

# Application Data Sheet

## No. 12

### GC

Gas Chromatography

## Analysis of Alcohol Compounds in Blood (1)

Measurements of oxygenated compounds and alcohols, primarily ethanol, in blood, are frequently performed in forensic medicine, emergency medicine, and other fields. In forensic medicine, such measurements are utilized to determine levels of intoxication from alcohol consumption and to evaluate criminality. In emergency medicine, they are utilized to distinguish between alcohol consumption and other medical cases. For such analyses, systems equipped with a headspace sampler and GC-FID detector are often used.

These measurements must be convenient and quick, and provide high-accuracy results. The Shimadzu HS-20 headspace sampler is capable of meeting these demands. When the HS-20, GC-2010 Plus, and a workstation (LabSolutions LC/GC) are used in combination, everything including the headspace conditions and GC conditions can be controlled from the workstation, which makes it easy to configure settings and operate the system.

This report examines the repeatability and linearity of a standard ethanol solution, and confirms the separation of other oxygenated compound standard solutions. The analysis presented here was performed using the HS-20 and the Rtx-BACPlus series of high-separation columns specifically designed for the analysis of alcohol in blood.

### Analysis Conditions

#### HS-20

##### Shared Conditions

Oven Temp.:	85 °C	Vial Agitation:	Off
Vial Warming Time:	15 min.	Vial Pressurization:	100 kPa
Vial Pressurization Time:	1 min.	Load Time:	0.5 min.
Injection Time:	0.5 min.	Needle Flash Time:	0.5 min.
Sample Line Temp.:	150 °C	Transfer Line Temp.:	150 °C
Vial Volume:	20 mL		

#### GC-2010 Plus AF + LabSolutions LC/GC

Columns:	(1) Rtx-BAC Plus 2, 0.32 mm × 30 m, d.f. 0.6 μm (for screening)		
	(2) Rtx-BAC Plus 1, 0.32 mm × 30 m, d.f. 1.8 μm (for separation confirmation)		
Column Temp.:	40 °C		
Carrier Gas Pressure:	100 kPa (helium pressure mode)	Split Ratio:	1:20
FID Temp.:	250 °C	Hydrogen:	40 mL/min.
Makeup Gas:	30 mL/min. (helium)	Air:	400 mL/min.

### Results

Table 1 shows area value repeatability for standard solutions (0.1 mg/mL) of ethanol and t-butanol, and area ratio repeatability for ethanol and t-butanol, both obtained using the Rtx-BAC Plus 2 column. Fig. 1 shows overlapping chromatograms for ethanol in the standard solution. As indicated, favorable repeatability was obtained, with an ethanol area value repeatability of 1.42 %, and area ratio repeatability of 0.62 %.

Fig. 2 shows the linearity of ethanol in the 0.1 mg/mL to 1.6 mg/mL range. It is evident that favorable linearity was obtained, with  $R = 0.9999$  or higher.

Fig. 3 shows an example of the separation of a standard solution with seven oxygenated components, including alcohol, using the Rtx-BAC Plus 2. All peaks were successfully separated within 3 minutes. Fig. 4 shows the separation of a standard solution with seven oxygenated components using the Rtx-BAC Plus 1. The separation pattern shown is different from that with the Rtx-BAC Plus 2, so qualitative ability is increased by performing analysis with both columns.

Fig. 5 shows the separation of a standard solution with 10 oxygenated components using the Rtx-BAC Plus 1. The 10 oxygenated components were successfully separated in 5 minutes.

