GLOBAL ANALYTICAL SOLUTIONS

GERSTEL

AppNote 1/2005

Blood Alcohol Analysis Using an Automated Static Headspace Method

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Abstract

Forensic laboratories face the need to analyze many samples of human blood and body fluids for alcohol content. The large number of samples that require quantification of ethanol in these facilities creates a challenge for the methodology employed. Factors that need to be considered are sample throughput, resolution, and carryover. A successful method for these analyses should be fast, precise, and accurate.

Current methods used in these analyses use a gas chromatograph coupled to a static headspace sampler and flame ionization detector (FID). The x, y, z robotic autosampler used in this study has a capacity of up to 128 headspace samples, which is a distinct advantage compared to other samplers commercially available.

Results obtained with the instrument and methodology described in this report meet the specifications set by the California Department of Justice Blood Alcohol Operating Procedures (Title 17). A dual-column, dual-FID blood alcohol analysis system that can be used for confirmation of ethanol peaks was also tested and produced results with good precision (below 5 % RSD).

INTRODUCTION

Headspace gas chromatography (HS-GC) for determination of ethanol content of blood is widely used by forensic labs to test automobile drivers charged with DUI (driving under the influence). The method originates from 1964 when G. Machata [1] published the first use of HS-GC for quantitative analysis.

The method includes the use of an internal standard (IS) compound. Tert-butanol or n-propanol may be used as internal standards for the alcohol in blood determinations. The choice of which internal standard to use depends on the type of column utilized in the GC instrument.

Blood is a very complex matrix that varies depending on the individual. The salt or lipid content may be different and headspace analysis with the use of an IS provides fast measurements that can be automated. In this study, a GERSTEL MPS 2 robotic autosampler with a headspace gas-tight syringe was used to analyze ethanol solutions in different concentrations.

In this study, we developed a method that meets the specifications set by the California Department of Justice Blood Alcohol Operating Procedures (Title 17) [2]. We also configured and tested a separate dual-column/dual-FID system that adds confirmation because of the different elution order of ethanol on each column.

EXPERIMENTAL

Instrumentation

Analyses were performed on a 6890 GC equipped with single or dual FID's (Agilent Technologies), and a GERSTEL MPS 2 multipurpose sampler configured for static headspace injection.

Reagents

- Ethyl alcohol, absolute, 200 proof, 99.5%, A.C.S. reagent grade
- Methyl alcohol, 99.8%, A.C.S. reagent grade
- Acetone 99.5%, A.C.S. reagent grade
- n-Propanol (1-propanol) 99.5% A.C.S. reagent grade (IS)
- Isopropanol (2-propanol), 99.5%, A.C.S. reagent grade
- Blood alcohol mix resolution control standard (Restek, # 36256). 0.100 g/dL in water of 8 compounds: acetaldehyde, acetone, acetonitrile, ethanol, ethyl acetate, isopropanol, methanol and methyl ethyl ketone (MEK).

Preparation of standards

Secondary standard (SS). 0.25 mL of absolute (200 proof) ethanol and 0.125 mL of n-propanol pipetted into a 100mL volumetric flask and diluted with bottled water.

Quality control standard (QC). 0.15 mL of absolute (200 proof) ethanol and 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.

Resolution standard (RS). 0.25 mL of absolute (200 proof) ethanol, 0.1 mL methanol, 0.1 mL isopropanol, 0.01 mL acetone and 0.125 mL n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.

Blank standard. 0.125 mL of n-propanol pipetted into a 100 mL volumetric flask and diluted with bottled water.

All standards above were diluted 1:6 in bottled water prior to use. 500 µL of standard was then pipetted into a 20 mL headspace vial.

1 mL of 1000 µg/mL internal standard (n-propanol) and 1mL of the blood alcohol mix resolution control standard (Restek, # 36256, Lot# A034323) was diluted in 18 mL bottled water. 4 mL of standard was then pipetted into a 20 mL headspace vial.

All vials were crimp-capped using blue silicone/PTFE septa.

Calculations

$$K = \frac{(AIK \times CKE)}{AKE}$$

$$CO = \frac{(AUE \times AIK \times CKE)}{(AIU \times AKE)} \quad \text{or} \quad CO = \frac{AUE \times K}{AIU}$$

Where:

K = response factor

CO = concentration of ethanol in the unknown sample

AUE = peak area of ethanol peak in unknown sample

AIK = peak area of internal standard peak in calibration standard

CKE = concentration of ethanol in secondary alcohol standard

AIU = peak area of internal standard peak in unknown sample

AKE = peak area of secondary alcohol standard

Quality control criteria for California compliance

- Calibration runs consist of 6 secondary standards followed by a resolution standard. The calibration constant K is then calculated for each of the 6 secondary standards and the mean is calculated. The value of the K constant for each of the six determinations must fall within ± 1.5 % of the mean value.
- The results for the resolution standard shall indicate a resolution of 0.01% acetone in the presence of 0.20% ethanol.
- Analysis runs consist of a Blank (water, no IS) and standards (SS, QC and RS) followed by the sample set (2 replicas per sample) followed by two additional standards (QC and SS).
- The result of the blank sample should be less than 0.01%.

