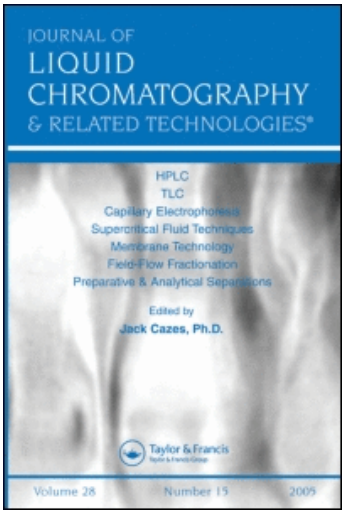

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High-Performance Liquid Chromatographic Determination of Trace Quantities of Azinphos Methyl and Azinphos Methyl Oxon in Various Water Sources by Direct Injection and Trace Enrichment

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To cite this Article Bushway, R. J.(1982) 'High-Performance Liquid Chromatographic Determination of Trace Quantities of Azinphos Methyl and Azinphos Methyl Oxon in Various Water Sources by Direct Injection and Trace Enrichment', Journal of Liquid Chromatography & Related Technologies, 5: 1, 49 — 62
To link to this Article: DOI: 10.1080/01483918208068818
URL: http://dx.doi.org/10.1080/01483918208068818

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(1), 49-62 (1982)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TRACE QUANTITIES OF AZINPHOS METHYL AND AZINPHOS METHYL OXON IN VARIOUS WATER SOURCES BY DIRECT INJECTION AND TRACE ENRICHMENT

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method is described for the determination of trace amounts of the organophosphate insecticide, azinphos methyl and its degradation product, azinphos methyl oxon, by direct injection and by trace enrichment. The compounds were analyzed on a uBondapak C_{18} column with UV detection at 224 nm. The mobile phase for the analysis was acetonitrile-water (50:50) at a flow rate of 1.3 ml/min. Ten minutes were required for the chromatographic analysis. Water from three sources, public water supply, stream, and ocean was analyzed for azinphos methyl and azinphos methyl oxon at concentrations as low as 11.9 and 11.3 ppb, respectively, without a clean-up, concentration or derivatization step. Azinphos methyl was quantitated at 0.29 ppb and azinphos methly oxon at 0.27 ppb by employing a concentration step involving a C_{18} Sep-Pak cartridge. The coefficients of variation for all determinations ranged from 0.77 to 9.06%. Peak heights were used for quantitation. Several other pesticides have been shown not to interfere with the analysis of either compound.

INTRODUCTION

Azinphos methyl (0,0-Dimethyl S- 4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate is an

organophosphate pesticide that is extensively used because of its effectiveness on sucking and chewing insects. Toxicity studies performed on whole animals thus far have demonstrated that azinphos methyl is one of the more toxic of the organophosphate pesticides (1,2). Also azinphos methyl is one of the more persistent organophosphates (3,4). In sunlight or in water the compound decomposes to N-methyl benzazimide, benzazimide, anthranilic acid, azinphos methyl oxon and other compounds (4,5). Of all these degradation products the oxygen analog is the most toxic. Because of its possible toxicity, persistence and wide use near water supplies, a rapid, accurate and sensitive method is needed to determine azinphos methyl and its degradation product azinphos methyl oxon in water.

Present methods for the determination of trace amounts of these compounds include colorimetric, fluorimetric, gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) procedures. The colorimetric (6,7) fluorimetric (8) and gas chromatographic (9-13) methods are time consuming due to lengthy extractions, column clean-up and/or derivatization steps. Futhermore except for the GC procedures these methods are nonspecific. Since azinphos methyl and azinphos methyl oxon are heat labile, analysis by GC is difficult. These disadvantages in the other methods can be circumvented by using HPLC. However, the HPLC procedures developed thus far are not applicable to water (14-18). This paper presents a rapid, accurate and sensitive HPLC method for the determination of azinphos methyl and azinphos methyl oxon in water at low ppb levels either by direct injection or trace enrichment using a C_{18} Sep-Pak cartridge.

