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Note

High-performance liquid chromatographic determination of azinphos methyl and azinphos methyl oxon in fruits and vegetables

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Azinphos methyl or Guthion (O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] phosphorodithioate) is currently registered for use on a variety of food crops in the U.S.A. The Environmental Protection Agency (EPA) tolerances range from 0.2 to 5 ppm. In sunlight or in water the compound decomposes to N-methylbenzazimide, benzazimide, anthranilic acid, azinphos methyl oxon, and other compounds[1,2]. Of all these degradation products, the oxygen analogue is the most toxic.

Present methods for the determination of residues of these compounds include colorimetric, fluorimetric, gas chromatographic (GC), and high-performance liquid chromatographic (HPLC) procedures. The colorimetric[3,4], fluorimetric[5], and GC[6–10] methods are time consuming due to lengthy extractions, column cleanup and/or derivatization steps. The HPLC procedures are not readily applicable to fruits and vegetables[11–14]. This paper presents an HPLC method for analysis of residues of azinphos methyl and its oxygen analogue in tomatoes, green beans, potatoes, apples and blueberries.

EXPERIMENTAL

Solvents and pesticides

HPLC-grade acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Azinphos methyl and the oxygen analogue were obtained from Mobay (Kansas City, MO, U.S.A.). All other pesticides were provided by the Environmental Protection Agency (Research Triangle Park, NC, U.S.A.) with purities ranging from 98 to 99.9%. HPLC-grade acetonitrile was used to dissolve all standards and to elute azinphos methyl and the oxygen analogue from the C_{18} Sep-Pak (Waters Assoc., Milford, MA, U.S.A.). HPLC-grade methanol and water were employed to activate the Sep-Pak. Reagent-grade methanol was the extracting solvent and HPLC-grade methylene chloride was used in the partitioning step.

Apparatus

The HPLC system consisted of a Waters Assoc. 6000A pump, a U6K injector, and a differential refractometer along with a Schoeffel (Westwood, NJ, U.S.A.) variable-wavelength UV detector and a Houston Instruments (Austin, TX, U.S.A.)

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dual-pen recorder. The C_{18} μBondapak column (Waters Assoc.) was 30 cm × 4 mm I.D., packed with a 10-μm packing. Operating conditions were: mobile phase, acetonitrile–water (50:50); flow-rate, 1.3 ml/min; column temperature, ambient; wavelength, 224 nm; attenuation, 0.04 a.u.f.s.; and chart speed, 0.4 in./min.

Preparation of standard

Two standard solutions, each containing both compounds, were prepared by serial dilution, one with a concentration of 80 μg/ml and the other with a concentration of 800 ng/ml. For samples containing 100–500 ppb* of the compounds, the standard curve was prepared by injecting 50 μl of a 0.08, 0.16, 0.24, and 0.32 μg/ml solution while for samples in the 500 ppb–1 ppm range, 10 μl of a 0.4, 0.8, 1.2, 1.6 and 2.0 μg/ml solution were injected. For samples with concentrations greater than 1 ppm, 5 μl of a 1.6, 3.2, 5.12 and 10.24 μg/ml solution were injected. Since peak height *vs.* concentration of each compound was linear, peak height was used to calculate the concentration.

Preparation of sample

A 50-g sample was weighed into a 1-quart jar-size Waring blender and extracted for 10 min with 100 ml of methanol. The extract was filtered through a Büchner funnel with suction, and the filtrate was concentrated to *ca.* 15–20 ml using a rotary evaporator set at a temperature of 45°C. Then the concentrate was poured into a separatory funnel containing 130 ml of water, to which 50 ml of methylene chloride were added. This step was repeated with 30 ml of methylene chloride. The methylene chloride fractions were combined, and evaporated to dryness on a rotary evaporator set at 45°C. Acetonitrile was used to dissolve the sample and bring it to volume in a 50-ml volumetric flask. The sample was cleaned up by passing 4 ml of the sample through an activated C_{18} Sep-Pak. The first 2 ml of the sample were discarded, while the second 2 ml were saved. The C_{18} Sep-Pak was activated by first passing 2 ml of methanol through it followed by 5 ml of water.

