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Application Data Sheet

No.140

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Volatile Toxic Substances Using Headspace GC/MS Part.2 - Cyanide and Azide -

Forensic toxicologists in the police laboratories and forensic medicine departments of university measure a variety of volatile substances in the course of investigating accidents, crimes, and other incidents.
Used in industrial applications, cyanide and azide are compounds that are relatively easy to procure. This has resulted in incidents of contamination by these toxic substances. After these incidents, testing regimes for poisons were enhanced in order to determine the cause of such incidents, i.e. crime or suicide.
Blood alcohol and paint thinner can be measured relatively simply using headspace sampler, with measurements performed on a routine basis at police laboratories and in university forensic departments.
There have been reports of the headspace method being used to measure cyanide and azide. These compounds are normally measured by performing PFB derivatization, solvent extraction, and then liquid injection for GC/MS analysis. However, the derivatization and extraction steps of this method are labor-intensive.

This two-part application presents details on the investigation of using headspace GC/MS to measure cyanide and azide, as well as information on optimizing column conditions to allow simultaneous measurement of alcohol and paint thinner. Of these two investigations, results obtained from measuring cyanide and azide are presented below. Please see Application Data Sheet No. 139 for part one.

Sample Preparation

Aiming to carry out the same procedures as during blood alcohol analysis whenever possible, 1-propanol was used as the internal standard.

Standard Solutions
The standard solutions below were prepared as required.
- Aqueous solution of 1-propanol (0.5 mg/mL)
  5 mg of 1-propanol was weighed out, diluted in ultrapure water and made up to 10 mL in a measuring cylinder.
- Aqueous solution of ascorbic acid (0.1 M)
  1.76 g of L-ascorbic acid was weighed out, diluted in ultrapure water and made up to 10 mL in a measuring cylinder.
- Aqueous solution of phosphoric acid (50 %)
  Concentrated phsophoric acid (85 %) was diluted 1.7-fold in ultrapure water.
- Cyanide ion (CN-) standard solution (1 mg/mL)
  250 mg of potassium cyanide (KCN) was mixed with an aqueous solution of 0.1 M NaOH to make up 100 mL in a measuring cylinder.
- Azide ion (N3-) standard solution (1 mg/mL)
  155 mg of sodium azide (NaN_3) was diluted in ultrapure water and made up to 100 mL in a measuring cylinder.

Sample Preparation
Standards for calibration curve of cyanide and azide were prepared by adding each compound to hemolyzed equine blood to concentrations of 0.15, 0.5, 1.5, 5.0, and 10.0 μg/mL. 0.5 mL of each mixture of hemolyzed equine blood was then added to a 20 mL headspace vial, after which 0.5 mL of internal standard (aqueous solution of 1-propanol [0.5 mg/mL]) was added to each vial. 0.2 mL of 0.1 M ascorboc acid solution (aqueous) was then added, 0.2 mL of 50 % phosphoric acid was added to the inner wall, and the vial was quickly sealed with a headspace cap and agitated.

Analytical Conditions

Table 1 shows the headspace and GC/MS analytical conditions. Alcohol, cyanide, and azide were all measured using the same headspace and GC/MS conditions, and only paint thinner was measured with different conditions after changing the split ratio and detector voltage. With the HS-20 headspace sampler, even when GC/MS analysis conditions are changed, measurements can be performed within the same batch file by switching methods, as long as the same headspace conditions are used.

For this application, results were collected using the GCMS-TQ™8040 GC-MS/MS device, though the same results can be obtained using the GCMS-QP™2020 single-GC/MS device.

