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Note

Simple and sensitive method for the determination of acetaldehyde in blood by gas chromatography

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Acetaldehyde, an intermediate metabolite of ethanol, is suspected to be a mediator of a number of both the acute and chronic actions of ethanol. There has been considerable interest in assaying acetaldehyde in blood, and a number of sensitive and specific methods, apparently using similar head-space analysis, have recently been described [1–4]. However, the level of acetaldehyde in blood from normal healthy individuals given ethanol is extremely low. In fact, it is usually even below the detectable limit of the hydrogen flame ionization detector used in the head-space method. Various difficulties are also inherent in attempting to determine blood acetaldehyde levels, owing to acetaldehyde formation and disappearance when blood samples containing ethanol are treated in practice.

Pentafluorobenzyloxylamine (PFBOA), on the other hand, has been found to be an excellent derivatizing agent in the gas chromatographic (GC) determination of low-molecular-weight carbonyl compounds, such as aldehydes and ketones, in aqueous solution [5, 6]. The derivatives are stable, very volatile and extremely sensitive to eletron-capture detection (ECD).

The present paper describes a reliable and sensitive GC–ECD method for assaying low levels of acetaldehyde in blood samples from its O-pentafluorobenzyloxime (O-PFBO) derivative.

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EXPERIMENTAL

Blood samples

Male human blood was taken from an antecubital vein and was heparinized. Fresh blood was used for all experiments.

Reagents

Acetaldehyde (99.5%) was obtained from E. Merck (Darmstadt, F.R.G.) and was redistilled in a cold room before use. Stock solutions of acetaldehyde (90 μM) were prepared with isotonic saline. PFBOA hydrochloride (m.p. 115°C) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The aqueous solution was prepared with 0.1 *M* acetate buffer (pH 4). All other reagents were of the highest analytical grade available. Ethanol was redistilled before use.

Apparatus and conditions

In the assay, a Shimadzu GC-7A gas chromatograph equipped with a 10-mCi ^{63}Ni electron-capture detector and a 2-m glass column packed with 2% OV-17 on 60–80 mesh Chromosorb W AW-DMCS, with a column temperature of 90°C, a detector temperature of 150°C and a chart speed of 5 mm/min, were used. The peak areas were recorded by a Shimadzu Chromatopack C-R1A.

Standard procedure

To a 10-ml centrifuge tube, 0.6 ml of acetonitrile was added, followed by the addition of 0.5 ml of human blood containing various concentrations (0–18 μM) of acetaldehyde. The mixture was then shaken for 30 s with a mechanical shaker and allowed to stand in an ice-bucket for at most 2 min to precipitate proteinic substances. Then 0.25 ml of the supernatant was immediately transferred to a second centrifuge tube and 0.1 ml of aqueous PFBOA solution (1 mg/5 ml as the hydrochloride) was added. The components were mixed and the tube was placed in the ice-bucket for 60 min, except for the study of time course. After incubation, one drop of 9 *M* sulphuric acid and 2 ml of *n*-hexane were added to the reaction mixture, and the resulting O-PFBO derivative of acetaldehyde was extracted with *n*-hexane. The hexane layer was removed within 1 min and dried over anhydrous sodium sulphate. A 1-μl aliquot of the solution was injected onto the GC column. Quantitation was carried out by the absolute standard method.

Recovery study

A known concentration of acetaldehyde was added to blood without changing the volume by more than 10%. Then the blood was immediately treated according to the procedure described above. To investigate the possible effect of ethanol oxidation on the recovery of acetaldehyde, ethanol was added to the blood containing 9 μM acetaldehyde to a final concentration of 20 m*M*.

RESULTS AND DISCUSSION

It has been reported that the reaction of carbonyl compounds with PFBOA proceeds readily in weakly acidic media (pH 2–5) to yield derivatives extractable

