Static Headspace Blood Alcohol Analysis with the G1888 Network Headspace Sampler

Application

Forensics

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Abstract

A G1888 Network Headspace Sampler coupled to a 6890N gas chromatograph was used for the determination of forensic blood alcohols. Standard mixtures in water were used to demonstrate the analyses. Two headspace systems, based on 0.53-mm and 0.32-mm id columns, are described. Isothermal analyses with cycle times below 5 min are easily achieved with sufficient resolution to avoid common interferences. A new automated headspace Sampler with 70-sample tray and inert flow path is introduced in this application. Total system control from the GC ChemStation is possible with new 21 CFR Part 11 compliant software specific for headspace sampling.

Introduction

Blood alcohol analysis is a widely used, high-throughput application in forensic laboratories. The use of static headspace sampling has many well known advantages for the determination of volatiles in a variety of less than ideal matrices. Blood or other biological fluids are certainly not the cleanest of matrices and, therefore, are well suited for headspace sampling. In terms of GC analysis, some of the advantages of automated headspace include reduced inlet and column maintenance, better quantitation, limited sample preparation, and increased throughput. The G1888 Network Headspace Sampler employs a completely inert flow path, uniform heated zones, and unique vent line purging capability. When taken together, these attributes lead to a reduction in carryover with improved repeatability.

Dual-column systems offer an advantage in that elution order of ethanol and some other common metabolites differ on the DB-ALC1 and DB-ALC2 stationary phases. This provides added confirmation and a potential reduction in possible inferences or co-elutions with ethanol. See Table 1 for a listing of instrument settings.

Experimental

Table 1. Instrument Conditions

0.53-mm Column System

6890N GC

Injection port	Split/Splitless
Temperature	250 °C
Split ratio	10:1
Carrier gas	Helium
Carrier flow	12 mL/min
Detector	FID, 300 °C

GC Oven Program

Initial temperature	40 °C
Initial time	5 min

G1888A Headspace Sampler

Loop size	1 mL
Vial pressure	9.0 psig
Headspace oven	70 °C
Loop temp	80 °C
Transfer line temp	90 °C
Equilibration time	10 min, high shake
GC Cycle time	4 min
Pressurization	0.2 min
Vent (loop fill)	0.2 min
Inject	0.5 min

Columns

DB-ALC1	30 m × 0.53 mm × 3.0 µm
DB-ALC2	30 m × 0.53 mm × 3.0 µm
Guard column	0.15 m × 0.53 mm deactivated fused silica

Y splitter, deactivated Agilent part no. 5181-3398

0.32-mm Column System

6890N GC

Injection port	Split/Splitless
Temperature	150 °C
Split ratio	5:1
Carrier gas	Helium
Inlet pressure	18.8 psi
Detector	FID, 300 °C

GC oven program

Initial temperature	35 °C
Initial time	7 min

G1888A Headspace Sampler

Loop size	1 mL
Vial pressure	11.5 psig (supplied by GC EPC Aux)
Headspace oven	60 °C
Loop temp	70 °C
Transfer line temp	80 °C
Equilibration time	15 min, high shake
GC cycle time	6 min
Pressurization	0.15 min
Vent (loop fill)	0.15 min
Inject	0.5 min

Columns

DB-ALC1	30 m × 0.32 mm × 1.8 µm
DB-ALC2	30 m × 0.32 mm × 1.2 µm

Two-hole ferrule Agilent part no. 5062-3580

Two columns are connected to one split/splitless injection port in both systems. This allows simultaneous injection into both columns with each connected to an flame ionization detector (FID). A glass Y connector/retention gap and two-hole ferrule are used for the 0.53-mm and 0.32-mm systems, respectively. After connection, initial experiments using n-propanol were conducted to ensure an equal split between the columns. Areas recorded on both channels agreed to within 5%.

The G1888 Headspace Sampler was interfaced to the split/splitless inlet by cutting the carrier line near the inlet weldment and then connecting a zero dead volume (ZDV) union to the headspace transfer line and inlet carrier at the weldment. The supply end of the cut carrier line is then connected to the electronic pneumatic control (EPC) carrier inlet bulkhead at the back of the G1888 Headspace Sampler. Therefore, an inlet EPC channel from the 6890N was used to control carrier flow.