High-performance liquid chromatography

Depending on the concentrations of the compound in the sample, 5–50 μl was injected. The concentration of the unknown samples was determined from the standard curve.

RESULTS AND DISCUSSION

The chromatograms of the separation of azinphos methyl and azinphos methyl oxon in potatoes, beans, tomatoes, apples, and blueberries spiked at 0.16 ppm are shown in Fig. 1. Retention times for the two compounds are 4.05 cm for azinphos methyl oxon and 8.9 cm for azinphos methyl. The azinphos methyl peak is well resolved in all commodities, but the azinphos methyl oxon peak is not. Although baseline separation is not achieved in any of the products spiked at the 0.16-ppm level, it is possible to quantitate green beans, potatoes and apples at that level by drawing the baseline diagonally. The reproducibility for quantitation at that level in

* Throughout this article, the American billion (10^9) is meant.

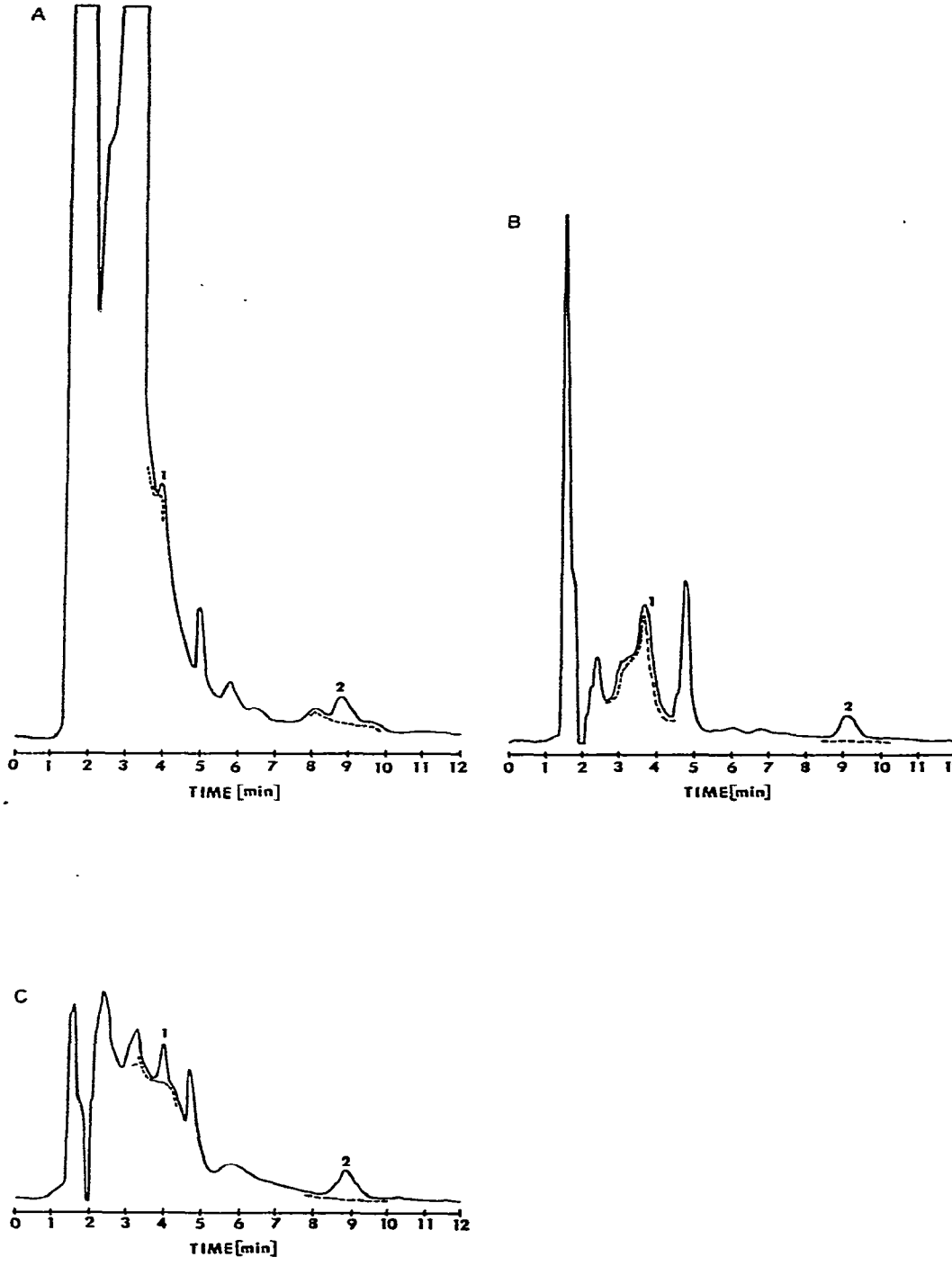
A
1
2
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME [min]
B
1
2
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME[min]
C
1
2
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME[min]

