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Short Communication

Gas chromatographic method for simultaneous determination of acephate and methamidophos in bark, cone and seed samples of conifers

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ABSTRACT

A convenient, simple and rapid gas chromatographic method is described to quantify simultaneously acephate and methamidophos from the bark, cones and seeds of black spruce, *Picea mariana* (Mill.), by ethyl acetate solvent extraction, concentration and charcoal–cellulose microcolumn cleanup. Average recoveries from the matrices fortified with 0.10, 1.0 and 5.0 μg/g ranged from 88.8 to 99.0% with overall relative standard deviations of 1.1 to 6.5%. The limits of detection and quantification of the two compounds from the three matrices were 3 and 10 ng/g, respectively.

INTRODUCTION

Acephate (O,S-dimethyl acetylphosphoramidothioate) is the active component of Acecap-97 implants (Creative Sales, Inc., Fremont, NE, U.S.A.) that are used in controlling certain destructive pests of diptera and lepidopterous species in high-volume conifers and seed orchards [1]. Recent studies have shown that trunk implants in black spruce, *Picea mariana* (Mill.), trees with Acecap is effective in controlling cone and seed insects, protecting cone crops from insect attack and increasing the seed procurement to meet regeneration requirements of spruce trees [2].

In plants, acephate is converted into metabolic products, primarily methamidophos (O,S-dimethyl phosphoramidothioate), a potent anticholinesterase agent [3,4]. The insecticidal action of acephate has been related to its conversion to methamidophos or to a combined anticholinesterase effect of the parent and its primary metabolite [5].

As part of studying the efficacy of Acecap implants, we are required to measure the upward mobility of acephate, its accumulation, persistence and metabolic conversion to methamidophos in bark, cones and seeds of black spruce at varying times after implantation. At present, no suitable residue method is available in the literature to analyze acephate and methamidophos from these complex matrixes containing terpenes, hydroxy acids, lipoidal materials and esters. Gas chromatographic (GC) [6–13], GC–mass spectrometric [14] and high-performance liquid chromatographic (HPLC) [15,16] methods have been reported for the analysis of ace-

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phate and methamidophos. Most of these methods are primarily for agricultural commodities, foods, biological samples and formulated products. The procedures by Sundaram and Hopewell [8], Szeto *et al.* [9] and Richmond *et al.* [12], although relevant to our present needs, are somewhat laborious and expensive due to high volume sample extraction followed by evaporation or centrifugation, solvent partition and macrocolumn cleanup consuming time and large quantities of solvents. This paper describes a convenient, simple and rapid method to quantify simultaneously acephate and methamidophos from the bark, cones and seeds of black spruce by using solvent extraction, concentration, microcolumn cleanup and GC analysis of the purified extracts.

EXPERIMENTAL

Chemical standards

Samples of acephate and methamidophos (purity > 98%) were provided, as a free gift, by Chevron (Richmond, CA, USA). Stock solutions of the standards were made accurately in ethyl acetate at 100 μg/ml and stored at 4°C in tightly sealed volumetric flasks. Dilute solutions at 0.10, 1.0 and 5.0 μg/ml in ethyl acetate were made from the standards in amber coloured volumetric flasks and stored similarly at 4°C. These dilute solutions were used for spiking samples and for the preparation of analytical standards to calibrate the GC.

Reagents

Ethyl acetate was pesticide grade supplied by Caledon Labs., Georgetown, Canada. Nuchar SN charcoal, supplied by Fisher Scientific (Cat. No. C-177), Unionville, Canada, was acid washed [17] and used. Whatman CF-11 cellulose powder, supplied by Fisher was used as received. Sodium sulphate (reagent grade) and Pyrex glass wool were obtained from Fisher, cleaned [18] and used.

Apparatus

The gas chromatograph was a Hewlett-Packard (HP) (Analytical Division, Palo Alto, CA, USA) Model 5890A fitted with a nitrogen–phosphorus detector, HP 7673A autosampler and HP 3292A computerized integrator for area and height measurements of the peaks. The column was DB-17, 15 m × 530 μm with 1.0 μm film thickness. The other instrument parameters were: *Gases*: carrier (helium), 15 ml/min; split flow, 80 ml/min; septum purge, 5 ml/min; detector (hydrogen), 3.5 ml/min; air, 120 ml/min; make-up (helium), 15 ml/min. *Temperatures*: capillary injection port 200°C, detector 250°C. *Oven programme*: initial 60°C at 0.5 min, ramp rate 70°C/min; final 190°C at 8 min. *Injection mode*: splitless, inlet purge on at 0.5 min. *Injection volume*: 2 μl for standards and samples. *Retention time*: Methamidophos 5.3 min, acephate 7.4 min.

All other equipment used in the study was similar to the ones described earlier [13].

