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Gas Chromatograph Brevis™ GC-2050

Application News

# Dual-Column Analysis of Blood Alcohol Content (BAC) Using Nitrogen Carrier Gas with Brevis GC-2050

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## User Benefits

- Dual-column gas chromatography, where 2 columns with different separation characteristics are connected, improves qualitative performance.
- The Brevis GC-2050 can use nitrogen, an inexpensive, alternative carrier gas, to analyze blood VOCs while maintaining good separation.
- The Brevis GC-2050 is compact, so it occupies less space in the laboratory.

## ■ Introduction

Analysis of blood alcohol content (BAC) and other volatile organic compounds (VOCs) is essential for law enforcement agencies to determine alcohol intoxication and its role in traffic accidents, assaults, and injuries, as well as to identify the causes of certain criminal behaviors, poisonings, and so on.

Typically, this analysis is performed by a gas chromatograph (GC) that is combined with a headspace sampler (HS) and a flame ionization detector (FID). Since accurate results are essential with BAC analysis, 2 columns can be used with different separation characteristics so that cross-checking can be performed. By using an integrated HS-GC system equipped with 2 FIDs connecting to a dual-column configuration, cross-checking can be performed in a single analysis. In this system, the columns are directly connected to the transfer line using a 2-hole ferrule, which minimizes sample losses due to adsorption.

In addition to the high-end Nexis™ GC-2030, Shimadzu also offers the Brevis™ GC-2050 as a compact gas chromatography solution, and in this Application News, it is used with an HS-20 NX headspace sampler (Fig. 1) to perform BAC analysis. Hydrogen was used as the carrier gas in a similar experiment in Application News 01-00570, but in this experiment, nitrogen was used because it is a cheaper alternative carrier gas. Nitrogen generally has an adverse effect on separation, so this experiment also investigated the conditions for separation for 10 types of blood VOCs.

Fig. 1 Brevis™ GC-2050 and HS-20 NX
(Specially-Designed Space-Saving Transfer Line for GC-2050)

Table 1 List of Consumables for Column Connection

| Nos. in Figs 2 & 3 | Name | P/N | Remarks |
|---|---|---|---|
| (1) | 1/16″ FITTINGS GVF16(2)-004 10/PK | 225-19056 | • 2-hole ferrule<br>• 10 pc per unit |
| (2) | 1/16″ MALE/FEMALE CONNECTOR | 225-19057 | 1 pc |
| (3) | SMI Union Kit | 227-35024-02 | 0.4 mm–0.5 mm |
| (4) | Restrictor tubing | 227-35023-02 | Cut 2 m tube into two 100 lengths of 100 mm |

## ■ Instrument Setup

In this experiment, the GC-2050, which was equipped with 2 flame ionization detectors (FID) and 2 columns with different separation characteristics, was coupled with a headspace (HS) sampler system (Fig. 2). This system's dual-line simultaneous analysis improves both the qualitative performance and productivity. 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 as the analytical columns due to their suitability for blood alcohol content (BAC) analysis.

A list of consumables required for dual-column simultaneous analysis is shown in Table 1, and the column connection modules for HS coupling are shown in Fig. 3. Each column was connected to restrictor tubing (0.22 mm I.D., 100 mm) with an SMI Union Kit (0.4–0.5 mm). The HS connector nut set comprised a 1/16″ FITTINGS GVF16(2)-004 10/PK and a 1/16″ MALE/FEMALE CONNECTOR. A 0.5 mL loop (P/N: 225-21889-85) was used for the HS-20 NX sample loop.

