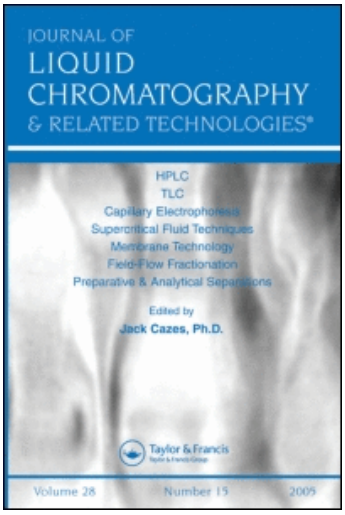

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ANALYSIS OF PHOSMET AND AZINPHOS-METHYL IN APPLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatography (HPLC) method has been developed to analyze two organophosphate insecticides (phosmet and azinphos-methyl) in apples. The procedure includes a novel extraction whereby whole apples are sonicated for 2 min in 100 ml of MeOH to remove the pesticides. Reversed-phase HPLC separation was accomplished with an Ultremex C18 column and acetonitrile:methanol:water as the eluent. Detection was at 224 nm for phosmet and 300 nm for azinphos-methyl. For both pesticides the limit of detection was 0.5 ppb and the linearity was from 1 to 405 ng injected. Average recoveries were 80% for phosmet and 86% for azinphos-methyl. Thirteen apple varieties comprising 240 apples were analyzed from supermarkets and roadside stands for phosmet (amount found ranged from none detected to 1233 ppb) and azinphos-methyl (amount found ranged from none detected to 388 ppb). Confirmation of phosmet and azinphos-methyl was made by UV spectral scans.

INTRODUCTION

Phosmet (0,0-dimethyl S-(N-phthalimidomethyl)phosphorodithioate) and azinphos-methyl (0,0-dimethyl S(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl)phosphorodithioate) are nonsystemic organophosphate insecticides that are used extensively on apples to control codling moth and other pests. Because of their widespread employment (1), possible health effects (2) and insufficient residue data (2), there is a need to monitor phosmet and azinphos-methyl in food commodities. Furthermore as the risk-benefit analysis process and toxicology become more sophisticated, there will be a need to know exact residue levels in food.

Previous analytical methods for these 2 insecticides have been primarily gas chromatography and gas chromatography/mass spectroscopy (3-11). HPLC procedures are nonexistent for simultaneous analysis in fruits. There have been two HPLC methods developed for azinphos-methyl analysis in fruits (12,13). This paper describes a reversed-phase HPLC method for the simultaneous analysis of phosmet and azinphos-methyl in apples that is 50 to 100 times more sensitive than the previous procedures and uses a novel sonication wash technique for extraction.

EXPERIMENTAL

Materials and Chemicals

Apples were obtained from supermarkets and roadside stands located within a 100 mile radius of Bangor, ME during the fall of 1990 and spring of 1991.

Solvents were HPLC grade (VWR, Boston, MA 02101) except for the MeOH (ACS grade, Fisher Scientific, Fairlawn, NJ 35666) employed for the sonication. Azinphos-methyl and phosmet were obtained from the Environmental Protection Agency, Research Triangle Park, NC 27711.

Apparatus

The HPLC system consisted of a Waters Associates (Milford, MA 01757) 510 pump, a Valco pneumatic injector (VICI Instruments, Houston, TX 77255), a Hewlett Packard (Avondale, PA 19311) 1040A Photodiode Array detector, Waters Associates 490 UV-VIS detector, and a Houston Instruments (Austin, TX) dual pen recorder.

Chromatography

An Ultremex 3 ODS column (stainless steel, 15 cm x 4.6 mm i.d.) (Phenomenex, Torrance, CA 90501) was employed for the separation along with a mobile phase of acetonitrile-methanol-water (40:16:44) at a flow-rate of 1.1 ml/min. When the HP 1040A detector was in line then 25 μl injections were made while 10 μl were injected when the Waters 490 detector was employed. Azinphos-methyl was monitored at 300 nm while phosmet was monitored at 224 nm with the sensitivity for both wavelengths set at 0.04 AUFS. Linearity (peak height and area) for each pesticide was from 1 ng to 405 ng injected.

