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Application News

No. G286A

Gas Chromatography

Analysis of Carbon Monoxide in Blood

Carbon monoxide (CO) is known as a toxic gas produced from the incomplete combustion of organic compounds. Since CO is responsible for many cases of poisoning, the carboxyhemoglobin saturation level is measured to be used as an index to determine whether poisoning by carbon monoxide has occurred. Gas chromatography thermal conductivity detectors (GC-TCD) employ an indirect measurement method that isolates carbon monoxide in blood for analysis, but sensitivity is not very high. On the other hand, barrier discharge ionization detectors (BID) are able to detect most compounds, with the exception of helium and neon, at high sensitivity compared to TCD. BID analysis is useful because measuring at higher sensitivities allows the volume of a blood sample used in testing to be reduced, enabling any remaining blood in the sample to be used in other tests. This article introduces an example of measuring carbon monoxide in blood using GC-BID.

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Analysis Method

The pretreatment method was performed as follows by referencing "Quantitative Testing 1-2 (2)" under "II-1 Toxic Gas Testing Methods" in "Testing Methods and Annotation for Toxic Pharmaceuticals 2006".

1. Preparation of potassium ferricyanide aqueous solution (oxidizing agent)
   20 g of potassium ferricyanide and 5 g of saponin were dissolved in distilled water to precisely obtain a volume of 100 mL.
2. Preparation of sample solution
   0.25 mL of blood sample, 0.5 mL of distilled water, and 0.25 mL of oxidizing agent were added to a 9-mL vial and the vial was sealed immediately.
3. Measurement
   The blood sample was kept warm at 30 °C for 90 minutes and then measurement was performed by injecting 0.1 mL of headspace gas into the GC using a gas-tight syringe. The Rt-Msieve 5A column was used.

Table 1 Analysis Conditions

Model	: Tracera™ (GC-2010 Plus + BID-2010 Plus)
Column	: RESTEK Rt-Msieve 5A (30 m × 0.53 mm I.D., df = 50 µm) with Particle Trap 2.5 m
Column Temp.	: 100 °C
Inj. Mode	: Split 1:7
Inj.Temp	: 250 °C
Carrier Gas	: He 45 cm/sec (constant linear velocity mode)
Det. Temp.	: 280 °C
Discharge Gas	: 50 mL/min (He)
Inj. Volume	: 0.1 mL

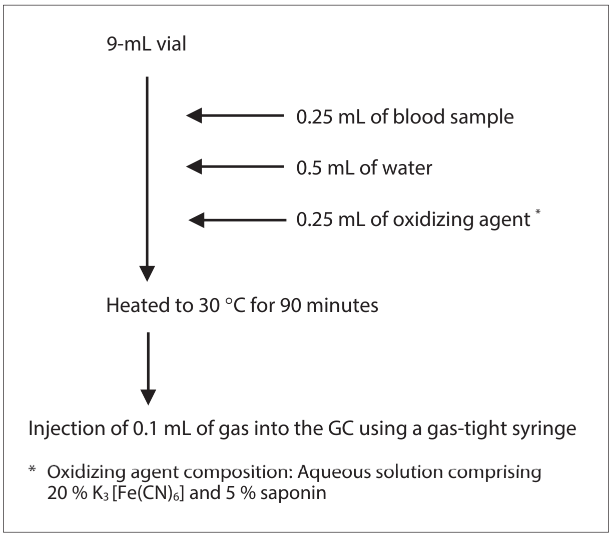

Fig. 1 Example of Sample Pretreatment

Measurement of Blood Sample Saturated with Carbon Monoxide

A blood sample saturated with carbon monoxide was created by bubbling 10 mL of CO through a 25 mL blood sample and mixing, and this process was repeated nine times. An untreated blood sample and the blood sample saturated with carbon monoxide were analyzed according to steps 2 and 3 of the analysis method and the resulting chromatograms are shown in Fig. 2.

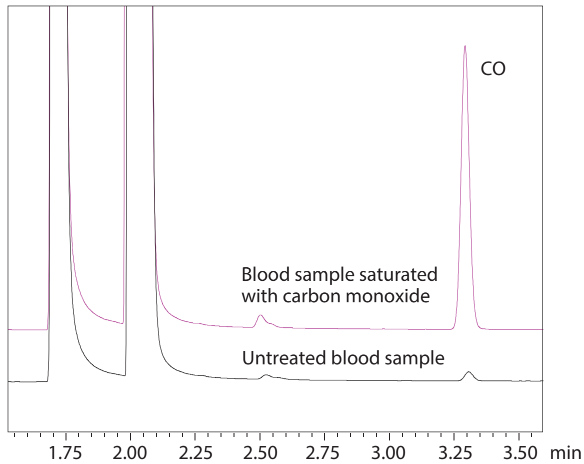

Fig. 2 Comparison of Untreated Blood Sample and Blood Sample Saturated with Carbon Monoxide

Linearity of Calibration Curve

A calibration curve from 2 to 3900 ppm was created by diluting carbon monoxide standard gas with air. Fig. 3 shows the calibration curve. There is sufficient sensitivity even with an extremely low concentration of 2 ppm, indicating that detection is possible at low concentrations which cannot be detected using a TCD.