Results and Discussion

California DOJ Blood Alcohol Operating Procedure (Title 17). Figure 1 shows the instrument used in this study. It consists of a GERSTEL MPS 2 autosampler that can be programmed to be used with headspace syringes. For this study we used a 2.5 mL syringe that can also be programmed to inject different volumes (recommended volumes from 0.25 mL up to 2.5 mL). The headspace syringe adaptor is heated and can be controlled to optimize the syringe temperature.

Figure 1. MPS 2 Headspace autosampler coupled to a 6890 Agilent GC.

Incubation of the samples is carried out in a heated agitator with six sample positions. GERSTEL MASter software will “prep-ahead” samples if equilibration times are longer than GC analysis times so there are always up to six samples incubating. This results in great time savings and excellent sample throughput. For this analysis we selected an incubation temperature of 65 ºC and a syringe temperature of 70 ºC. It is recommended to use a slightly higher syringe temperature as the sample is transferred from the vial to the syringe to avoid condensation.

Secondary and resolution standards were analyzed. An example chromatogram is displayed in Figure 2. It can be seen that there is no ethanol carryover in the blank and the IS reproduces well. Using the gas chromatograph in the isothermal mode, we were able to separate the alcohols present in the SS and also the compounds present in the resolution standard in approximately 3.5 minutes (Figure 3). The updated chromatographic conditions include the use of a capillary GC column instead of the packed column currently used for these analyses in California.

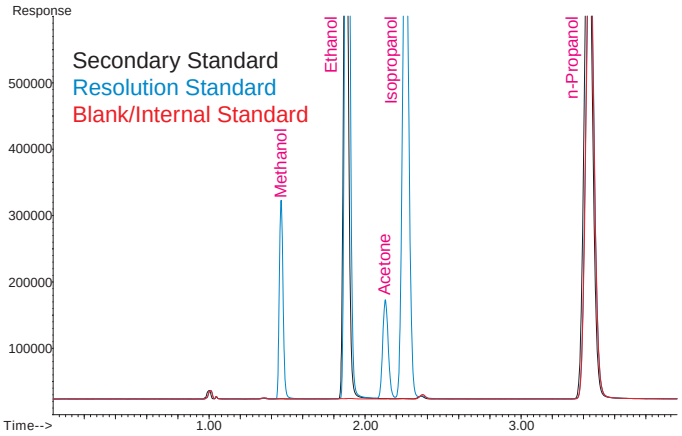

Figure 2. FID overlay of Blank/Internal Standard (IS), Resolution Standard and Secondary Standard (SS) for California Compliance.

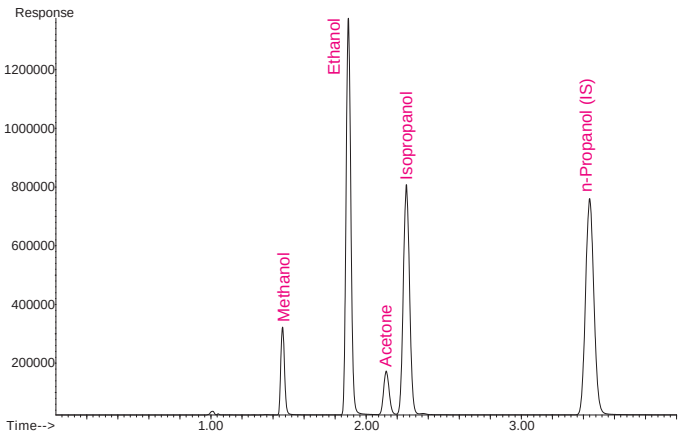

Figure 3. FID trace of Resolution Standard for California Compliance.

In order to test the robustness of the method, 48 calibration sequences were run with the MPS 2 over a three month period using the parameters listed in Table 1. Using the K factor criteria all 48 sequences passed. Perturbations of the system during this 3 month period include changing the GC column, changing the syringe, and two power outages. An example of the K factor check is shown in Table 2. To test the accuracy of the method, reference samples with known alcohol concentrations were prepared; the alcohol content was calculated and found to be accurate.