Table 1 Repeatability (n = 7) of Peak Area Values for 0.1 mg/mL of Ethanol and t-Butanol

	1	2	3	4	5	6	7	mean	RSD%
Ethanol	57886	58682	57373	57753	58193	56139	57141	57595	1.422
t-Butanol	969772	980873	964274	958311	969829	949515	953278	963693	1.128
Ethanol/ t-Butanol	0.05969	0.059826	0.059499	0.060265	0.060003	0.059124	0.059942	0.059764	0.622

Table 2 Repeatability (n = 7) of Retention Times for 0.1 mg/mL of Ethanol and t-Butanol

	1	2	3	4	5	6	7	mean	RSD%
Ethanol	1.691	1.691	1.691	1.692	1.691	1.692	1.691	1.691	0.0230
t-Butanol	2.117	2.117	2.117	2.118	2.117	2.118	2.117	2.117	0.0180

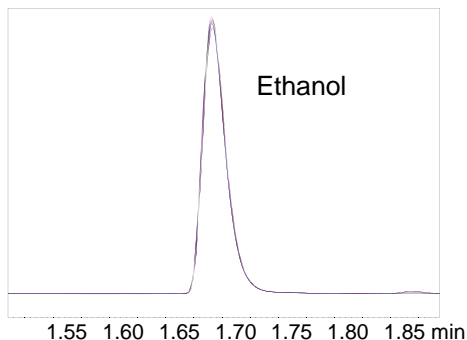


Fig. 1 Repeatability (n = 7) for a 0.1 mg/mL Standard Solution of Ethanol

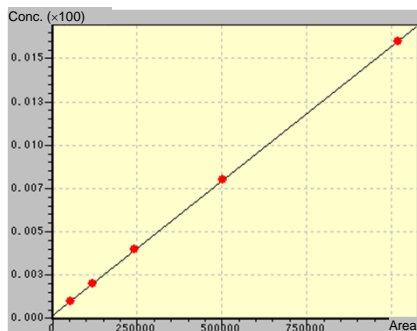


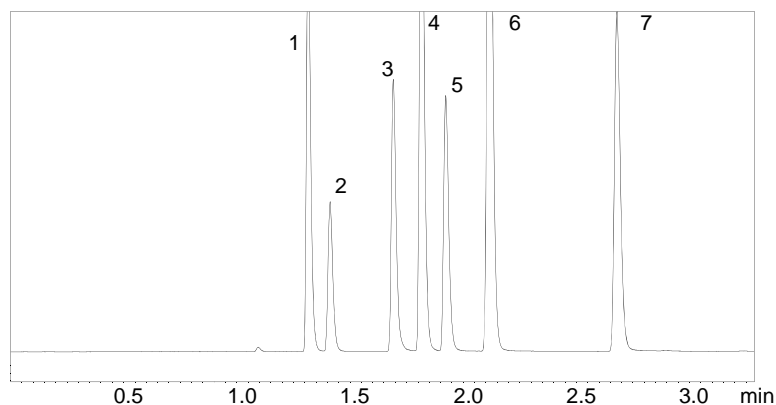
Fig. 2 Linearity of 0.1 mg/mL to 1.6 mg/mL Standard Solutions of Ethanol

$$Y = aX + b$$

$a = 1.553005e-006$   
 $b = 1.974794e-002$   
 $R^2 = 0.9999422$   
 $R = 0.9999711$

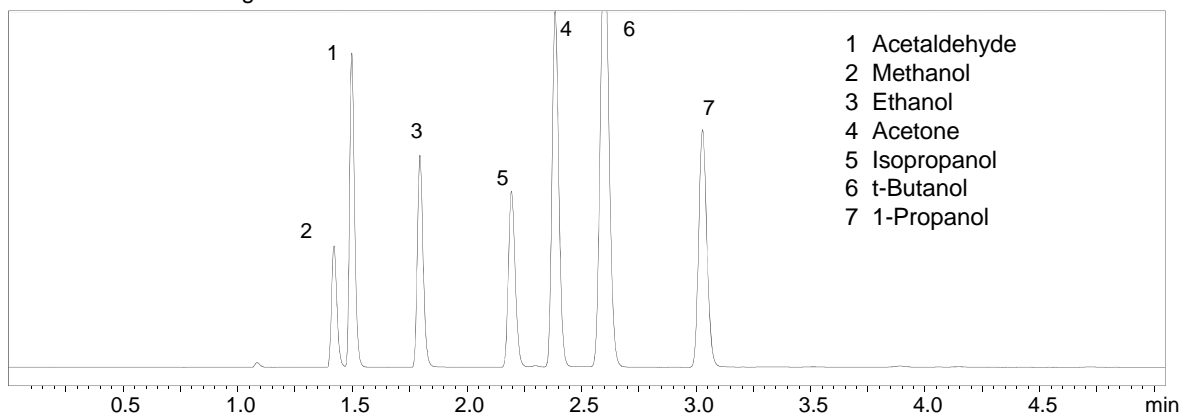
External standard method  
Calibration curve: Straight line  
Through the origin: No  
Weighting: None