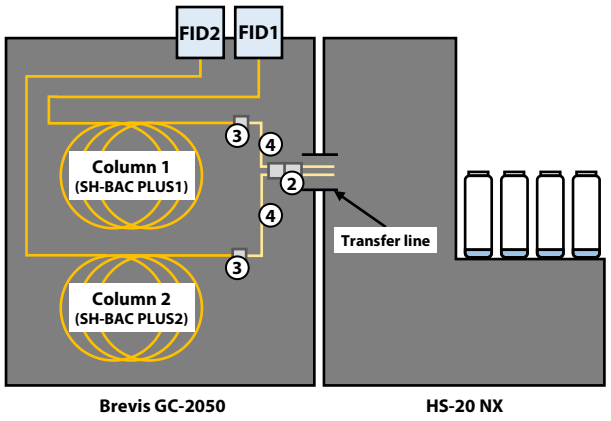


Fig. 2 Schematic Illustration of HS-GC Dual-Column System

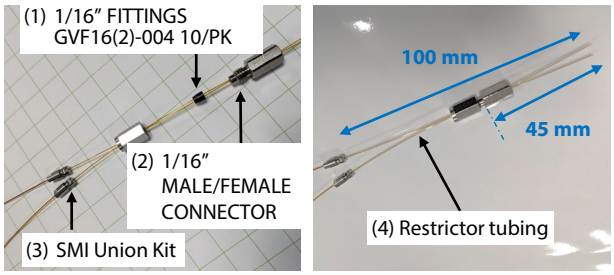


Fig. 3 Column Connection Modules for HS Coupling

## ■ Analysis Conditions

The analysis conditions are shown in Table 2.

Table 2 Analysis Conditions

| | |
|---|---|
| GC: | Brevis GC-2050 |
| HS: | HS-20 NX |
| [HS-20 NX] | |
| Oven Temp. | : 65 °C (20 min) |
| Sample Line Temp. | : 90 °C |
| Transfer Line Temp. | : 100 °C |
| Shaking Level | : 3 |
| Pressurizing Time | : 0.5 min |
| Pressure Equilibration Time | : 0.1 min |
| Load Time | : 0.5 min |
| Load Equilibration Time | : 0.1 min |
| Injection Time | : 0.5 min |
| Needle Flush Time | : 3 min |
| GC Cycle Time | : 9.1 min |
| Pressurize Gas Pressure | : 100 kPa, $N_2$ |
| Sampling Volume | : 0.5 mL |
| [GC-2050] | |
| Carrier Gas | : $N_2$ |
| Flow Control Mode | : Linear velocity constant (30 cm/sec) |
| Purge Flow | : 2 mL/min |
| Split Ratio | : 20 |
| Columns | : SH-BAC PLUS1 (P/N: 227-36260-01)<br>(30 m × 0.32 mm I.D., 1.8 µm)<br>SH-BAC PLUS2 (P/N: 227-36263-01)<br>(30 m × 0.32 mm I.D., 0.6 µm) |
| Column Temp. | : 50 °C (8.5 min) |
| FID Temp. | : 250 °C |
| FID $H_2$ Flow | : 32 mL/min |
| FID Makeup Flow | : 24 mL/min, $N_2$ |
| FID Air Flow | : 200 mL/min |

## ■ Sample Preparation

Standard solutions for the ethanol calibration curve were prepared for the final concentrations of 0.1, 0.5, 1.0, and 2.0 mg/mL. tert-butanol was used as the internal standard solution, and it was prepared to a final concentration of 1.0 mg/mL.

After spiking a 20 mL volume HS vial with 480 µL of water or blood, followed by 20 µL of calibration standard and then 100 µL of internal standard, then the vial was capped and mixed. In this experiment, commercially available human whole blood was used. When analyzing blood for a forensic investigation, 500 µL of blood and 100 µL of internal standard would presumably be inserted into the HS vial.

## ■ Analysis Process

The recommended procedure for analyzing blood samples is shown in Fig. 4.

High-concentration samples were analyzed first as a precondition to improve analytical repeatability. Polar components, such as ethanol and methanol, tend to be readily absorbed by the sample line, resulting in poor detection of the peak area immediately after the start of analysis, and this may gradually increase with subsequent runs. Therefore, filling the adsorption points by initially analyzing high-concentration samples promotes analytical stability by inhibiting fluctuations in detected peak areas.

A mixed solution containing 10 mg/mL each of ethanol and tert-butanol was used as the high-concentration sample. After analyzing it, 2 serial injections of water were analyzed as blanks. The resulting chromatograms demonstrated that there were no traces of ethanol or tert-butanol in the blank analysis.

After this preconditioning process, 5 calibration solutions were analyzed, followed by the blood samples. To determine the change in peak area over time, an aqueous ethanol standard with a final vial concentration of 1 mg/mL was analyzed for each of the 5 blood sample analysis runs.

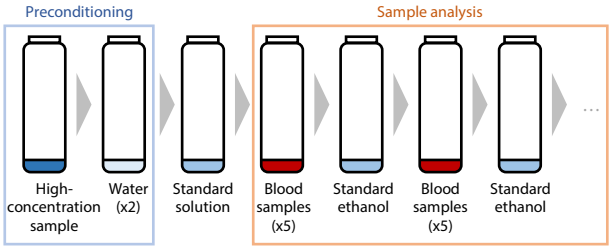


Fig. 4 Recommended Analytical Process

## ■ Linearity of the Calibration Curve

Serial dilutions were prepared with blood and water solutions, and calibration curves were created. The results compared with the calibration curve are shown in Fig. 5. Even with solutions prepared from blood and water, the coefficient of determination ($R^2$) exceeded 0.999, which demonstrates good linearity.

