showing this configuration.

Consumables

Table 1 lists consumables used in this Application Note. Table 2 lists the ethanol standards offered by Agilent. These standards can be combined to create accurate calibration standards, or used singly to check the accuracy of a calibration curve.

Table 1. List of consumables used for this application.

Consumable	Part number
10 mL clear, crimp top, headspace vials	5190-2285
Ultra Inert, low pressure drop, split inlet liner with glass wool	5190-2295
Inert tee for capillary flow technology	G3184-60065
Column inlet ferrules (graphite, 0.4 mm, for 0.25 mm columns)	500-2114
FID ferrules (graphite, 0.5 mm, for 0.32 mm columns)	5080-8853
CFT ferrules (UltiMetal Plus Flexible Metal ferrules, 0.32 mm id)	G3188-27502
Agilent J&W DB-BAC1 UI (30 m × 320 µm, 1.8 µm)	123-9334UI
Agilent J&W DB-BAC2 UI (30 m × 320 µm, 1.2 µm)	123-9434UI
Deactivated fused silica for precolumn to inert tee (30 m × 250 µm id × 350 µm od)	160-2255-30

Table 2. Ethanol calibration standard concentrations and corresponding part numbers.

Standard	Part number
20 mg/dL	5190-9756
50 mg/dL	5190-9757
80 mg/dL	5190-9758
100 mg/dL	5190-9759
150 mg/dL	5190-9760
200 mg/dL	5190-9761
300 mg/dL	5190-9762
400 mg/dL	5190-9763

Experimental parameters

Table 3 lists the method parameters for both the GC and headspace sampler used in this Application Note.

Sample preparation

Calibration standards were prepared in vials to achieve ethanol concentrations in the range of 10 to 320 mg/dL. The internal standard of *n*-propanol was added to achieve a final concentration of 100 mg/dL.

To determine repeatability, ethanol standards (n = 10) were prepared at 15 and 60 mg/dL with the internal standard at 100 mg/dL.

Table 3. GC and headspace sampler parameters.

GC parameter	8860 GC
Carrier gas	Helium
Inlet type	Split/splitless
Inlet temperature	110 °C
Inlet pressure	24 psi
Split ratio	10:1
Oven program	40 °C for 4.5 minutes
FID	250 °C
Headspace parameter	7697A headspace sampler
Vial pressurization gas	Helium
Loop size	1 mL
Vial size	10 mL
Vial shaking	Off
Caps	PTFE lined
Vial standby flow	20 mL/min
Vial equilibration time	7.00 minutes
Inject time	1.00 minutes
GC cycle time	4.50 minutes
Oven temperature	70 °C
Loop temperature	80 °C
Transfer line	0.53 mm id, deactivated fused silica through septum
Transfer line temperature	90 °C
Vial fill pressure	15 psi
Loop fill mode	Custom
Loop ramp rate	30 psi/min
Loop final pressure	1.5 psi
Loop equilibration time	0.05 minutes
Post injection purge	200 mL/min for 3 minutes
Carrier gas controlled by GC	

Calibration/linearity

Calibration was performed on both the J&W DB-BAC1 UI and J&W DB-BAC2 UI columns, first on the individual columns, then in the dual-column, dual-FID configuration. In both cases, a six-level calibration curve was collected at 10, 20, 40, 80, 160, and 320 mg/dL ethanol in

water with *n*-propanol at 100 mg/dL as an internal standard. Replicate injections ($n = 3$) were done at each level. Figures 2 and 3 show calibration curves for ethanol on the J&W DB-BAC1 UI and J&W DB-BAC2 UI columns in the single-column configuration.

After calibrating the columns individually, an inert tee was installed to determine simultaneous calibration curves. Figures 4 and 5 show the calibration curves for the dual-column, dual-FID configuration.