The calibration curve shows good linearity with a correlation coefficient (R^2) of 0.999 or greater in the 2 to 3900 ppm concentration range.

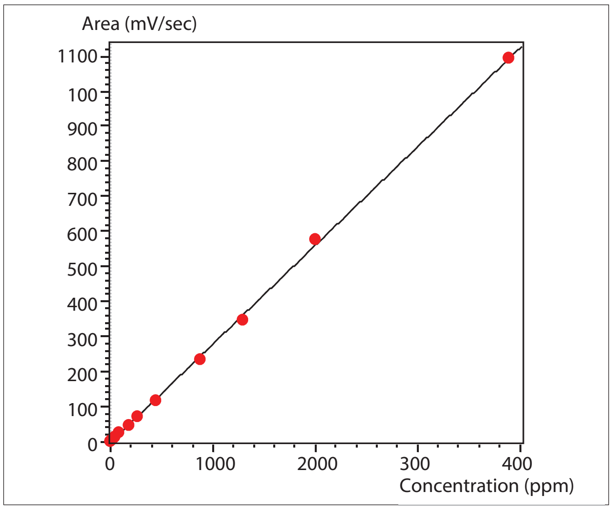

Fig. 3 Calibration Curve

Analysis of Carbon Monoxide in Blood

Fig. 4 shows the results of analyzing carbon monoxide in the blood of a smoker and non-smoker. We can observe a significant difference in CO concentration between the smoker and non-smoker.

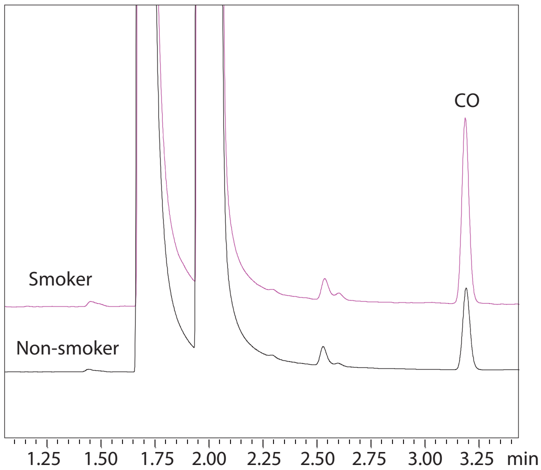

Fig. 4 Comparison of a Smoker and Non-Smoker

Calculating Carboxyhemoglobin Saturation Levels

The percentage of carboxyhemoglobin saturation (hereafter CO-Hb (%)) must be determined because CO-Hb (%) relates to the degree of CO poisoning. The concentration of carbon monoxide in the blood of six smokers and six non-smokers was determined and the CO-Hb (%) calculation results are listed in Table 2.

Table 2 Calculating Carboxyhemoglobin Saturation Levels

		1	2	3	4	5	6
Smoker	Analysis quantitative value (ppm)	414	452	285	240	339	318
	CO-Hb binding amount (μmol)	0.133	0.146	0.092	0.077	0.109	0.102
	CO-Hb max. binding amount (μmol)	2.191	2.412	2.558	2.601	2.586	2.657
	CO-Hb (%)	6.084	6.034	3.587	2.971	4.211	3.854
Non-smoker	Analysis quantitative value (ppm)	146	158	218	188	207	255
	CO-Hb binding amount (μmol)	0.047	0.051	0.07	0.061	0.067	0.082
	CO-Hb max. binding amount (μmol)	2.617	2.357	2.613	2.530	2.395	2.766
	CO-Hb (%)	1.794	2.156	2.689	2.393	2.777	2.964

* The CO-Hb maximum binding amount (μmol) was determined using a spectrophotometer.

Equations

CO-Hb binding amount (μmol) = total CO amount in headspace
= A * B / 0.082 / 303 / 1000
CO-Hb max. binding amount (μmol) = total hemoglobin in blood sample
= C * D * 4 * 369.2 * 1000 / 64500
CO-Hb (%) = CO-Hb binding amount / CO-Hb max. binding amount * 100

A : CO quantitative value (ppm)
B : Headspace volume (mL)
C : Absorbance at 540 nm, according to "Quantitative Testing 1-2 (2)" under "II-1 Toxic Gas Testing Methods" in "Testing Methods and Annotation for Toxic Pharmaceuticals 2006"
D : Used blood sample volume (mL)

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References :

The Pharmaceutical Society of Japan: Testing Methods and Annotation for Toxic Pharmaceuticals 2006 - Analysis, Toxicity, and Coping Methods

The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

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