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Determination of fluoroacetate in aqueous samples by headspace gas chromatography

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Abstract

A headspace (HS) GC method was developed for the determination of fluoroacetate in water samples. Fluoroacetate in samples dried under vacuum was derivatized to its ethyl ester by HS incubation with sulfuric acid and ethanol, detected by GC using a 5% phenyl–methylpolysiloxane fused-silica capillary column with flame ionization detection, and confirmed by GC–MS. Quantification was performed by the internal standard method using toluene. The HS conditions were optimized for incubation time and temperature and volumes of sulfuric acid and ethanol. The calibration graph was linear in the range of 5–200 μg and the limit of quantification was 0.5 μg ($S/N = 14$).

Keywords: Headspace analysis; Food analysis; Fluoroacetate; Pesticides

1. Introduction

Fluoroacetate, which after metabolic conversion into fluorocitric acid inhibits the aconitase activity in the Krebs cycle [1], is very toxic to animals and man. Because its sodium salt is widely used as a rodenticide, there have been many cases of poisoning of domestic and farm animals. GC has been used for the determination of fluoroacetate, and various kinds of esterification reagents have been adopted to convert this non-volatile organic acid into volatile derivatives [2–4]. However, derivatization procedures are tedious, GC separations sometimes suffer from severe interferences and also quantitative accuracy cannot be expected. Headspace (HS) GC is useful for the determination of volatile substances [5,6]. By addition of esterifying reagents to the HS reaction mixture, even non-volatile organic acids have been detected by GC [7–11]. In this work, a method was developed for the determination of fluoroacetate in water samples, using the HS-GC method in conjugation with sulfuric acid and ethanol as esterifying reagents.

2. Experimental

2.1. Reagents

Sodium fluoroacetate and ethyl fluoroacetate (EFA) were purchased from Wako (Osaka,

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Japan). Other chemicals were of analytical reagent grade.

2.2. Apparatus

The instrument used for GC analysis was a Model 14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with flame ionization detector and split injector. A CBP10 5% phenylmethylpolysiloxane fused-silica capillary column (25 m × 0.33 mm I.D., film thickness 0.5 μm) (Shimadzu) was used with helium as the carrier gas at a flow-rate of 3 ml min^{-1}. The injection port, detector and column oven were maintained at 200, 200 and 50°C, respectively. The splitting ratio was adjusted to 30.

For GC–MS analysis, a QP 1100EX GC mass spectrometer (Shimadzu) was used. A CBJ1 dimethylpolysiloxane fused-silica capillary column (15 m × 0.53 mm I.D., film thickness 1.5 μm) (Shimadzu) was used with helium as the carrier gas at a flow-rate of 14 ml min^{-1}. The injection port, interface, ion source and column oven were maintained at 200, 250, 250 and 40°C, respectively. A jet separator was used as an interface between the megobore capillary column and the ionization source. Electron impact (EI) ionization (ionization energy 70 eV) and chemical ionization (CI) (ionization gas isobutane) were used for ionization. The acquisition mass ranges were 50–220 for EI and 80–250 for CI, respectively, and the sampling rate was 1 scan s^{-1}.

2.3. Headspace conditions

HS equilibration was performed in a screw-capped septum vial (10 ml). A 1.0-ml volume of sample solution was placed in a vial and evaporated to dryness under vacuum. In order to establish a calibration graph, 1.0 ml of sodium fluoroactate solution of known concentration was evaporated. A 30-μl volume of concentrated sulfuric acid and 70 μl of an ethanol solution containing toluene (200 ppm, v/v) as internal standard (I.S.) were introduced into the dried vial and sealed with the septum. The mixture was vortex mixed and allowed to stand at 60°C. After incubation for 15 min, 1.0 ml of the gas phase was subjected to GC or GC–MS.

2.4. Sample treatment

A piece of meat (10 mg) suspected to contain poison was minced with 3 ml of distilled water. The extracted aqueous solution was centrifuged and the resulting supernatant was evaporated to dryness and subjected to the HS-GC method.

