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Application News

No. GC-006

Gas Chromatography

Dual Channel Blood Alcohol Content (BAC) Analysis

■ Introduction

The analysis of ethanol (alcohol) concentration in blood is routinely carried out in forensic labs. The generally accepted method to accurately determine blood alcohol content (BAC) utilizes static headspace sampling and dual column separation by gas chromatography followed by flame ionization detection (GC/FID). Typically, this test is carried out isothermally at 40 °C. High linear velocity of carrier gas coupled with a high split ratio is commonly employed to achieve a short analysis time, but this translates into huge consumption of helium carrier gas. For example, a typical analysis with two 0.32 mm ID columns operating at the linear velocity of 80 cm/sec each and a split ratio of 30:1 consumes over 200 mL of helium carrier gas per minute! Furthermore, the column flow rate (7 mL/min) is well outside of the optimal range for capillary columns (1-3 mL/min) and significant peak tailing is observed under these conditions.

Using the Shimadzu GC-2010 Plus and HS-10 headspace sampler, this application note demonstrates that by moderately increasing column temperature to 50°C and using the optimal flow rate for capillary columns, BAC analysis can be completed in less than 3.5 min without sacrificing peak shapes. More importantly, helium carrier gas consumption was reduced to less than 80 mL/min at the same split ratio (30:1). In addition, good resolution and linearity as well as excellent reproducibility of ethanol concentration measurements have been observed with this set-up.

■ Standards and Sample Preparation

Blood alcohol resolution standard n-P was purchased from Restek, #36010. Ethanol standards (0.010, 0.040, 0.10, 0.40 and 1.00 g/dL ethanol) were prepared by serial dilution from 200 proof ethanol with deionized water to specified concentrations. An internal standard (IS) of 0.020 g/dL *n*-propanol was prepared by diluting *n*-propanol (Sigma, 34817) with deionized water. Aliquots for analyses were prepared by mixing 50 µL of sample with 500 µL of IS solution in 20 mL headspace vials (Shimadzu, 220-94796-01) and sealed with screw caps with PTFE/silicone septa (Shimadzu, 220-94796-02). Deionized water was used as the blank solution.

■ Instrumentation

A Shimadzu GC-2010 Plus equipped with an advanced flow controller (AFC), a split/splitless injector (SPL) and two Flame Ionization Detectors (FID) was used for this study. An HS-10 static headspace sampler with heated transfer line was used for sample preparation and introduction into the GC through the SPL equipped with a 2-way capillary column adaptor (Shimadzu, 221-56222-91). Effluent from HS-10 was split between two columns (SH-Rtx-BAC Plus1, 0.32 mm × 30 m × 1.8 µm, 227-36260-01 and SH-Rtx-BAC Plus2, 0.32 mm × 30 m × 0.6 µm, 227-36260-01). Each column was connected to a separate FID and analyzed simultaneously (Figure 1).

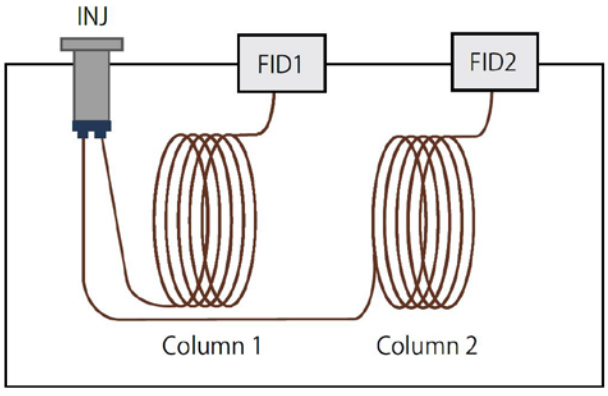


Figure 1: Schematic drawing of the dual column configuration.

■ Analytical Conditions

GC-2010 Plus

- SPL Temp = 150 °C
- Column Temp = 50 °C isothermal (unless specified otherwise in text)
- FID Temp = 250 °C, H_2 flow = 40 mL/min, Air flow = 400 mL/min, Makeup flow = 25 mL/min.
- Carrier gas: Helium
- Flow control mode: constant linear velocity @ 40 cm/sec (unless specified otherwise in text)
- Column flow = 2.5 mL/min
- Split ratio = 30
- Purge flow = 1 mL/min
- Injection volume: 1 mL headspace
- GC run time: 3.5 min

