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Application News

Gas Chromatography

Analysis of Blood Alcohol Using the Shimadzu GC-2010 Plus and HS-10 Headspace Sampler

No. GC-004

■ Introduction

The analysis of ethanol (alcohol) concentration in blood is routinely carried out in forensic labs and the medical field. The generally accepted method to accurately determine blood alcohol content (BAC) uses static headspace sampling and dual column separation by gas chromatography followed by flame ionization detection (GC/FID). This application note demonstrates a fast, accurate and reproducible BAC analysis carried out with an HS-10 static headspace sampler and a Shimadzu GC-2010 Plus Gas Chromatograph (Figure 1).

■ 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 a transfer line was used for sample preparation and introduction into the GC through a single SPL. Effluent from the HS-10 was divided between two columns (Rtx-BAC1, 0.53mm × 30m × 3.0µm, Restek 18001 and Rtx-BAC2, 0.53mm × 30m × 2.0µm, Restek 18000) using a Y-connection (Restek 20406), presumably at a 1:1 ratio. Each column was connected to a separate FID and analyzed simultaneously.

Figure 1: HS-10 headspace sampler and GC-2010 Plus

■ Standards and Sample Preparation

Blood alcohol resolution standard n-P was purchased from Restek, #36010. Calibration standards (0.0200 g/dL, 0.0400 g/dL, 0.100 g/dL, 0.200 g/dL and 0.400 g/dL ethanol) and BAC control standard (0.0800 g/dL ethanol) were prepared by serial dilution from 200 proof ethanol (Fisher, BP2818) with deionized water to specified concentrations. An internal standard of 0.0200 g/dL *n*-propanol (IS) was prepared by diluting *n*-propanol (sigma, 34817) with deionized water. Aliquots for analyses were prepared by mixing 1.00mL of IS solution with 100µL of individual resolution, calibration or control standard in 20mL screw cap headspace vials (Shimadzu, 220-94796-01). For blank samples, 100µL deionized water is mixed with 1.00mL of IS solution.

■ Analytical Conditions

GC-2010 Plus

- SPL Temp = 150°C
- Column Temp = 40°C
- FID Temp = 250°C, H_2 flow = 40 mL/min, Air flow = 400 mL/min, Makeup flow = 20.0mL/min.
- Carrier gas: Helium
- Flow control mode: Pressure @ 88.0kPa, total column flow = 21.85 mL/min, split ratio = 10.0, purge flow = 0.5mL/min
- Injection volume: 1mL headspace
- GC run time: 3 min

HS-10

- Vial equilibration: 15min @ 80°C
- Sample Pathway Temp = 95°C
- Transfer Line Temp = 105°C
- Vial Pressurization: 1.00 min @160kPa
- Loop Load Time = 0.50 min
- Injection time = 1.00 min

■ Results

Resolution of components in BAC mixture

The headspace sample of BAC resolution standard n-P was split between the Rtx-BAC1 and Rtx-BAC2 column in a nominal 1:1 ratio and analyzed simultaneously. All six components in the mixture eluted in less than three min with satisfactory resolution (Figure 2). Note that elution order of the BAC2 column differs from that of the BAC1 column, which in practice is used to confirm results from BAC1.

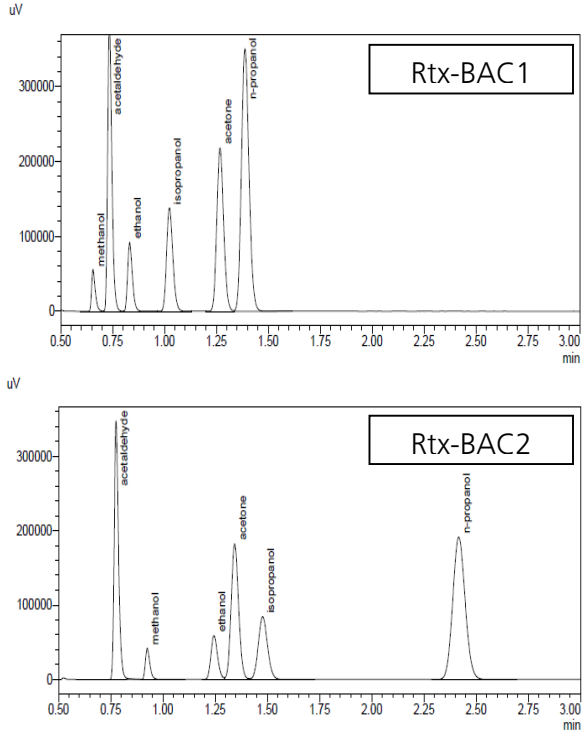

Figure 2: Analysis of BAC resolution standard n-P on Rtx-BAC1 (top chromatogram) and Rtx-BAC2 (bottom chromatogram).

