Application Note

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Blood Alcohol Analysis with the Integrated Agilent 8697 Headspace Sampler on 8890 GC-Dual FID System

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Abstract

Legal blood alcohol concentration (BAC) limits for operating motor vehicles vary around the globe, with many countries setting intoxication levels at either 0.05 or 0.08%. The statistical assessment of the Agilent 8697 headspace sampler connected to the Agilent 8890 GC shows excellent ethanol linearity between 0.02 to 0.4%. The instrumentation features a dual column, dual-FID configuration, and vial-to-vial reproducibility at the 0.05% concentration, with less than 2% RSD for most alcohol and carbonyl compounds in a mix.

Introduction

BAC analysis by GC is an established test in legal, forensic, and diagnostic scenarios. Blood is an extremely complex mixture of largely nonvolatile material. This presents significant challenges for direct injections of whole blood samples in traditional GC approaches. Low-molecular-weight alcohols and/or alcohol metabolites, present in cases of intoxication or exposure, are much more volatile than the rest of the blood matrix, creating an opportunity for headspace sampling.

Incorporating a sampler to extract the volatile content of a sample minimizes the inconvenience of both extensive sample preparation and human error. The 8697 headspace sampler builds on the valve and loop technology successfully demonstrated in the past, adding improved pneumatic control, full integration into the GC driver, and several user improvements. These improvements include step-by-step diagnostics, available through the browser interface or touch-screen interactions, indicator lights to confirm correct tray placement, and a reinforced transfer line. Similarities in parameter zones create a seamless migration from existing 7697A headspace parameters.[1,2,3]

Experimental

An Agilent 8890 GC, configured with a split/splitless inlet and dual flame ionization detectors (FID), was used to receive the sample from the Agilent 8697 headspace sampler. Additional system details are provided in Figure 1, with analytical standards and consumables used in testing provided in Table 1.

Method conditions are shown in Table 2. A 1 L batch of water (Millipore dispensed) containing 0.1% (v/v) *t*-butanol was prepared for the internal standard and solvent. Headspace vials for testing contained 0.5 mL of sample volume, with 50 µL of standard added to 450 µL of water/*t*-butanol solution.

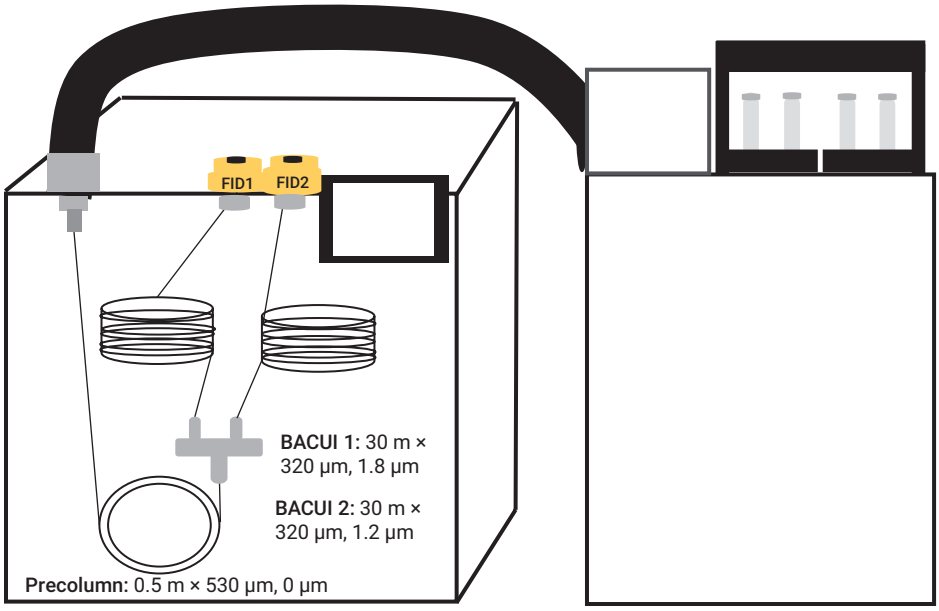

Figure 1. Configuration details of headspace/GC system used in testing.

Table 1. Instrument consumables and standards used in testing.

