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Application News

Gas Chromatograph Brevis™ GC-2050

Dual-Column Analysis of Blood Alcohol Content (BAC) with Brevis GC-2050

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User Benefit

- Employing two different types of columns makes BAC analysis more productive.
- Employing Brevis GC-2050 achieves less occupied space in the laboratory.
- Employing Hydrogen is also available in this system instead of Helium. This makes the total run-time quite shorter.

■ Introduction

Analysis of blood alcohol content (BAC), as well as other volatile organic compounds (VOCs) analysis, is essential technique in forensic field to determine the degree of intoxication caused by alcohol consumption and to evaluate criminality.

Generally, this analysis is performed with gas chromatograph (GC) coupled with headspace sampler (HS) and frame ionization detector (FID). To get accurate results, this analysis needs two columns for cross-check. Both accuracy and quickness are required in this analysis, so connecting a single HS-GC system to two columns leading to each FID fulfills the requests with remarkably smart way.

Our new GC named Brevis GC-2050 is designed to be more compact than ever, which can be utilized for this BAC analysis as well as Nexis™ GC-2030 that is our high-end model. The width of GC-2050 is approximately 35% smaller than existing GC model. Moreover, GC-2050 can hold two columns despite its smaller size, which contributes more productivity in the laboratory as well. This application news introduces BAC analysis with HS-20 NX and Brevis GC-2050 (Fig.1). Using the specialized column series SH-BAC PLUS, linearity and repeatability of ethanol were evaluated, as well as separation of VOCs. Hydrogen is employed as carrier gas in this system, which achieves faster runtime.

* Sterile sheep blood which is commercially available was used in this experiment.

Fig. 1 Brevis™ GC-2050 and HS-20 NX
(Specially designed space-saving transfer line for GC-2050)

Table 1 Consumables list for column equipment

ID in Fig. 2,3	Name	P/N	Note
①	2-hole Ferrule	225-19056	10 pc per unit
②	Twin MS Kit	225-20201-91	10 pc per unit
③	SMI Union Ferrule	227-35025-02	0.4 mm - 0.5 mm, 10 pc per unit
④	Restrictor Tubing	227-35023-02	Cut a 2 m tube into two sets of 100 mm

■ Preparation of the Instrument

In this experiment, two different types of columns were equipped to HS, which are connected to two FIDs, respectively (shown in Fig. 2). Simultaneous dual-lines analysis achieves higher throughput as well as qualification accuracy. SH-BAC PLUS1 (30 m × 0.32 mm I.D., 1.8 µm) and SH-BAC PLUS2 (30 m × 0.32 mm I.D., 0.6 µm) were used for analytical columns.

Table 1 shows the list of consumables for this dual-column analysis, and Fig. 3 shows the schematic illustration image of the system. Both columns are connected to "Restrictor Tubing" (0.22 mm I.D.、100 mm) with "SMI Union Ferrule" (0.4 mm - 0.5 mm). "2-hole Ferrule" and "Twin MS Kit" were utilized in nut connection part in HS. 0.5 mL loop (P/N: 225-21889-85) was set for sample loop. Hydrogen and Nitrogen were employed as carrier gas and as pressuring gas, respectively.

