SHIMADZU

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

*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.

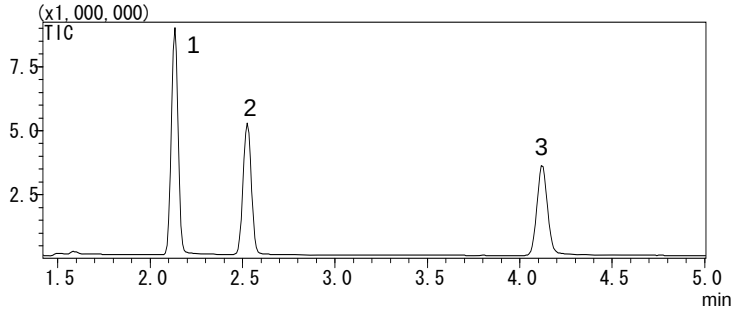

Fig. 1: Total Ion Current Chromatogram
1 = Ethanol; 2 = 2-propanol; 3 = 1-propanol

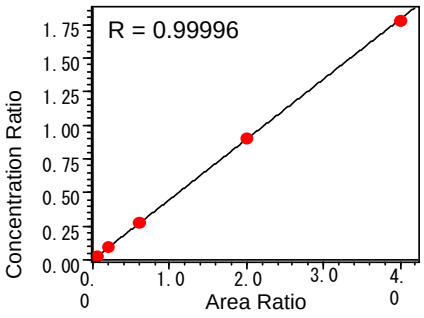

Fig. 2: Ethanol Calibration Curve
Ethanol concentration: 0.03 mg/mL; 0.1 mg/mL; 0.3 mg/mL; 1.0 mg/mL; 2.0 mg/mL
1-propanol concentration: 0.5 mg/mL

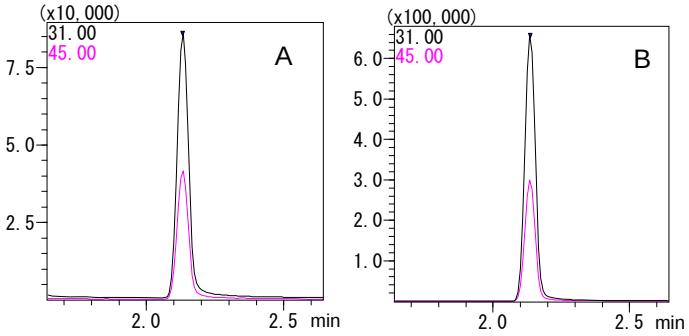

Fig. 3: Mass Chromatograms for (A) Aqueous Ethanol Solution (0.03 mg/mL) and (B) Blood Spiked with Ethanol (0.3 mg/mL)

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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