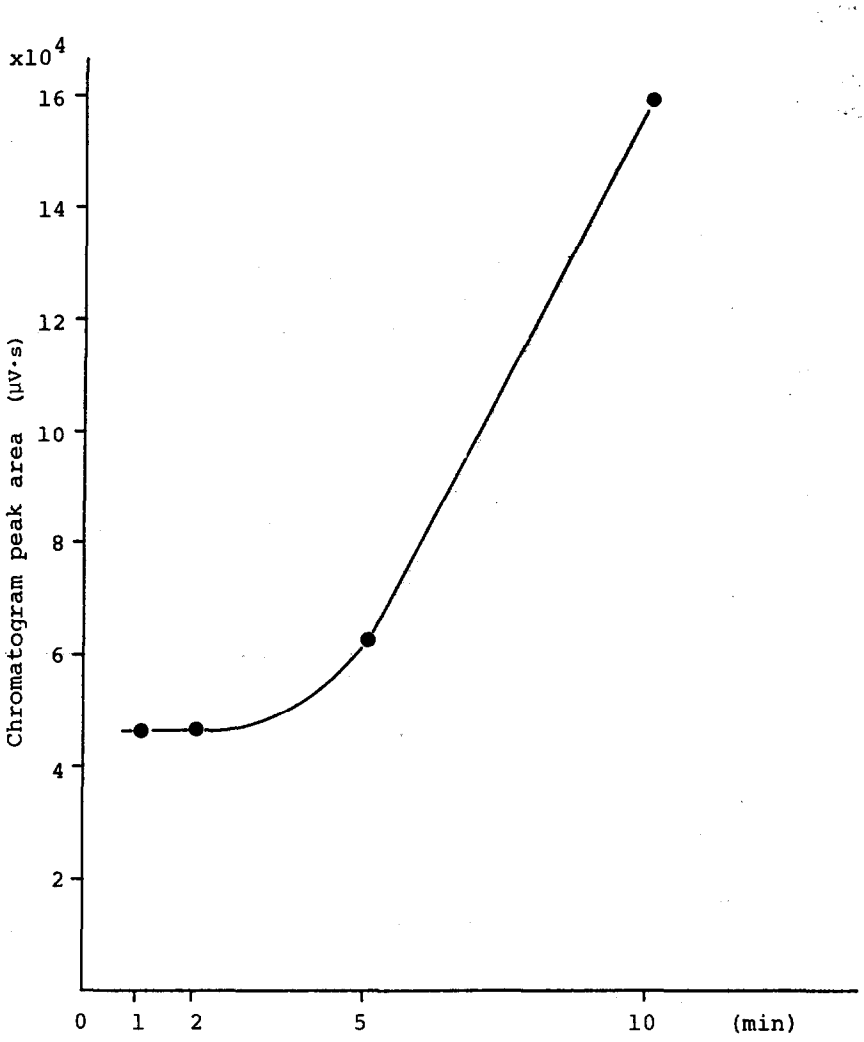

Fig. 1. Effect of period after mixing blood with acetonitrile on the peak area of acetaldehyde. Blood samples contain 18 μM acetaldehyde and 20 mM ethanol.

from aqueous solution with organic solvent [5, 6]. Taking into account the suitable pH (2–5) for the condensation reaction, whole blood was deproteinized with acetonitrile instead of perchloric acid, which is used in the traditional methods. As acetaldehyde is an intermediate metabolite of ethanol, it always coexists with ethanol. One of the major problems associated with currently employed methods is the spontaneous formation of acetaldehyde from ethanol during the deproteinizing process with perchloric acid [7, 8]. When this happens, significant amounts of acetaldehyde form within several seconds. After deproteinization with acetonitrile, the peak area of acetaldehyde also increased with time, although the level showed no change within 1–2 min after mixing (Fig. 1). No increase was observed in samples of blood containing only acetaldehyde and no ethanol, or in acetal-