Table 1: Analytical Conditions

HS: HS-20
GC-MS: GCMS-TQ™8040

[HS]		[GC]			
Headspace mode:	Loop	Column:	Rtx™-BAC2 (length: 30 m, 0.32 mm I.D., df = 1.2 µm, Leistec Corporation)		
Oven temp.:	60 °C	Column oven temp.:	40 °C (5 min) → (40 °C/min) → 200 °C (1 min)		
Sample line temp.:	100 °C	Carrier gas:	Helium		
Transfer line temp.:	150 °C	Carrier gas control:	Linear velocity (62.5 cm/sec)		
Vial pressurization gas pressure:	70 kPa	Injection mode:	Split		
Vial warming time:	10 min	Split ratio:	10:1 (alcohol, cyanide, azide)		
Vial pressurization time:	0.5 min		30:1 (paint thinner)		
Loading time:	0.5 min	[MS]			
Loading equalization time:	0 min	Interface temp.:	230 °C	Ion source temp.:	200 °C
Injection time:	0.5 min	Solvent elution time:	0.7 min	Data acquisition time:	1 - 10 min
Needle flush time:	5 min	Measurement mode:	Scan	Mass range:	*m/z* 10 - 300
GC cycle time:	18 min	Event time:	0.2 sec	Emission current:	60 µA (standard)

*Note: The detection voltage and other conditions must be optimized since they can differ depending on equipment status.

Analytical Results of Blood Cyanide

Fig. 1 shows the mass chromatograms obtained when analyzing a blank and 0.15 µg/mL standard. The mass spectrum is shown in Fig. 2. The ion that appears at m/z 27, which is the HCN ion, was used for quantification. The calibration curve obtained by internal standard calibration (0.15, 0.5, 1.5, 5 and 10 µg/mL) is shown in Fig. 3. The calibration curve correlation coefficient (R) was 0.9999 or above, showing that linearity was good.

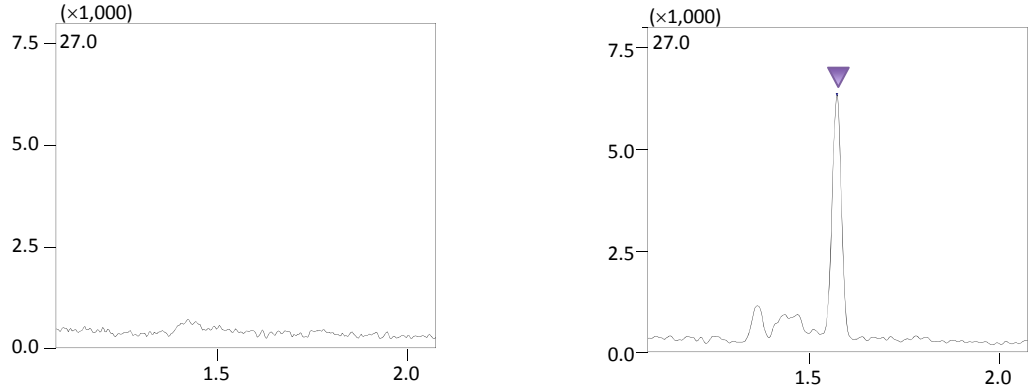

Fig. 1: Mass Chromatogram of Cyanide when Analyzing a Blank and 0.15 µg/mL Standard
Left: Blank, Right: 0.15 µg/mL Standard Sample

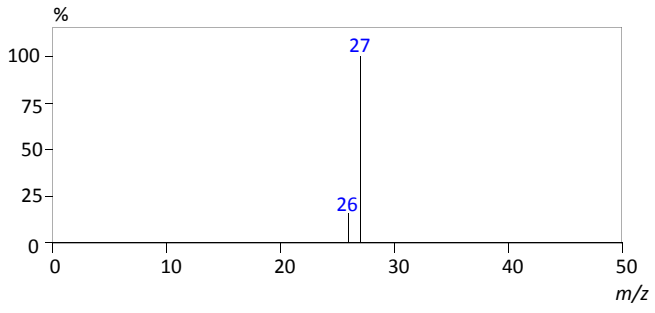

Fig. 2: Mass Spectrum of Cyanide

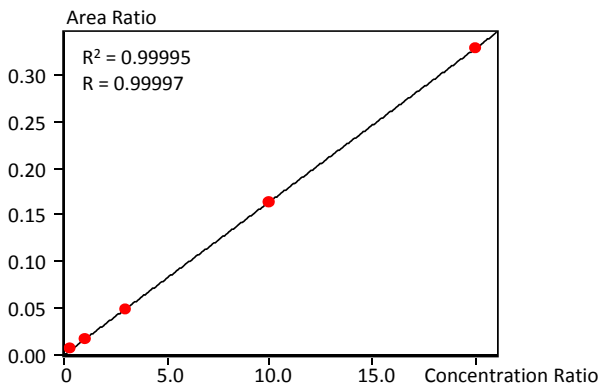

Fig. 3: Cyanide Calibration Curve
(Concentration: 0.15 - 10 µg/mL)