3. Results

3.1. Gas chromatographic separation and mass spectrometric confirmation of ethyl fluoroacetate

As shown in Fig. 1, the GC peak derived from fluoroacetate (3.9 min) was well separated from toluene, ethanol, diethyl ether (condensation product of ethanol) and the other impurities found in the HS gas, and eluted at the same position as the standard EFA. The EI mass spectrum of the peak (Fig. 2A) showed the same fragmentation pattern as EFA. The CI mass spectrum of the peak (Fig. 2B) showed the MH^+ ion (m/z 107), which was identically obtained with EFA.

3.2. Headspace conditions

The I.S. method was adopted for raising the quantitative accuracy, and toluene was chosen as the I.S. because of its chemical stability and similar GC retention time and similar boiling temperature (110.6°C) to those of EFA (121.6°C). The areas of both the EFA and toluene peaks were measured by changing the incubation times, temperatures and volumes of sulfuric acid and ethanol. As shown in Fig. 3, almost the identical areas of the EFA and toluene peaks, and also the area ratios of the EFA and I.S. peaks, were obtained for the incubations from 10 to 25 min. Incubation for 15 min was chosen for reasons of rapidity and

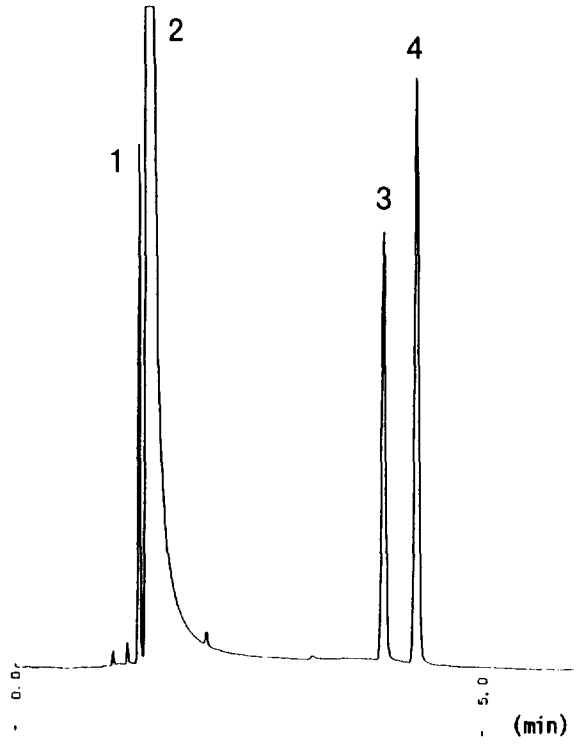

Fig. 1. Headspace gas chromatogram of ethyl fluoroacetate. Sodium fluoroacetate (100 μg) was incubated with 30 μl of sulfuric acid and 70 μl of ethanol containing 200 ppm (v/v) toluene at 60°C for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. Peaks: 1 = diethyl ether, 2 = ethanol; 3 = EFA; 4 = toluene.

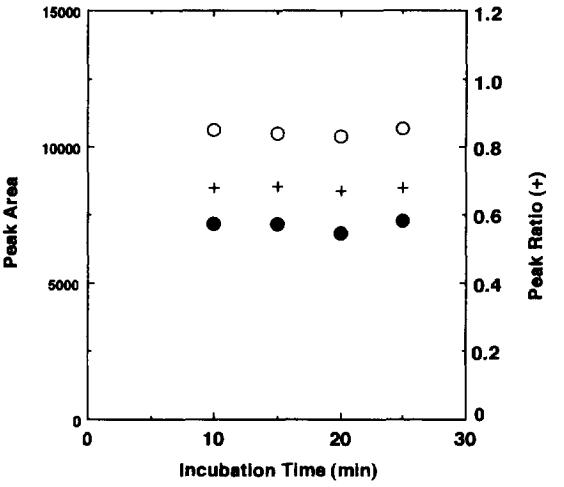

Fig. 3. Effect of headspace incubation times on the areas of the ethyl fluoroacetate and toluene peaks. Sodium fluoroacetate (100 μg) was incubated with 30 μl of sulfuric acid and 70 μl of ethanol containing 200 ppm (v/v) toluene at 60°C for an appropriate time in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. Areas of the (●) EFA and (○) toluene peaks and (+) the area ratios of EFA to toluene peaks are plotted against incubation time.

accuracy of analysis. As shown in Fig. 4, with increase in HS temperature the areas of both the EFA and toluene peaks were increased, and the peak-area ratios were also increased. Considering the boiling temperature of ethanol (78.5°C) and the increasing variation of the peak areas due to the increased gas pressure, incubation at 60°C was chosen.