EXPERIMENTAL

Solvents and Pesticides

Acetonitrile and water were of HPLC grade and were purchased from Fisher Scientific Co. (Pittsburgh,PA). Azinphos methyl and azinphos methyl oxon both with a purity of 98.9% were obtained from Mobay Chemical Co. (Kansas City,MO). All other pesticides were obtained from the Environmental Protection Agency (Research Triangle Park,NC) with purities ranging from 98 to 99.9%. Pesticide standards were dissolved in ACS grade methanol. HPLC grade acetonitrile was used to elute azinphos methyl and azinphos methyl oxon from the C_{18} Sep-Pak cartridge (Waters Assoc.,Milford,MA). ACS grade methanol and HPLC grade water were employed to activate the Sep-Pak.

Water Samples

Water samples were of three types--drinking, stream and salt water. The drinking water was obtained at the laboratory; the stream water was collected in Orono, ME and the salt water from the Atlantic Ocean in Sullivan, ME.

Standard Preparation

Stock solutions of azinphos methyl and azinphos methyl oxon were prepared at concentrations of 1.19 mg /ml and 1.13 mg/ml, respectively. One ml of each stock solution was added to a 100 ml volumetric flask and brought to volume with methanol. All solutions used for direct analysis and trace enrichment were prepared by appropriate dilutions of this 100 ml solution.

Apparatus

The HPLC system consisted of a Waters Assoc. 6000A pump, a U6K injector, a Schoeffel (Westwood,NJ) variable-wavelength UV detector and a Houston Instruments

(Austin,TX) dual pen recorder. The column (30 cm x 4 mm I.D.) was a μBondapak C_{18} (Waters Assoc.; particle size 10 μm). 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.

For trace enrichment studies a Fluid Metering (Oyster Bay,NY) pump (Model RP-SY) was employed. Connections at the outlet and inlet ends of the pump were made with Teflon tubing and a C_{18} Sep-Pak cartridge was connected to the outlet end. The flow rate was set at 22.2 ml/min.

Analytical Procedure

Direct analysis: Water samples as received and spiked were filtered (2 ml) through a 0.45 μm Millipore aqueous filter (Waters Assoc.) and injected (250 μl) directly into the HPLC system.

Trace enrichment: Water samples (250 ml) were spiked at a level of 0.29, 2.62, 5.24 and 10.48 ppb for azinphos methyl and 0.27, 2.49, 4.98 and 9.96 ppb for azinphos methyl oxon. For all spiking levels except 10.48 and 9.96 ppb (for which 100 ml were used) 250 ml of water were passed through an activated C_{18} Sep-Pak cartridge using the Fluid Metering pump. The Sep-Pak was activated by prewetting the cartridge with 4 ml of methanol followed by 5 ml of water. To elute the azinphos methyl and azinphos methyl oxon adsorbed on the packing, 2 ml of acetonitrile was passed through the cartridge; a 100 μl aliquot was injected into the HPLC system. When ocean water was analyzed by trace enrichment, the salt water trapped in the Sep-Pak was removed by passing 4 ml of HPLC grade water through the cartridge before the acetonitrile elution step.

RESULTS AND DISCUSSION

Results from the direct injection of three types of spiked water samples-- salt, stream and drinking-- are given in Table 1. Samples varied in concentration from 11 ppb to 90-95 ppb azinphos methyl and azinphos methyl oxon. All water samples were chromatographed without clean-up, concentration or derivatizing steps. Such a procedure is not only rapid and simple, but also is quite precise with coefficients of variation ranging from 0.98 to 6.11% for all types of water at each spiking level. Futhermore there are only two coefficients above 5% (Table 1). In order to obtain the most accurate results, the working standard for each compound should be prepared in a water matrix as near as possible in composition to the sample being analyzed. Also the standard should be prepared each day. Although the lowest concentration detected in this study by direct injection of water was 11 ppb for both compounds, it should be possible to detect and quantify 5.5 ppb of each (by injecting 500 ul) provided that the sample matrix is free of co-chromatographing substances. Typical chromatograms of stream water samples are shown in Figures 1 and 2. There are no interfering peaks. Even though the peak heights are small at the lower concentrations, the precision is not adversely affected Table 1).