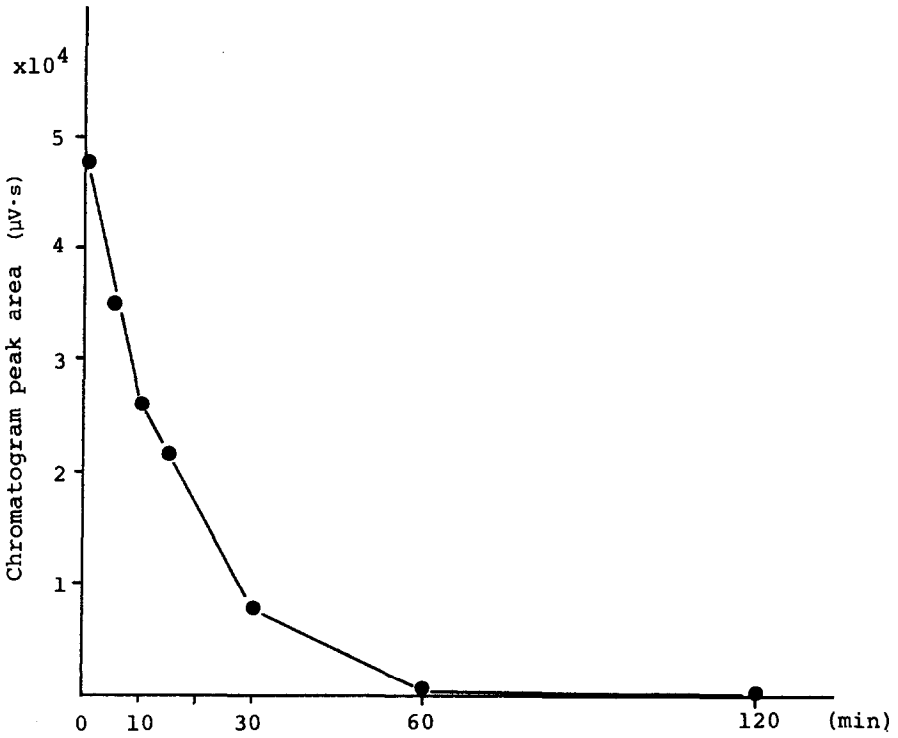

Fig. 2. Time-lapse changes in the acetaldehyde level of blood samples left standing at 26°C; 18 μM acetaldehyde and 20 mM ethanol were added to the blood samples just before the experiment began.

dehyde solution prepared in isotonic saline. Stowell et al. [8] demonstrated that the non-enzymic production of acetaldehyde occurs mostly during deproteinization of human blood samples containing ethanol. Although it is not clear why the peak area increases spontaneously, formation was negligible when the supernatant was pipetted within 2 min after mixing with acetonitrile.

On the contrary, the possibility exists that some acetaldehyde can bind to erythrocytes or plasma proteins. Aldehyde dehydrogenase in erythrocytes has the capacity to oxidize acetaldehyde [9]. As shown in Fig. 2, 18 μM acetaldehyde added to blood decreased rapidly and disappeared within 60 min regardless of the presence of ethanol. Therefore, accurate determination of low concentrations of acetaldehyde requires that blood samples be treated as soon as possible.

As can be seen in Fig. 3, the condensation reaction was complete in 60 min at 0°C, after which the values were constant up to 6 h. This result conformed with that obtained from the blood samples containing ethanol (data not shown).

The blood samples spiked with 3 and 9 μM acetaldehyde were recovered 94.7 and 99.8%, respectively. The mean recovery of the blood samples spiked with 9 μM of acetaldehyde and 20 mM ethanol was 96.8%. Ethanol had no significant effect on the recovery of acetaldehyde. The standard curve exhibited a good linearity with correlation coefficients 0.997 and the detection limit was 1 μM. The reproducibility was determined by carrying out five determinations from blood