Calibration

Duplicates of each calibration standard were run to generate the calibration curves (Figure 3). A blank sample (deionized water with IS) was run after the highest calibration concentration of ethanol to address potential carryover issue. As shown below, a calibration curve with excellent linearity was generated in either analytical line (Figure 4). Furthermore, no significant carryover was detected (Figure 3).

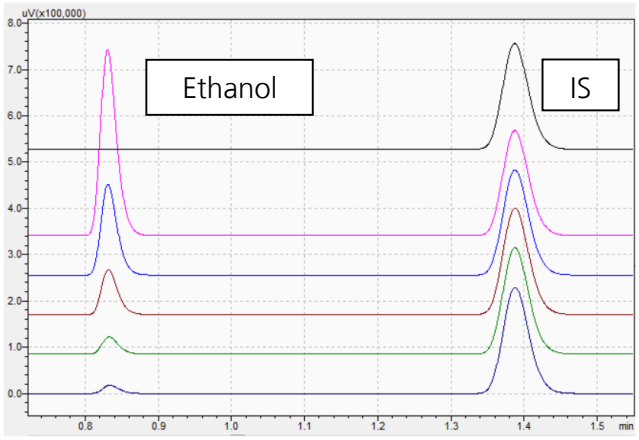

Figure 3: Chromatograms of calibration standards and a blank.

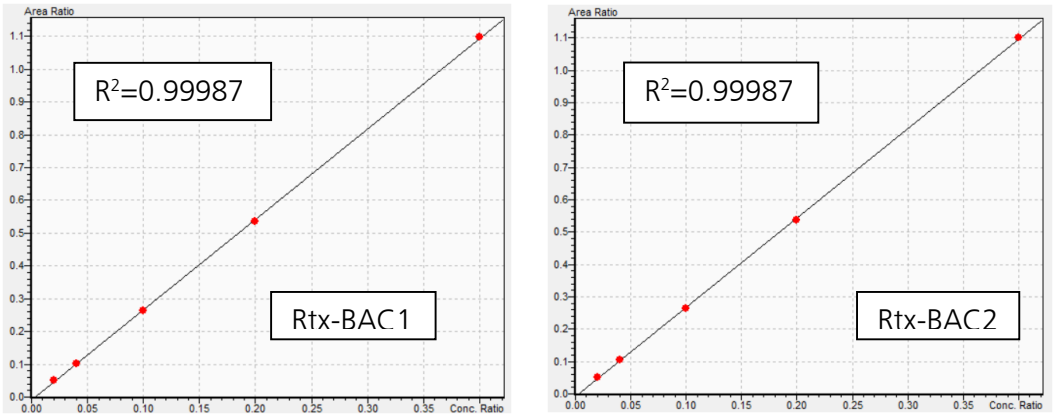

Figure 4: Five-point calibration curve for ethanol from indicated analytical line.

Reproducibility

Six aliquots of BAC control standard were run consecutively and the results are shown in Table 1. Highly reproducible measurements were observed from both analytical lines: the % RSD for concentration is 0.570 from Rtx-BAC1 and 0.500 from Rtx-BAC2.

<< FID >>
ID#1 Compound Name: Ethanol

Title	Ret. Time	Height	Area	ISTD Area	Area Ratio	Conc.	Unit
BAC 021116 14.gcd	0.832	74872	122085	582261	0.210	0.0795	g/dL
BAC 021116 15.gcd	0.832	73912	120790	576113	0.210	0.0795	g/dL
BAC 021116 16.gcd	0.832	75385	122896	589209	0.209	0.0791	g/dL
BAC 021116 17.gcd	0.832	74233	120821	580367	0.208	0.0789	g/dL
BAC 021116 18.gcd	0.832	73169	120269	578194	0.208	0.0788	g/dL
BAC 021116 19.gcd	0.832	75053	122842	581954	0.211	0.0800	g/dL
Average	0.832	74437	121617	581349	0.209	0.0793	
%RSD	0.000	1.107	0.937	0.774	0.579	0.570	
Standard Deviation	0.000	824	1139	4501	0.00121	0.0005	

Table 1: Quantitative results of BAC control standard from Rtx-BAC1 analytical line. Similar results were obtained from Rtx-BAC2 analytical line.

■ Conclusions

In this study, BAC analysis was carried out successfully with a Shimadzu HS-10 static headspace sampler and a GC-2010Plus. This straightforward automated set up demonstrates good resolution, short run times as well as excellent reproducibility of ethanol concentration measurements, thus serves as a cost effective choice for routine BAC analysis.

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