Bark, cone and seed samples

Bark and cone samples from untreated control spruce trees were sampled from the forest areas in Newfoundland, Canada and stored in plastic bags at 4°C until analysis. All seeds were removed by breaking the cones into small pieces and vigorously shaking them in plastic vials using a mechanical shaker.

Sample preparation and extraction

The bark and cone samples were cut into small pieces using clean hand pruners. Before fortification, the seeds, cones and bark samples were pulverized individually using a Sorvall Omni-mixer. Aliquots (5 g) of each sample were taken in glass jars, fortified with 0.10, 1.0 and 5.0 μg/g acephate and methamidophos, allowed to equilibrate for 30 min and homogenized (Polytron PT-40, speed 6000 rpm) thrice, using 75 ml of ethyl acetate each time. Each homogenate was filtered under suction through a tube funnel plugged with a small wad of glass wool at the bottom topped with sodium sulphate (25 g) to remove the moisture. The crude extract was flash evaporated to a small volume, transferred quantitatively to a graduated centrifuge tube and the volume adjusted to 5.0 ml by a Meyer N-Evap so that 1 g of the matrix was equal to 1 ml of the extract.

Microcolumn cleanup

The packing of the microcolumn (Pasteur pipette 15 cm × 0.8 cm I.D.) consisted of a wad of glass wool at the bottom, 1 cm of anhydrous Na_2SO_4 followed by 3 cm of a mixture containing 20% Nuchar charcoal and 80% cellulose and topped with 1

TABLE I

RECOVERIES OF ACEPHATE AND METHAMIDOPHOS RESIDUES FROM FORTIFIED BLACK SPRUCE SAMPLES

Substrate	Fortification level (μg/g)	No. of replicates	Acephate			Methamidophos		
			Recovery (%)	S.D. (±)	R.S.D. (%)	Recovery (%)	S.D. (±)	R.S.D. (%)
Bark	0.10	3	95.6	5.5	5.8	93.6	4.6	4.9
	1.00	3	89.7	3.2	3.6	94.9	5.0	5.3
	5.00	3	92.8	4.9	5.3	90.3	3.0	3.3
	0.10–5.00	9	92.7	4.8	5.2	92.9	4.2	4.5
Cones	0.10	3	91.9	4.3	4.7	91.4	3.8	4.2
	1.00	3	99.0	1.1	1.1	94.1	2.1	2.2
	5.00	3	92.0	5.4	5.9	95.8	2.8	2.9
	0.10–5.00	9	94.3	5.0	5.3	93.8	3.2	3.4
Seeds	0.10	3	91.4	5.9	6.5	88.8	3.6	4.1
	1.00	3	96.6	2.0	2.1	96.4	2.9	3.0
	5.00	3	95.2	4.3	4.5	90.6	3.8	4.2
	0.10–5.00	9	94.4	4.4	4.7	91.9	4.6	5.0

cm of Na_2SO_4. After thoroughly washing the column with 20 ml of ethyl acetate, 1.0 ml of the extract was transferred to the column, eluted with 10.0 ml of ethyl acetate and the eluate concentrated using the N-Evap to 1.0 ml for GC analysis. The sorbent size and elution volume necessary to desorb the analytes were determined by spiking the columns with standards, eluting and analyzing the eluates.

Gas chromatographic analysis

A volume of 2 μl of the final extract was injected into the gas chromatograph and concentrations of acephate and methamidophos were determined by comparing the area counts obtained by the integrator with those obtained by injections of analytical standard solutions. Control samples and reagent blanks did not give any interference peaks corresponding to the analytes.

RESULTS AND DISCUSSION

The solvent for sample extraction was chosen after several options (acetonitrile [11,14], acetone [6], methanol and dichloromethane [15]) were tested. Similarly, the use of different adsorbents (alumina, silica and Florisil) in microcolumn cleanups and eluting solvents and solvent mixtures [3,8,9] were tested. The resultant chromatograms after column cleanups were unsatisfactory due to high background noise, interference peaks and late eluting peaks. Best results were obtained using ethyl acetate as extracting solvent and Nuchar–cellulose microcolumns for cleanup with ethyl acetate as eluent. Occasionally, the excessive amount of lipids in the concentrated crude extracts of spruce seeds, caused the column capacity to exceed altering the elution pattern. In such cases, the crude extracts equivalent to < 1.0 g of the matrix was used or a liquid–liquid (hexane–acetonitrile) partition [8,11] step was introduced to remove the waxy impurities prior to column cleanup.

Table I shows the recovery data of fortified bark, cone and seed samples. The mean recoveries from the three matrixes at three fortification (0.10, 1.0 and 5.0 μg/g) levels were within the range of 88.8–99.0% with overall relative standard deviations (R.S.D.s) of 1.1–6.5%. Considering the complexity of the matrixes, the recovery of the two analytes were quantitative and the low standard deviations (S.D.s) and R.S.D.s indicate that the method is precise.