Preparation of Standards

Ten mg each of azinphos-methyl and phosmet were weighed into a 50 ml volumetric flask and brought to volume with acetonitrile. Aliquots of 10, 30, 90, 270, and 810 μl were removed and placed into separate 10 ml volumetric flasks. Each flask was brought to volume with 50:50 acetonitrile-water.

Extraction Procedure

The apple was weighed and then placed into a 600 to 800 ml glass beaker. One hundred ml of MeOH were added and the apple was sonicated in a Branson 2200 ultrasonic bath (Danbury, CT 06810) for 2 min making sure that the entire surface came in contact with the MeOH. The MeOH was decanted into a 250 ml round bottom flask and rotary evaporated at 35°C. The residue was dissolved in

5 ml of methyl tert-butyl ether (MTBE) and transferred to a scintillation vial before drying under nitrogen. To this residue 1 ml of 50:50 acetonitrile:water was added and the mixture was sonicated and filtered through a 0.45 μ nylon syringe filter (Gelman Corp., Ann Arbor, MI 68173). Before injecting, samples were usually diluted 1:5 with 50:50 acetonitrile-water depending upon the pesticide concentration on the apples.

RESULTS AND DISCUSSION

It was observed that phosmet is not stable in MeOH and especially a MeOH:water mixture. Degradation begins after a couple of hours so it is important that the sonicated apples are evaporated immediately to prevent degradation. Furthermore, the samples and standards are dissolved in acetonitrile:water to stop degradation. Similar results were observed by Holland and McGhie (3) using MeOH on kiwifruit. Although Holland and McGhie observed that it was an acidic MeOH solution that caused 7% degradation of phosmet over a 24 hr period, our observations indicated that all MeOH caused breakdown of phosmet.

Typical HPLC chromatograms of apple sonications are shown in Figures 1 (phosmet) and 2 (azinphos-methyl). Chromatographic time is rapid with 6.8 min for azinphos-methyl and 7.2 min for phosmet. As for peak confirmation, UV spectra from 190 to 350 nm were taken for each pesticide peak at the up slope, pinnacle and down slope using a photodiode array system. From these spectra, it was learned that the best wavelength for azinphos-methyl analysis was 300 nm even though 224 nm was the most sensitive wavelength. The reason being was that captan co-elutes with azinphos-methyl. Since captan does not absorb at 300 nm and azinphos-methyl has another UV maxima at 300 nm, then 300 nm was chosen for azinphos-methyl quantification. As for phosmet, all 3 spectra for each apple were the same as the standard. Thus the UV max at 224 nm was employed for its determination.

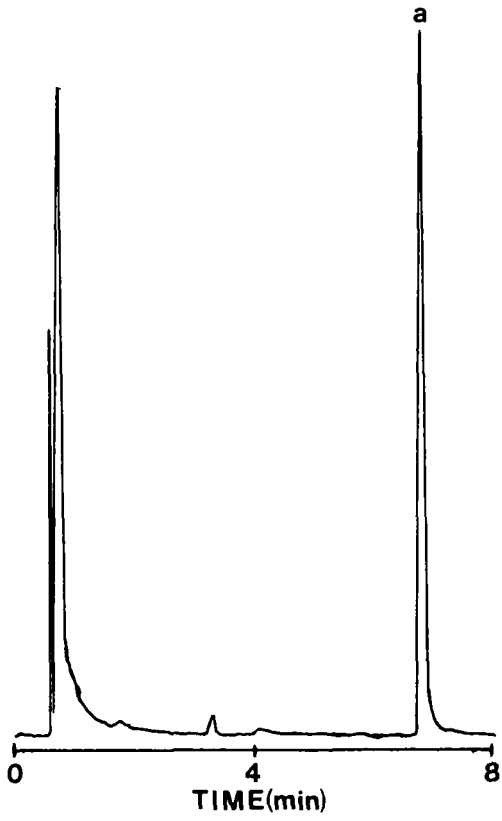

Figure 1. Typical chromatogram of phosmet in apples. Peak a = phosmet. Conditions given in text.