The volume of sulfuric acid had significant effects on the peak areas (Fig. 5A). Generally in HS equilibrium, the analyte concentration in the gas phase is correlatively decreased when the volume of the liquid phase is increased. However, irrespectively of ethanol volume, the area of the toluene peak was simply increased as the volume of sulfuric acid was increased, and these

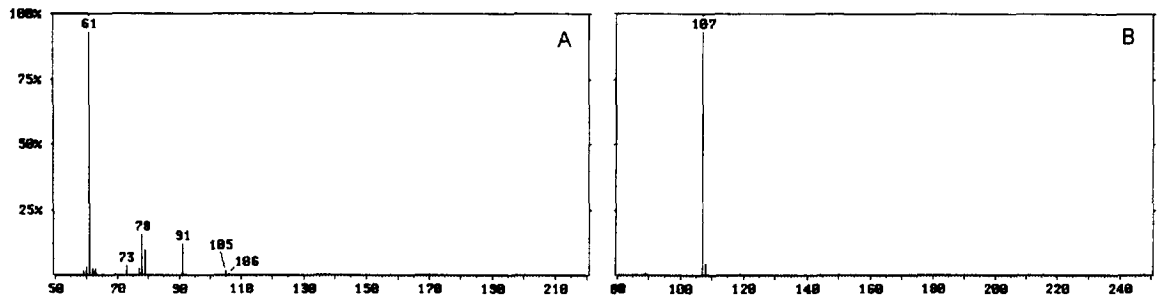

Fig. 2. Mass spectra of ethyl fluoroacetate peak. The HS gas obtained as in Fig. 1 was subjected to GC–MS. Ionization mode: (A) electron impact and (B) chemical ionization.

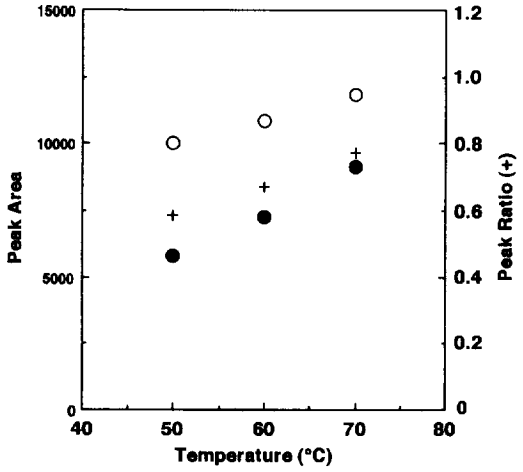

Fig. 4. Effect of headspace temperature on the areas of the ethyl fluoroacetate and toluene peaks. Sodium fluoroacetate (100 μg) was incubated with 30 μl of sulfuric acid and 70 μl of ethanol containing 200 ppm (v/v) toluene at an appropriate temperature for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. Areas of (●) EFA and (○) toluene peaks and (+) the peak-area ratios are plotted against temperature.

responses reached a maximum or rather decreased slightly only in the case of 100 μl of ethanol. In contrast, the area of the EFA peak increased, reached a maximum and then decreased, and the sulfuric acid volume giving the highest peak area increased as the ethanol volume increased (Fig. 5A). The solvent ratio of sulfuric acid to ethanol (from 20 to 30%) gave the highest EFA area in HS gas per fluoroacetate originally added to the liquid phase. This can also be observed for the area ratios, which decreased as the volume of sulfuric acid increased (Fig. 5B). A 30-μl volume of sulfuric acid was chosen as it gave reproducible and high EFA peak areas.