Fig. 1.

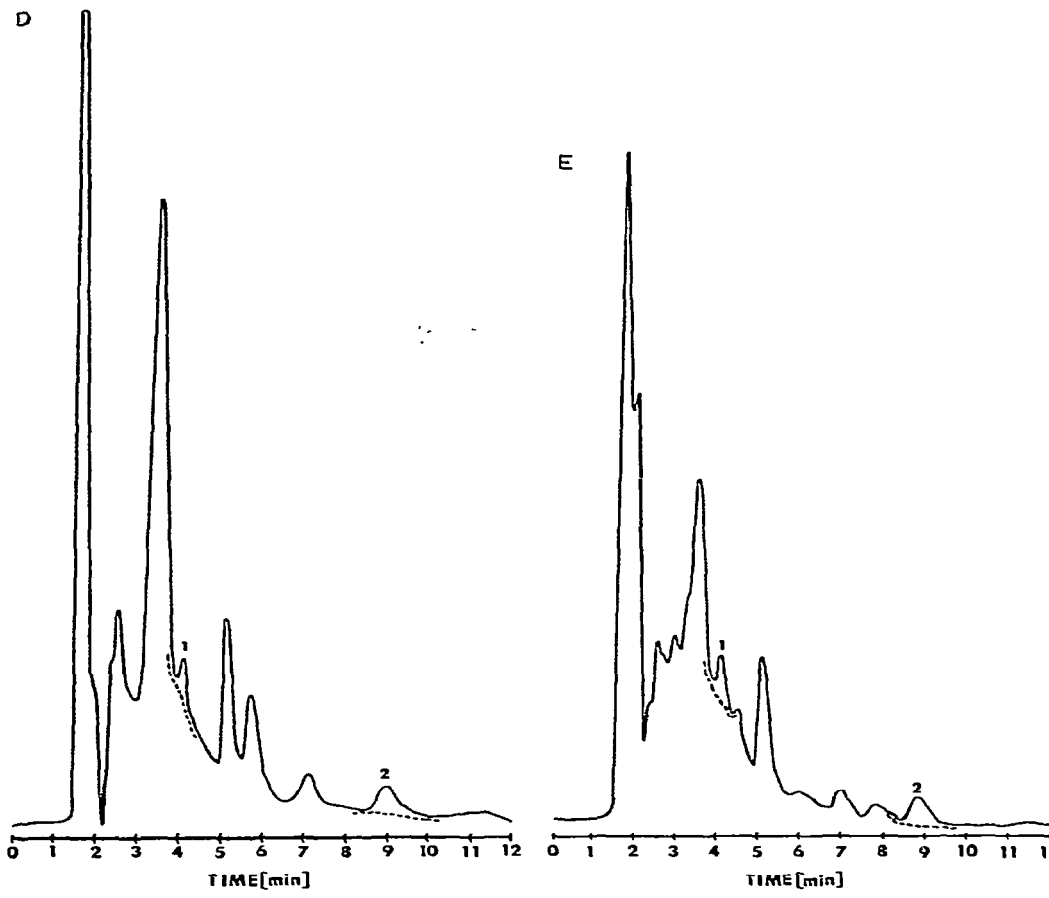

Fig. 1. Liquid chromatograms of azinphos methyl oxon (1) and azinphos methyl (2) spiked at 0.16 ppm in blueberry (A), tomato (B), green beans (C), apple (D), and potato (E). Chromatographic conditions are described in the text. Dashed lines stand for background due to the sample blank.

those three foods is good for a residue level method (Table I). At the higher spiking concentrations, the oxygen analogue, as is the azinphos methyl, is well resolved with no interferences (Fig. 2). Total analysis time, including sample preparation, is approximately 45 min.

