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Note

Improved analysis of acephate and methamidophos in biological samples by selective ion monitoring gas chromatography–mass spectrometry

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Acephate is a water-soluble, broad spectrum organophosphate (OP) insecticide used for the control of agricultural and forestry pests. It is produced by the N-acetylation of methamidophos, which is a highly toxic OP insecticide. Although the insecticidal properties of acephate and methamidophos are similar, acephate is 40–50 times less toxic to mammals than methamidophos (LD_{50} for acephate is 900 mg/kg and for methamidophos it is 20 mg/kg). The use of acephate as a domestic insecticide has increased rapidly due to its low mammalian toxicity. It has been proposed that acephate could be metabolized in certain plants and animals to produce methamidophos[1]. A simple and sensitive method for the quantification of acephate and methamidophos is required to monitor the fate of acephate in exposed animals. Several gas chromatographic (GC) methods have been developed for the analysis of these insecticides[2,3]. The GC methods are time-consuming, since they require cleanup of crude extract by either silica gel or charcoal columns[4]. In this paper, a convenient, simple, and rapid gas chromatographic–mass spectrometric (GC–MS) method is described for the analysis of acephate and methamidophos in biological samples. Unlike GC methods, this method does not require the cleanup of crude extract.

EXPERIMENTAL

Material

The GC–MS device used was a Hewlett-Packard Model 5993C-OP-95 with an electron impact ionizer and an integral GC. The column used was a 15 m × 0.25 mm I.D. capillary, HP-SE 54 coated, fused-silica column. Acephate and methamidophos were provided as a free gift by Chevron (San Francisco, CA, U.SA.). Other reagents were purchased from Fisher Scientific. Dog blood and brain samples were spiked with acephate and methamidophos before extraction and analysis.

Method

Acephate and methamidophos were analyzed by monitoring selected ions by the GC–MS apparatus.

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Sample preparations

Blood. Blood samples containing various concentrations of acephate and methamidophos were mixed thoroughly with acetonitrile (25 ml/ml blood). After centrifugation, the clear supernatant was collected in a round-bottom flask and evaporated to dryness by flash evaporation at 40°C. The residues were redissolved in 1–5 ml of ethyl acetate. Sodium sulfate (1 g) was added to the ethyl acetate to remove moisture. The mixture was centrifuged and 1–3 μl of clear ethyl acetate layer was injected directly into the GC–MS apparatus.

Saline. Procedure for the extraction of acephate and methamidophos from exposed saline or other aqueous solution was similar to the blood.

Brain. Brain samples containing acephate and methamidophos were mixed with acetonitrile (1:25, w/v) and homogenized. The homogenate was centrifuged and the clear supernatant was transferred into a 150-ml round-bottom flask. The remaining tissue sediment was homogenized twice with 15 ml of acetonitrile and the supernatants were collected. The combined supernatant was flash-evaporated to dryness. The dry residue was redissolved in 1–5 ml of ethyl acetate. Sodium sulfate (1 g) was added to the extract to remove the moisture. A volume of 1–3 μl of clear ethyl acetate layer was injected into the GC–MS apparatus.

GC–MS conditions. GC–MS conditions were selected to yield a short run time with clean separation of acephate and methamidophos and with no interfering ions. The GC–MS conditions were:

inlet temperature = 180°C; oven temperature = 100°C for 2 min, then increasing 25°C/min to 200°C; source chamber pressure = $6 \cdot 10^{-6}$ torr; electron impact voltage = 70 eV. Mass detection was initiated 1.5 min after sample injection to allow the elution of solvent and to protect the ion detector. The ions selected for monitoring acephate were *m/z* 94, 136 and for methamidophos *m/z* 94.

The extraction efficiency was determined by adding known amounts of these insecticides to the blood and brain samples and then determining the amount recovered. In order to monitor the formation of methamidophos from acephate, 200 pmole of acephate was added to one ml of blood or saline. Samples were extracted at 6, 24 and 48 h after the addition of acephate. A volume of 1–3 μl of ethyl acetate layer was injected into the GC–MS apparatus. The concentrations of acephate and methamidophos were determined.