Ten mL headspace vials, each with 2-mL water solution, were used throughout. The resolution check samples were prepared by adding 100 µL of a 0.1 g/dL standard to a 10-mL vial.

Results and Discussion

In this application note, the G1888 Automated Headspace Sampler was used. This sampler features an inert Siltek™ sample path for maximum inertness and minimal carryover. Sample-path

tubing, sampling needle, transfer line, and vent lines are all deactivated. Care was taken to minimize cold spots reducing the possibility of unwanted condensation. Initial setup of the blood alcohol systems first involved verification of proper column installation by checking for uniform sample split between the two columns, followed by a resolution check. To check resolution and peak symmetry, an eight-component sample (Restek #36256) was used. The resulting chromatograms are shown in Figure 1. In the United States, n-propanol, or isopropanol, are commonly used as the internal standards (ISTD) for gas chromatographic blood alcohol determinations. However, in postmortem work, methyl-ethyl ketone (MEK) is commonly used since n-propanol can be a degradation product.

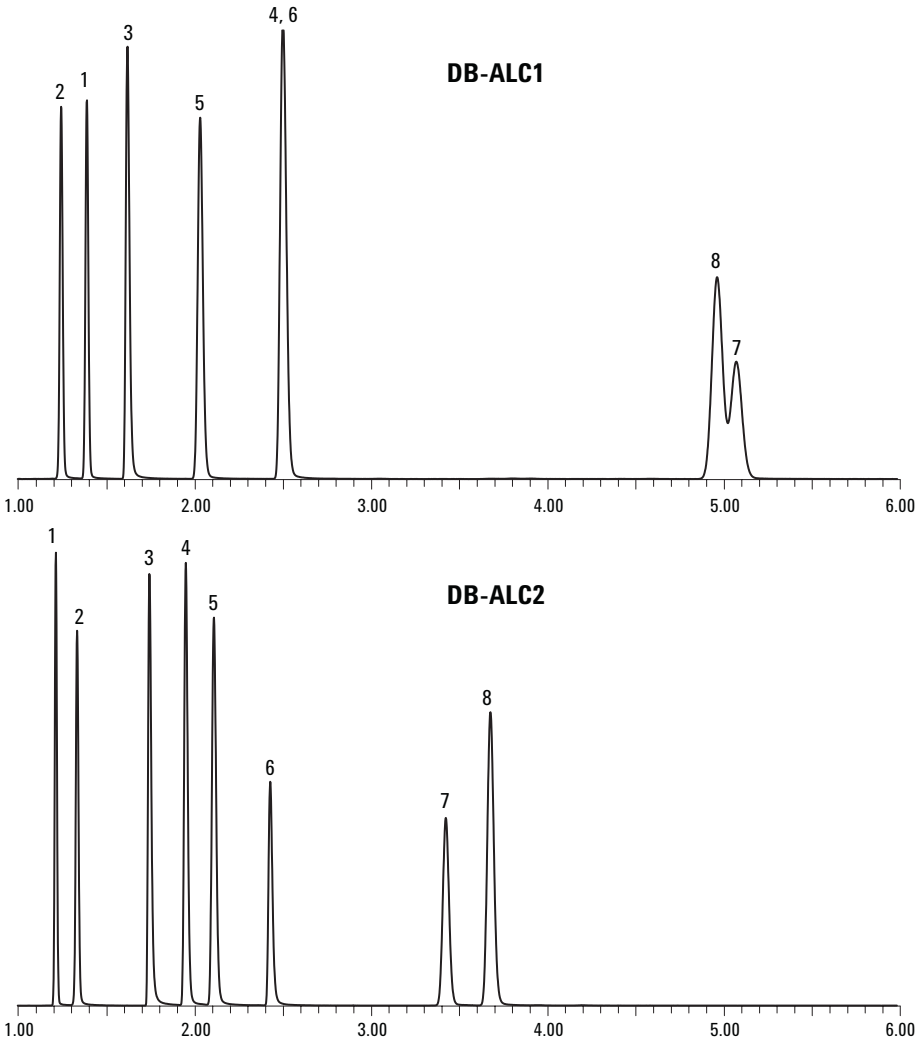

Figure 1. Resolution check standard on 0.32-mm column system at 35 °C. Peak identifications: 1. Acetaldehyde, 2. Methanol, 3. Ethanol, 4. Acetone, 5. 2-propanol, 6. Acetonitrile, 7. Ethyl acetate, and 8. MEK.