Fig. 6 shows the effect of ethanol volume on

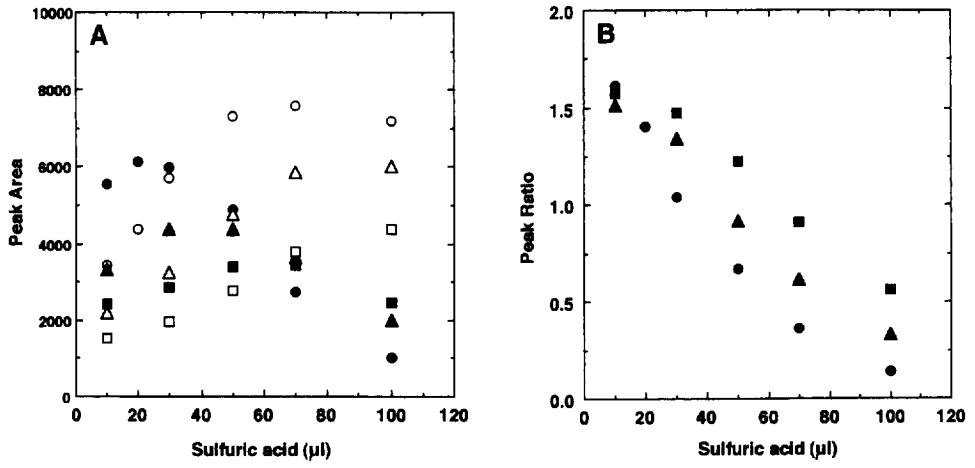

Fig. 5. Effect of the volume of sulfuric acid on the areas of the ethyl fluoroacetate and toluene peaks. Sodium fluoroacetate (100 μg) was incubated with an appropriate volume of sulfuric acid and ethanol (circle = 100 μl; triangle = 150 μl; square = 200 μl) containing 10 nl of toluene at 60°C for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. (A) Areas of (closed symbols) EFA and (open symbols) toluene peaks and (B) the peak-area ratios are plotted against sulfuric acid volume.

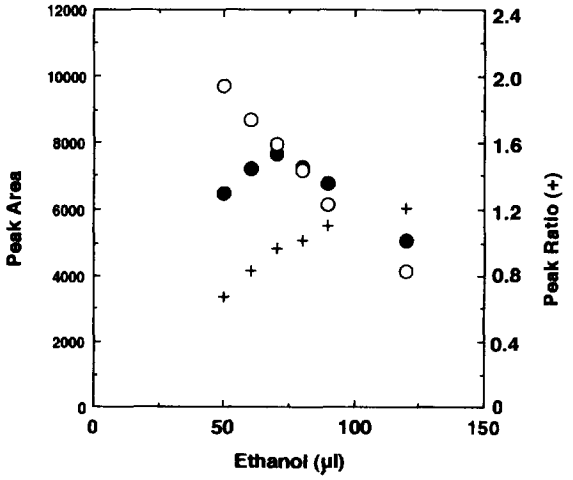

Fig. 6. Effect of the volume of ethanol on the areas of ethyl fluoroacetate and toluene peaks. Sodium fluoroacetate (100 μg) was incubated with 30 μl of sulfuric acid and an appropriate volume of ethanol containing 10 nl of toluene at 60°C for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. Areas of the (●) EFA and (○) toluene peaks and the peak-area ratios (+) are plotted against ethanol volume.

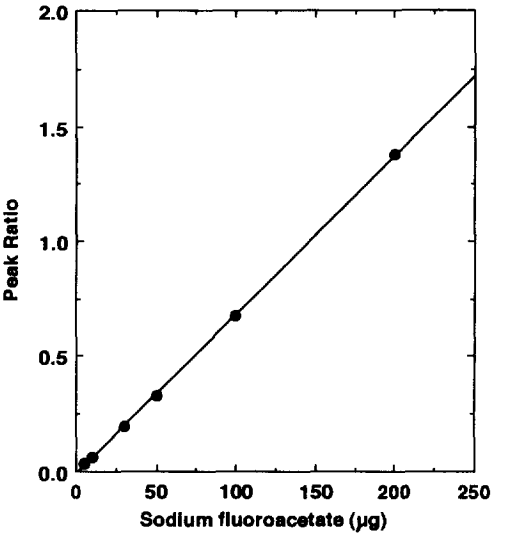

Fig. 7. Calibration graph for fluoroacetate. An appropriate amount of sodium fluoroacetate was incubated with 30 μl of sulfuric acid and 70 μl of ethanol containing 200 ppm (v/v) toluene at 60°C for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC. Area ratios of EFA to toluene peaks are plotted against the amount of sodium fluoroacetate.

