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DETERMINATION OF SODIUM MONOFLUOROACETATE IN SOIL AND BIOLOGICAL SAMPLES AS THE DICHLOROANILIDE DERIVATIVE

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SUMMARY

A method is described for the determination of trace amounts of sodium monofluoroacetate (MFA-Na) in soil and biological samples. Soil samples were sonicated with distilled water in the presence of basic magnesium carbonate. Biological samples were extracted with distilled water by sonication and the extracts were coagulated by addition of equal volumes of alcohol and centrifuged. MFA-Na in each sample solution was adsorbed on Dowex 1-X8 anion-exchange resin and eluted with 2% (w/v) sodium chloride. The eluate was acidified with hydrochloric acid and treated with 2,4-dichloroaniline and N,N′-dicyclohexylcarbodiimide. The dichloroanilide derivative of MFA-Na was extracted with ethyl acetate and quantified by gas chromatography with electron-capture detection and gas chromatography–mass spectrometry. The detection limits were 0.0015 and 0.003 μg/g in 20 g of soil and 10 g of biological sample, respectively.

INTRODUCTION

Sodium monofluoroacetate (MFA-Na), a highly toxic compound inhibiting a certain step in the tricarboxylic acid cycle, is used for the control of field mice. The potassium salt of monofluoroacetic acid (MFA) is known as a toxic constituent of the South African poisonous plant *Dichapetalum cymosum* ("Gifblaar")[1]. The LD_{50} of MFA-Na is variable in different species and, for example, is 0.06–0.1 mg/kg in canines[2]. The potential toxicity of MFA-Na to non-target animals and the possibility of secondary poisoning require a sensitive method for determining MFA-Na in biological samples.

For chromatographic analysis, MFA-Na is often converted into various ester derivatives with appropriate chromatographic properties[3–12]. However, the concomitant water generally interferes with esterification of MFA-Na. Recently, we have developed a method for determining trace amounts of MFA-Na in water samples by gas chromatography, where MFA-Na in an aqueous solution acidified with hydrochloric acid is converted to the dichloroanilide derivative (MFA-DCA) by reaction with 2,4-dichloroaniline (DCA) and N,N′-dicyclohexylcarbodiimide (DCC)[13]. We have applied this method to the determination of trace amounts of MFA-Na in various

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environmental water samples. We expected this method to be applicable to the determination of MFA-Na in environmental samples such as soil and biological samples. However, the aqueous extracts of these samples contain large amounts of various impurities, which made the direct application of this method difficult.

In this paper, we report a practical method for the determination of MFA-Na in soil and biological samples. The new method involves extraction of the sample with water, ion-exchange pretreatment of the extract, the anilide derivatization of MFA-Na in aqueous solution and quantification of the anilide derivative by gas chromatography with electron-capture detection (GC–ECD). Gas chromatographic–mass spectrometric (GC–MS) analysis of the anilide derivative by using a capillary column for a better separation from impurities and more specific detection is also described.

EXPERIMENTAL

Apparatus

A Model UT-20 ultrasonic cleaner (300 W, 26 kHz; Kokusai Electric, Tokyo, Japan) was used for sample extraction. A Shimadzu Model GC-3BE gas chromatograph equipped with a ^{63}Ni electron-capture detector and a JMS-D-300 mass spectrometer (JEOL, Tokyo, Japan) were used for analyses.

Reagents and materials

MFA-Na was obtained from Wako (Osaka, Japan), DCA from Tokyo Kasei Kogyo (Tokyo, Japan), DCC from Kanto Chemical (Tokyo, Japan) and Wako-gel S-1 silica gel from Wako. Organic solvents and other reagents used were the same as used previously[13]. Basic magnesium carbonate, ammonium sulphate and sodium hydroxide were of analytical-reagent grade. GS25 glass-fibre filter-paper (Toyo Roshi, Tokyo, Japan) was used. Dowex-1-X8 strongly basic anion-exchange resin (50–100 mesh) was treated prior to use as follows. The resin was immersed in distilled water and packed into a glass column. The packed resin was successively washed with 10-fold volumes of 2 *M* sodium hydroxide, 1 *M* ammonium sulphate, 1 *M* hydrochloric acid and distilled water until neutral. A glass column (10 mm I.D.) packed with 10 ml of the resin was used for each experiment.