A six-component mix using the wide-bore column system is shown in Figure 2.

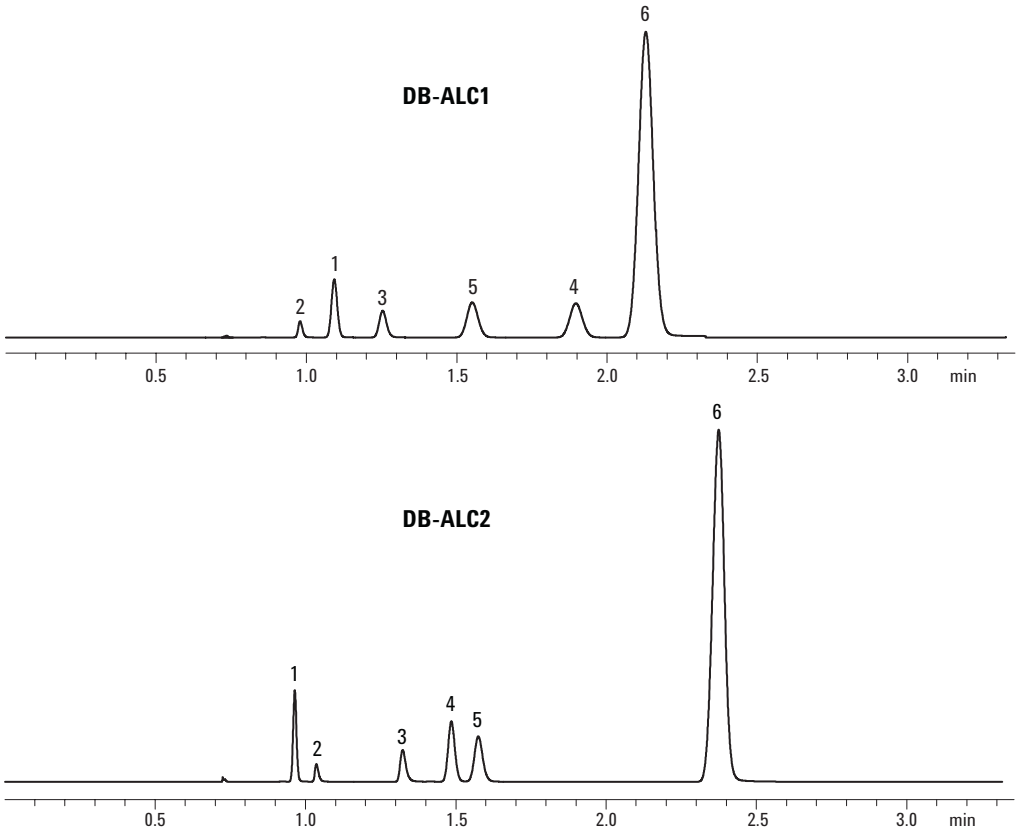

Figure 2. Chromatograms using the 0.53 mm DB-ALC1 and DB-ALC2 columns and the six-component standard at 35 °C. Peak identifications: 1. Acetaldehyde, 2. Methanol, 3. Ethanol, 4. Acetone, 5. Isopropanol, and 6. n-propanol.

Repeatability

Repeatability results for the eight-component standard and the 0.32-dual column and 0.53-dual column systems are shown in Tables 2 and 3, respectively.

Table 2. Repeatability (RSD) of the 0.32-mm Column System; 18 Runs Each of 0.1 g/dL and 0.15 g/dL Calibration Solutions. For DB-ALC1 (0.15 g/dL Runs), the Maximum k Was 0.291 and the Minimum Was 0.285.

DB-ACL-	Conc. g/dL	Acetald.	Methanol	Ethanol	Acetone	Isopropanol	n-propanol	MEK	Calibration K Factor for EtOH (RSD)
1	0.1	0.78	2.32	2.11	1.12	1.75	1.83	0.82	0.36
2	0.1	0.72	2.77	2.10	1.72	1.72	1.85	0.95	0.34
1	0.15	0.86	3.11	2.68	1.31	2.34	2.50	0.93	0.58
2	0.15	0.84	3.48	2.69	1.33	2.31	2.50	0.93	0.55

Table 3. Repeatability (RSD) of the 0.53-mm Column System; 40 Runs of a 0.1% Solution