Consumables		Standards		Vendor	Test
20 mL vials and crimp caps	p/n 5190-2286	BAC resolution mix	5190-9765	Agilent	Resolution
GC liner (2 mm id)	p/n 5190-6168	Ethanol calibration	G3440-85035	Agilent	Linearity
Transfer line (deactivated fused silica)	0.53 mm id	*t*-Butanol, >99%	24127	Millipore/Sigma	
Columns		Custom solvents	Custom	Restek	Reproducibility
Precolumn: 0.5 m × 0.53 mm × 0 µm	p/n 160-2535-10				
Column 1: Agilent J&W DB-BAC1 UI (30 m × 0.32 mm × 1.8 µm)	p/n 123-9334UI				
Column 2: Agilent J&W DB-BAC2 UI (30 m × 0.32 mm × 1.2 µm)	p/n 123-9434UI				

Table 2. Headspace and GC conditions used in testing.

8697 Headspace Conditions	
Oven Temperature	70 °C
Loop Temperature	80 °C
Transfer Line Temperature	90 °C
Vial Equilibration	7 min
Injection Time	1 min
Vial Size	20 mL
Vial Fill Mode	Default
Fill Pressure	15 psi
Pressurization Gas	Nitrogen
Loop Fill Mode	Custom
Final Loop Pressure	1.5 psi
Loop Equilibration	0.05
Loop Volume	1 mL
8890 GC Conditions	
Inlet Temperature	150 °C
Split Ratio	10:1
Mode	Constant pressure
Inlet Pressure	21 psi (helium)
Oven	40 °C, isothermal for 5 minutes
FID Temperature (Both)	250 °C
Air Flow	400 mL/min
Hydrogen Flow	30 mL/min
Makeup (N_2)	25 mL/min

Results and discussion

Three tests were used to evaluate the 8697 headspace sampler performance: ethanol linearity, vial-to-vial reproducibility, and resolution of the BAC mix standard.

Ethanol linearity

A calibration curve was created using six data points between 0.02 and 0.4 mg/dL. Individual plots from a calibration test are provided in Figure 2. The curve regression was generated using the internal standard, *t*-butanol, and comparing relative ratios with no weighting. Several replicates of the ethanol linearity test were performed, with all R^2 values exceeding 0.9995 over this range.