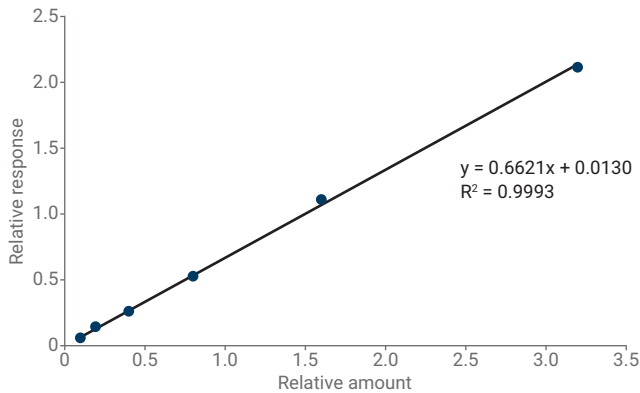


Figure 2. Calibration curve for ethanol relative to the internal standard on the J&W DB-BAC1 UI column in the single-column configuration.

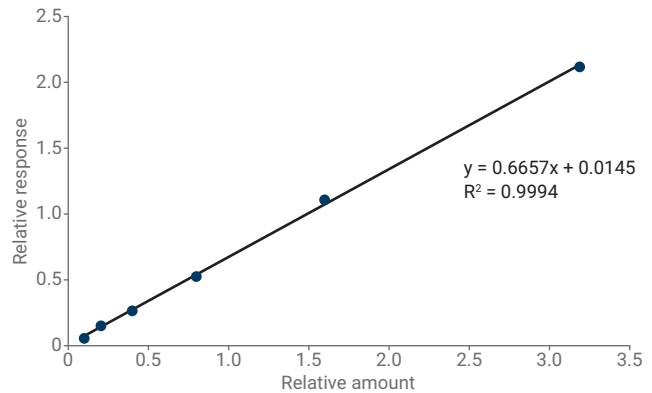


Figure 3. Calibration curve for ethanol relative to the internal standard on the J&W DB-BAC2 UI column in the single-column configuration.

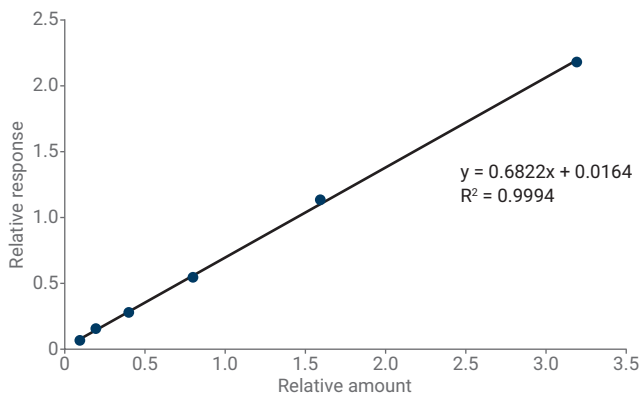


Figure 4. Calibration curve for ethanol relative to the internal standard on J&W DB-BAC1 UI in the dual-column configuration.

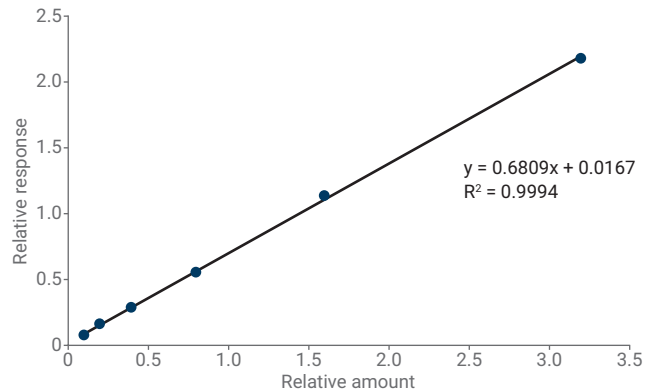


Figure 5. Calibration curve for ethanol relative to the internal standard on J&W DB-BAC2 UI in the dual-column configuration.

Repeatability

Area and retention time repeatability were determined at 15 and 60 mg/dL, respectively (Tables 4 to 6).

Table 4 shows that RSDs for each concentration and column are less than 2 %.

The retention times for all combinations of compound, column, and concentration stayed essentially constant at 0.07 % or less.