Using the final extraction volume of 5.0 ml, the limit of detection (LOD) [19] (3 × S.D. of blank

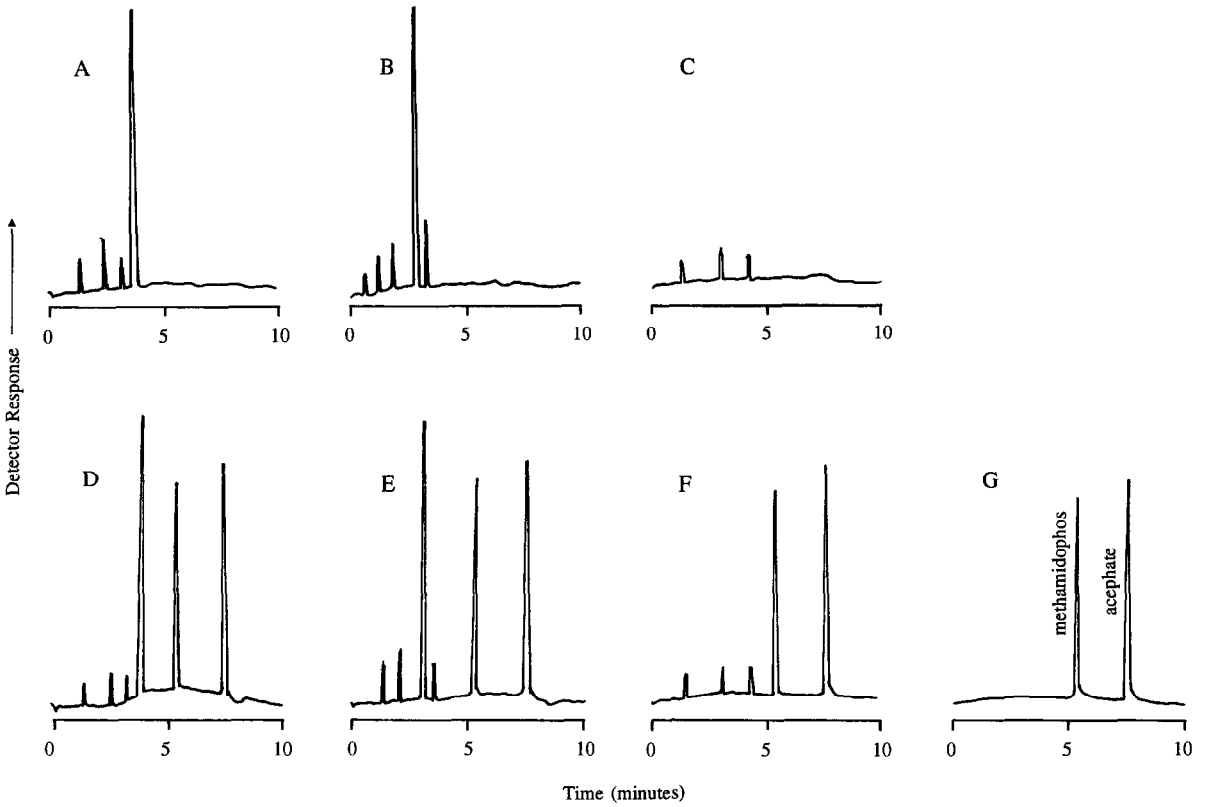

Fig. 1. Typical GC chromatograms of fortified black spruce samples: (A) bark control; (B) seeds control; (C) cones control; (D) bark fortified at 1.0 μg/g; (E) seeds fortified at 1.0 μg/g; (F) cones fortified at 1.0 μg/g; (G) acephate and methamidophos mixed standard solution (2 ng each) at GC conditions used in analysis of black spruce samples (integrator attenuation 2^9 at time 0 min; 2^7 at time 6.5 min).

response) for the three matrixes was 3 ng/g. The limit of quantification (LOQ) (10 × S.D. of blank response) was 10 ng/g. The sensitivity may be improved by using a smaller final extraction volume.

Typical chromatograms obtained for a mixed standard of acephate and methamidophos, unfortified blank samples of bark, cone and seed and the three of them each fortified separately with 1.0 ppm of the mixed standard are given in Fig. 1. The chromatographic peaks are sharp and well resolved with no chromatographic interferences in the vicinity of the retention times of methamidophos (5.3 min) and acephate (7.4 min). The total chromatographic analysis time amounted to 10 min and the total analysis time on average is less than 1.2 h/sample.

The method developed provides the basis for a sensitive, rapid and inexpensive method for the analysis of acephate and methamidophos in conifer bark, cone and seed samples. The high recoveries and low R.S.D.s indicate that the method is applicable to other forestry matrixes.

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