SHIMADZU
Excellence in Science

GC-MS GCMS-QP™2020 NX, HS-20 NX

Application News

Simultaneous Analysis of Blood Alcohol and Volatile Toxic Substances Using Headspace GC-MS

Yuki Sakamoto

User Benefits

- Headspace GC-MS enables simultaneous analysis of blood alcohol and volatile toxic substances, such as cyanide and azide.
- The HS-20 NX is designed to provide sample flow path inertness and the shortest possible flow path, which reduces carryover and enables simultaneous and simple analysis of high ethanol concentrations, such as those present in blood alcohol analysis, and trace amounts of volatile toxic substances.

■ Introduction

Police laboratories and university forensic medicine departments analyze a variety of volatile toxic substances in the course of investigating accidents, crimes, and other incidents.

Blood alcohol (ethanol) levels are analyzed for evidence of intoxication in traffic accidents involving drunk driving, physical assault, and injuries inflicted while under the influence of alcohol and acute alcohol poisoning. Paint thinners containing mainly toluene, methanol, and ethyl acetate have anesthetic and stimulant properties, and the abuse of thinners by inhalation has become a social issue that is now regulated by legislation aimed at preventing abuse. Meanwhile, cyanides and azides are chemical compounds with industrial applications, making them relatively accessible to those in certain industries. This easy access has led to the major social issue of contamination incidents caused by abuse of these toxic substances. Therefore, systems for testing major poisons and toxic substances have been enhanced to better identify the causes of suicide and various crimes.

The headspace technique enables relatively simple measurements of blood alcohol and paint thinner concentrations, so it is routinely used by police laboratories and university forensic medicine departments. Cyanides and azides are typically measured using GC-MS on samples that have undergone PFB derivitization and solvent extraction followed by liquid injection. However, this technique is cumbersome due to the need for pre-treatment of samples, including the derivitization and extraction steps.

This Application News describes using the headspace GC/MS technique to simultaneously analyze alcohol and paint thinner—which are typically measured with the headspace technique—together with cyanide and azide.

■ Sample Preparation

Equine hemolysate was used for the blood samples, and 2-methyl-1-propanol and 2-propanol-d8 were used as the internal standards. An internal standard mix solution was also prepared by dissolving the internal standards in ultrapure water to obtain 0.2 mg/mL 2-methyl-1-propanol and 0.005 µg/mL 2-propanol-d8.

An ascorbic acid solution (0.1 M) was prepared to a volume of 100 mL after dissolving 1.76 g of L-ascorbic acid in ultrapure water. An aqueous phosphoric acid solution (50 %) was prepared using a 1.7-fold dilution of commercially-available phosphoric acid (85 %) in ultrapure water. A cyanide ion (CN^-) standard solution (1 mg/mL) was prepared to a volume of 100 mL after dissolving 250 mg of potassium cyanide (KCN) in 0.1 M aqueous NaOH solution. An azide ion ($N3^-$) standard solution (1 mg/mL) was prepared to a volume of 100 mL after dissolving 155 mg of sodium azide (NaN_3) in ultrapure water.

Calibration curve standards for cyanide, azide, and ethanol were prepared by spiking blood samples immediately before analysis to obtain cyanide concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 µg/mL, azide concentrations of 2.0, 5.0, 10.0, 20.0, and 50.0 µg/mL, and ethanol concentrations of 0.03, 0.1, 0.3, 1.0, and 2.0 mg/mL. Aliquoted 0.5 mL samples of blood spiked with the respective concentrations of cyanide, azide, and ethanol were added to 20 mL headspace vials, and 0.5 mL of internal standard mix solution was then added. Next, 0.2 mL of 0.1M aqueous ascorbic acid solution was added, and then 0.2 mL of 50 % phosphoric acid was added by pouring it along the inner wall of the vial. Each vial was then sealed with the headspace cap and agitated. For the paint thinner analysis, 5 µL of paint thinner stock solution was added to the 20 mL headspace vial and immediately sealed with the headspace cap.