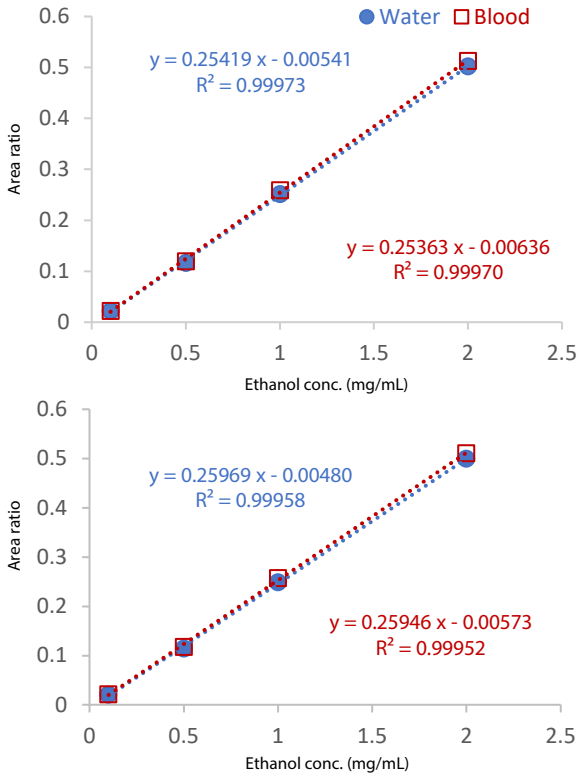


Fig. 5 Calibration Curve of Ethanol Standard Solution
(Top: SH-BAC PLUS1, Bottom: SH-BAC PLUS2)

## ■ Accuracy and Repeatability of Spiked Blood Samples

To demonstrate the quantitative accuracy of the blood sample analysis, blood samples prepared to predetermined concentrations were quantified with calibration curves created from blood and water solutions, and the area ratio repeatability of each concentration (%RSD) was determined. The results are shown in Table 3 below. Each concentration was consecutively analyzed 5 times by the recommended analytical process (Fig. 4). To determine the change in peak area over time, 1 mg/mL of standard water solution was analyzed for each of the 5 blood sample analysis runs. Peak area repeatability of 1 mg/mL standard water solution was found to be good at %RSD=1.51.

Table 3 Concentration and Peak Area Repeatability %RSD (n=5)

| Prepared concentration | Using water calibration curve | Using blood calibration curve | Area ratio repeatability (%RSD) |
|---|---|---|---|
| | Conc. (mg/mL) | Conc. (mg/mL) | |
| 0.1 mg/mL | | | |
| SH-BAC PLUS1 | 0.114 | 0.118 | 1.93 |
| SH-BAC PLUS2 | 0.114 | 0.119 | 1.89 |
| 0.5 mg/mL | | | |
| SH-BAC PLUS1 | 0.512 | 0.499 | 0.50 |
| SH-BAC PLUS2 | 0.511 | 0.497 | 0.52 |
| 1 mg/mL | | | |
| SH-BAC PLUS1 | 1.04 | 1.019 | 1.23 |
| SH-BAC PLUS2 | 1.05 | 1.019 | 1.21 |
| 2 mg/mL | | | |
| SH-BAC PLUS1 | 2.11 | 2.059 | 1.60 |
| SH-BAC PLUS2 | 2.11 | 2.059 | 1.60 |

Table 4 Retention Time and Peak Area Repeatability %RSD (n=5)

| ID | Compound | SH-BAC PLUS1 | | SH-BAC PLUS2 | |
|---|---|---|---|---|---|
| | | Retention time (min) | Area ratio repeatability (%RSD) | Retention time (min) | Area ratio repeatability (%RSD) |
| 1 | Methanol | 2.107 | 1.61 | 1.713 | 1.12 |
| 2 | Acetaldehyde | 2.114 | 0.98 | 1.642 | 0.87 |
| 3 | Ethanol | 2.409 | 0.69 | 1.945 | 1.03 |
| 4 | 2-Propanol | 2.816 | 0.41 | 2.134 | 1.36 |
| 5 | Acetonitrile | 2.939 | 0.85 | 2.365 | 1.02 |
| 6 | Acetone | 3.023 | 0.59 | 2.080 | 1.34 |
| 7 | tert-Butanol | 3.226 | I.S. | 2.229 | I.S. |
| 8 | 1-Propanol | 3.629 | 0.42 | 2.736 | 1.50 |
| 9 | Methyl ethyl ketone | 5.020 | 0.49 | 2.977 | 1.31 |
| 10 | Ethyl acetate | 5.314 | 0.70 | 2.833 | 1.08 |