Ion-exchange pretreatment

MFA-Na was separated from aqueous samples with a 13 cm × 1 cm I.D. ion-exchange column packed with 10 ml of the activated resin, Dowex 1-X8. A 20-μg amount of MFA-Na were loaded on the ion-exchange column and elution of MFA from the resin was investigated in order to choose the eluent.

The influence of the flow-rate of MFA-Na solution through the column on adsorption was examined. A solution of 20 μg of MFA-Na in 100 ml of water was passed through the column at flow-rates in the range 2–18 ml/min. The influence of the pH of MFA-Na solution on adsorption was also examined. The pH of an aqueous solution containing 20 μg of MFA-Na in 100 ml of water was adjusted in the range 2.6–11, and the solution was passed through the column.

Procedure for soil

A mixture of a 20 g of sample, 1 g of basic magnesium carbonate and 50 ml of

distilled water in a 200-ml beaker was sonicated for 20 min. The mixture was allowed to stand for about 15 min, the supernatant was decanted and the residue was extracted with 50 ml of distilled water in the same way. The combined extracts were centrifuged for 15 min at 3000 rpm and filtered by suction through a glass-fibre filter-paper. The filtrate and the washings were combined and passed through the ion-exchange column. The resin was washed with 50 ml of distilled water. MFA was eluted from the column with 50 ml of 2% (w/v) sodium chloride solution. The eluate was subjected to derivatization and instrumental analysis.

Procedure for biological samples

A mixture of 10 g of homogenized animal tissue or plant material or a 2-ml blood sample and 30 ml of distilled water in a centrifuge tube was sonicated for 20 min. After being allowed to stand for a while, the mixture except for the blood sample was centrifuged for 15 min at 2000 *g* and the upper suspension was decanted. The residue was again extracted with 30 ml of distilled water in the same way.

To the combined extract or diluted blood after sonication, an equal volume of ethanol was added. The mixture was stirred slowly, allowed to stand for about 30 min and centrifuged for 15 min at 3000 rpm. The supernatant was filtered by suction through a glass-fibre filter-paper and the filtrate was passed through the ion-exchange column. The resin was washed with 50 ml of distilled water. MFA was eluted from the column with 50 ml of 2% (w/v) sodium chloride solution.

Derivatization[13]

The eluate from the ion-exchange column as mentioned above was transferred into a 100-ml separating funnel. To the eluate 0.25 ml of 10 *M* hydrochloric acid, 2 ml of 0.5 *M* DCA in ethanol, 0.8 ml of 1 *M* DCC in ethanol and 15 ml of ethyl acetate were added. The mixture was shaken vigorously for 1 h on a reciprocating shaker. The aqueous layer was separated after the addition of 5 g of sodium chloride and extracted again with 5 ml of ethyl acetate. The combined organic layer was washed with 5 ml of 3 *M* hydrochloric acid, saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in 2 ml of benzene, loaded on to a silica gel (3 g) column slurry-packed with benzene and washed with 50 ml of benzene. The MFA-DCA derivative was eluted with 100 ml of *n*-hexane–diethyl ether (95:5). The eluate was concentrated and subjected to instrumental analysis.

Gas chromatographic and gas chromatographic–mass spectrometric analysis

GC–ECD analysis of the dichloroanilide derivative was performed on a 2.1 m × 3 mm I.D. glass column packed with equal lengths of 5% DEGS–1% H_3PO_4 on Chromosorb W (60–80 mesh) and 5% Apiezone L grease–2% H_3PO_4 on Chromosorb W (60–80 mesh)[13]. The column and detector temperatures were 175°C and the injector temperature was 195°C. The flow-rate of the carrier gas (nitrogen) was 20 ml/min.

For calibration, amounts of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 μg of MFA-Na were dissolved in 50 ml of distilled water and 1 g of sodium chloride was added to each solution. The solution containing sodium chloride was subjected to the derivatization reaction described above.