HS-10

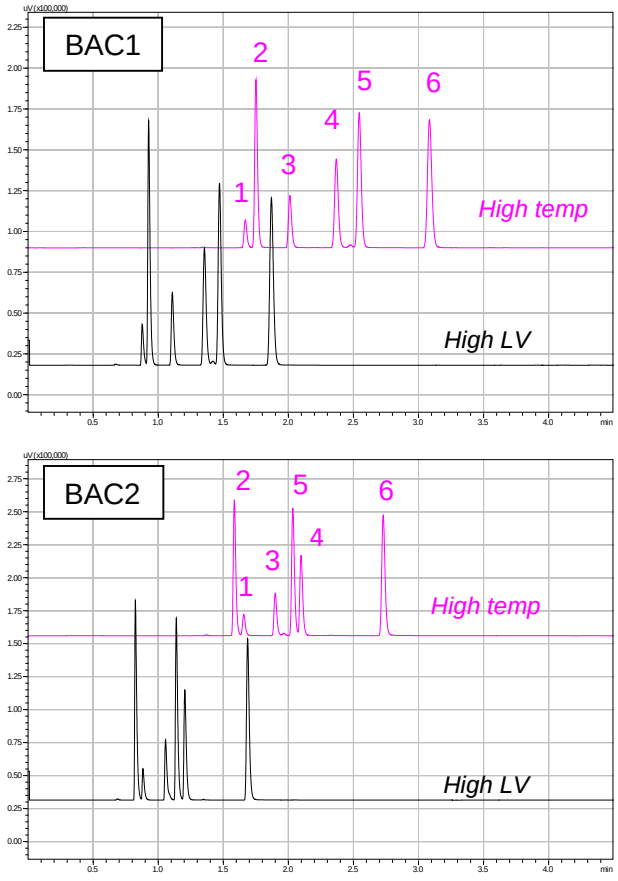
- Vial equilibration: 15 min @ 80 °C
- Sample Pathway Temp = 95 °C
- Transfer Line Temp = 105 °C
- Vial Pressurization: 1.00 min @75 kPa
- Loop Load Time = 0.50 min
- Injection time = 1.00 min

■ Results

Resolution of components in BAC mixture

The headspace sample of a BAC resolution standard was split between SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 column in a nominal 1:1 ratio by using a 2-way capillary column adaptor at the end of the injection port. Compounds eluted from both columns were analyzed simultaneously by two FIDs (Figure 1). The system was operating at the linear velocity of 40 cm/sec so that the column flow rate was within the optimal column flow rates for capillary columns (1-3 mL/min). Column temperature was raised from the standard 40°C to 50°C to shorten the analysis time.

As shown in Figure 2, all six components in the mixture were well resolved and eluted in less than 3.5 minutes using this method. For comparison, the BAC resolution standard was also assayed using the standard high linear velocity (High LV) method. Although shorter analysis was achieved using the high LV method (under 2 min), much better peak shape and overall better resolution were obtained by using the high temp method (Table 1).



1. Methanol
2. Acetaldehyde
3. Ethanol
4. 2-propanol
5. Acetone
6. *n*-Propanol (internal standard)

Figure 2: Analysis of BAC resolution standard on SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 using high temperature method (*High temp,* linear velocity = 40 cm/sec, column temp = 50 °C) or high linear velocity method (*High LV,* linear velocity = 80 cm/sec, column temp = 40 °C). Note that the elution order is the same for either method on the same column, but the elution order is different for SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 columns.

BAC1 analytical line

Peak#	Name	Tailing Factor		Resolution	
		High LV	*High temp*	*High LV*	*High temp*
1	Methanol	1.682	1.277	--	--
2	Acetaldehyde	1.518	1.285	1.625	1.999
3	**Ethanol**	**1.441**	**1.261**	**5.482**	**6.078**
4	2-Propanol	1.294	1.200	6.269	7.273
5	Acetone	1.265	1.178	2.669	3.328
6	*n*-Propanol	1.231	1.172	8.336	9.335

BAC2 analytical line

Peak#	Name	Tailing Factor		Resolution	
		High LV	*High temp*	*High LV*	*High temp*
1	Methanol	1.740	1.243	2.052	1.928
2	Acetaldehyde	1.772	1.372	--	--
3	**Ethanol**	**2.088**	**1.364**	**5.913**	**6.110**
4	2-Propanol	1.563	*n.d.*	2.060	1.560
5	Acetone	1.619	1.337	2.680	3.396
6	*n*-Propanol	1.390	1.265	13.585	14.272

Table 1: Comparison of peak shape and resolution of components in BAC resolution standard assayed under different GC conditions. Tailing factor S is calculated as the following: $S = \frac{W_{0.05}}{2 \times a_{0.05}}$ ($W_{0.05}$ is the peak width at 5% peak height and $a_{0.05}$ is the width of the front half of the peak at 5% peak height). Thus a symmetric peak has a tailing factor of 1.