the areas of the EFA and toluene peaks when the sulfuric acid volume was fixed at 30 μl. Although the area of toluene peak simply decreased as the ethanol volume increased, that of EFA increased, reached a maximum at 70 μl ethanol and then decreased. The area ratios also increased. A 70-μl volume of ethanol was chosen as it gave the greatest EFA peak area.

3.3. Calibration

Under the optimized HS conditions, the calibration graph (Fig. 7) was linear for sodium fluoroacetate concentrations ranging from 5 to 200 μg ml^{-1} ($r^2 = 0.999$; r = linear correlation coefficient). The limit of quantification was 0.5 μg ml^{-1} ($S/N = 14$).

3.4. Application to forensic examination

This method was applied to the analysis of forensic samples. As shown in Fig. 8, fluoroacetate extracted from the piece of meat was confirmed by HS-GC–MS, and its concentration was calculated to be ca. 13 mg g^{-1}.

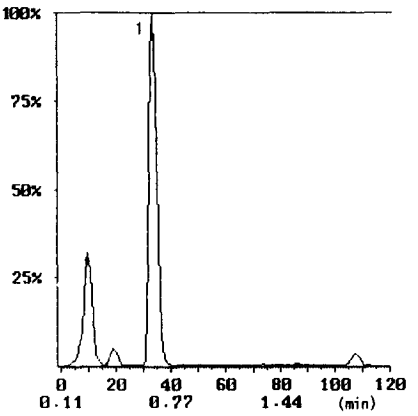

Fig. 8. Headspace gas chromatogram of an aqueous meat extract. The aqueous extract of a piece of meat (10 mg) suspected to contain poison was evaporated to dryness, incubated with 30 μl of sulfuric acid and 70 μl of ethanol at 60°C for 15 min in a 10-ml vial and 1.0 ml of HS gas was subjected to GC–MS with EI ionization. The trace is the total ion chromatogram and the peak 1 represents EFA.

4. Discussion

Organic acids can be derivatized to their volatile esters under strongly acidic and alcoholic conditions [6]. The reaction mechanism seems to be acid-catalysed condensation with alcohol [12], and so the reagent concentrations should influence the reaction efficiency. The volatility of the ester produced can be affected by the liquid phase matrix, where the production of ester is in equilibrium with the decomposition. Therefore, the peak areas of the derivatized ester found in the HS gas should be controlled by both derivatization efficiency and HS behaviour, which can be determined by the HS temperature and liquid-phase components.

The partition coefficient of toluene, a non-polar solvent, was not affected by the liquid-phase volume of the polar solvent ethanol (Fig. 6), but the volatility was markedly enhanced by sulfuric acid with a salting-out effect (Fig. 5). Almost constant areas of the EFA peak were obtained even with incubation for 10 min (Fig. 3), indicating a rapid HS equilibration and derivatization reaction. Raising the temperature led to an increase in the EFA peak area greater than that of toluene, but it is not clear whether this greater response is due to the temperature-promoted derivatization efficiency or more favourable volatility. The highest EFA area in the HS gas was required for the condition of a solvent ratio (sulfuric acid to ethanol) of 20–30%. Below 20%, a lower peak area can probably be expected because of either a lower derivatization efficiency or lower volatility owing to the low sulfuric acid level, indicating an insignificant salting-out effect. Above 30%, a lower peak area may also be expected, probably because of a lower derivatization efficiency (or increased decomposition of the ester produced). We chose 30 μl of sulfuric acid and 70 μl of ethanol, in consideration of the GC sensitivity. Too little liquid phase may not be insufficient to match the large scale of dried samples.

This HS-GC procedure is simple and does not need a tedious extraction procedure. The limit of quantification is almost equal to those of other GC methods [1–3]. This method was successfully applied to water samples, and may be applicable to biological samples, if the matrix effect is correctly considered.

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