The extraction efficiency and reproducibility of the method were determined by preparing three samples on each of two different days at each of the spiking levels. All samples were spiked at one or more levels below and at one level above the tolerance levels for that particular food crop as established by the EPA. Results of the recovery study are shown in Table I. Reproducibility and extraction efficiency were good with recoveries ranging from 75.2 to 105% and coefficients of variation from 2.4 to 15% with only one coefficient above 8.9%.

To determine the reproducibility of using such small peak heights for quantification, six consecutive 50-μl injections of standards for the 0.16-ppm spiking level were made. The coefficients of variation were 1.51 and 3.87% for azinphos methyl

TABLE I

RECOVERY OF AZINPHOS METHYL AND AZINPHOS METHYL OXON ADDED TO FIVE FOODS

Values are given as mean percentage recovery ± coefficient of variation. A = Azinphos methyl; AO = azinphos methyl oxon.

Spiking level	*Potatoes*		*Apples*		*Blueberries*		*Green beans*		*Tomatoes*	
	A	*AO*	*A*	*AO*	*A*	*AO*	*A*	*AO*	*A*	*AO*
0.16 ppm	97.3 ± 8.2	84.0 ± 8.2	95.2 ± 6.2	88.5 ± 15.0	84.2 ± 8.6	−*	102.5 ± 5.9	100.2 ± 6.3	102.7 ± 4.3	−*
0.80 ppm	96.3 ± 8.9	97.8 ± 2.4	90.0 ± 4.3	105.0 ± 4.1	75.2 ± 6.3	93.3 ± 2.4	89.5 ± 4.5	94.8 ± 6.2	94.3 ± 3.5	92.8 ± 8.4
5.12 ppm			90.4 ± 8.1	93.8 ± 3.3			89.2 ± 3.1	92.1 ± 3.2	88.5 ± 2.5	97.4 ± 3.9
10.24 ppm					81.8 ± 4.4	85.2 ± 4.3				

* Interfering peak; azinphos methyl oxon could not be detected.

