

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.

S. Uchiyama

■ 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 | : Tracer TM (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 |

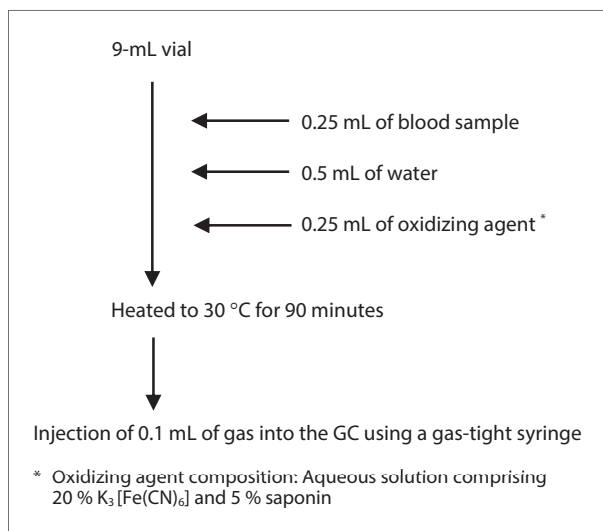


Fig. 1 Example of Sample Pretreatment

■ 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.

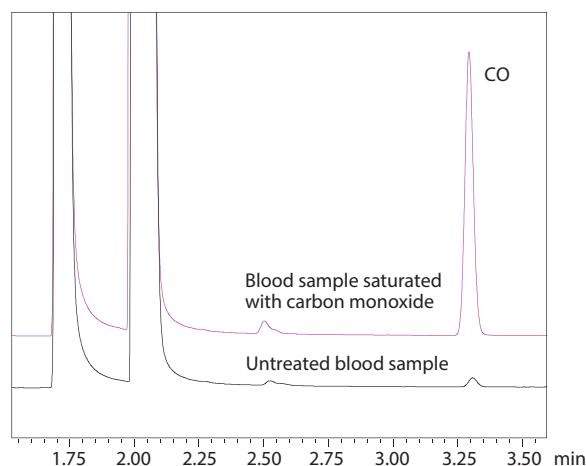


Fig. 2 Comparison of Untreated Blood Sample and Blood Sample Saturated with Carbon Monoxide

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.

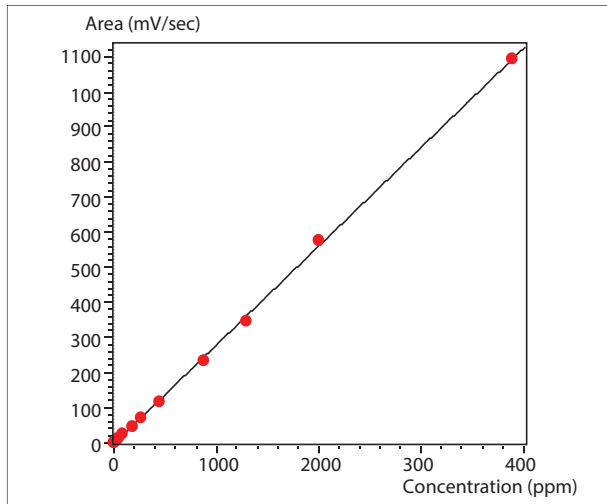


Fig. 3 Calibration Curve

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.

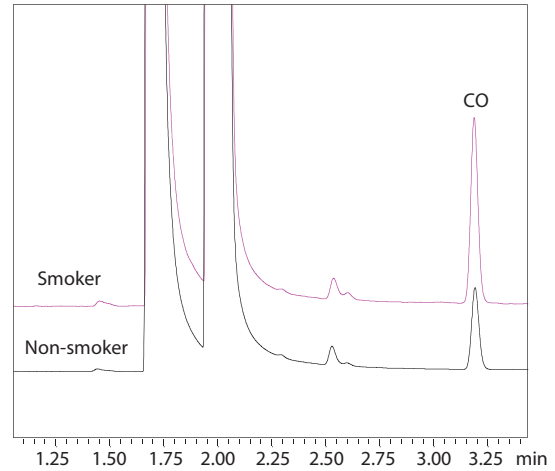


Fig. 4 Comparison of a Smoker and Non-Smoker

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)

Acknowledgments : We would like to thank Takeshi Omori and Yasuo Seto at the National Research Institute of Police Science for providing and creating the data that was used to produce this issue of Application News.

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.

Tracera is a trademark of Shimadzu Corporation in Japan and/or other countries.

Rt is a registered trademark of Restek Corporation in the United States.



Shimadzu Corporation

www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, product/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation or its affiliates, whether or not they are used with trademark symbol "TM" or "®". Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services. Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

First Edition: Mar. 2017
 Second Edition: Feb. 2019