Because azinphos-methyl and phosmet are nonsystemic insecticides, a novel extraction procedure was tried on apples. Instead of the normal blender extraction, which requires samples to be extensively cleaned-up to even obtain quantitation levels at 50 to 100 ppb, the whole apple was sonicated in 100 ml of MeOH for 2 min. This sonication extraction was rapid and allowed for greater sensitivity because of the elimination of any co-eluting substances.

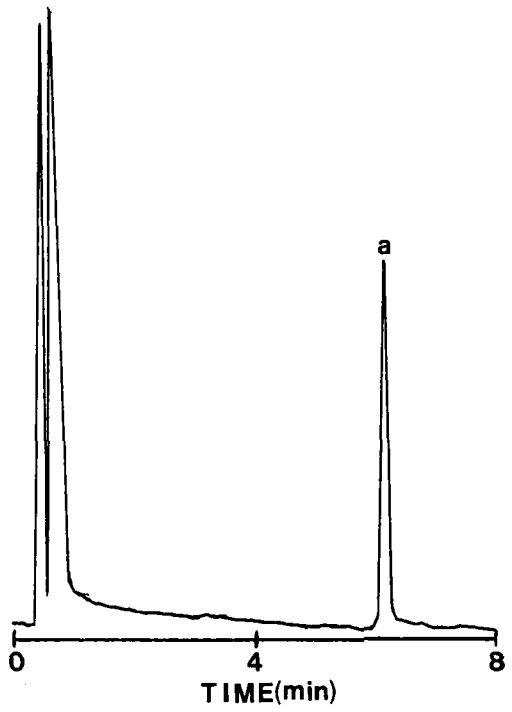

Figure 2. Typical chromatogram of azinphos-methyl in apples. Peak a = azinphos-methyl. Conditions given in text.

Since the apples were extracted by washing, it was difficult to test for recovery by spiking. Therefore, actual field samples were used. Apples were sonicated for 2 min then peeled. The peel was extracted for any remaining pesticide residue by the Luke (14) extraction procedure. Also the flesh was analyzed for pesticide residues using the Luke (14) method. The results are given in Tables 1 and 2. Twenty-four apples consisting of 5 different varieties were used for the phosmet sonication recovery study. Percent recoveries ranged from 56 to 100 with the average being 80 (Table 2). The percent coefficient of variation (% CV) was 20.9 which was excellent considering the sensitivity of this method.

TABLE 1.
Recovery of Phosmet in Field Apples

Apple Variety	----------μg Phosmet Found---------- Sonication	Acetone Extract Peel	Flesh	% Recovery from Sonication
McIntosh 1	8.3	ND	ND	100
McIntosh 2	1.8	0.6	ND	74
McIntosh 3	8.9	7.0	0.4	56
McIntosh 4	3.1	0.2	ND	90
McIntosh 5	1.5	ND	ND	100
Red Delicious 1	8.2	ND	0.4	96
Red Delicious 2	2.5	ND	ND	100
Red Delicious 3	2.0	1.0	ND	69
Red Delicious 4	8.6	0.8	0.2	85
Red Delicious 5	4.2	2.8	ND	60
Red Delicious 6	5.7	4.6	ND	56
Red Delicious 7	0.5	ND	ND	100
Red Delicious 8	0.5	ND	ND	100
Nova EZ Grow 1	4.1	2.4	ND	63
Nova EZ Grow 2	5.0	1.0	0.6	78
Nova EZ Grow 3	2.9	0.8	0.4	71
Nova EZ Grow 4	3.6	0.6	ND	87
Nova EZ Grow 5	5.5	3.4	ND	62
Cortland 1	1.1	0.4	ND	93
Cortland 2	1.5	1.0	ND	70
Cortland 3	1.5	ND	ND	61
Cortland 4	0.8	ND	ND	100
Cortland 5	1.1	0.6	0.2	59
Wolf River 1	3.1	0.3	ND	91