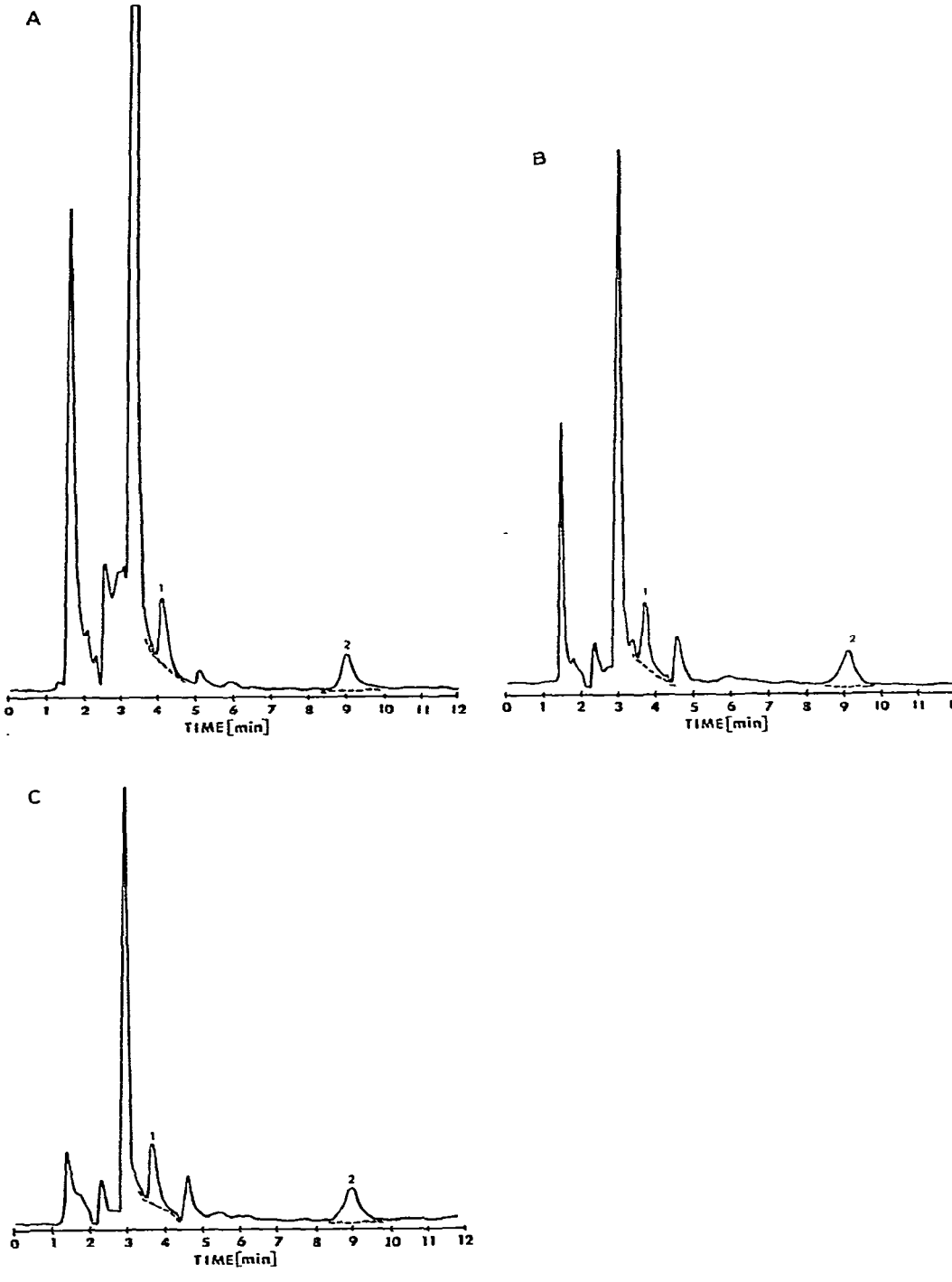

Fig. 2.

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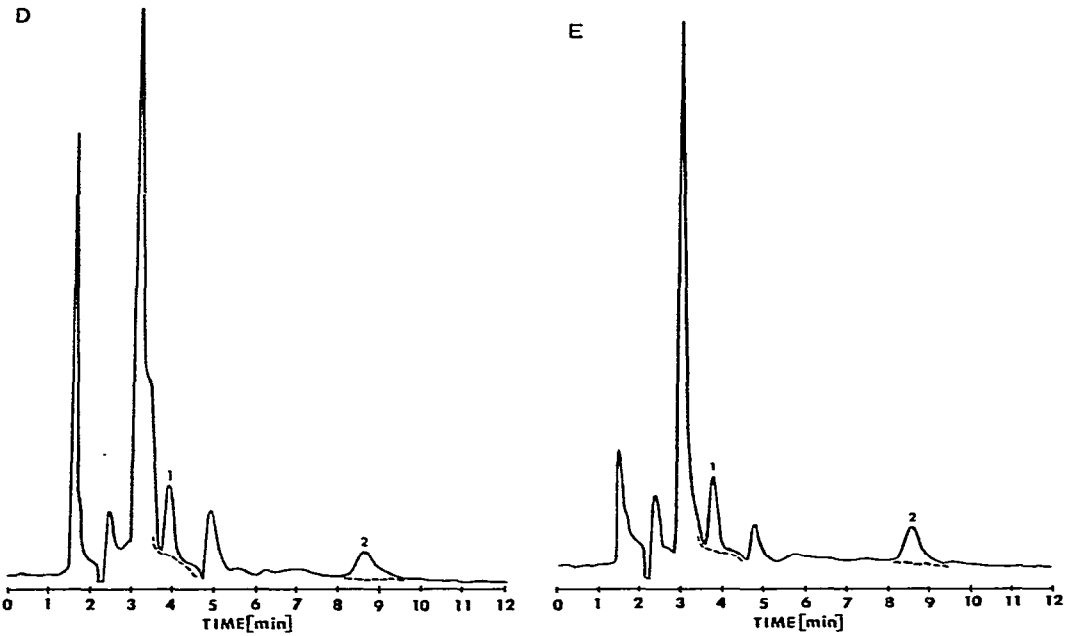