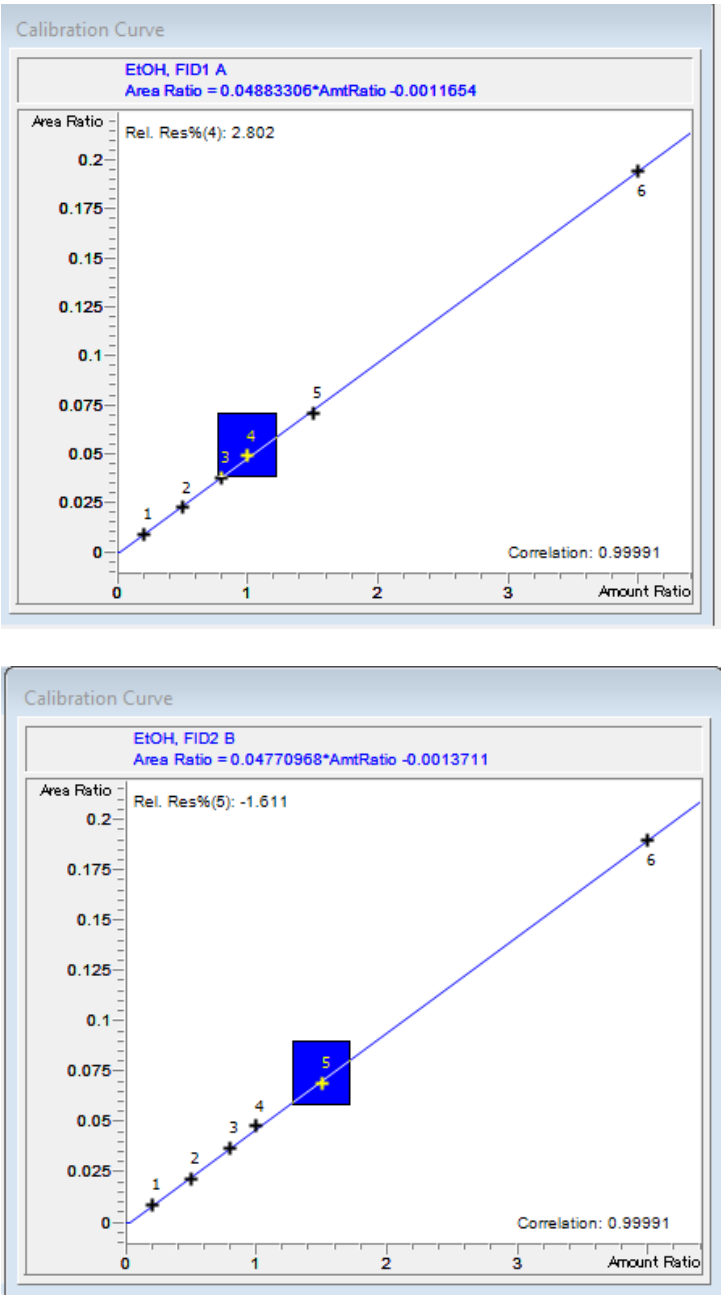

Figure 2. Ethanol calibration plots for both FIDs across the range of 0.02 to 0.4 mg/dL.

Reproducibility

A repeatability study was executed using a multicomponent solution added to the water/ISTD to create a 0.05 mg/dL solution concentration in each headspace vial. Table 3 details retention time and the reproducibility of each compound referenced to the internal standard, *t*-butanol, across 12 sequential injections.

Resolution

Using the column confirmation pair of Agilent J&W DB-BAC1 and DB-BAC2 UI provides significant resolution under these conditions. The flow can be accelerated or slowed, depending on the individual compound list and resolution requirements. Chromatograms for both columns are provided in Figure 3, with retention time and resolution data in Table 4.

Table 3. Vial-to-vial reproducibility of BAC mix standard.

Compound	FID 1		FID 2	
	RT	RRF	RT	RRF
Methanol	0.03%	2.06%	0.03%	1.72%
Acetaldehyde	0.04%	2.09%	0.00%	2.11%
Ethanol	0.00%	2.16%	0.02%	1.69%
Isopropanol	0.02%	1.49%	0.03%	1.34%
Acetone	0.02%	0.74%	0.00%	1.01%
1-Propanol	0.04%	1.90%	0.02%	1.61%
2-Butanone	0.04%	2.74%	0.02%	2.68%

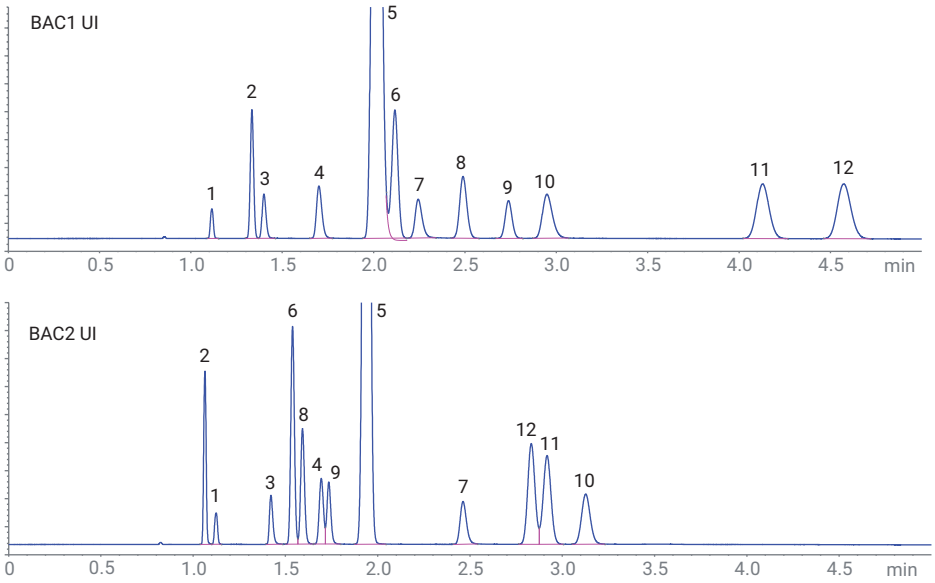

Figure 3. FID chromatograms of Agilent BAC resolution mix on Agilent J&W DB-BAC1 UI and DB-BAC2 UI columns.