Intra-day reproducibility and Inter-day reproducibility were evaluated by repeating analysis of a 1.5 μg/mL standard (in hemolyzed equine blood) eight times each day for five days. Intra-day reproducibility (n = 8) on the first day is shown in Table 2, and Inter-day reproducibility over five days is shown in Table 3. The accuracy of intra-day repeatability of eight repeated measurements was 97.2 - 103.6 % (average 100.5 %), with a reproducibility relative standard deviation (%RSD) of about 2 %. The inter-day reproducibility of quantitative measurements of concentration performed over five days was within 5 %, which shows good quantitative performance.

Table 2: Intra-Day Reproducibility of Area Ratio and Concentration (n = 8, First Day)

	Area Ratio	Concentration (μg/mL)	Accuracy (%)
1st	0.053	1.546	103.0
2nd	0.054	1.555	103.6
3rd	0.053	1.519	101.2
4th	0.052	1.503	100.2
5th	0.051	1.476	98.4
6th	0.051	1.457	97.2
7th	0.053	1.520	101.3
8th	0.052	1.486	99.1
Average	0.052	1.508	100.5
Standard Deviation (SD)	0.001	0.034	2.251
%RSD	2.101	2.240	2.240

Table 3: Inter-Day Reproducibility of Qualitative Results Over Five Days

	Average Area Ratio	Average Concentration (μg/mL)	Concentration %RSD (Intra-Day Reproducibility)
1st Day	0.052	1.508	2.240
2nd Day	0.051	1.463	1.577
3rd Day	0.056	1.610	1.126
4th Day	0.055	1.603	2.287
5th Day	0.057	1.645	1.359
Inter-day Reproducibility (%RSD)	4.614	4.907	

Analytical Results of Blood Azide

Fig. 4 shows the mass chromatograms obtained after analyzing a blank and a 1.5 μg/mL standard. The mass spectrum is shown in Fig. 5. The ion that appears at *m/z* 43, which is the HN_3 ion, was used for quantification. The azide peak has a broad leading edge that reduces sensitivity compared to other volatile toxic substances, but sensitivity is deemed sufficient based on a reported blood concentration of about 5 μg/mL or above in cases of death. The calibration curve obtained by internal standard calibration (1.5, 5 and 10 μg/mL) is shown in Fig. 6. The calibration curve does not pass through the origin, but has a correlation coefficient (R) of 0.999 or above that shows linearity is good.

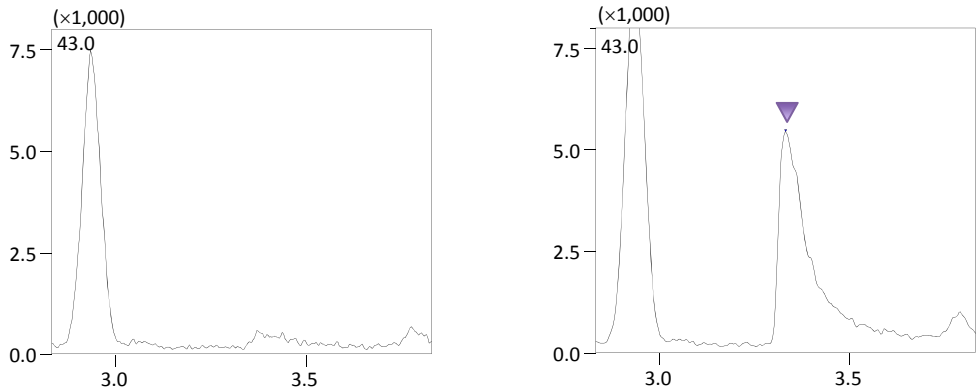

Fig. 4: Mass Chromatogram of Azide when Analyzing a Blank and a 1.5 μg/mL Standard
Left: Blank, Right: 1.5 μg/mL Standard Sample