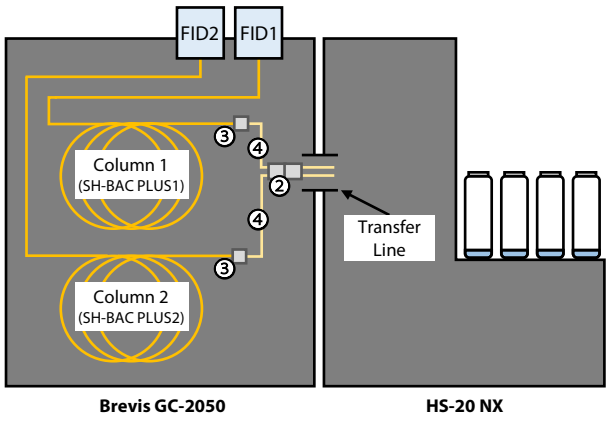

Fig. 2 Schematic illustration of HS-GC dual-column system

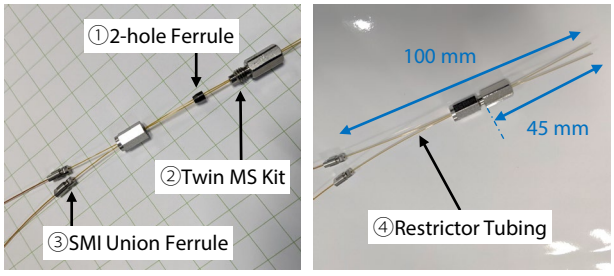

Fig. 3 Column connection modules for the system

■ Analytical Condition

Table 2 shows the analytical condition.

Table 2 Analytical condition

GC Model	: Brevis GC-2050
Headspace Sampler	: HS-20 NX
[HS-20 NX]	
Oven Temp.	: 65 °C (20min)
Sample Line Temp.	: 90 °C
Transfer Line Temp.	: 100 °C
Vial Stirring	: 3
Vial Pressurization Time	: 0.5 min
Pressure Equalization Time	: 0.1 min
Loading Time	: 0.5 min
Load Equalization Time	: 0.1 min
Injection Time	: 0.5 min
Needle Flush Time	: 3 min
GC cycle Time	: 4 min
Vial Pressure	: 80 kPa, Nitrogen
Sampling Volume	: 0.5 mL
[GC-2050]	
Carrier Gas	: Hydrogen
Carrier Gas Control	: Constant Linear Velocity (60 cm/sec)
Purge Flow	: 0 mL/min
Injection Mode	: Split 1:13
Column	: SH-BAC PLUS1 (P/N：227-36260-01) (30 m × 0.32 mm I.D., 1.8 µm) SH-BAC PLUS2 (P/N：227-36263-01) (30 m × 0.32 mm I.D., 0.6 µm)
Column Temp.	: 40 °C (3 min)
FID Temp.	: 250 °C
FID H2 Flow Rate	: 32 mL/min
FID Make up Flow Rate	: 24 mL/min, Nitrogen
FID Air Flow Rate	: 200 mL/min

■ Sample Preparation

Fig. 4 shows the schematic illustration for how to put the sample to the HS vial. 480 µL of water or blood was induced in 20 mL HS vial, followed by adding 20 µL of spiking standard solution and 100 µL of internal standard (IS) solution. The lid was hermitically sealed. For practical experiment, adding 500 µL of blood and 100 µL of IS solution are assumed. Table 3 shows the concentration of each standard solution.

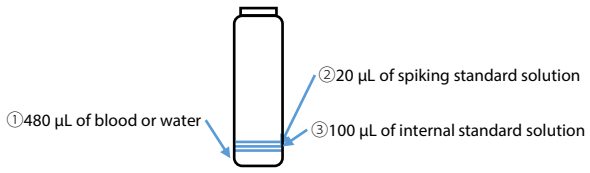

Fig 4 Scheme of sample preparation

Table 3 Concentration of each standard solution

Compound	Stock solution conc.	Final conc. (except IS solution)
Spiking standard solution for calibration curve (Ethanol)	600 mg /mL	20 mg /mL
	3000 mg /mL	100 mg /mL
	6000 mg /mL	200 mg /mL
	12000 mg /mL	400 mg /mL
IS solution（tert-Butanol）	600 mg /mL	100 mg /mL

■ Analysis Process

Fig. 5 shows schematic illustration of an example of analytical process. For better repeatability, a highly concentrated sample was injected for conditioning prior to the whole experiment. Highly polar compounds like Ethanol and Methanol are likely to adsorb to the sample line. It causes underestimation of the peak area for several initial injections and the peak area typically becomes higher during serial injections. Therefore, filling the adsorption points up with high concentrated compounds beforehand will promotes the analytical stability.

As a high concentrated sample, each 1000 mg/dL of Ethanol and tert-Butanol was used. After injecting a high concentrated sample, two serial injections of water were performed as blanks. It was confirmed that no carryover was detected during the blank analysis.