Fig. 1 HS-20 NX and GCMS-QP™ 2020 NX

■ Analytical Conditions

Table 1 shows the headspace (HS) and GC/MS analytical conditions. Alcohol, cyanide, and azide were all analyzed using the same HS and GC/MS conditions. The paint thinner was analyzed after changing the split ratio and the detector voltage. When using the HS-20 NX, provided that the HS conditions are identical, measurements with different GC/MS analytical conditions can be performed by switching the methods in the same batch files. Changing the split ratio is effective for analyzing cyanides and azides requiring quantitative assay of trace concentrations and for high concentrations of paint thinner stock solution.

Table 1 HS-20 NX and GCMS-QP2020 NX Analytical Conditions

HS:	HS-20 NX				
GC-MS:	GCMS-QP2020 NX				
[HS]		[GC]			
Headspace Mode:	Loop	Column:	SH-BAC2 (length: 30 m, 0.32 mm I.D., df = 1.2 µm)		
Oven Temp.:	60 °C		P/N: 227-36262-01		
Sample Line Temp.:	100 °C	Column Oven Temp.:	40 °C (5 min)→(40 °C/min)→200 °C (1 min)		
Transfer Line Temp.:	150 °C	Carrier Gas:	Helium		
Pressurizing Gas Pressure:	70 kPa	Flow Control Mode:	Linear velocity (62.5 cm/sec)		
Equilibrating Time:	10 min	Injection Mode:	Split		
Vial Pressurization Pressurizing Time:	0.5 min	Split Ratio:	10:1 (alcohol, cyanide, azide)		
Pressure Equilibration Time:	0.1 min		30:1 (paint thinner)		
Load Time:	0.5 min	Carrier Gas Save Mode:	Split ratio 5 (1 min)		
Load Equilibration Time:	0 min	[MS]			
Injection Time:	0.5 min	Interface Temp.	230 °C	Ion Source Temp.:	200 °C
Needle Flush Time:	5 min	Solvent Cut Time:	0.7 min	Data Acquisition Time:	1–10 min
GC Cycle Time:	19 min	Analysis Mode:	Scan	Mass Range:	*m/z* 10–300
		Event Time:	Scan 0.1 sec, SIM 0.2 sec		

Note: The detector voltage needs to be optimized as it differs according to status of the device.

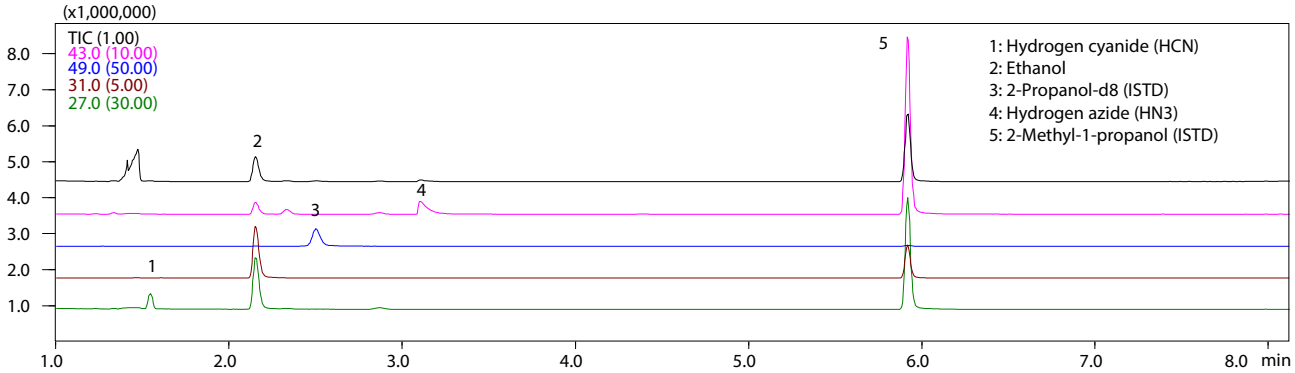

Fig. 2 Total Ion Current Chromatogram (Hydrogen Cyanide: 1 µg/mL, Ethanol: 0.3 mg/mL, Hydrogen Azide: 10 µg/mL)