## ■ Separation of 10 Blood VOCs

A solution of 10 VOCs spiked with blood (0.1 mg/mL) was analyzed to determine the separation of methanol, acetaldehyde, ethanol, isopropyl alcohol, acetonitrile, acetone, tert-butanol, 1-propanol, methyl ethyl ketone, and ethyl acetate. The resulting chromatogram is shown in Fig. 6. All peaks were detected within 6.0 minutes in the BAC PLUS1 column and within 3.5 minutes in the BAC PLUS2 column, indicating good separation. The elution sequence of the 10 blood VOCs differed significantly between the columns, which enhanced the system's qualitative capability. Table 4 shows the retention time of each compound and the repeatability (n=5) of the peak area ratio for the tert-butanol internal standard.

## ■ Conclusion

This experiment involved dual-column analysis of blood alcohol content (BAC) by the compact Brevis GC-2050 and the HS-20 NX. Dual-column configuration enables cross-checking in a single analysis. In addition, nitrogen was used as the carrier gas instead of helium. While hydrogen enables high-speed analysis, it also requires careful handling, so from the perspective of safety, installation of various optional tools such as hydrogen sensor and stop valve have been recommended. Nitrogen gas is relatively inexpensive and easy to handle, and this experiment demonstrated that it also provides reliable results while maintaining good separation.

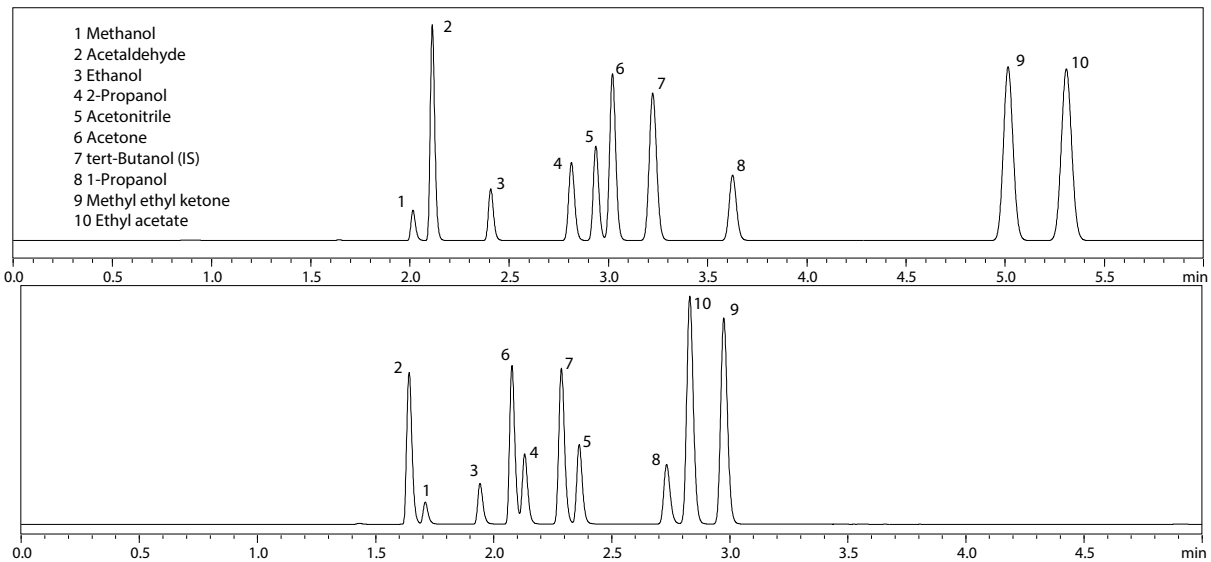


Fig. 6 Chromatograms of Mixed Solution Containing 10 Blood VOCs
(Top: SH-BAC PLUS1, Bottom: SH-BAC PLUS2)



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