Analysis time is average taking approximately 8 min before both compounds are eluted. A shorter analysis time probably would not be possible since azinphos methyl oxon elutes near interfering compounds under the present system.

Thirty four commonly used pesticides were injected to determine if they co-chromatographed with azinphos methyl or the oxygen analog (Table 2). None of the

TABLE 1

Direct Analysis of Azinphos Methyl and Azinphos Methyl Oxon

	Azinphos Methyl		Azinphos Methyl Oxon		
Water Source	Level Spiked (ppb)	CV (%)	Level Spiked (ppb)	CV (%)	Blank for Both
Salt	11.9	4.00	11.3	5.45	0
Salt	23.8	6.11	22.6	4.27	0
Salt	47.8	2.31	45.2	2.62	0
Salt	95.2	3.15	90.4	4.44	0
Stream	11.9	3.33	11.3	2.17	0
Stream	23.8	1.97	22.6	3.02	0
Stream	47.6	2.42	45.2	1.36	0
Stream	95.2	2.06	90.4	0.98	0
Drinking	11.9	4.59	11.3	3.83	0
Drinking	23.8	1.31	22.6	1.44	0
Drinking	47.8	2.47	45.2	2.17	0
Drinking	95.2	3.60	90.4	0.98	0

CV=Coefficient of variation

Each value represents the mean of six samples analyzed

TABLE 2

Retention Times of Pesticides and Azinphos Methyl Oxon Relative to Azinphos Methyl

Pesticide	Retention Time Relative To Azinphos Methyl Ratio
Azinphos Methyl	1.00
Azinphos Methyl Oxon	0.45
2,4-D	0.20
2,4,5-T	0.20
Amitrole	0.29
Atrazine	0.67
Benomyl	1.39
Captan*	0.99
Carbaryl	0.64
Carbofuran	0.60
Chlorpropham	1.22
Chlorpyrifos	4.76
Coumaphos	2.52
Dicamba	0.18
Diuron	0.71
Ethyl Parathion	2.10
Famphur*	0.97
Fenitrothion	1.49
Fenthion	2.03
Folpet	1.37
Methomyl	0.38
Methyl Parathion	1.23
Monuron	0.53
1-Naphthol	0.69
PCNB	3.65
PCP	0.23
Picloram	0.18
Pirimicarb	0.63
Pirimiphos-Methyl	2.54
Promecarb*	0.98
Prometon	0.75
Prometryne	1.20
Propanil	0.86
Propazine	0.87
Propham	0.80
Simazine	0.55

*compounds that could interfere with azinphos methyl

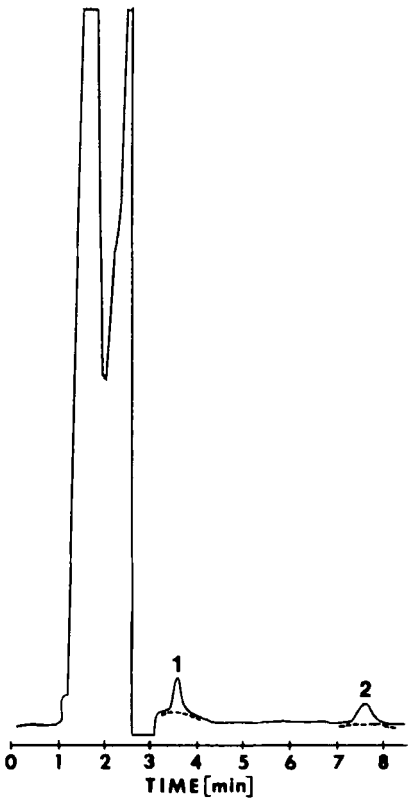

Figure 1. HPLC chromatogram of stream water spiked with azinphos methyl at 11.9 ppb and azinphos methyl oxon at 11.3 ppb: direct injection. Solvent system, acetonitrile -water (50:50); flow rate, 1.3 ml/min; detector sensitivity, 0.04 a.u.f.s.; wavelength, 224 nm; chart speed, 0.4 in/min; amount injected, 250 ul. Peaks: 1= azinphos methyl oxon; 2= azinphos methyl. Dashed lines stand for background due to the sample blank.

