

# Application Data Sheet

## No. 7

### GCMS

Gas Chromatograph Mass Spectrometer

## Measurement of Ethanol in Blood Using Headspace GC-MS

Ethanol in blood is analyzed in the context of scientific crime detection with respect to traffic accidents caused by drinking, and violence, injuries, and homicides associated with alcohol[1,2]. The concentration of ethanol in blood is an important factor in understanding the extent of intoxication. Under the Road Traffic Act and the Order for Enforcement of the Road Traffic Act, an individual is considered to be under the influence of alcohol if the blood ethanol level is 0.3 mg/mL or more. While packed column GC-FID and capillary GC-FID are recognized measurement methods, a GC-MS is often used instead. This datasheet investigates the ability of Headspace GC-MS to measure ethanol in blood.

### Experiment

#### Reagents

An aqueous solution of 1-propanol added to a blood sample as an internal standard was prepared by dissolving 1-propanol (special grade reagent from Wako Pure Chemical Industries) in milliQ distilled water. The standard aqueous ethanol solution used to create the calibration curve was prepared by incrementally diluting ethanol (special grade reagent from Wako Pure Chemical Industries) in milliQ distilled water to prepare samples at each concentration of 0.03 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 1.0 mg/mL, and 2.0 mg/mL. In addition, to confirm that the 2-propanol used for sterilization during blood sampling is separated in the chromatogram from the ethanol and 1-propanol, aqueous solutions exclusively for confirmation of separation were prepared with regard to ethanol, 2-propanol, and 1-propanol, respectively. In terms of actual samples, blood was collected from an individual who had not been drinking and was stored in a Spitz tube containing a blood anticoagulant. A prescribed amount of the blood was measured out, ethanol was added, and the mixture was agitated with Vortex to create the blood sample spiked with ethanol (0.3 mg/mL).

#### Treatment

For the standard sample's calibration curve, 0.5 mL of standard aqueous ethanol solutions (0.03 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 1.0 mg/mL, and 2.0 mg/mL) were added to headspace vials (22 mL). Next, 0.5 mL of the internal standard aqueous 1-propanol solution (0.5 mg/mL) was added, and the vials were immediately sealed with headspace caps (aluminum cap: butyl rubber/PTFE). Similarly, 0.5 mL of the blood spiked with ethanol was added to a headspace vial. Next, 0.5 mL of internal standard aqueous 1-propanol solution (0.5 mg/mL) was added, and the vial was immediately sealed with a cap.

#### Instrument

A direct connection between headspace and the GC-MS was adopted to minimize carrier gas consumption, and a Press-Tight connector (P/N: 221-38102-91) was used for the connection between columns. The analysis conditions are shown in Table 1.

Table 1: Analysis Conditions

HS	: TurboMatrix HS		
GC-MS	: GCMS-QP2010 Ultra		
[HS]		[GC]	
Headspace mode	: constant	Vaporization chamber temperature	: 200°C
Injection time	: 0.05 min *	Column	: Rtx®-BAC2 (30 mL × 0.32 mm I.D., 1.2 μm Restek)
Zone temperature settings	: (O/N/T)	Column oven temperature	: 40°C (5min) -> (40°C/min) -> 200°C (1min)
Oven temperature	: 60°C	Carrier gas	: Helium
Needle temperature	: 100°C	[MS]	
Transfer temperature	: 150°C	Interface temperature	: 230°C
Sample shaker	: OFF	Solvent elution time	: 0.7 min
GC cycle time	: 20 min	Measurement mode	: Scan
Pressurization time	: 1 min	Event time	: 0.5 sec
Uptake time	: 0 min	Detector voltage	: -0.1 kV(relative value) *
Warming time	: 15 min		
HS carrier gas pressure	: 70 kPa		
		Ion source temperature	: 200°C
		Data sampling time	: 1 to 10 min
		Mass range	: m/z 29-300
		Emission current	: 150 μA (high sensitivity)

\*Note: The headspace sampler injection time and detection voltage must be optimized since they can differ depending on equipment status.

## Results

The total ion current chromatogram obtained from the mixed sample is shown in Fig. 1. It can be confirmed that the 2-propanol, which was used for sterilization when sampling the blood, was completely separated in the chromatogram and had no effect on the quantitative values. The calibration curve obtained via internal standard calibration (0.03 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 1.0 mg/mL, and 2.0 mg/mL) is shown in Fig. 2. Utilizing this calibration curve, Fig. 3 and Table 2 show the mass chromatograms and repeatability obtained from the results of measuring the blood spiked with ethanol and an aqueous solution with an ethanol concentration equivalent to 1/10 the 0.3 mg/mL blood ethanol concentration considered as under the influence.