Fig. 2. Liquid chromatograms of azinphos methyl oxon (1) and azinphos methyl (2) spiked at 0.8 ppm in blueberry (A), tomato (B), green beans (C), apple (D), and potato (E). Chromatographic conditions are described in the text. Dashed lines stand for background due to the sample blank.

and the oxygen analogue, respectively. It is probable that peak area determination by electronic integration would decrease the variation.

Limits of detection for the method were determined to be 0.16 ppm for azinphos methyl in all the food products analyzed and 0.4 ppm for the oxygen analogue. The value of 0.4 ppm was deduced by looking at the chromatograms for the 0.8-ppm analyses (Fig. 2). A peak height half of those shown in the chromatograms could be measured consistently. Based on this, reproducible quantitation at the 0.4-ppm level would be possible.

To test the effect of the volume injected on the peak height of the compounds, three standard curves were used in this study. It was determined that the injection volume had no effect on peak height. Based on this finding, it would be possible to prepare only one standard curve to analyze samples from 0.16 ppm to 10 ppm. The most useful standard curve would be prepared with the concentrations for the second standard curve (0.50–1 ppm) given in the Experimental section.

Thirty-nine commonly used pesticides were injected to determine if they cochromatographed with azinphos methyl or the oxygen analogue. None of the thirty-nine interfered with the oxygen analogue, but three —captan, promecarb, and famphur— could interfere with azinphos methyl if they were present in the samples at certain concentrations. If the presence of any of those three compounds is expected, a higher percentage of water could be added to the mobile phase to achieve separation between one of those three compounds and azinphos methyl. Table II lists the pesticides and the oxygen analogue and the ratio of their retention times to that of azinphos methyl.

TABLE II

RETENTION TIMES OF PESTICIDES AND AZINPHOS METHYL OXON RELATIVE TO AZINPHOS METHYL

Pesticide	*Retention time relative to azinphos methyl*	*Pesticide*	*Retention time relative to azinphos methyl*
Azinphos methyl	1.00	Methyl parathion	1.23
Azinphos methyl oxon	0.45	Monuron	0.53
2,4-D	0.20	1-Naphthol	0.69
2,4,5-T	0.20	PCNB	3.65
Amitrole	0.29	PCP	0.23
Atrazine	0.67	Picloram	0.18
Benomyl	1.39	Pirimicarb	0.63
Captan*	0.99	Pirimiphos-methyl	2.54
Carbaryl	0.64	Promecarb*	0.98
Carbofuran	0.60	Prometon	0.75
Chlorpropham	1.22	Prometryne	1.20
Chlorpyrifos	4.76	Propanil	0.86
Coumaphos	2.52	Propazine	0.87
Dicamba	0.18	Propham	0.80
Diuron	0.71	Ronnel	3.68
Ethyl parathion	2.10	Simazine	0.55
Famphur*	0.97	Terbacil	0.52
Fenitrothion	1.49	Thiram	0.71
Fenthion	2.03	Trifluralin	0.17
Folpet	1.37	Ziram	1.17
Methomyl	0.38		

* Compounds that could interfere with azinphos methyl.

The C_{18} Sep-Paks used in this study to clean up the plant material were very effective. If Sep-Paks are not available, minicolumns could be packed with C_{18} packing to achieve the same cleanup effect. All samples were filtered through a 0.45-μm organic filter (Waters Assoc.) before being injected.

This method is a fast and reliable means of determining azinphos methyl and azinphos methyl oxon in foods. In addition, it is sensitive to well below the tolerance levels for the pesticide in the fruits and vegetables used in this study.

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