After this pre-conditioning process, five points calibration samples were analyzed, followed by a blood sample. For checking the stability of the peak area, 100 mg/dL of standard Ethanol sample was analyzed after every five blood injections.

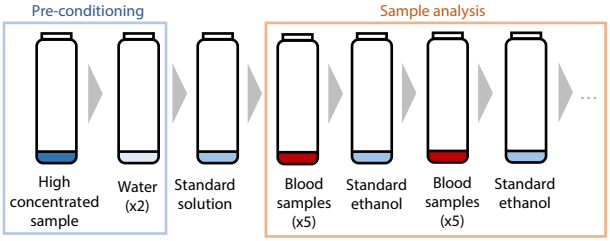

Fig. 5 Recommended analytical process

■ Linearity of the Calibration Curve

Fig. 6 shows the calibration curve that was created with mean values of six repeated analysis of each calibration points. Difference was evaluated when using water and blood as solvent. Both Ethanol and Butanol peaks were counted higher in blood solvent than water solvent, and the slope of the calibration was slightly different from each other. This should be because the matrix compounds containing in blood should interfere the liquid-gas equilibrium condition inside the vial. The effect should differ from compounds. Correlation coefficients for both water and blood calibration curve were more than 0.999.

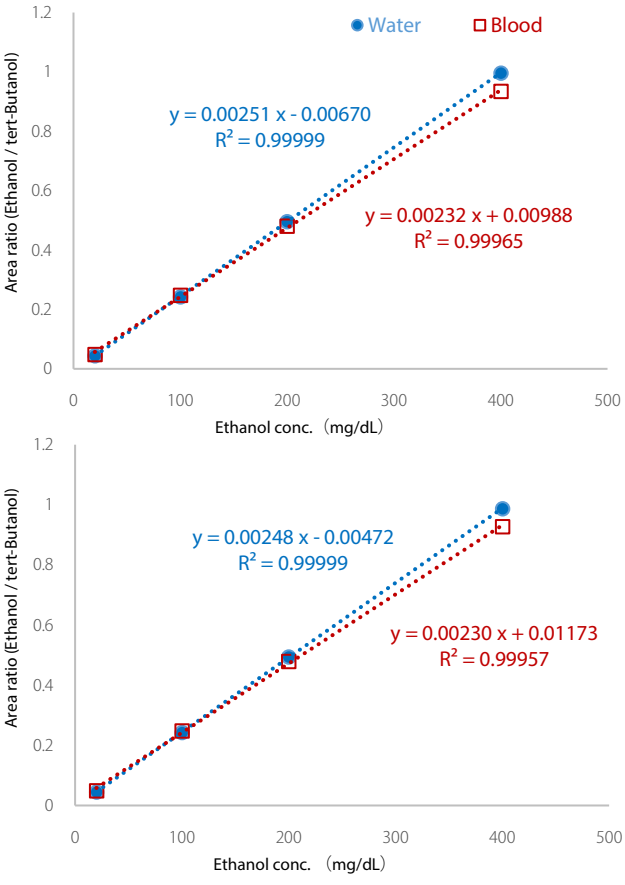

Fig. 6 Calibration curve of Ethanol
(Upper: SH-BAC PLUS1, Lower: PLUS2)

■ Analysis of Blood Sample

Blood was assumed as the field sample and was added to the standard solutions shown in Table 3 (Final conc. of 0~400 mg/dL except IS solution). The obtained chromatograms are shown in Fig. 7. The quantification results of these samples are shown in Table 4. Errors from the spiked concentration were all within 10%, even under using either calibration curve whose solvent is water or blood. Although blood calibration curve should be preferable, water calibration curve should be also available under the difference was fully evaluated like shown in this experiment.

Repeatability of the 100 mg/dL standard solution analyzed after every five blood samples analysis was also evaluated. The %RSD was 1.29 (n=6) that is satisfying result.