thirty four interfered with the oxygen analog, 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 these three compounds is expected, a higher percentage of water could be added to the mobile phase to

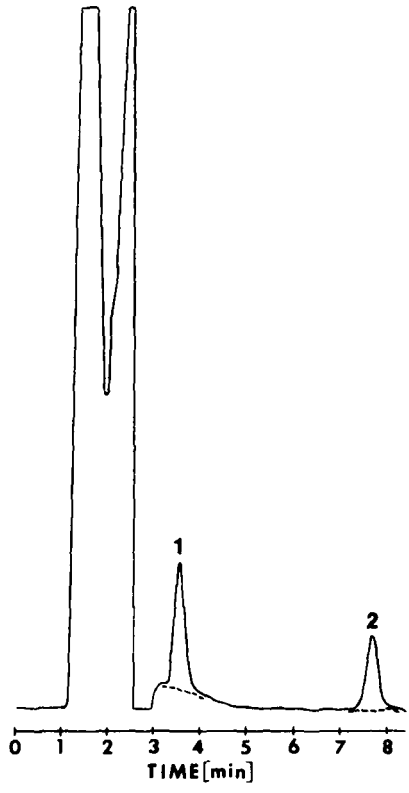

Figure 2. HPLC chromatogram of stream water spiked with azinphos methyl at 23.8 ppb and azinphos methyl oxon at 22.6 ppb: direct injection. Conditions the same as in Figure 1. Peaks: 1= azinphos methyl oxon; 2= azinphos methyl. Dashed lines stand for background due to the sample blank.

achieve separation between the different pesticides. A list of the thirty four pesticides and the oxygen analog of azinphos methyl along with the ratio of their retention times to that of azinphos methyl are given in Table 2.

In order to detect and quantify azinphos methyl and azinphos methyl oxon in water at concentrations lower than those possible by direct injection, trace

TABLE 3

C_{18} Sep-Pak Enrichment of Azinphos Methyl and Azinphos Methyl Oxon

	Azinphos Methyl			Azinphos Methyl Oxon		
Water Source	Amount (ppb)	Recovery (%)	CV (%)	Amount (ppb)	Recovery (%)	CV (%)
Salt	0.29	93	3.41	0.27	88	9.06
Salt	2.62	96	3.94	2.49	98	2.66
Salt	5.24	95	0.77	4.98	99	1.49
Salt	10.48	103	4.12	9.96	100	3.45
Stream	0.29	88	6.82	0.27	98	4.50
Stream	2.62	95	4.08	2.49	96	4.01
Stream	5.24	96	2.33	4.98	96	2.01
Stream	10.48	98	1.27	9.96	99	1.28
Drinking	0.29	98	4.49	0.27	100	3.83
Drinking	2.62	94	3.92	2.49	92	5.33
Drinking	5.24	96	1.02	4.98	98	0.87
Drinking	10.48	96	1.79	9.96	97	2.86

CV=Coefficient of variation

Each value represents the mean of six samples analyzed

enrichment using a C_{18} Sep-Pak cartridge was developed. The results are shown in Table 3. Water samples were spiked with azinphos methyl at a concentration range of 0.29 to 10.48 ppb and azinphos methyl oxon at a concentration range of 0.27 to 9.96 ppb. Recoveries ranged from 88 to 103% with coefficients of variation from 0.77 to 9.06%. Typical chromatograms of the trace enrichment of azinphos methyl and azinphos methyl oxon are shown in Figures 3 and 4. With the use of direct analysis and trace enrichment azinphos methyl and azinphos methyl oxon can be quantified in water from approximately 0.27 to 95 ppb.

A study was conducted to determine how many times a Sep-Pak cartridge could be reused. Under our conditions, each cartridge could be used three times. Obviously the number of times a cartridge could be used would have to be determined for each water source analyzed.