GC–MS analysis was performed on a 15 m × 0.53 mm I.D. OV-1701 wide-bore

capillary column (Gasukuro Kogyo, Tokyo, Japan). The injector temperature was 250°C, the column oven temperature was 200°C, the ion source temperature was 250°C and the separator temperature was 250°C. Helium was used as the carrier gas at a flow-rate of 15 ml/min. The ionization voltage was 27.5 eV, the ionization current was 300 μA and the ion multiplier voltage was 2.0 kV. The fragment ion peak at m/z 186 was monitored for analysis. Aliquots of 2 μl were injected directly. Amounts of 0.1, 0.2, 0.4, 0.6 and 0.8 μg of MFA-Na were used for calibration.

RESULTS AND DISCUSSION

Pretreatment of sample extract

When MFA-Na was extracted from soil or biological samples with water, soil components such as humic substances or tissue constituents such as protein were also extracted in large amounts into the aqueous solution. If the extract is directly subjected to derivatization with DCA and DCC, no successful result is obtained. Purification of MFA-Na in the water extract is essential prior to the derivatization.

Richard and co-workers have successfully used an anion-exchange resin prepared from XAD to separate and concentrate organic acids from aqueous solution[14], and also reported good recoveries of C_2–C_{10} aliphatic carboxylic acids at the ppm level from oil shale process water[15]. Peterson[5] has separated MFA-Na from aqueous solution with Amberlite IR-45 in a batch process. McGary and Meloan[16] used a Bio-Rad AG1-X8 strongly basic resin for the same purpose.

We investigated the separation of MFA-Na from water samples with the strongly basic anion-exchange resin Dowex 1-X8. MFA was adsorbed quantitatively on the resin at flow-rates through the column in the range 2–18 ml/min. Even MFA-Na solution containing 500 mg/l of sodium chloride gave the same result and no influence on the adsorption of MFA-Na was observed. The pH of MFA-Na solution in the range 2.6–11 was also observed to have no influence on adsorption.

Elution of MFA from the resin with various eluents is shown in Table I. Dilute sodium hydroxide solution eluted MFA from the resin, but a large volume of the eluent was required, which is unsuitable for concentration of MFA-Na; for the subsequent derivatization step, an elution volume of less than 50 ml is desirable.

TABLE I

ELUTION OF MFA-Na FROM ANION-EXCHANGE COLUMN

MFA-Na (20 μg) was adsorbed on a Dowex 1-X8 (Cl^-) (50–100 mesh) column (13 cm × 1 cm I.D.) and eluted.

Eluent	*Concentration (M)*	*Recovery (%)*	
		~ 50 ml[a] *(1st fraction)*	*~ 100 ml*[a] *(2nd fraction)*
NaOH	0.1	2	10
	0.2	13	45
NaCl	0.1	46	54
	0.25	97	3
	0.5	98	2

[a] The eluate was fractionated into 50-ml volumes.

Sodium chloride solution at concentrations of more than 0.25 *M* is satisfactory for the quantitative elution of MFA from the resin within an elution volume of 50 ml. In derivatizing with DCA and DCC, sodium chloride concentrations in the range *ca.* 1–4% (w/v) gave constant recoveries[13] and higher concentrations of sodium chloride reduced the recovery. Therefore, the eluent for stripping MFA-Na from the ion-exchange resin was selected as 2% (w/v) sodium chloride solution. A 50-ml volume of the eluent could recover MFA-Na quantitatively from the column and the eluate could be subjected to the derivatization procedure without initial addition of sodium chloride.

Extraction from soil sample

The aqueous extract of soil is muddy and contains various inorganic and organic substances such as humic substances. Direct application of this extract to the ion-exchange column, even after filtration, often choked the column owing to precipitation of brown materials.

Calcium hydroxide and magnesium carbonate have been used as reagents to coagulate such materials and precipitate water-soluble organic substances and ions[17,18]. Aqueous extraction of soil by sonication in the presence of the reagents afforded an extract which contained little coloured and supended substances, and this was suitable for subsequent loading on to the ion-exchange column. Basic magnesium carbonate posseses a low water solubility and the extract obtained was weakly basic. The extract obtained by using calcium hydroxide absorbed ambient carbon dioxide, forming a film of calcium carbonate on the surface. This was disadvantageous for the subsequent loading on the ion-exchange column.