■ Analysis Results

1. Alcohol, Cyanide, and Azide

Fig. 2 shows the total ion current chromatogram from the equine blood standards for the medium-concentration calibration curve, and Fig. 3 shows the SIM chromatograms from the blank samples and the standards for the lowest-concentration calibration curve. The respective correlation coefficients (R) exhibited good linearity at ≥0.9992 (Fig. 4).

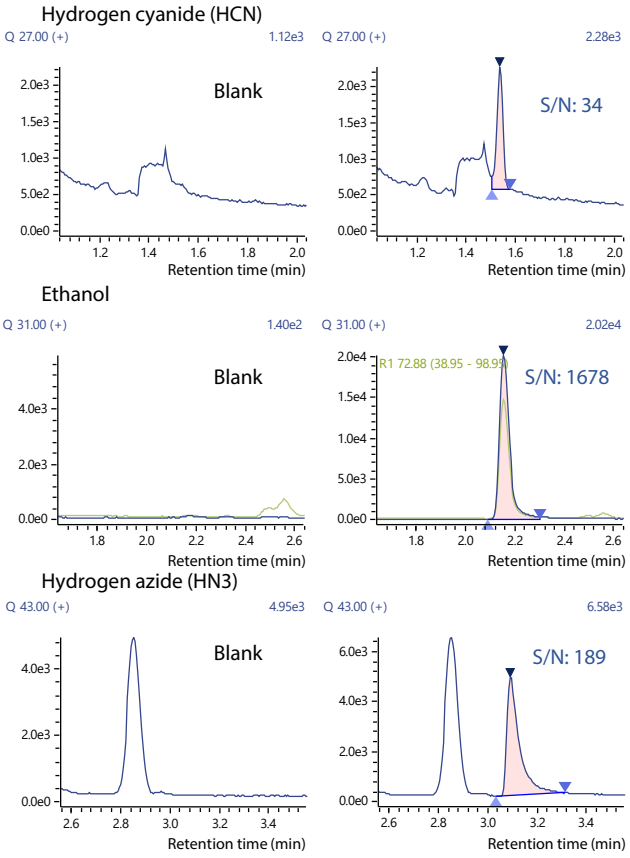

Fig. 3 SIM Chromatograms of Each Analyte Compound

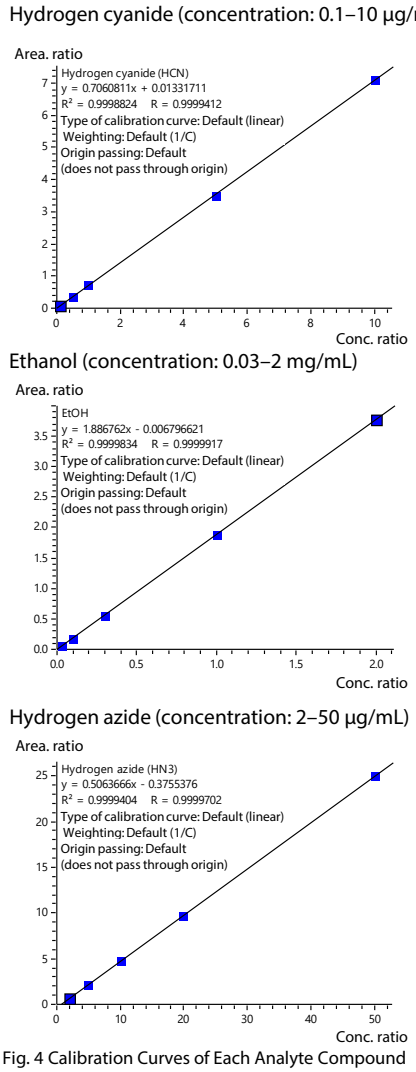

Fig. 4 Calibration Curves of Each Analyte Compound

Table 2 Intra-Day Repeatability (n=7) of Calibration Curve LV1 Concentrations

	Hydrogen Cyanide		Ethanol		Hydrogen Azide	
	Concentration (µg/mL)	Accuracy (%)	Concentration (mg/mL)	Accuracy (%)	Concentration (µg/mL)	Accuracy (%)
1st	0.105	104.8	0.032	106.6	1.819	91.0
2nd	0.099	99.0	0.032	107.5	1.820	91.0
3rd	0.105	105.1	0.033	108.7	1.658	82.9
4th	0.103	103.5	0.033	109.8	1.618	80.9
5th	0.103	103.3	0.032	106.1	1.469	73.5
6th	0.107	107.5	0.033	109.0	1.573	78.6
7th	0.104	103.7	0.032	107.5	1.541	77.1
Mean	0.104	103.8	0.032	107.9	1.643	82.1
Standard deviation (SD)	0.003	2.567	0.000	1.347	0.135	6.729
%RSD	2.472	2.472	1.249	1.249	8.192	8.192

Table 3 Inter-Day Repeatability of Calibration Curve LV1 Concentrations (5 Days)

	Hydrogen Cyanide		Ethanol		Hydrogen Azide	
	Concentration (µg/mL)	Accuracy (%)	Concentration (mg/mL)	Accuracy (%)	Concentration (µg/mL)	Accuracy (%)
Day 1	0.094	94.5	0.033	108.7	1.910	95.5
Day 2	0.105	105.2	0.033	110.2	1.999	99.9
Day 3	0.103	103.1	0.033	110.9	1.851	92.5
Day 4	0.100	100.2	0.033	109.1	2.140	107.0
Day 5	0.099	99.2	0.033	111.0	2.132	106.6
Mean	0.100	100.4	0.033	110.0	2.006	100.3
Standard deviation (SD)	0.004	4.080	0.000	1.032	0.130	6.490