Acetone extract of the peel and flesh was done by the Luke (14) method
ND = none detected at a detection limit of 0.05 μg total phosmet

TABLE 2.
Recovery of Azinphos-methyl in Field Apples

Apple Variety	----μg Azinphos-methyl Found---- Sonication	Acetone Extract Peel	Flesh	% Recovery from Sonication
Sweet apple 1	1.7	ND	ND	100
Sweet apple 2	2.6	ND	ND	100
McIntosh 1	4.1	1.0	ND	80
McIntosh 2	1.4	0.3	ND	82
McIntosh 3	2.0	1.5	ND	57
Cortland	18.8	0.4	ND	98

Acetone extract of the peel and flesh was done by the Luke (14) method
ND = none detected at a detection limit of 0.05 μg total azinphos-methyl

Azinphos-methyl results are given in Table 2. Since phosmet and azinphos-methyl have similar chemical structures only 6 apples (3 different varieties) were used for this recovery study. The average percent recovery was 86 with a % CV of 19.4. It should be noted that care must be taken when sonicating the apple to make sure that all the skin comes in contact with the MeOH. Also apples that contain synthetic wax cannot be extracted effectively by MeOH sonication for these pesticides.

To determine the effect of evaporation on phosmet and azinphos-methyl recovery, an experiment was designed whereby organic apples were sonicated and then spiked with the pesticides at 100 ppb. It was found that 94% recoveries were obtained on three different samples for both pesticides. Thus, evaporation had little effect on the recoveries.

Also from Tables 1 and 2, it can be seen that if the apples are peeled at least 90% of phosmet and azinphos-methyl can be removed. This 90% removal factor was identical to what Love et al. (4) found with kiwifruit.

TABLE 3.
Phosmet and Azinphos-methyl Levels in Apples From Supermarkets and Roadside Stands by Sonication

Apple Variety	Number Analyzed	----------range in ppb---------- Azinphos-methyl	Phosmet
McIntosh	106	ND-387	ND-1233
Cortland	12	ND	2.2-209
Red Delicious	42	ND	0.7-989
Wolf River	8	ND	1.2-7.0
Red Paula	8	ND	4.6-126
Liberty	21	ND	0.7-181
Sweet Apple	8	5.3-267	ND-6.8
Nova EZ Grow	14	ND-4.1	41.6-397
Jona Free	10	ND	10.9-130
Macoon	1	20.6	3.9
Spencer	8	ND	5.4-28.2
Empire	1	ND	69.3
Rome	1	ND	98.3

Total of 240 apples from 10 different roadside stands and supermarkets
ND = none detected at a detection limit of 0.5 ppb

Two hundred and forty apples consisting of 13 varieties from several roadside stands and supermarkets were analyzed for their azinphos-methyl and phosmet content. The results are summarized in Table 3. Of these 240 samples 89% were positive for phosmet and 25% for azinphos-methyl. Sixty-nine percent of the positive phosmet apples contained less than 50 ppb phosmet while 80% of the positive azinphos-methyl apples contained less than 50 ppb azinphos-methyl. The highest concentration of phosmet found was 1233 ppb while for azinphos-methyl it was 388 ppb. Considering the tolerances are 2 ppm for azinphos-methyl and 10 ppm for phosmet, these apples are well below the tolerance levels.

At least 24 samples can be run through the complete method per day. The limiting step is the evaporation, but with the new type of spinning evaporators

being developed evaporation may not be limiting and as many as 50 samples could be done in a day. Since the tolerances for both pesticides on apples are in the ppm level, it may seem ridiculous to develop a method with such a low detection limit (0.5 ppb). However, there is an increased interest to know what the actual amounts of pesticides are in food so that the toxicologists and epidemiologists will have accurate data to design and do their studies. Presently, scientists dealing with the toxicity of pesticides in food assume that each pesticide used on food is present at its tolerance level and as can be seen (Table 3) this may not be the case. Thus wrong assumptions can lead to incorrect conclusions.

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