Table 4. Peak IDs, retention times, and resolution (USP) calculations on BAC1 UI and BAC2 UI columns.

Peak Index	Compound Name	RT BAC1 UI (min)	Rs BAC1 UI	RT BAC2 UI (min)	Rs BAC2 UI
1	Methanol	1.071		1.081	2.7
2	Acetaldehyde	1.284	7.9	1.022	
3	Ethanol	1.348	2.0	1.371	11.5
4	Isopropanol	1.644	7.2	1.640	3.1
5	*t*-Butanol	1.953	5.2	1.881	5.3
6	Propanal	2.048	1.3*	1.485	4.1
7	*n*-Propanol	2.175	2.0*	2.388	10.6
8	Acetone	2.412	3.8	1.538	1.8
9	Acetonitrile	2.65	3.9	1.674	1.1
10	2-Butanol	2.867	3.0	3.043	3.3
11	Ethyl acetate	4.027	12.4	2.84	1.6
12	2-Butanone	4.456	4.1	2.753	7.1

The tabulated value for resolution is the comparative value of the target peak and the compound peak immediately preceding the target. The resolution values for propanal and *n*-propanol were manually calculated. To maintain a consistent *t*-butanol (ISTD) response between ethanol calibration and resolution mix standards, a tail skim was applied in the integration parameters. This modification disabled the automated calculation of the tangential peak width, which is required for USP resolution calculations (Figure 4). Because the elution order is not identical between the two columns, the low resolution compounds on column 1 are better separated on column 2, effectively demonstrating the complementary nature of this confirmation analysis. Alternatively, *n*-propanol can be used as an internal standard.

Peak attributes such as peak width and peak symmetry can be helpful indicators of the overall health of the system and consumables. The 8890 and Intuvo 9000 GC systems offers a feature called Peak Evaluation[4] that can be enabled to monitor key features of an application, notifying the user about slight differences before any loss of productivity is encountered. A simple firmware upgrade enables users of existing qualified GC mainframes with this feature.

$$R = \frac{2(t_{R2} - t_{R1})}{W_1 + W_2}$$

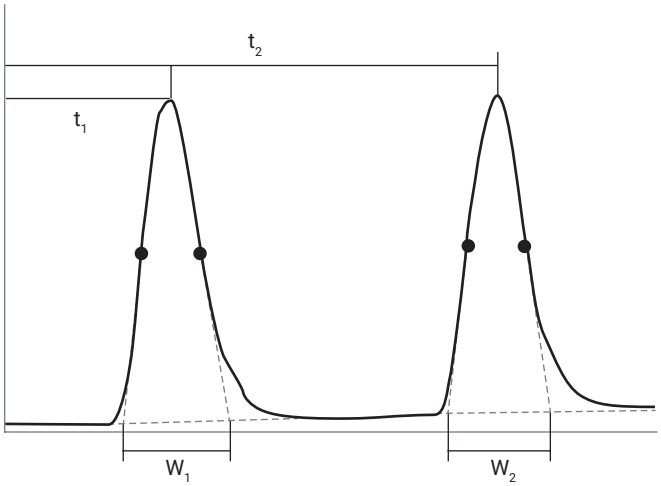

Figure 4. USP calculation for resolution as determined by Agilent OpenLab ChemStation edition, version C.1.10.

Conclusion

The Agilent 8697 headspace sampler lends itself to a simple method transfer and equivalent performance to the Agilent 7697A sampler. What the 8697 brings forward is an expansion of the GC intelligence in a fully integrated driver, improved user-guided diagnostics, and at-your-fingertips resources to walk the user through maintenance, operation, and troubleshooting to promote efficiency.

References

1. Analysis of Blood Alcohol Concentration with an Agilent Intuvo 9000 GC System. *Agilent Technologies application note*, publication number 5991-8999EN, **2020**.
2. Abercrombie, V. 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. *Agilent Technologies application note*, publication number 5991-8206EN, **2017**.
3. Wieder, L.; Pan, J.; Veeneman, R. Forensic Analysis of Blood Alcohol Concentration. *Agilent Technologies application note*, publication number 5994-0443EN, **2019**.
4. Agilent Technologies: GC Intelligence. https://www.agilent.com/en/product/gas-chromatography/gc-intelligence (accessed 16 February 2021).

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