Table 1. Method parameters for California compliance.

Agilent 6890

Inlet:	Split/splitless, 100°C
	Split 1:5
Column:	30m DB-ALC2 (Agilent)
	d_i= 0.53mm, d_f= 2.0µm
Oven:	isothermal, 40°C

MPS 2 Headspace

Incubation:	65°C (15 min)
Syringe:	2.5 mL, 70°C
Injection:	1 mL (500 µL/s)

Table 2. Example of calculation and check of K factor.

File name	EtOH Peak Area	n-Propanol (IS) Peak Area	K factor	-1.5% / +1.5%
7230002	113206882	106875141	0.1794	Pass / Pass
7230003	116621856	109083941	0.1777	Pass / Pass
7230004	114897369	107497317	0.1778	Pass / Pass
7230005	111194673	104605854	0.1787	Pass / Pass
7230006	115948256	109036988	0.1787	Pass / Pass
7230007	114896941	107920646	0.1785	Pass / Pass
Average	114460996	107503314	0.1785	0.176 / 0.181
StD	1975014	1662204	0.0006	
% RSD	1.73	1.55		

Blood Alcohol Dual-Column Confirmation Method. We configured a system with dual complimentary alcohol columns from a single inlet and dual FIDs for blood alcohol analysis [3]. The dual system has an advantage since the order of elution is different on each column and therefore it adds confirmation of the peak identification. In order to verify the precision of the splitter (Figure 4) we installed two identical columns and checked the response of the secondary standard on both columns.

