II.1.6 Chloroform and dichloromethane

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Introduction

Chloroform exerts a suppressing effect on the central nervous system. It had been used as a general anaesthetic since the 19th century, but it disappeared, because of its hepatotoxicity and arrhythmia-inducing effects. It is now being used for industrial purposes, such as a solvent, extracting reagent, refrigerant and chemical material. Chloroform poisoning can be seen in accidental, suicidal [1] and homicidal cases.

Dichloromethane (methylene dichloride, methylene chloride) is also being widely used in industries as a solvent and refrigerant like chloroform, and causing many poisoning cases due to accidents and suicides [2]. Recently, dichloromethane has become of interest as a substitute of chlorofluorocarbon, because the latter was found to accelerate the depletion of the ozone layer and is in the line of being abolished completely.

Since both chloroform and dichloromethane are volatile compounds, their analysis is usually made by the headspace extraction and GC detection. In this chapter, a simple headspace GC method, using dichloromethane as internal standard (IS) for assays of chloroform and *vice versa*, is presented [3].

Reagent and their preparation

i. Reagents

Chloroform, dichloromethane and methanol of special grade can be purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan and many other manufacturers.

ii. Preparation

A 337-μL volume of chloroform and a 377-μL volume of dichloromethane are separately dissolved in methanol to prepare each 100 mL solution as stock solutions. Each solution is diluted 5-fold with methanol; a 10-μL volume containing 10 μg of each compound is added to 0.5 mL whole blood as IS and mixed well.

GC Conditions[a]

GC column[b]: a DB-1 fused silica wide-bore capillary column (30 m × 0.53 mm i. d., film thickness 5 μm, J&W Scientific, Folsom, CA, USA).

GC conditions: an HP 5890 Series gas chromatograph (Agilent Technologies, Palo Alto, CA, USA)[c]; detector: FID; column (oven) temperature: 50 °C (10 min) → 20 °C/min → 280 °C; injection temperature: 250 °C; detector temperature: 280 °C; carrier gas: He; its flow rate: 10 mL/min; injection mode: splitless.

Procedure

i. A 0.5-mL volume of whole blood[d], 10 μL of dichloromethane solution (IS, containing 10 μg) for measurement of chloroform and 0.5-mL of distilled water are placed in a 7-mL volume glass vial with a Teflon-septum screw cap, immediately capped and mixed gently.
ii. The vial containing the mixture is heated at 55 °C for 20 min on a heat block or in a water bath. A 0.5-mL volume gas-tight syringe to be used can be simultaneously heated on the same heat block.
iii. A needle[e] of 23 G is used for the syringe. After heating, a 0.5-mL volume of the headspace vapor is drawn into the syringe and injected into GC immediately.
iv. More than 4 vials are prepared to construct a calibration curve; to each vial, a 0.5-mL volume of whole blood obtained from a healthy subject, 10 μL of IS (10 μg of dichloromethane) and one of the various concentrations of chloroform are added, and the following procedure is exactly the same as described above. The calibration curve is composed of chloroform concentration on the horizontal axis and the peak area ratio of chloroform to dichloromethane (IS) to enable the calculation of chloroform concentration in a test specimen.
v. In case of the analysis of dichloromethane, chloroform is used as IS, conversely, and the procedure is exactly the same as above.

Assessment of the method

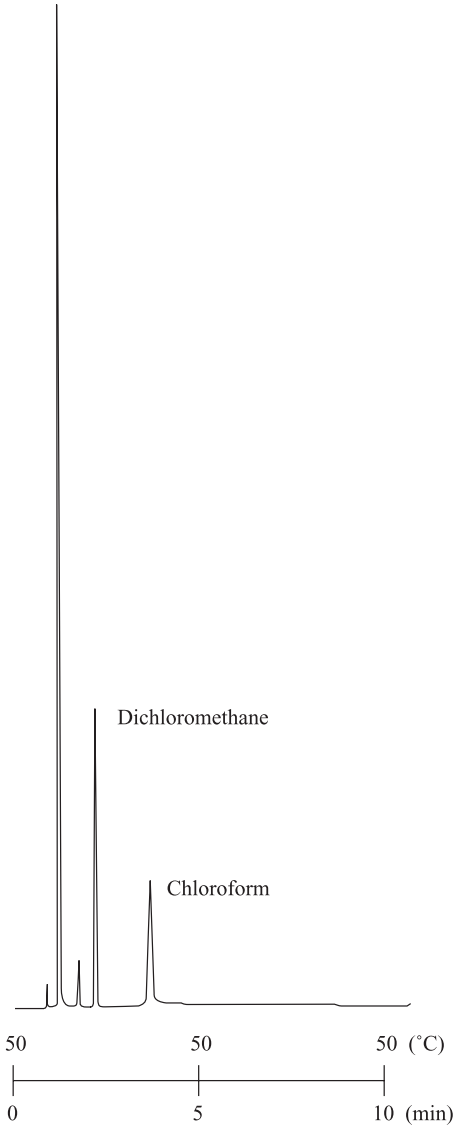
Figure 6.1 shows a gas chromatogram obtained from human whole blood (0.5-mL) containing both chloroform and dichloromethane. With the DB-1 column, the peaks of chloroform and dichloromethane appeared separated well, and were not interfered with by any impurity peak. The methanol used as vehicle appeared around 1 min of retention time as a big peak, but did not interfere with that of dichloromethane.