Soil with a relatively low ignition loss has a low content of organic substances. Addition of an organic solvent such as an alcohol to the aqueous extract obtained by sonication of such a soil sample caused brown material to precipitate to a certain extent. The extract obtained in this way could be loaded on the column. However, this method was not applicable to soil with a high ignition loss.

Extraction from biological samples

Extraction of MFA-Na from biological samples such as animal tissues, stomach contents, plants and baits has been performed with water or hydrophilic solvents under wet conditions[3–12,18]. Subsequently efforts have often been made to separate MFA from water. In this work, MFA-Na was subjected to derivatization in aqueous solution and no separation from water was required. However, aqueous extracts of animal tissue, blood, and plant samples contained large amounts of protein, etc., and was incapable of being loaded on to the ion-exchange column. Addition of an organic solvent is generally suitable for deproteinization. In this work, ethanol was chosen as a non-ionic protein coagulating agent.

To the aqueous extract of biological samples an equal volume of ethanol was added and the mixture was allowed to stand for about 30 min. Subsequently, precipitates were removed by centrifugation and filtration. The filtrate was applied to the ion-exchange column, and MFA was adsorbed on the resin. Inadequate deproteinization in this step caused the ion-exchange column to choke and gave a low final recovery of MFA-Na.

TABLE II

RECOVERY DATA FOR MFA-Na ADDED TO SOIL SAMPLES

Sample No.	*Ignition loss[a] (%)*	*Amount added (μg)*	*Recovery (μg)*	*Average recovery (%)*	*Relative standard deviation (%)*
1	3.5	0.40	0.40, 0.40, 0.41, 0.37, 0.40	99	3.8
2	8.4	0.50	0.38, 0.39, 0.39, 0.37	77	2.5

[a] 600°C, 2 h.

Recovery test

A recovery test on MFA-Na was carried out on several samples according to the analytical procedure. Portions of 20 g of soil were spiked with MFA-Na and analysed. The results are given in Table II. For soil that contained relatively large amounts of organic substances, more than 70% of MFA-Na was recovered. Fig. 1 shows a chromatogram obtained from soil spiked with MFA-Na. The peak of MFA-DCA derivative was well separated from others and successfully determined. However, additional peaks appeared at retention times longer than 10 min, some of which were considered to correspond to short-chain aliphatic carboxylic acids. The relative retention times for the acetic, propionic, formic, and butyric acid derivatives were about 1.4, 1.8, 1.8 and 2.3, respectively, on the column used[13]. The appearance of later peaks required 60–90 min for one GC–ECD analysis.

Portions of 10 g of homogenized biological samples were spiked with MFA-Na and analysed by GC–ECD. The results are given in Table III. Typical chromatograms obtained from these samples are shown in Figs. 2 and 3, which indicate a good

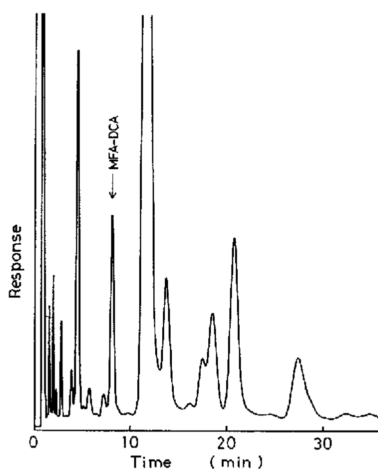


Fig. 1. Gas chromatogram for soil spiked with 0.02 μg/g of MFA-Na.

TABLE III

RECOVERY DATA FOR MFA-Na ADDED TO BIOLOGICAL SAMPLES

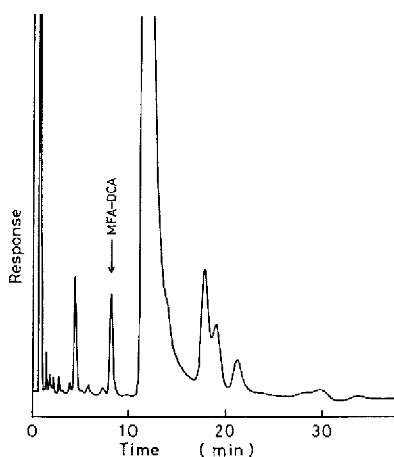
Sample	*Amount added (μg)*	*Recovery (μg)*	*Average recovery (%)*	*Relative standard deviation (%)*
Fish meat	0.50	0.40, 0.37, 0.45	81	10
Fish internal organs[a]	0.50	0.50, 0.48, 0.47	97	3.2
Liver (pig)	0.50	0.42, 0.42, 0.41, 0.39	82	3.4
Bovine blood[b]	0.10	0.090, 0.086, 0.091	89	3.0