Mean RF: 1.674826e-006  
RF standard deviation: 1.019928e-007  
RF relative standard deviation: 6.089755



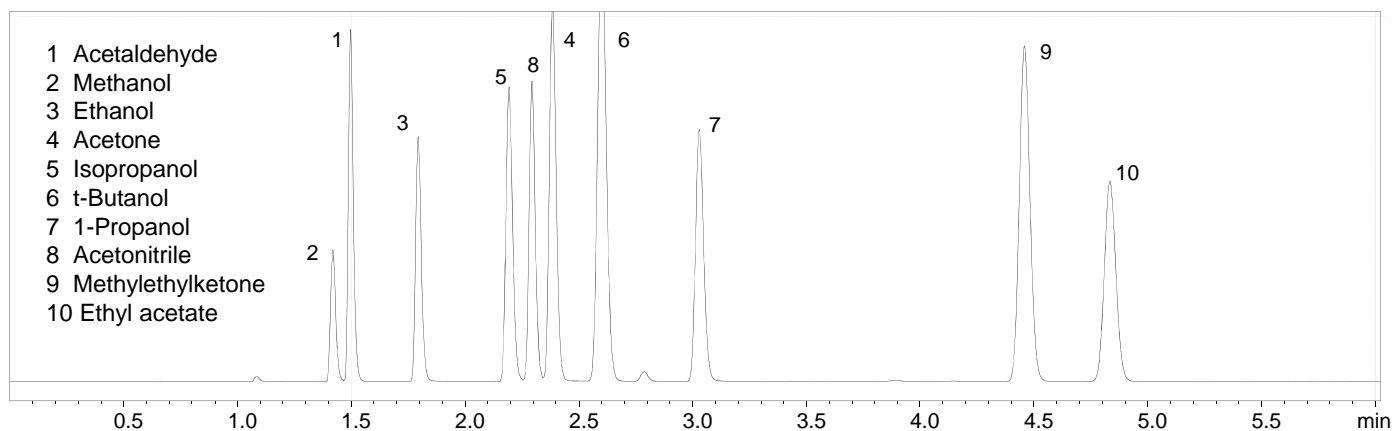
- 1 Acetaldehyde
- 2 Methanol
- 3 Ethanol
- 4 Acetone
- 5 Isopropanol
- 6 t-Butanol
- 7 1-Propanol

Fig. 3 Chromatograms for Seven Components (0.1 mg/mL each) Obtained Using the Rtx-BAC Plus 2



- 1 Acetaldehyde
- 2 Methanol
- 3 Ethanol
- 4 Acetone
- 5 Isopropanol
- 6 t-Butanol
- 7 1-Propanol

Fig. 4 Chromatograms for Seven Components (0.1 mg/mL each) Obtained Using the Rtx-BAC Plus 1



- 1 Acetaldehyde
- 2 Methanol
- 3 Ethanol
- 4 Acetone
- 5 Isopropanol
- 6 t-Butanol
- 7 1-Propanol
- 8 Acetonitrile
- 9 Methyl ethyl ketone
- 10 Ethyl acetate

Fig. 5 Chromatograms for 10 Components (0.1 mg/mL each) Obtained Using the Rtx-BAC Plus 1