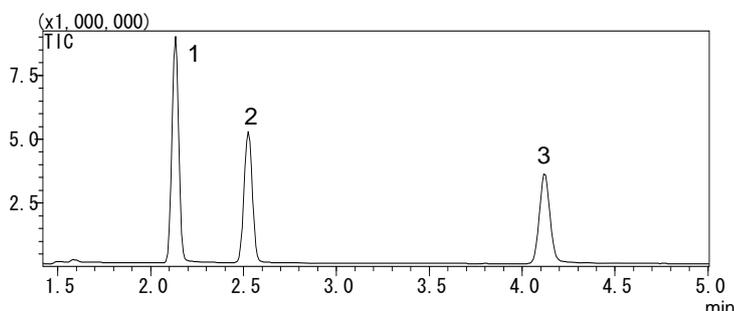


Fig. 1: Total Ion Current Chromatogram  
1 = Ethanol; 2 = 2-propanol; 3 = 1-propanol

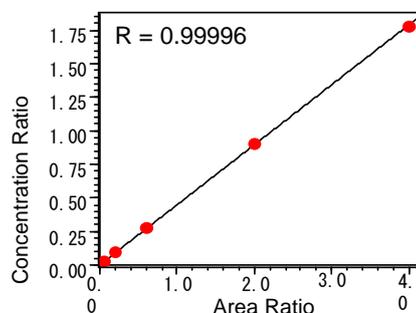


Fig. 2: Ethanol Calibration Curve  
Ethanol concentration: 0.03 mg/mL; 0.1 mg/mL;  
0.3 mg/mL; 1.0 mg/mL; 2.0 mg/mL  
1-propanol concentration: 0.5 mg/mL

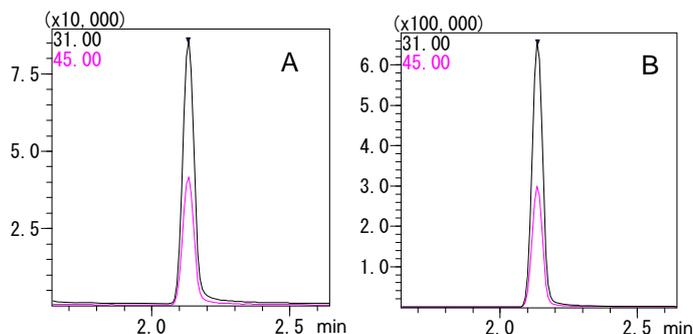


Fig. 3: Mass Chromatograms for (A) Aqueous Ethanol Solution (0.03 mg/mL) and (B) Blood Spiked with Ethanol (0.3 mg/mL)

Table 2: Quantitative Results and Repeatability

	Aqueous Ethanol Solution	Blood Spiked with Ethanol
1	0.0299	0.296
2	0.0293	0.303
3	0.0293	0.302
4	0.0304	0.303
5	0.0294	0.296
6	0.0295	0.293
7	0.0291	0.291
Average	0.0296	0.298
%RSD	1.5563	1.697
Maximum	0.0304	0.303
Minimum	0.0291	0.291
Standard D	0.0005	0.005

Units mg/mL, n = 7

## Summary

While ethanol concentrations in blood differ depending on the degree of intoxication, it has been reported that, in general, a value of 3.5 mg/mL or higher will cause most people to fall into a comatose state, and deaths from paralysis of the respiratory center[1,2]. For this reason, in this investigation, a calibration curve was created ranging from a concentration of 0.03 mg/mL, which is equivalent to 1/10 the 0.3 mg/mL blood ethanol concentration considered as under the influence as per the Road Traffic Act, all the way to a concentration of 2.0 mg/mL. Satisfactory linearity is indicated in the results, with a correlation coefficient of 0.99996. In addition, satisfactory quantitative results were obtained from measuring the aqueous ethanol solution (0.03 mg/mL) and blood spiked with ethanol (0.3 mg/mL), with average values of 0.0296 mg/mL (1.56 % CV) and 0.298 mg/mL (1.70 % CV), respectively.

### References

- [1] Osamu Suzuki, Mikio Yashiki, editors: Dokuyakubutsu Bunseki Jissen Handbook – Chromatography-wo-cyushin-toshite (Practical Handbook for the Analysis of Toxic Pharmaceuticals, with a Focus on Chromatography) Jiho, Inc., Tokyo 2002.  
[2] The Pharmaceutical Society of Japan, editors: Dokuyakubutsu-shiken-ho-to-cyukai 2006 – Bunseki, Dokusei, Taisho-ho (Testing Methods and Annotation for Toxic Pharmaceuticals 2006 – Analysis, Toxicity, and Coping Methods) Tokyo Kagaku Dojin, Tokyo, 2006.

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