%RSD	4.063	4.063	0.939	0.939	6.470	6.470

Seven assay replicates were performed each day for 5 days on the standard solution (equine hemolysate) with the LV1 concentration on the calibration curve to evaluate intra-day and inter-day repeatability. Intra-day repeatability on Day 1 is shown in Table 2, and inter-day repeatability after 5 days is shown in Table 3.

The accuracy of intra-day repeatability after 7 replications was 99.0–107.5 % (mean: 103.8 %) for hydrogen cyanide, 106.1–109.8 % (mean: 107.9 %) for ethanol, and 73.5–91.0 % (mean: 82.1 %) for hydrogen azide. The low accuracy for hydrogen azide was attributed to high adsorption, which was suggested by the peak tailings. Intra-assay precision was ≤10 % for all the compounds.

The accuracy of inter-day repeatability and intra-assay precision after 5 days was 94.5–105.2 % (mean: 100.4 %; %RSD: 4.063) for hydrogen cyanide, 108.7–111.0 % (mean: 110.0 %; %RSD: 0.939) for ethanol, and 95.5–106.6 % (mean: 100.3 %; %RSD: 6.470) for hydrogen azide, thus yielding good inter-day repeatability results for all 3 compounds.

2. Paint Thinner Components

Fig. 5 shows the total ion current chromatogram obtained from analyzing the main components of paint thinner, namely methanol, ethyl acetate, and toluene, using the same analytical conditions. The analytical conditions used in this analysis enabled the separation of the 3 main paint thinner components in 10 minutes.

Fig. 6 shows the SIM chromatograms of blood samples spiked with each of the main paint thinner components at a concentration of 0.1 µg/mL. The limit of detection (LOD) of each main component (the concentration at which peak-to-peak S/N=3) was 0.03 µg/mL for methanol, 0.01 µg/mL for ethyl acetate, and 0.003 µg/mL for toluene.

