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Note

Simplified method for determining acephate and methamidophos residues in several substrates

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Acephate (O,S-dimethyl acetylphosphoramidothioate) is a broad-spectrum insecticide, introduced by the Chevron Chemical Company under the trade name Orthene® in 1971 (ref. 1). The uses of this chemical for pest control in agriculture, forestry, and for domestic purposes have increased rapidly in the past because of its effectiveness against a wide range of pest insects, such as aphids, thrips, leaf miners, sawflies and lepidopterous larvae and because of its low toxicity to higher animals, especially fish. However, acephate is partially metabolized in some plants and animals to yield the highly toxic methamidophos (O,S-dimethyl phosphoramidothioate), an excellent insecticide in its own right, and marketed under the trade names Monitor® and Tamaron® in North America and Europe, respectively.

In order to monitor the fate of acephate and methamidophos following their application for purposes of insect pest control, sensitive, reliable, and relatively simple analytical methods for these compounds are essential. A gas chromatographic (GC) method for the analysis of residues of these compounds in crops was described by Leary[2] and modified for citrus foliage by Nigg *et al.*[3]. According to these authors, plant tissues are extracted with ethyl acetate or acetonitrile by blending. The crude extracts are cleaned by eluting acephate and methamidophos from silica gel columns with 10% (v/v) methanol–ether prior to analysis by gas chromatography–alkali flame-ionization detection (GC–AFID). This fraction still contains considerable amounts of extraneous substances, such as plant pigments and other co-extractants, which are deleterious to the GC columns and reduce sensitivity progressively[4].

An activated coconut-charcoal column was described for the extraction and clean-up of acephate and methamidophos from natural waters[5], a method sensitive to sub-ppb concentrations. However, the preparation and activation of the coconut charcoal, as well as the elution of the samples from the charcoal columns are exceedingly tedious and cricital for consistent results.

This paper describes simpler and less time-consuming methods for the analyses

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of acephate and methamidophos residues in natural waters, sediments, and tissues from plants (asparagus ferns and spears) and animals (trout).

MATERIALS AND METHODS

Analytical grades of acephate (99.3%) and methamidophos (99.6%) were obtained from the Chevron Chemical Company. Standard solutions for sample fortifications and gas–liquid chromatographic (GLC) analyses were prepared by appropriate dilutions with acetone or ethyl acetate. Charcoal (Nuchar C, Fisher Scientific) was acid-washed prior to use[6]. Pyrex glass wool and Whatman CF-11 cellulose were used in clean-up columns. Glass-distilled, pesticide-grade solvents were used throughout.

Asparagus ferns and spears were obtained from the Research Station, Agriculture Canada, Summerland, B.C., Canada. Rainbouw trout (*Salmo gairdneri*), mean weight 5 g, were purchased from a trout farm in the Lower Fraser Valley, east of the city of Vancouver, B.C. Water and sediment samples were collected from a pond, located in the Coastal Mountains, approximately 50 km north-east of Vancouver.

Prior to fortification with acephate and methamidophos to give concentrations of either 1.0 or 0.01 ppm, plant tissues were macerated; sediments, placed in Büchner funnels, were stripped of excess water by aspiration; whole fish, one per sample, were weighed and cut into small pieces. Water was treated as received.

Extraction and clean-up

Tissue and sediment samples were extracted, three times each, according to Table I (solvents: EA = ethyl acetate; An = acetonitrile. Na_2SO_4 = anhydrous sodium sulphate.

TABLE I

EXTRACTION PROCEDURES

Sample	Asparagus	Fish	Sediment
Sample size (g)	10	5	50
Solvent (ml per extraction)	EA, 100	An, 100	An, 100
Time (min per extraction)	5	1	5
Na_2SO_4 (g)	20	10	50
Apparatus	Omni-Mixer	Polytron PT20	Omni-Mixer

The Na_2SO_4 was added before the first extraction; the Sorvall Omni-Mixer was equipped with threaded 400-ml jars.

The asparagus and fish extracts were filtered through glass-fibre disks in Büchner funnels, the sediment extracts through Pyrex glass wool plugs in filter funnels. The three fractions from each sample were combined in measuring cylinders and the volumes recorded. Aliquots of crude fish extracts, equivalent to 2 g of body weight, were transferred to 250-ml separatory funnels and extracted three times with 20 ml of hexane to remove hexane-soluble lipids. The combined hexane phases were back-extracted with 20 ml of acetonitrile before being discarded.