General resolution test with BAC checkout mix

To document the performance of the J&W DB BAC1-UI and J&W DB BAC2-UI columns, the Agilent BAC checkout mix (p/n 5190-9765) was analyzed using the dual-column configuration. Figure 6 shows chromatograms displaying the separation of the compounds in the mix on both the J&W DB-BAC1 UI and J&W DB-BAC2 UI columns.

The chromatograms in Figure 6 show that all 12 compounds in the BAC checkout mix are resolved from each other on both columns, including acetaldehyde and ethanol. All peaks also show symmetry on both columns.

Table 4. Relative standard deviations (RSD %) across 10 injections for the ethanol response relative to the internal standard response at 15 and 60 mg/dL on each column.

Column/concentration of ethanol	15 mg/dL	60 mg/dL
J&W DB-BAC1 UI	1.33 %	1.08 %
J&W DB-BAC2 UI	1.25 %	1.10 %

Table 5. RSD% across 10 injections of the retention time of ethanol at 15 and 60 mg/dL.

Column/concentration of ethanol	15 mg/dL	60 mg/dL
J&W DB-BAC1 UI	0.07 %	0.03 %
J&W DB-BAC2 UI	0.07 %	0.03 %

Table 6. RSD% across 10 injections of the retention time of *n*-propanol for the data sets with ethanol at concentrations of 15 and 60 mg/dL.

Column/concentration of ethanol	15 mg/dL	60 mg/dL
J&W DB-BAC1 UI	0.05 %	0.02 %
J&W DB-BAC2 UI	0.05 %	0.05 %

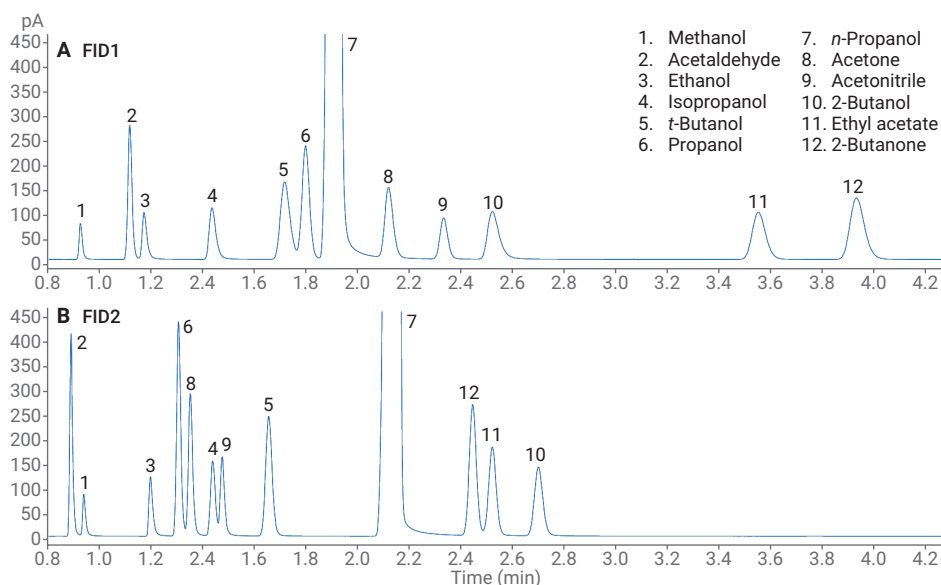


Figure 6. Chromatograms of BAC checkout mix separated using the J&W DB-BAC1 UI (A) and J&W DB-BAC2 UI (B) columns.

Conclusion

The 8860 GC system equipped with a 7697A headspace sampler allows for identification, quantitation, and confirmation of blood alcohol analytes in a single run, and meets most method requirements. Linearity on both columns is excellent in both the single and dual-column configurations, as is the precision for both area and retention time and the ability to effectively separate all compounds of interest from each other. The 8860 GC provides an economical yet effective method of determining ethanol concentration in routine analyses.

Reference

1. Boswell, H. A.; Dorman, F. L. Uncertainty of Blood Alcohol Concentration (BAC) Results as Related to Instrumental Conditions: Optimization and Robustness of BAC Analysis Headspace Parameters. *Chromatography* **2015**, 2, 691–708.

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