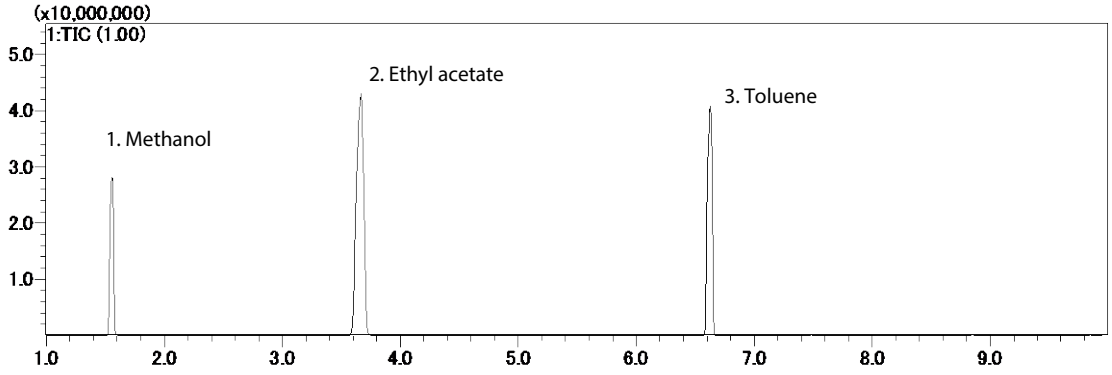

Fig. 5 Total Ion Current Chromatogram of Main Components of Paint Thinner

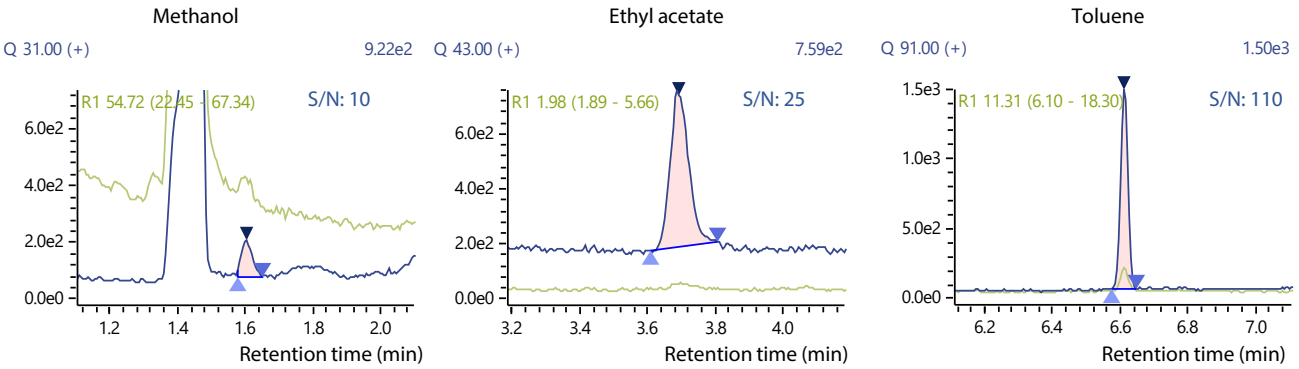

Fig. 6 SIM Chromatograms of Blood Samples Spiked with Main Components of Paint Thinner (Concentration: 0.1 µg/mL)

■ Conclusion

Analysis of the volatile toxic substances cyanide and azide was performed using the same column and analytical conditions as those used for measuring blood alcohol (ethanol) levels. By simply spiking the test samples with aqueous ascorbic acid solution and phosphoric acid, it was possible to analyze cyanide and azide using the same analytical conditions as those used to test blood alcohol levels.

The HS-20 NX headspace sampler is designed to provide outstanding vial incubation performance, sample flow path inertness, and the shortest possible flow path, which reduces carryover and enables simple analysis of high ethanol levels, such as those present in blood alcohol analysis, and trace amounts of cyanides and azides.

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

01-00667-EN First Edition: Feb. 2024