Crude extracts, equivalent to 2 g of asparagus or fish or 10 g of sediment, were

used for clean-up. In 100-ml round-bottom flasks, they were evaporated just to dryness by flash evaporation at 38°C. The residues were picked up in 1 ml of ethyl acetate, followed by the addition of 4 ml of hexane. Glass columns (30 × 1.1 cm I.D.) with PTFE stop-cocks were packed, from the bottom up, with a glass wool plug, 2 cm of Na_2SO_4, 4 cm of a 2:5 (w/w) mixture of Nuchar C and Whatman CF-11, 2 cm of Na_2SO_4 and a glass wool plug. The packed columns were pre-washed with 10 ml of ethyl acetate, followed by 10 ml of hexane. Then the redissolved extracts were transferred quantitatively from the round-bottom flasks to the clean-up columns. The resulting eluates were discarded. Finally, acephate and methamidophos were eluted from the columns with 40 ml of ethyl acetate. These eluates were suitably concentrated for GLC analysis.

Water samples (5 ml) were mixed with approximately 75 ml of acetonitrile, to form an azeotropic mixture, and then evaporated just to dryness in a flash evaporator at 38°C. The residues were picked up in appropriate volumes of acetone for GLC analysis, no further clean-up being required.

GLC analysis

Three Pyrex glass columns were used: (1) 36 cm × 4 mm I.D., Ultra-Bond II, 100–120 mesh; (2) 75 cm × 2 mm I.D., 1% OV-225 on Ultra-Bond 20M, 100–120 mesh; (3) 36 cm × 3 mm I.D., 1% Carbowax 20M TPA on Chromosorb W HP, 100–120 mesh. Columns 1 and 2 were operated isothermally at 150 and 140°C, respectively, with the carrier gas flow-rate at 40 ml/min; column 3 was programmed from 145 to 190°C at 30°C/min, carrier gas flow-rate at 60 ml/min.

The operating parameters were as follows: air 120 ml/min, hydrogen 3.5 ml/min and helium as carrier 40 ml/min with columns 1 and 2 and an alkali flame-ionization detector (Tracor 702-NP) at 240°C; hydrogen 200 ml/min, air 150 ml/min and nitrogen as carrier 60 ml/min with column 3 and a flame photometric detector (phosphorus mode) at 170°C; inlet and outlet temperature 210°C.

Detector response was calibrated daily before and after sample analysis with authentic reference-grade standards. Quantification of acephate and methamidophos was based on average peak heights of these external standards, injected before and after sample analysis.

RESULTS AND DISCUSSION

Performance of the GC columns

All three columns gave good separation of acephate and methamidophos; absolute retention times are given in Table II. Columns 1 (Ultra-bond II) and 2 (1% OV-225 on Ultra-Bond 20M) are recommended because good separation of acephate and methamidophos can be obtained isothermally at relatively low temperature, and because of the inertness of these packings[7]. It was observed, however, that moisture has deleterious effects on these columns, as well as on column 3, and that, in order to maintain their high performance, it is extremely important to analyse on these columns moisture-free extracts only. Fig. 1 shows typical chromatograms obtained with these columns.

Recoveries of acephate and methamidophos

Recoveries from the four substrates (plant tissue, fish tissue, sediments and

TABLE II

ABSOLUTE RETENTION TIMES OF ACEPHATE AND METHAMIDOPHOS

GC column	*Temperature*	*Retention times (min)*	
		Acephate	*Methamidophos*
(1) Ultra-Bond II	150°C	1.25	0.75
(2) 1% OV-225 on Ultra-Bond 20M	140°C	1.76	0.88
(3) 1% Carbowax 20M TPA on Chromosorb W HP	145–190°C (30°C/min)	1.05	0.47

natural water) were 85%, or higher, following fortification with both acephate and methamidophos to give either 1.0 or 0.01 ppm of both (Table III). The exceedingly high recovery of methamidophos from water treated to contain 0.01 ppm, namely 116 ± 7.2%, is difficult to explain. De-acetylation of acephate was not indicated since recovery of acephate from these samples was not correspondingly lower (Table III). Methamidophos-resembling responses in control samples, discussed below, evidently were not responsible either.