Calibration

Ethanol standards of concentrations from 0.01 to 1 g/dL (0.01-1%) were assayed to generate the calibration curves. A blank sample was run after the highest calibration standard (1% ethanol) to address potential carryover issue. As shown below, no carryover was detected (Figure 3). Calibration curves with excellent linearity spanning two orders of magnitude in concentration were obtained for both analytical lines (Figure 4).

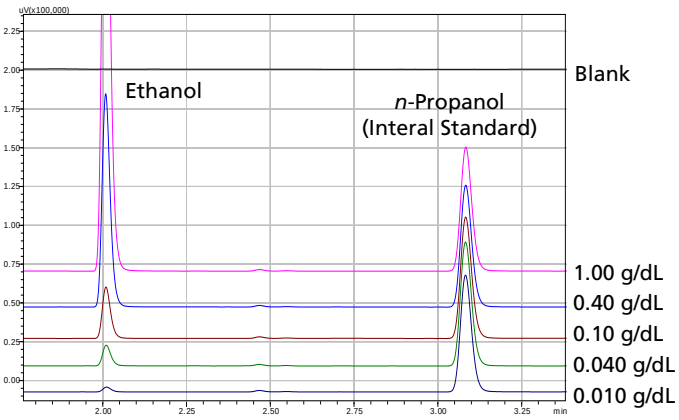


Figure 3: Chromatograms of calibration standards and a blank from BAC1 analytical line. Similar results were obtained from BAC2 analytical line.

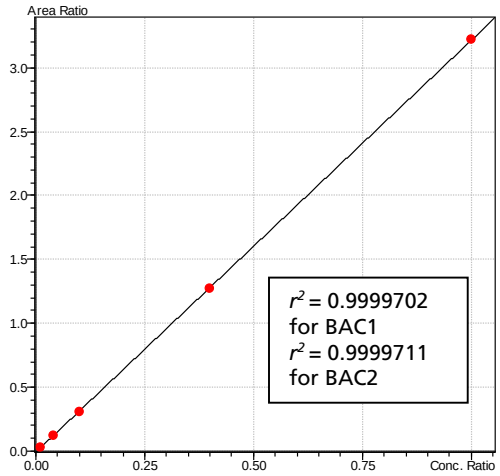


Figure 4: Five-point calibration curve for ethanol from BAC1 analytical line. Each standard (0.010, 0.040, 0.10, 0.40 and 1.00 g/dL) was run in duplicates. Internal standard quantification method was used. Similar results were obtained from BAC2 analytical line. Correlation coefficient (r^2 values) are shown in the inset for both analytical lines.

Reproducibility

30 samples of 0.080 g/dL (0.080%) ethanol control standard were assayed and the internal standard quantification method was used to determine the ethanol concentration. As shown in Table 2, the average (mean) ethanol concentration obtained is 0.0798 g/dL from BAC1 analytical line and 0.0794 g/dL from BAC2 analytical line. And the relative standard deviation (*RSD or coefficient of variation*) is 0.736 % for BAC1 analytical line and 0.983 % for BAC2 analytical line, demonstrating excellent accuracy and repeatability in both cases.

	BAC1	BAC2
AVERAGE RETENTION TIMES (MIN)	2.012	1.899
% RSD (% CV) FOR RET. TIME	0.061	0.072
AVERAGE ETHANOL CONC. (G/DL)	0.0798	0.0794
% RSD (% CV) FOR CONC.	0.736	0.983
STANDARD DEVIATION FOR CONC. (G/DL)	0.000587	0.000780

Table 2: Statistical results for ethanol control standard (*n=30*).

■ Conclusions

In this study, an improved BAC analysis was carried out using the Shimadzu HS-10 static headspace sampler and GC-2010 Plus gas chromatograph. By reducing the linear velocity and increasing column oven temperature to 50 °C, the GC separation was completed in less than 3.5 minutes. In addition, superior peak symmetry and compound resolution as well as excellent linearity and reproducibility of ethanol concentration measurements were obtained for both analytical lines.

Moreover, the amount of carrier gas required was much reduced from the commonly employed high linear velocity method. The gas saver function of the GC-2010 Plus can be turned on to further reduce helium consumption during GC idling time.

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