RESULTS AND DISCUSSION

Selection of ions for monitoring acephate and methamidophos

The fragmentation pattern for acephate and methamidophos is shown in Fig. 1. The highest mass number observed for acephate and methamidophos were ions at *m/z* 183 and *m/z* 146, respectively. However, the ions present in greatest abundances were *m/z* 136, 94 for acephate and *m/z* 94 for methamidophos. Ions at *m/z* 42 and 43 were not considered unique to these insecticides since they were also present in the solvent peak. Molecular ions were not detected for both acephate and methamidophos. Ions at *m/z* 136 and 94 were selected for monitoring these insecticides due to their uniqueness and high abundances. Other ions, such as *m/z* 95 and 125 could also be included in ion-monitoring. However, in our experience, increasing the number of ions monitored decreased the sensitivity of the assay. Our capillary column

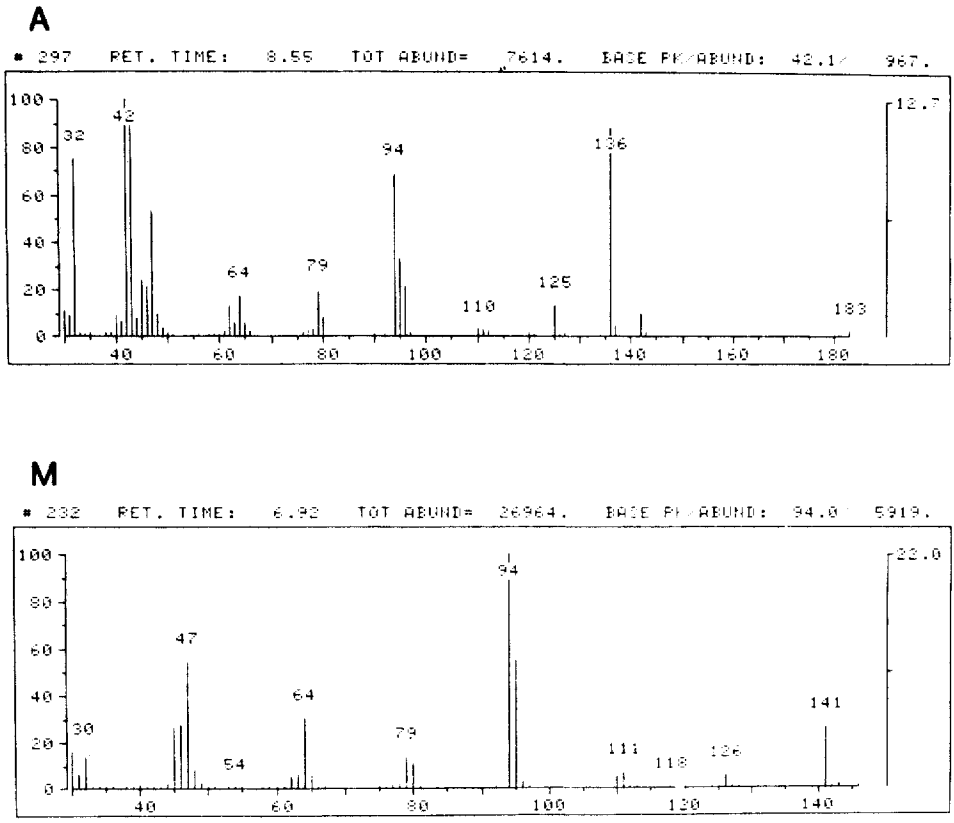

Fig. 1. (A) Mass spectra for acephate. Base peak is *m/z* 42. The ionic abundance of acephate peak was 12.7% of the total ionic abundance. Ions at *m/z* 94 and 136 were selected for monitoring acephate. (B) Mass spectra for methamidophos. Base peak is *m/z* 94. The ionic abundance of methamidophos peak was 22% of the total ionic abundance.

TABLE I

RECOVERY OF ACEPHATE AND METHAMIDOPHOS FROM BLOOD AND BRAIN SAMPLES

Values are mean ± S.D., $n = 4$.

Amount added (pmole/ml blood, pmole/g brain)	*Amount recovered (pmole/ml blood, pmole/g brain)*			
	Acephate		*Methamidophos*	
	Blood	*Brain*	*Blood*	*Brain*
10	8.1 ± 2.0	8.5 ± 1.0	7.9 ± 2.0	8.7 ± 1.3
20	16.0 ± 2.0	17.9 ± 3.0	16.3 ± 2.0	18.3 ± 1.9
40	30.0 ± 3.0	34.1 ± 4.3	32.0 ± 4.0	36.0 ± 3.8
80	64.3 ± 6.0	66.2 ± 4.0	68.0 ± 6.0	70.0 ± 9.6