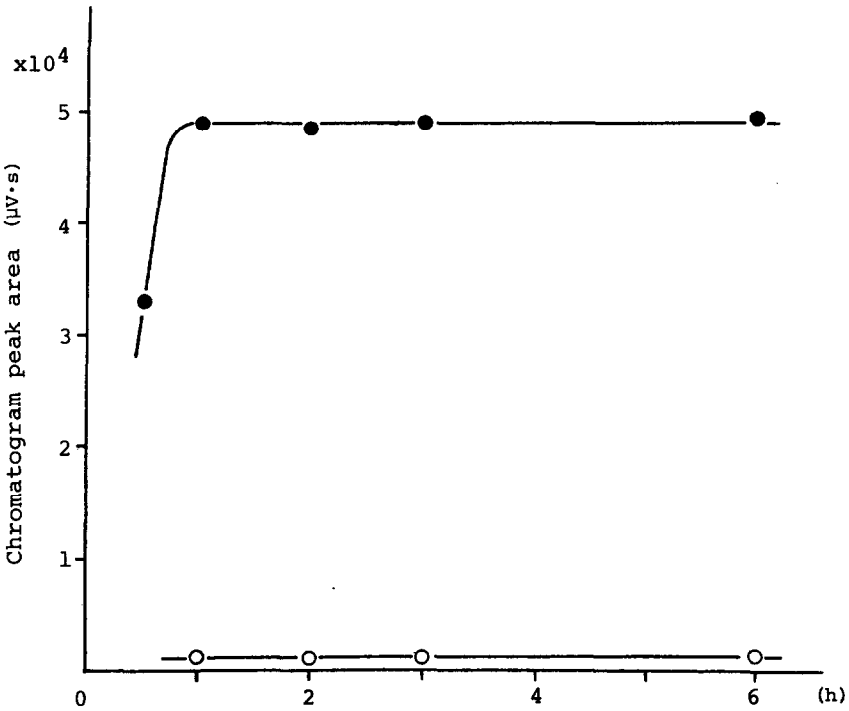

Fig. 3. Effect of reaction period on the condensation reaction with PFBOA. Blank human blood (○) and human blood spiked with 18 μM acetaldehyde (●) were examined after deproteinization with acetonitrile.

samples containing 18, 5 and 3 μM acetaldehyde. As the result, the coefficients of variation were 3.11, 5.91 and 4.75%, respectively.

Typical gas chromatograms of blank human blood and human blood spiked with 9 μM acetaldehyde are shown in Fig. 4. The retention time for the O-PFBO derivative of acetaldehyde was ca. 5.5 min, and a small peak was found in the blank human blood. The condensation reaction of acetaldehyde with PFBOA is considered to result in double peaks corresponding to *syn*- and *anti*-isomers. With the 2% OV-17 column, however, a single peak was obtained. The peak with retention time at ca. 2.8 min was identical with that of the O-PFBO derivative of formaldehyde. There is one more problem in the present assay, namely that blank values of formaldehyde were found to be relatively high. The origin of the high peak in the blank human blood is not evident. It may be caused by contamination of formaldehyde in air or in solutions.

We used 0.5 ml of blood in this experiment, but if a smaller amount of *n*-hexane is used for extraction, a relatively reduced amount of blood is sufficient. This method appears to be suitable for determining the acetaldehyde level in blood taken from individuals given ethanol with respect to specificity, sensitivity and simplicity under the constant experimental conditions described in *Standard procedure*.

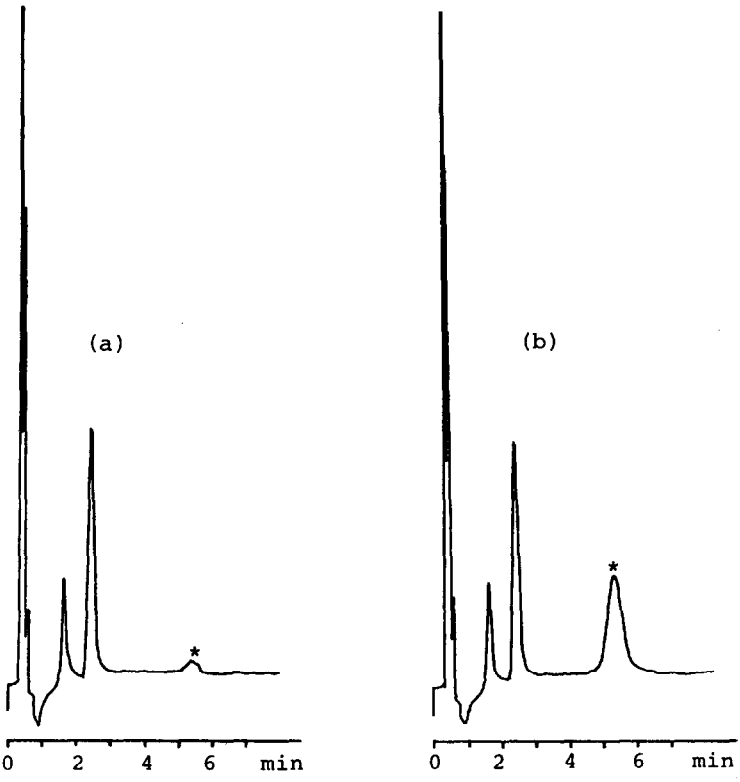

Fig. 4. Gas chromatograms of (a) blank human blood and (b) human blood spiked with 9 μM acetaldehyde. Conditions according to text. The elution position of acetaldehyde is marked with an asterisk.

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