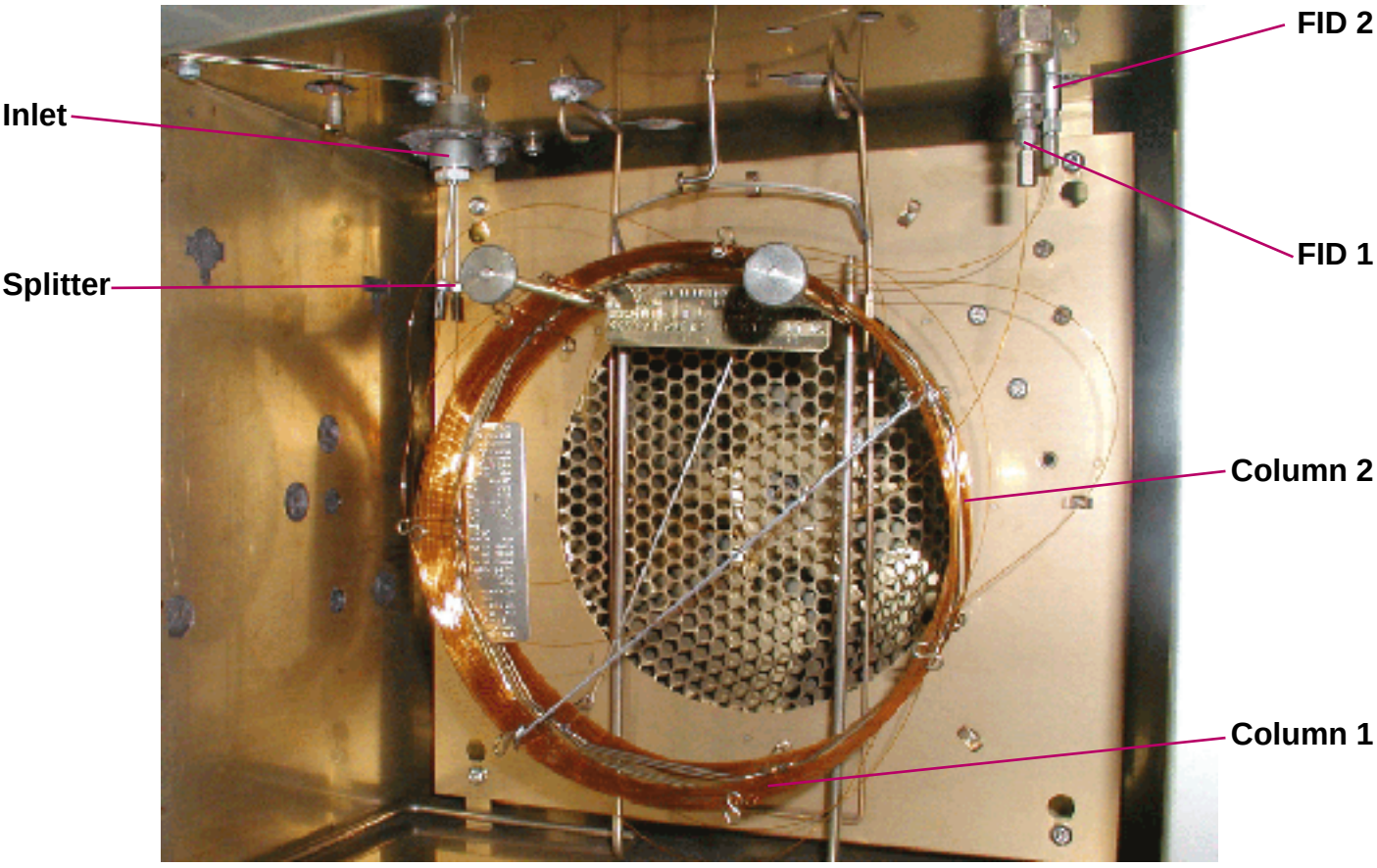

Figure 4. Dual-column dual-FID system used in the study.

Figure 5 shows that both retention time and peak area reproduce well and therefore the splitter appears to split the sample uniformly between the two columns. We tested 6 sequences; each sequence consisted of 1 blank, 6 SS and another blank. Using the K factor criteria all 6 sequences passed on both columns.

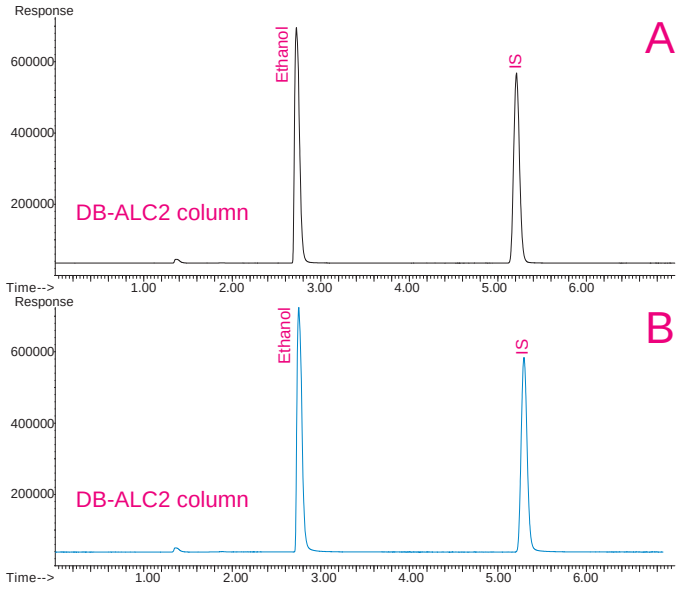

Figure 5. Dual-FID traces of Secondary Standard (SS) using two identical columns DB-ALC 2.

Once we were satisfied with the system performance, we used two columns with two different stationary phases. Using the parameters listed in Table 3 we analyzed the Restek resolution control standard. Figure 6 shows the chromatogram obtained for the resolution mix plus IS.

Table 3. Method parameters for dual-column dual-FID system.

Agilent 6890

Inlet:	Split/splitless, 150°C
	Split 1:5
Column:	30m DB-ALC1 (Agilent)
	d_i= 0.32mm, d_f= 1.8µm
Column:	30m DB-ALC2 (Agilent)
	d_i= 0.32mm, d_f= 1.2µm
Oven:	isothermal, 35°C

MPS 2 Headspace

Incubation:	65°C (15 min)
Syringe:	2.5 mL, 70°C
Injection:	1 mL (500 µL/s)