DB-ALC-	Acetald.	Methanol	Ethanol	Acetone	Isopropanol	Acetonitrile	n-propanol	Ethyl acetate	MEK
1	1.30	1.07	1.12	0.95	0.94	0.95	0.96	2.34	2.00
2	1.22	0.99	1.01	0.96	0.90	0.83	0.93	1.70	1.13

Many laboratories use a series of replicates at the 0.15 g/dL ethanol level for calibration and assessment of system performance. Chromatograms of this mixture are shown in Figures 3A and 3B, for DB-ALC1 and DB-ALC2, respectively

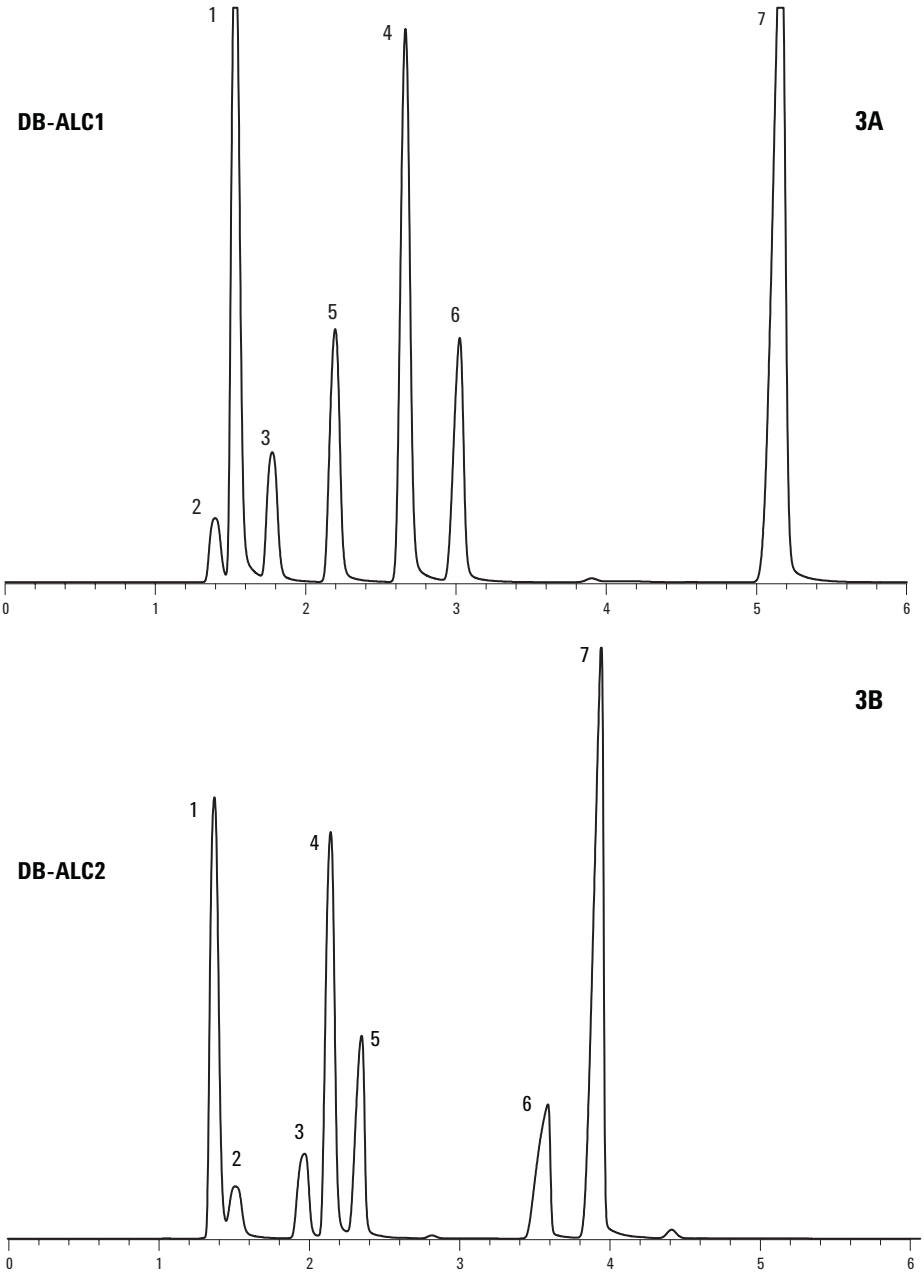

Figure 3. Blood alcohol standard at 0.15 g/dL on 0.32-mm DB-ALC1 (Figure 3A) and DB-ALC2 (Figure 3B) columns. Peak identifications: 1. Acetaldehyde, 2. Methanol, 3. Ethanol, 4. Acetone, 5. Isopropanol, 6. n-propanol, and 7. MEK.