The clean-up achieved with the charcoal–cellulose (Nuchar C–Whatman CF-11) column was excellent. Following elution from this column, extracts equivalent to 10 g of asparagus spear, 2 g of asparagus fern, 2 g of fish or 10 g of sediment were pigment-free and contained no other extraneous substances interfering with subsequent analysis by GLC of acephate and methamidophos using either of the columns, detectors or conditions described.

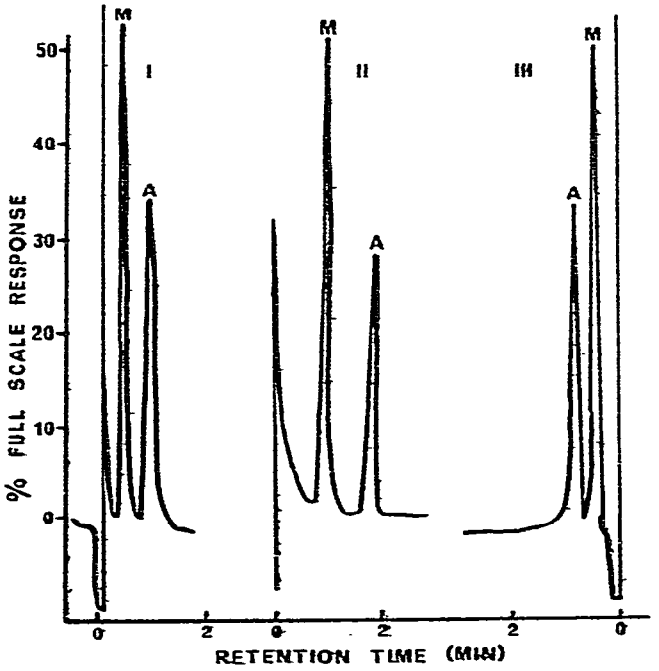

Fig. 1. Chromatograms of acephate (A) and methamidophos (M), 0.4 ng each, on (I) Ultra-Bond II, (II) 1% OV-225 on Ultra-Bond 20M and (III) 1% Carbowax 20M TPA on Chromosorb W HP.

TABLE III

RECOVERY OF ACEPHATE AND METHAMIDOPHOS FROM ASPARAGUS, FISH, POND WATER AND SEDIMENT

(Mean ± S.D., in %, $n = 4$)

Origin	*Fortification*			
	1.0 ppm		*0.01 ppm*	
	Acephate	*Methamidophos*	*Acephate*	*Methamidophos*
Asparagus	95.1 ± 2.3	93.1 ± 2.3	91.5 ± 2.3	86.8 ± 3.2
Fish	99.0 ± 7.9	84.8 ± 4.3	101 ± 7.5	84.6 ± 5.9
Sediment	105 ± 1.5	103 ± 1.7	96.7 ± 2.9	102 ± 6.9
Water	97.9 ± 1.9	95.5 ± 4.1	102 ± 8.2	116 ± 7.2

Figs. 2 and 3 show chromatograms of acephate and methamidophos, extracted from the four substrates following fortification to 0.01 ppm. Extracts of asparagus and sediment samples contained substances interfering with methamidophos analysis. These were removed, however, by discarding the first eluate following the transfer of the residue in ethyl acetate–hexane (1:4) to the Nuchar–cellulose clean-up column. Extracts without these interfering substances could be transferred to the clean-up columns in 5 ml of ethyl acetate (no hexane) and without discarding the resulting eluate.

In preparing the azeotropic mixtures, an excess of acetonitrile was added to the water samples to ensure complete removal of water during flash evaporation as trace