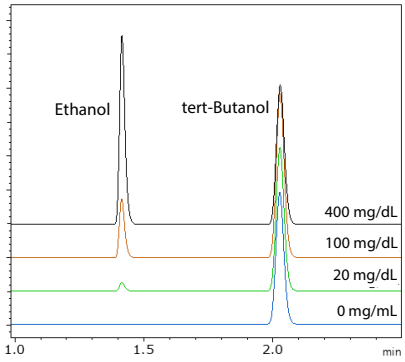

Fig. 7 Chromatograms of blood samples that contains both spiking and internal standards

Table 4 Difference of the obtained concentration values from the spiked concentration values (n=5)

Final conc. inside the vial (except IS solution)		Water calibration curve		Blood calibration curve	
		Conc. (mg/dL)	Error (%)	Conc. (mg/dL)	Error (%)
20 mg/dL	SH-BAC PLUS1	19.2	-4.0	18.5	-7.5
	SH-BAC PLUS2	18.7	-6.5	18.3	-8.5
100 mg/dL	PLUS1	98.9	-1.1	105.0	+5.0
	PLUS2	98.2	-1.8	104.7	+4.7
200 mg/dL	PLUS1	192.2	-3.9	206.4	-3.2
	PLUS2	190.7	-4.7	205.2	+2.6
400 mg/dL	PLUS1	373.6	-6.6	403.6	+0.9
	PLUS2	370.0	-7.5	400.1	+0.0

■ VOCs Separation

Seven VOCs (Methanol, Ethanol, 1-Propanol, 2-Propanol, Acetone, tert-Butanol and Acetaldehyde) that are typically identified in blood sample were analyzed. Resulted chromatograms from each 20 mg/dL (Final concentration in the vial) is shown in Fig. 8. All the compounds were eluted within 2.4 min and were fully separated. Accurate qualification should work because different separation pattern was obtained with each column. Table 4 shows repeatability (n=20) of the retention time and of the peak area ratio to tert-Butanol (internal standard) of each compound.

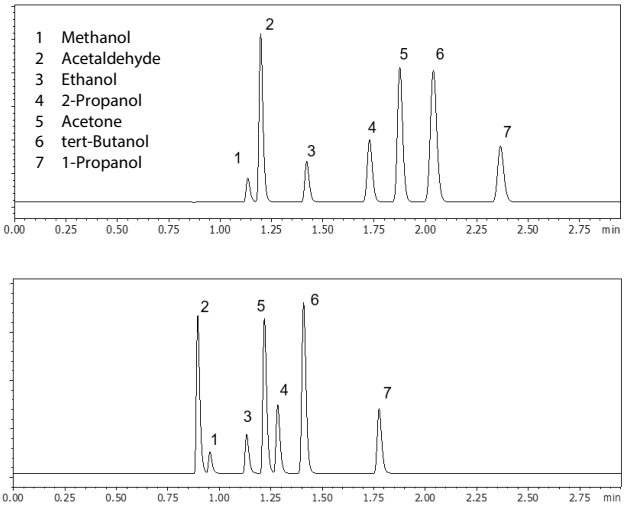

Fig. 8 Chromatogram of seven VOCs
(Upper：SH-BAC PLUS1, Lower：SH-BAC PLUS2)

Table 5 %RSD of the retention time (RT) and peak area ratio (n-=20)

ID	Compound	SH-BAC PLUS1		SH-BAC PLUS2	
		RT	Peak area ratio	RT	Peak area ratio
1	Methanol	0.0066	0.63	0.0165	0.57
2	Acetaldehyde	0.0077	1.24	0.0082	0.95
3	Ethanol	0.0067	0.48	0.0121	0.46
4	2-Propanol	0.0069	0.38	0.0080	0.47
5	Acetone	0.0060	0.70	0.0101	0.36
6	tert-Butanol	0.0062	-	0.0104	-
7	1-Propanol	0.0066	0.38	0.0010	0.36

■ Summary

BAC analysis was performed with Brevis GC-2050 coupled with HS-20 NX. Pre-conditioning kept the system stable and helped to obtain satisfying repeatability. The blood sample also worked to obtain adequate repeatability and calibration linearity.

This experiment employed Hydrogen as carrier gas instead of Helium. Rapid, compact, and reliable analytical system was accomplished by this analysis system.

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