Carryover

In blood alcohol analysis, negative or blank samples should show less than 1.0% ethanol as carryover. A 0.5% per component solution was used to demonstrate the lack of carryover, shown in Table 4. This concentration of ethanol, at several times the nominal expected level, is representative of a severe test for carryover and should show any significant weaknesses in the system. Ethanol carryover as measured by water/ISTD blank run made after 18 runs of a 0.15 g/dL standard gave a percent carryover of 0.6% (0.32-mm DB-ALC2).

A new feature of the G1888 allows users to set the vent purge time from the G1888 keyboard. This parameter is defined, as the time the vent valve is open beginning after valve injection is complete and can remain open up to a maximum of the cycle time setting. This additional purge time may provide a further reduction in carryover. The results shown in Table 4 used the default vent purge time of 30 seconds.

Table 4. Carryover Experiment. Areas are the Average of Six Consecutive Runs of a 0.5% per Component Mixture, Followed by a Water/ISTD Blank. The 0.53-mm Column System Was Used

DB-ALC1	Acetald.	Methanol	Ethanol	Acetone	Isopropanol	n-propanol
Average area	1764	7523	3268	5600	8116	1166
Blank area	31	57	47	*	*	1179
Area ratio	0.02	0.01	0.01	*	*	1.01

*Not measureable

Linearity

Flame ionization detectors are expected to show good linearity for all analytes of interest over the concentration ranges needed for blood alcohol systems. As shown in Figures 4 and 5, this has been verified for the wide-bore column system, and in Table 5 for the 0.32-mm column system. Regression coefficients for the 0.32-mm column system are indicated.

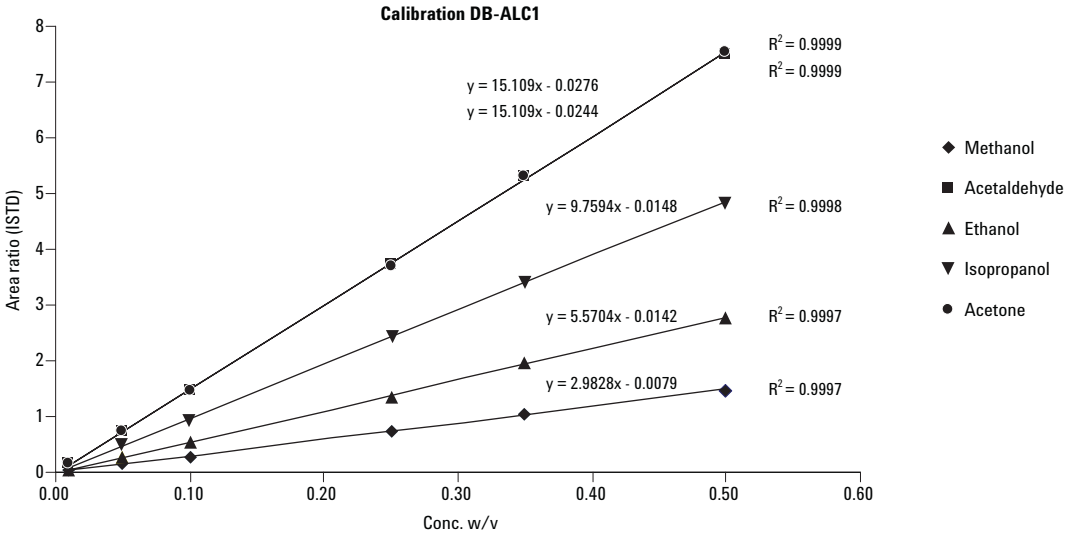

Figure 4. Calibration plots for the indicated standards using the 0.53-mm DB-ALC1 column.