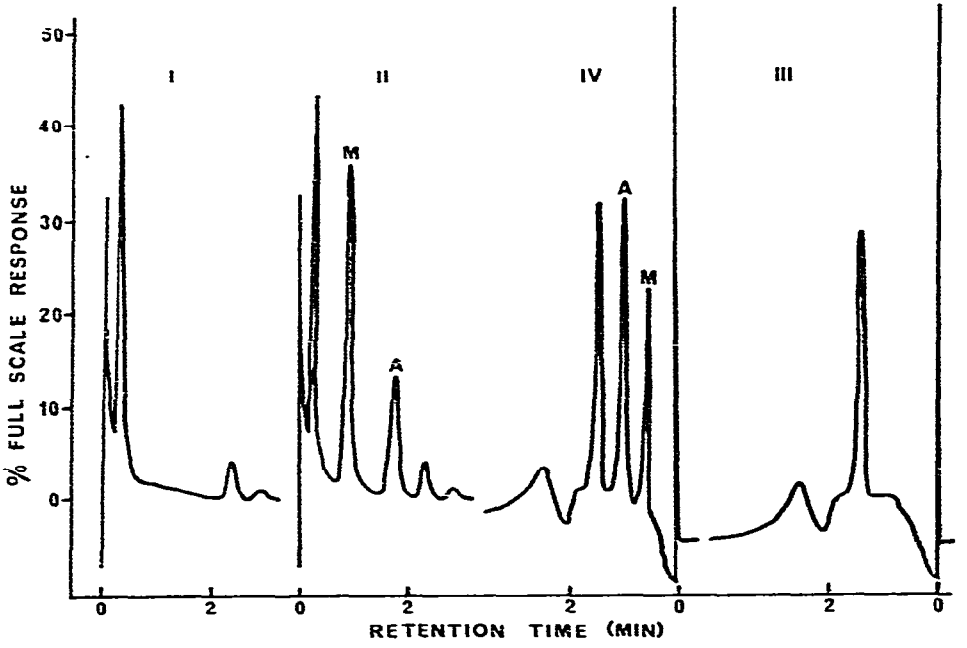

Fig. 2. Chromatograms of (I) asparagus blank, (II) asparagus with acephate (A) and methamidophos (M) at 0.01 ppm each, (III) fish blank and (IV) fish with A and M at 0.01 ppm each.

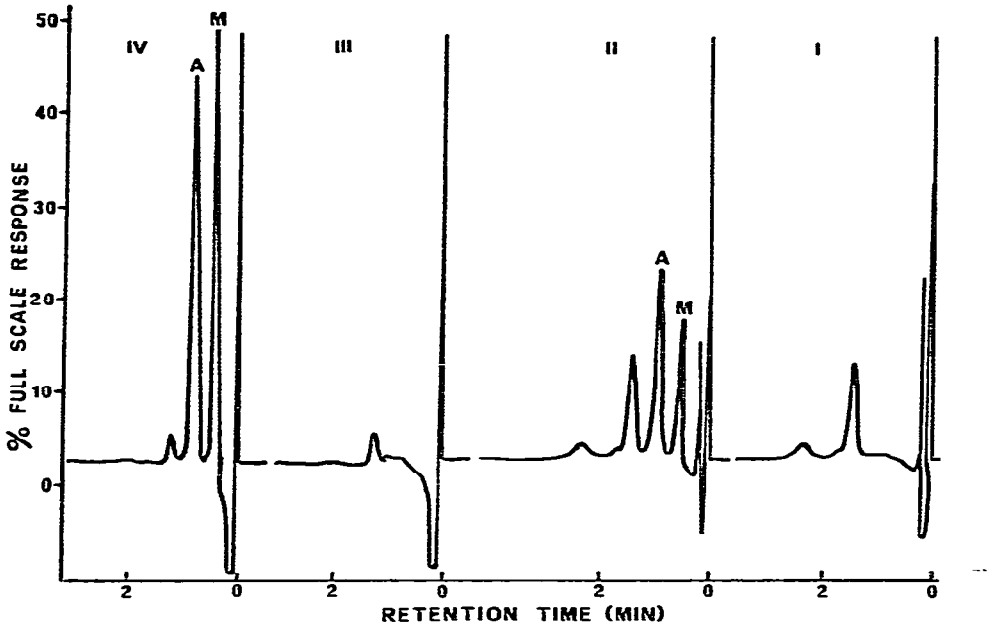

Fig. 3. Chromatograms of (I) sediment blank, (II) sediment with acephate (A) and methamidophos (M) at 0.01 ppm each, (III) water blank and (IV) water with (A) and (M) at 0.01 ppm each.

quantities of water present in the extracts drastically and rapidly shortened column life. This method for analysing acephate and methamidophos in water is rapid, simple, and reliable for sensitivities as low as 1–5 ppb. To improve the sensitivity levels, larger water samples and correspondingly larger volumes of acetonitrile would be required. Further clean-up, following evaporation of the azeotropic mixture, may also become necessary, rendering this method impractical and/or uneconomical if sub-ppb sensitivities are imperative. In that case, the coconut-charcoal method[5] might be more desirable.

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