[a] Without gall.
[b] Lyophilized, 2 ml.

separation of the MFA-DCA derivative. MFA-Na was successfully recovered from these biological samples. However, as observed with soil samples, additional peaks appeared later than the objective peak and about 90 min were required for one analysis.

A recovery test on bovine blood was conducted with a lyophilized sample. Portions of 2 ml of the blood were spiked with MFA-Na and analysed. In this instance, almost the same results were obtained.

The detection limits of this GC–ECD method were about 0.0015 and 0.003 μg/g with 20 g of soil and 10 g of biological sample, respectively.



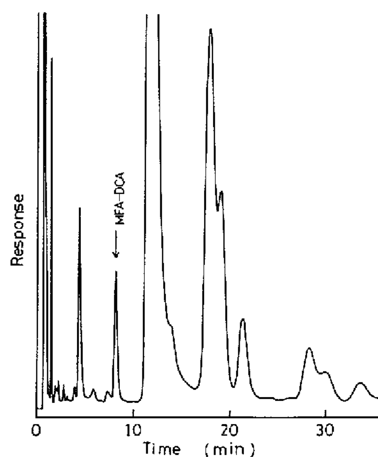


Fig. 2. Gas chromatogram for fish meat spiked with 0.05 μg/g of MFA-Na.

Fig. 3. Gas chromatogram for fish internal organs (without gall) spiked with 0.05 μg/g of MFA-Na.

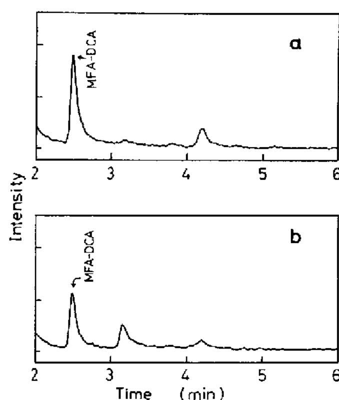


Fig. 4. Selected ion monitoring profiles for (a) soil and (b) pig liver spiked with 0.025 μg/g of MFA-Na. The mass monitored is the fragment ion peak at *m/z* 186.

GC–MS analysis

From the electron-impact mass spectrum of the MFA-DCA derivative[13], the molecular ion peak at *m/z* 221 and the fragment ion peak at *m/z* 186 were selectively monitored. The former was inferior to the latter with respect to quantification and contamination. Quantification was therefore effected by measurement of the area of the fragment ion peak at *m/z* 186. The calibration graph prepared with amounts of 0.1–0.8 μg of MFA-Na was linear.

Soil and pig liver were spiked with MFA-Na, treated according to the analytical procedure and analysed by GC–MS (selected ion monitoring mode). Fig. 4 shows typical chromatograms obtained, and demonstrates the usefulness of monitoring the ion peak at *m/z* 186. The time taken to analyse a sample was substantially reduced.

The recovery test was performed as in the analysis by GC–ECD, and the results are given in Table IV together with data obtained by GC–ECD. MFA-Na quantified by GC–MS was recovered satisfactorily with a lower precision compared with GC–ECD. The detection limit of the GC–MS method was similar to that of the GC–ECD method.

TABLE IV

RECOVERY OF MFA-Na BY GC–MS AND COMPARISON WITH GC–ECD

Sample[a]	*Amount added (μg)*	*GC–MS*		*GC–ECD*	
		Recovery (%)	*Relative standard deviation (%)*	*Recovery (%)*	*Relative standard deviation (%)*
Soil[b]	0.50	88	6.6	86	4.3
Liver (pig)	0.25	91	12	89	5.6

[a] Replicates ($n = 4$).
[b] Ignition loss (600°C, 2 h) = 4.1%.

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