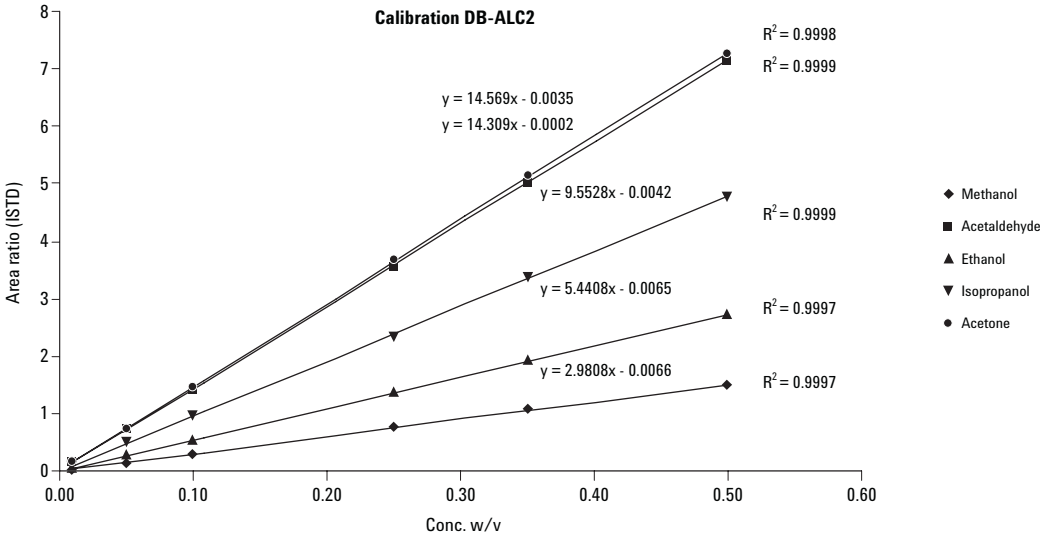

Figure 5. Calibration plots for the indicated standards using the 0.53-mm DB-ALC2 column.

Table 5. Linearity (R^2) of 0.32-mm Column System; Concentrations from 0.005 to 1.0 g/dL

DB-ALC-	Acedald.	MeOH	EtOH	Acetone	IsoPrOH.	n-PrOH.	MEK
1	0.99981	0.99946	0.99931	0.99993	0.99990	0.99983	0.99961
2	0.99985	0.99944	0.99962	0.99993	0.99990	0.99982	0.99946

System k Factor

A system k factor, or response factor, can be defined as (Area ISTD × Conc. EtOH in Std)/(Area of EtOH in Std). A system average k factor can be determined from 6 to 10 consecutive runs of a standard at 0.1 or 0.15 g/dL EtOH. The result for each sample should deviate from the average by no more than ±1.0%. See Table 2 for calibration k factor RSD's on the 0.32-mm column system. Limits are then placed on the run k factor determined for each sample. Run k values should fall within some specified allowable range established by the laboratory. Typically, ±3% of the average k is used.

The results shown in Table 6 illustrate the stability of the system for each column, after 40 runs and six different concentration levels. Calculated concentrations for run 40 differ from the initial concentration by less than 1%.

Table 6. Percent Deviation of Run 40 vs. Run 1 for Five Standards

Column	Nominal					
DB-ALC-	g/dL	MeOH	Acetald.	EtOH	IsoPrOH.	Acetone
1	0.01	0.65	–1.09	0.50	–0.08	–0.78
1	0.05	0.74	2.05	0.70	0.84	1.38
1	0.10	0.46	0.49	0.13	–0.19	–0.02
1	0.25	0.10	2.26	–0.17	0.10	1.08
1	0.35	0.23	0.00	–0.44	0.13	–0.06
1	0.50	0.27	0.73	–0.48	0.09	0.29
DB-ALC-						
2	0.01	–1.32	0.32	0.25	–0.80	–0.38
2	0.05	1.99	0.46	0.63	1.36	0.63
2	0.10	0.31	0.09	–0.40	–0.18	–0.43
2	0.25	2.15	–0.60	–0.15	1.02	0.05
2	0.35	–0.18	0.11	–0.16	–0.13	0.01
2	0.50	0.71	0.27	–0.12	0.20	–0.29

Calibration curves obtained on the first of 40 consecutive runs are based on the six concentrations shown.