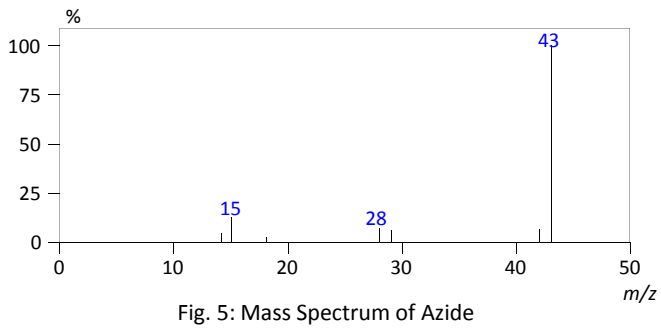

Fig. 5: Mass Spectrum of Azide

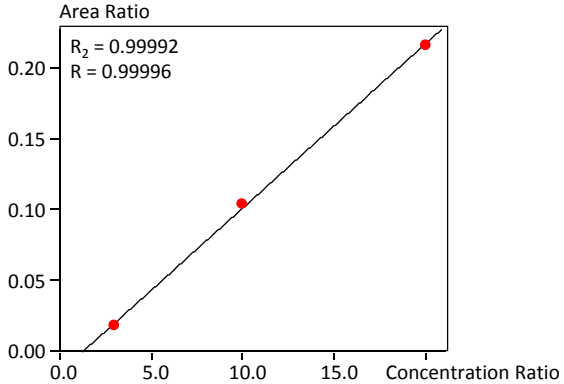

Fig. 6: Azide Calibration Curve
(Concentration: 1.5 - 10 μg/mL)

Intra-day reproducibility and Inter-day reproducibility were evaluated by repeating analysis of a 1.5 µg/mL standard (in hemolyzed equine blood) eight times each day for five days. Intra-day reproducibility (n = 8) on the first day is shown in Table 4, and Inter-day reproducibility over five days is shown in Table 5. The accuracy of intra-day repeatability of eight repeated measurements was 94.3 - 114.5 % (average 101.6 %), with a reproducibility relative standard deviation (%RSD) of about 7 %. The inter-day reproducibility of quantitative measurements of concentration performed over five days was within 2 %, which shows good quantitative performance.

Table 4: Intra-Day Reproducibility of Area Ratio and Concentration (n = 8, First Day)

	Area Ratio	Concentration (mg/mL)	Accuracy (%)
1st	0.018	1.420	94.7
2nd	0.018	1.449	96.6
3rd	0.020	1.513	100.9
4th	0.022	1.593	106.2
5th	0.023	1.623	108.2
6th	0.025	1.718	114.5
7th	0.019	1.464	97.6
8th	0.018	1.415	94.3
Average	0.020	1.524	101.6
Standard Deviation (SD)	0.003	0.110	7.307
%RSD	12.594	7.190	7.190

Table 5: Inter-Day Reproducibility of Qualitative Results Over Five Days

	Average Area Ratio	Average Concentration (mg/mL)	Concentration %RSD (Intra-Day Reproducibility)
1st Day	0.020	1.524	7.190
2nd Day	0.019	1.489	6.258
3rd Day	0.019	1.460	6.618
4th Day	0.019	1.463	7.077
5th Day	0.019	1.465	4.552
Inter-Day Reproducibility (%RSD)	3.295	1.839	

Summary

This application investigated whether the volatile toxic substances cyanide and azide could be measured using the same column and analytical conditions as used to analyze alcohol (ethanol) in blood. Cyanide and azide could be measured using the same analytical conditions as blood alcohol simply by adding ascorbic acid solution (aqueous) and phosphoric acid solution (aqueous) to the test sample.

The HS-20 headspace sampler provides high-performance vial heating and a sample loop of minimal length and inertness that reduces the carryover and allows for easy switching between analysis of high-concentration ethanol, such as in blood alcohol testing, and cyanide and azide that are present at trace level.

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