The efficiencies for extraction of chloroform and dichloromethane from the aqueous phase containing 0.5 mL whole blood were 12–20 % by the present headspace method.

The detection limit of this method is about 1 μg/mL whole blood for both compounds.

Dichloromethane is known to be converted into carbon monoxide (CO) *in vivo* [4], and thus in poisoning with dichloromethane, both carboxyhemoglobin (COHb) and dichloromethane concentrations were sometimes measured simultaneously [5]. However, the concentration of COHb is usually only around 10 %; it seems impossible to be killed only by CO poisoning after oral ingestion or inhalation of dichloromethane.

Figure 6.1



Detection of chloroform and dichloromethane from whole blood by wide-bore capillary GC (DB-1 column). The amount of chloroform and dichloromethane added to 0.5 mL of whole blood was equally 10 µg. The big peak appearing at an early stage is that of methanol used as vehicle.

Poisoning cases, and toxic and fatal concentrations

A chloroform-poisoning case [6]

A 27-year-old male was found in an unconscious state; it was estimated that he had ingested 4 ounces (114 mL) of chloroform orally. On arrival at a hospital, he was comatose, snoring and cyanotic. His physical conditions were: the pupils: dilated and not responsive to light; heart beat: 70/min; blood pressure: 140/90 mmHg; and respiration rate: 40/min. He was intubated and subjected to oxygen inhalation, and his stomach was lavaged. The gastric lavage solution gave a strong smell of chloroform. One hour after admission, the arterial blood showed pH at 7.29 and $PaCO_2$ at 50 mmHg. Therefore, 4.3 % glucose in saline and 0.9 % saline, 500 mL of each, were injected by intravenous drop infusion; 50 mL of 5 % $NaHCO_3$ was also injected to treat the metabolic acidosis. His electrocardiogram occasionally showed extrasystoles and slight lowering of ST within several hours after admission. Eleven hours after admission, the patient became responsive to a call and the intratracheal tube was removed. He complained of his chest pain upon swallowing and epigastric discomfort; he did not show any retrograde amnesia and remembered that he had ingested 4 ounces of chloroform. Twelve hours after admission, the clinical tests showed arterial blood at pH 7.4, $PaCO_2$ at 40 mmHg and bicarbonate ion at 21 mEq/L. Three days after admission, slight icterus and swelling of the liver appeared. On day 4, the symptoms of the liver disturbance were aggravated even more and he repeated vomiting. Such a serious state lasted for 2 days and the symptoms were gradually alleviated thereafter in about 10 days. He was discharged from the hospital on day 28. In this patient, almost no abnormality in electrolytes even during the acute stage was observed; while urea and bilirubin in his blood increased during 2–3 days of acute stage and decreased to normal values soon. The AST (GOT) reflecting liver function showed very high values during the acute stage, but gradually decreased until his discharge.

A dichloromethane-poisoning case [2]

A 63-year-old male was drying a big pump, after finishing unloading dichloromethane from a tanker; the hold of the tanker was filled with dichloromethane gas, which caused a collapse of his colleague working there. To rescue his colleague, he entered the hold, lost his consciousness for a short time by inhaling the same gas and was sent to an emergency room of a hospital. Upon arrival at the hospital, the level of his consciousness was JCS 1, and about 2 cm-sized contusion wound was found in the frontal region of his head. He complained of slight headache, but general conditions were stable without any abnormality in light reaction and in eye movement. As slight headache continued, COHb concentration was measured; it was 16.5 % 12 h after the exposure, followed by 7.6 % at 16 h and 2.3 % at 36 h. About 20 h after the exposure, the symptom was improved; he was discharged, about 40 h after exposure.

Toxic and fatal concentrations

After a single oral dose of 500 mg chloroform to 2 subjects, peak blood concentrations of about 1 and 5 μg/mL were attained in 1 h, respectively [7]. The fatal oral dose of chloroform is about

10 mL and the maximum permissible atmospheric concentration is 10 ppm. In fatal chloroform poisoning cases, its concentrations were 10–48 μg/mL in blood, 50.4–156 μg/g in the brain, 16–27 μg/g in the kidney, 6–86.2 μg/g in the liver and 0–60 μg/mL in urine [7].

Dichloromethane is very similar to chloroform in its structure; the toxic effects of the former is also considered similar to those of the latter. However, the anaesthetic effect of dichloromethane is much lower than that of chloroform. The maximum permissible atmospheric concentration of dichloromethane is 200 ppm. In a fatal dichloromethane poisoning case, its concentrations were 252 μg/mL in blood, 125 μg/g in the brain, 130 μg/g in the liver and 10 μg/mL in urine [8].

Notes

a) In this chapter, a usual headspace GC method using a fused silica wide-bore capillary column is described. If more sensitive detection of the compounds is necessary, a cryogenic oven trapping GC method using a medium-bore capillary column [3] is recommendable, because it gives the sensitivity 10–100 times higher.
b) Any wide-bore capillary column of a similar type can be used, regardless of its manufacturer.
c) Any GC instrument for a capillary column can be used for analysis.
d) Urine can be probably analyzed through the same procedure, because it is a much simpler matrix than whole blood.
e) As described in the chapter of ethanol, the author is using a 23 G needle of the gas-tight syringe with a 90°cut at its tip or with a conical cut to avoid the obstruction of the needle by a debris of a septum.

References

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