European Blood Alcohols

In the European Union, blood alcohol limits are either 0.5 or 0.8 g/L, although Sweden and Norway have a stricter limit of 0.2 g/L [1]. In addition, more restrictive levels have been mandated for young drivers in many countries. Principle means of measurement of alcohol include both breath and blood, with blood testing compulsory in a few countries. Unlike the U.S., the ISTD most often used for blood testing is t-butanol. Chromatograms are shown in Figures 6A and 6B, where t-butanol was used as the ISTD. Certain potential co-elutions need to be noted, however, including t-butanol/ acetonitrile/ acetone on DB-ALC1, and t-butanol/ acetonitrile on DB-ALC2.

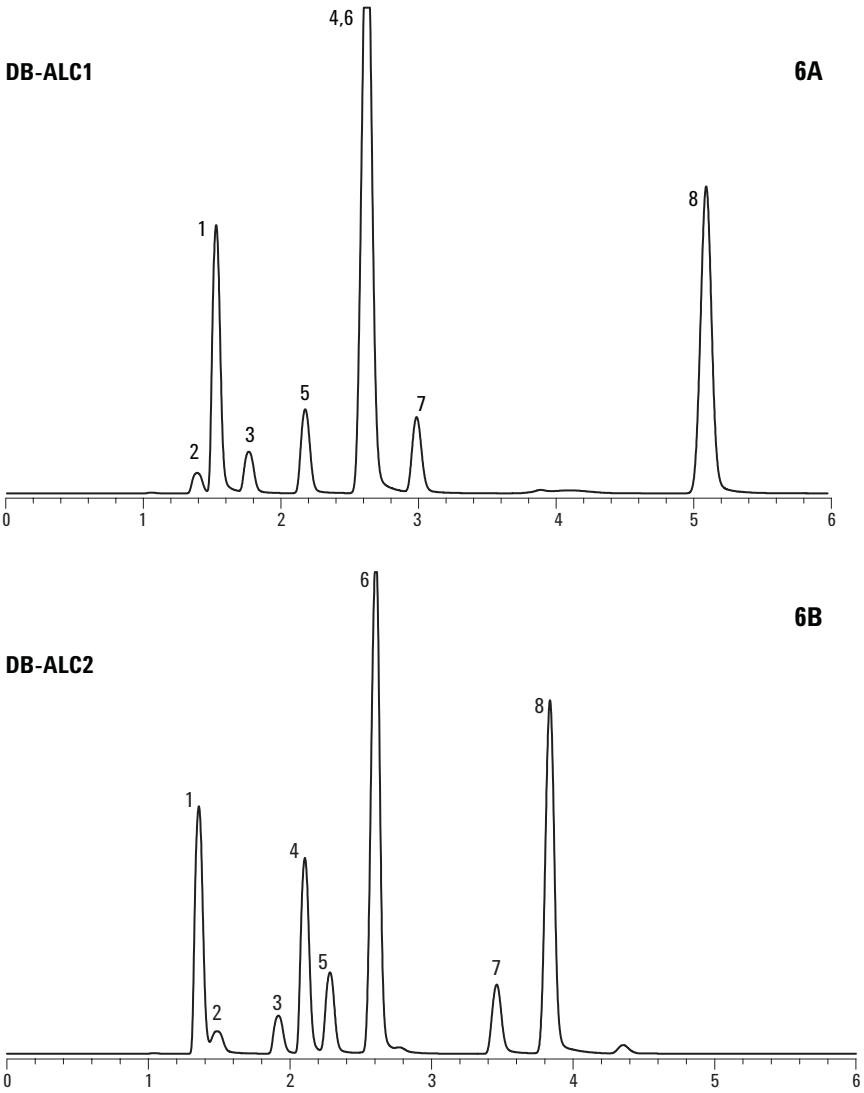

Figure 6. Blood alcohol chromatograms using t-butanol as the ISTD. Columns: 30 m × 0.32 mm DB-ALC1 (Figure 6A) and DB-ALC2 (Figure 6B). Peak identifications: 1. Acetaldeyde, 2. Methanol, 3. Ethanol, 4. Acetone, 5. Isopropanol, 6. t-Butanol, 7. n-propanol, and 8. MEK.

Inhalants in Blood

Although not demonstrated in the work, diethyl ether, hexane, chloroform, ethyl acetate, and toluene are also separated from the standard components of blood alcohol analysis on the dual column systems [2]. A change in the chromatographic program may be needed for optimization.