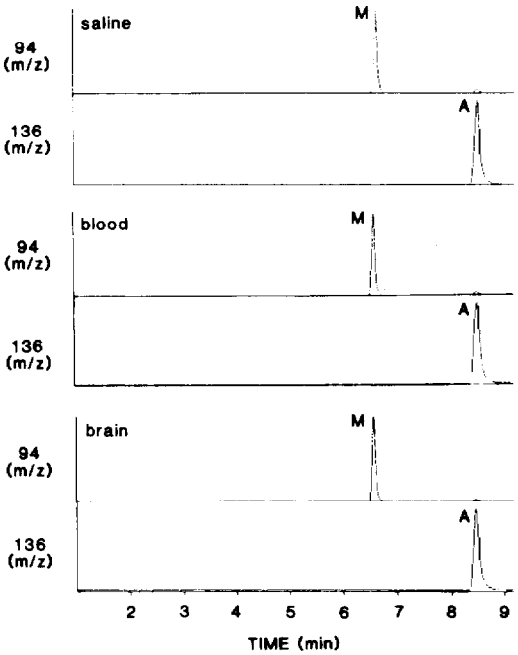

Fig. 2. Selected ion response chromatogram for acephate and methamidophos extracted from saline, blood, and brain samples. Ions at *m/z* 94 and 136 were monitored. Methamidophos eluted at 6.9 min and acephate at 8.55 minutes (A = acephate; M = methamidophos). Carrier gas: helium; flow-rate: 0.3 ml/min.

provided excellent separation of acephate and methamidophos in saline, blood and brain samples (Fig. 2). Although the samples were not cleaned on silica gel or charcoal column, the chromatogram was clean for both blood and brain samples (Fig. 2). Under the chromatographic conditions described, acephate was eluted at 8.5 min and methamidophos at 6.9 min.

TABLE II

CONCENTRATION OF ACEPHATE AND METHAMIDOPHOS IN BLOOD AND SALINE SAMPLES EXPOSED TO ACEPHATE (200 PMOLE/ML)

Values are mean ± S.D., $n = 4$.

Time after exposure (h)	*Concentration (pmole/ml)*			
	Acephate		*Methamidophos*	
	Blood	*Saline*	*Blood*	*Saline*
0	196.78 ± 7.8	201.0 ± 13.2	–	–
6	185.8 ± 8.0	194.0 ± 12.0	–	–
24	148.0 ± 4.0*,**	170.0 ± 9.7*	2.0 ± 0.8	–
48	106.0 ± 10.0*,**	147.0 ± 8.3*	5.0 ± 1.0	–

* $p < 0.05$ significant when compared with zero hour values.
** $p < 0.05$ significant when compared with corresponding saline values.

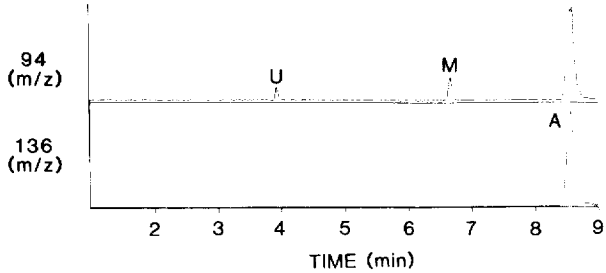

Fig. 3. Selected ion response chromatogram for acephate and methamidophos extracted from the blood exposed to 200 pmole of acephate (A = acephate; M = methamidophos; U = unknown). Ions at *m/z* 94 and 136 were monitored. Carrier gas: helium; flow-rate: 0.3 ml/min.

Recovery and extraction efficiency

The recovery of these insecticides from blood and brain samples is shown in Table I. A linear relationship was observed between the amount of insecticides added (10, 20, 40 and 80 pmoles) and the amount recovered from both blood and brain samples. The extraction efficiency was approximately 75–80% for both insecticides. Szeto *et al.*[1] have reported >90% recovery for acephate and methamidophos after extraction and charcoal cleanup of tissue samples.

Fate of acephate in the blood

The concentrations of acephate in the blood and saline samples at 0, 6, 24 and 48 h after addition of this insecticide are shown in Table II. The blood and saline acephate values were found to be 185.8 ± 8.0 and 195.0 ± 12.0 pmole/ml, respectively, at 6 h after addition of acephate to these samples. Thereafter, a gradual decrease in the concentrations of this insecticide in blood and saline samples was noted. At 24 and 48 h, the amount of acephate present in the blood was significantly lower than the amount present in the saline (Table II). An amount of 2 ± 0.8 and 5 ± 1.0 pmole/ml methamidophos was formed in the blood samples exposed to acephate and analyzed after 24 and 48 h, respectively (Fig. 3, Table II). Since the decay of acephate in the blood was faster than in the saline, and since methamidophos accumulation occurred only in the blood, it is proposed that blood might contain certain enzyme(s) capable of metabolizing acephate. At present this GC–MS technique is being used to study the kinetics of accumulation of methamidophos in animals exposed to acephate.

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