The detector response was linear for all concentrations employed. However, when performing trace enrichment determinations, the standard curve must be determined by using standards that are dissolved in 30 to 40 ml of HPLC grade water and passed through a C_{18} cartridge, followed by elution with 2 ml of acetonitrile. If the standards are not prepared in this manner, the concentrations of azinphos methyl and azinphos methyl oxon in the unknown samples will be incorrect, since the standards passed through the cartridge gave slightly higher peaks than those that were not. This discrepancy between peak heights was not due to contamination since the blank samples were clean.

CONCLUSIONS

The large extinction coefficients for azinphos methyl and azinphos methyl oxon at 224 nm and the relatively simple and pure matrix of water, permits the de-

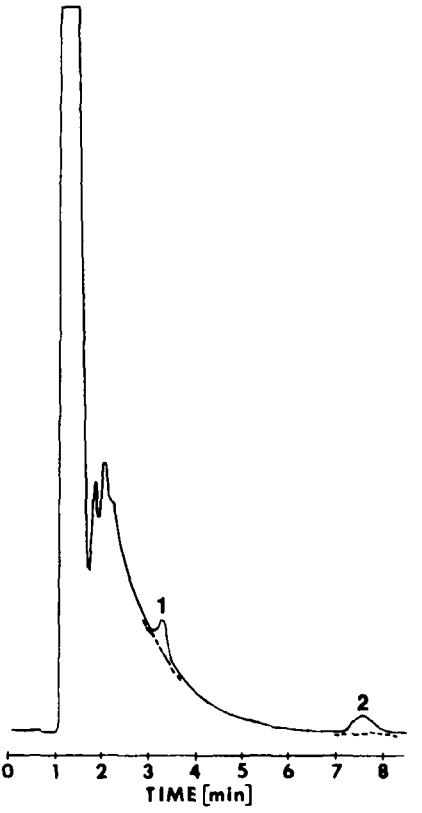

Figure 3. HPLC chromatogram of salt water spiked with azinphos methyl at 0.29 ppb and azinphos methyl oxon at 0.27 ppb: trace enrichment with C_{18} Sep-Pak. Amount injected, 100 µl; other conditions same as in Figure 1. Peaks: 1= azinphos methyl oxon; 2= azinphos methyl. Dashed lines stand for background due to the sample blank.

termination of these two compounds in water at low ppb levels by direct injection and/or trace enrichment. The method could also be used to analyze other organophosphate pesticides that have high extinction coefficients. This procedure is much faster and simpler than previously published methods for determining azinphos methyl and the oxon. It also offers the advantage of not using extracting solvents like methylene chloride.

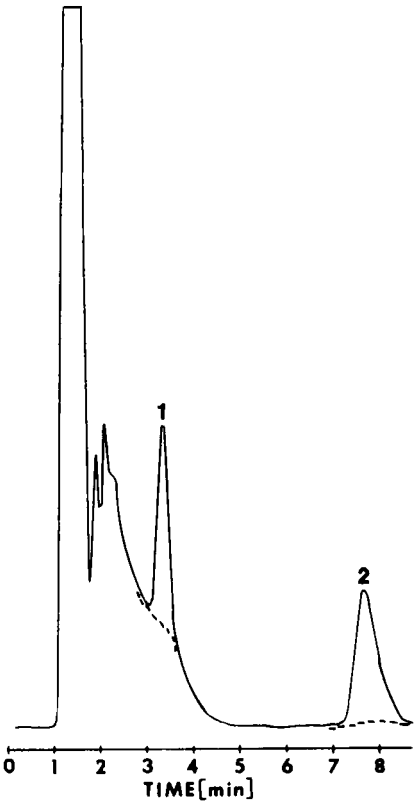

Figure 4. HPLC chromatogram of salt water spiked with azinphos methyl at 2.62 ppb and azinphos methyl oxon at 2.49 ppb: trace enrichment with C_{18} Sep-Pak. Amount injected, 100 ul; other conditions same as in Figure 1. Peaks: 1= azinphos methyl oxon; 2= azinphos methyl. Dashed lines stand for background due to the sample blank.

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