ChemStation Software

A software module has been developed for the G1888 Headspace Sampler that provides complete control of all instrument parameters and also uses the same sequence table as the Agilent liquid samplers. This software is available as an add-on product to the GC ChemStation (G2922A), providing fully integrated headspace control. ChemStation revision A.09.03 or later is required. An example of the sequence log table is shown in Figure 7.

Headspace Job Log: Agilent G1888 Headspace Sampler

Line	Vial#	Sample	Method	#Inj	Loaded	Prep'ing	Injecting	Finished	Analyzed	Time	Event
⊞ 1	1	Water blank	BAC35	1	☑	☑	☑	☑	☑		
⊟ 2	2	CAL Sample	BAC35	1	☑	☑	☑	☑	☑		
										15:45:32 Thu	Vial Loaded
										16:02:57 Thu	ChemStation Ready for Run
										16:05:32 Thu	Vial Equilibrated
										16:06:07 Thu	Sample Injecting
										16:06:37 Thu	Vial Unloaded
										16:21:07 Thu	ChemStation Completed Run
⊟ 3	3	QC SAMPLE	BAC35	1	☑	☑	☑	☑	☐		
										16:03:52 Thu	Vial Loaded
										16:21:15 Thu	ChemStation Ready for Run
										16:23:52 Thu	Vial Equilibrated
										16:24:26 Thu	Sample Injecting
										16:24:56 Thu	Vial Unloaded
⊟ 4	4	STD	BAC35	1	☑ 4	☐	☐	☐	☐		
										16:22:11 Thu	Vial Loaded
5	5	STD	BAC35	1	☐	☐	☐	☐	☐		
6	6	Blood1	BAC35	1	☐	☐	☐	☐	☐		
7	7	Blood2	BAC35	1	☐	☐	☐	☐	☐		
8	8	Blood3	BAC35	1	☐	☐	☐	☐	☐		
9	9	Blood4	BAC35	1	☐	☐	☐	☐	☐		
10	10	Blood5	BAC35	1	☐	☐	☐	☐	☐		
11	11	Blood6	BAC35	1	☐	☐	☐	☐	☐		
12	12	Blood7	BAC35	1	☐	☐	☐	☐	☐		
13	13	Blood8	BAC35	1	☐	☐	☐	☐	☐		
14	14	Blood9	BAC35	1	☐	☐	☐	☐	☐		
15	15	Blood10	BAC35	1	☐	☐	☐	☐	☐		
16	16	QC SAMPLE	BAC35	1	☐	☐	☐	☐	☐		
17	17	QC SAMPLE	BAC35	1	☐	☐	☐	☐	☐		
18	18	CAL SAMPLE	BAC35	1	☐	☐	☐	☐	☐		

Figure 7. An example of the time stamped sequence log table window using the Agilent G1888 Headspace Sampler. A sequence log file is also created.

All major events associated with vial processing are shown with a time stamp. Software is also 21 CFR Part 11 compliant with Agilent Security Pack installed. Setup of the sampler is handled with two pull-down menus, one for setting global parameters, such as LAN address and vial size, while the other opens a dialog box to set sample sequence timing and system temperatures.

Conclusions

Key parameters for blood alcohol analysis by headspace sampling include analysis time, resolution, repeatability, and carryover. The two isothermal systems described here offer fast cycle times, typically less than 5 minutes depending on the ISTD chosen. This provides good throughput when coupled to the 70-sample tray of the G1888. The DB-ALC1 and DB-ALC2 columns also provide good resolution, separating ethanol from common interferences. Deviation of the k factors from the average system k factor is below 1.5% in the experiments described here, at concentration levels ranging from 0.01 to 0.5 g/dL.

The headspace-GC system described here will give reliable determinations of forensic ethanol levels in blood and other biological matrices. Although a single column is usually adequate, the dual column approach gives additional confirmation and separation utility without an increase in analysis time. Carryover, a common problem in high-throughput laboratories, is reduced through a combination of inert flow path, improved thermal control, and programmable vent purge. The G1888 Headspace Sampler can be a valuable addition to forensic laboratories, as the US and other countries step up the enforcement of driving under the influence (DUI) laws.

References

1. Blood Alcohol Concentration Limits Worldwide, *ICAP Reports* 2002 **11**, May, www.icap.org.
2. E. Kuhn, M. Datta, and J. Ellis, “Separation and Identification of Blood Pollutants”, Agilent Technologies, publication B-0328 Rev.2, http://www.agilent.com/chem

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