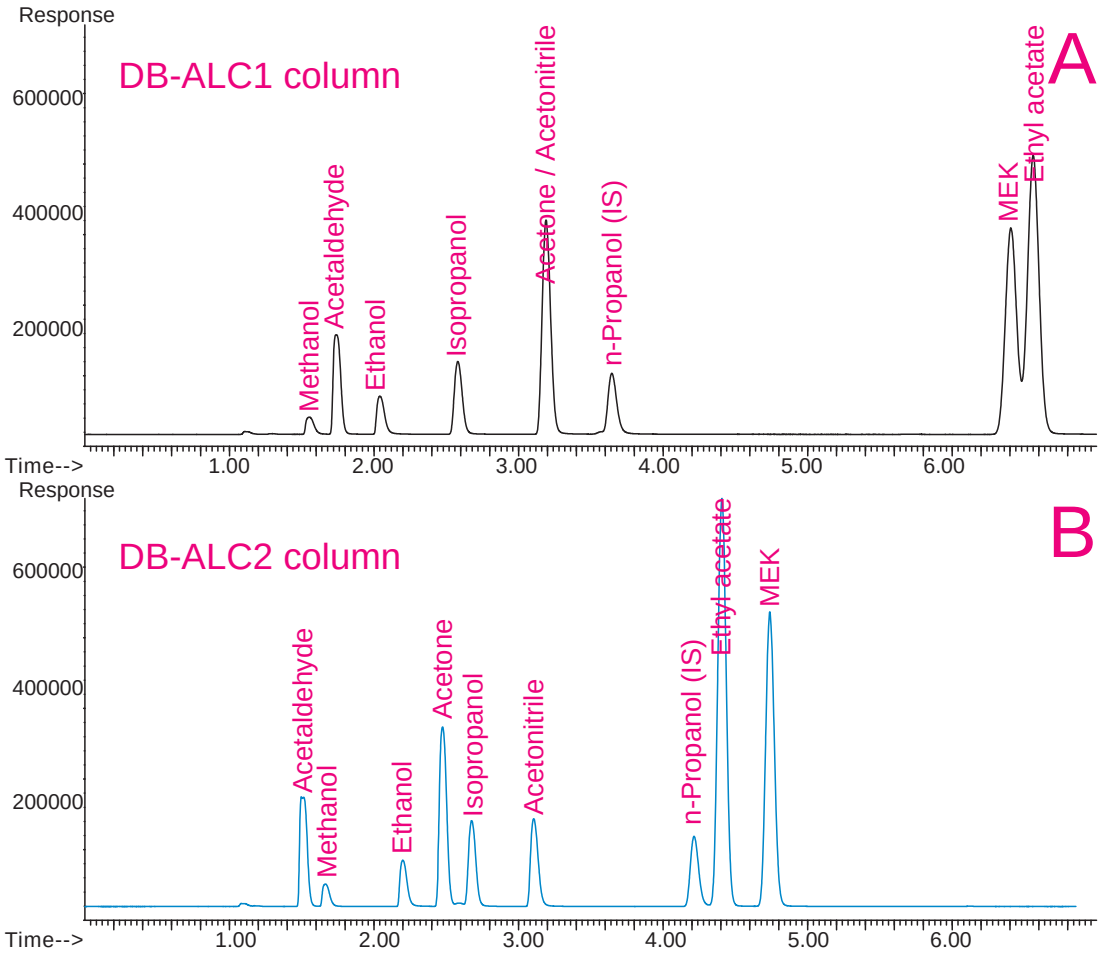

Figure 5. Dual-FID traces of Restek (#36256) Resolution Control Standard and Internal Standard (IS).

Precision for 12 replicas of this analysis are listed in Table 4. [Note: while analyzing the resolution standard we found a late-eluting breakdown product of acetaldehyde. If acetaldehyde is included in a standard, we recommend watching for a late eluting carryover peak and if necessary, extending the isothermal run to approximately 20 min to check for the presence of the breakdown product.

Table 4. Precision of 12 replicas using dual-column configuration.

Compound	Average peak area		Standard deviation		RSD [%]	
	ALC1	ALC2	ALC1	ALC2	ALC1	ALC2
Methanol	2321294	2628655	81681	93324	3.52	3.55
Acetaldehyde	12326272	13373526	534287	578415	4.33	4.33
Ethanol	4958271	5519370	180264	202302	3.64	3.67
Isopropanol	9719454	10594110	379814	418120	3.91	3.95
Acetone	31021477*	21991434	1350778*	962275	4.35*	4.38
Acetonitrile		11314551		481138		4.25
n-Propanol	9320684	9919804	344806	370832	3.70	3.74
MEK	39378050	42850828	1711203	1844065	4.35	4.30
Ethyl acetate	52394805	55868378	1904410	2026058	3.63	3.63

Conclusions

- The GERSTEL MPS 2 robotic autosampler is capable of delivering performance for blood alcohol analysis that meets or exceeds the California Title 17 Forensic Alcohol Analysis and Breath Alcohol Analysis performance criteria.
- Testing during a 3-month period showed good robustness and reproducibility.
- This instrumentation provides increased sample throughput by accommodating up to 128 samples and by using the “prep ahead” function to equilibrate samples.
- The GERSTEL MPS 2 autosampler also performed well when used with a dual column blood alcohol confirmation method.

References

[1] Machata, G. Mikrochim Acta, 1964, 262-271.

[2] California Code of Regulations Title 17, Division 1, Chapter 2, Subchapter 1, Group 8 “Forensic Alcohol Analysis and Breath Alcohol Analysis”.

[3] Firor, R.L., Meng, C-K, and Bergna, M., Agilent Technologies Application (publication number 5989-0959EN).

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