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Gas chromatographic determination of airborne residues of azinphosmethyl and azinphosmethyl-oxon by cool on-column injection

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Abstract

Gas chromatographic determination of azinphosmethyl and its thermally labile metabolite, azinphosmethyl-oxon, can be achieved with cool on-column injection and element-specific flame photometric detection. This paper presents a method of analysis for airborne residues of azinphosmethyl and azinphosmethyl-oxon in XAD-4 resin extracts using cool on-column injection. Calculated limits of quantitation for the analytical method are 8.1 ng/m^3 for azinphosmethyl and 10.2 ng/m^3 for azinphosmethyl-oxon. Average percentage recovery and percentage relative standard deviations for 10 replicates were $93\pm18\%$ for azinphosmethyl and $104\pm16\%$ for the oxon. This analytical method is rapid, robust and requires a minimum of gas chromatograph injection port maintenance. © 1997 Elsevier Science B.V.

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1. Introduction

Widespread use of organophosphate (OP) insecticides in agriculture, public health and residential pest control has prompted research into their environmental fate. Historically, researchers have been most interested in the OP dermal exposure of workers in agricultural settings. Exposure to azinphosmethyl (O,O-dimethyl S-{[4-oxo-1,2,3-benzotriazin 3(4H)-yl]methyl} phosphorodithioate) has been of particular concern due to its acute toxicity. Inhalation has often been overlooked as a component of agricultural worker exposure, but recently, interest in airborne pesticide residues as a component of exposure assessment has increased.

Azinphosmethyl (azm) and the bioactive analog azinphosmethyl-oxon (azm-o) are acetylcholinesterase inhibitors. Since dermal exposure to OPs and their environmental metabolite oxons has been suspected in citrus worker poisonings in California, analytical procedures that include OP oxon analogs must be pursued [1]. Thermal degradation of oxygen analogs of OP insecticides analyzed by GC has been reported [2]. HPLC–UV methods, which include the thermally labile azinphosmethyl-oxon, have been developed for dislodgeable foliar residues [3]. Using HPLC methodology avoids thermal degradation of the analyte that makes GC analysis difficult [4].

However, HPLC has several limitations when used to analyze airborne residues. The lack of commonplace, inexpensive and selective HPLC detectors can make analyte determination in air matrices difficult.

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Airborne pesticide residues have been determined using solid polymeric adsorbents such as XAD-4. XAD-4 is a macroreticular resin that is an efficient trapping medium for pesticides. Solid polymers allow the use of high flow-rate air samplers required for trace residue determination [5].

Raw XAD-4 requires extensive cleanup prior to use in air sampling. Pre-cleaned XAD-4 can retain impurities that are co-extracted with the pesticide residues, resulting in interferences that make HPLC–UV analyte determination difficult without further cleanup. However, OP pesticide residues may be determined directly in XAD-4 extracts by using selective GC detectors, such as flame photometric or nitrogen–phosphorous. UV determination is often less sensitive compared to selective GC detectors. Increased sample preparation time for additional cleanup of the XAD-4 co-extractants prior to HPLC–UV analysis can lead to lower sample throughput compared to GC methodology.

Previous work by this author has shown that azm-o undergoes sequential degradation in the hot split/splitless injection port of the GC. Fig. 1 shows thermal breakdown of azm-o in the hot split/splitless GC injection port. Fifty consecutive injections of composite azm and azm-o standards in solvent show reduced azm-o response. Stan and Goebel [6] and Stan and Muller [7] attribute OP pesticide losses in vaporizing injectors to thermal stress and liner adsorption of the analytes. Liner activation increases with each subsequent injection, thereby reducing analyte response over time. Injection port maintenance, which includes replacing the septum and glass inlet liner and cutting off the front end of the analytical or guard column, can restore response to satisfactory levels. As shown in Fig. 1, careful deactivation of the inlet liner with silanizing agents can eliminate azm degradation in the GC injection port. However, azm-o degradation is more severe, especially in environmental matrices.

Cool on-column injector technology allows some moderate thermally labile compounds to be quantitatively analyzed by GC. In cool on-column injection, the sample is deposited in the liquid phase directly inside the analytical or guard column. The injection port temperature, T_i, is held constant below the boiling point of the sample solvent. T_i is then rapidly increased to vaporize the solvent and analyte within

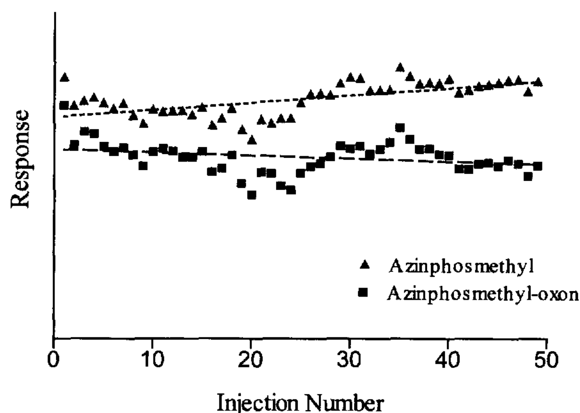

Fig. 1. Sequential (n=50) hot, splitless injections of 25 μg/ml standards of azinphosmethyl and azinphosmethyl-oxon in iso-octane. y-Axis shows relative NPD response calculated by area. A 1 m×0.25 mm ID deactivated guard column coupled with a capillary press-fit union to a 30 m×0.25 mm I.D. DB-5, 0.25-μm film thicknes, capillary column (J&W Scientific) was used for the separation. The carrier gas was He at 0.8 ml/min. Injection port and detector temperature were 190 and 300°C, respectively. The injection port liner was a 250-μl straight bore quartz glass model used without glass wool. The liner was carefully cleaned and deactivated with Sylon CT (Supelco) prior to use. The oven was programmed from an initial temperature of 90°C for 1 min to 265°C at 30°C/min. Injection volume was 2 μl.

the capillary column. Using the capillary column as the injection "port" avoids analyte contact with active surfaces, providing a more inert environment for sample vaporization [8].

2. Experimental

All solvents were Resi-Analyzed grade from J.T. Baker (Phillipsburg, NJ, USA). Analytical standards of azm, CAS 86-50-0, and azm-o were obtained from Bayer Agrochemicals Division (Kansas City, MO, USA). TFIA high-volume air samplers were used for residue collection (Staplex, Brooklyn, NY, USA). XAD-4 macroreticular resin was employed as the trapping medium for the samplers (Rohm and Haas, Philadelphia, PA, USA). A Turbovap II concentration workstation was employed for sample evaporation (Zymark, Hopkinton, MA, USA). Resin extractions were completed on a Model 75 wrist-action shaker (Burrel, Pittsburgh, PA, USA). Cameo 13F 13-mm,

0.45-μm syringe filters (MSI, Westboro, MA, USA) were used to prepare extracts for GC analysis.

Specific conditions for the application of Guthion and sample collection of azm and azm-o are described in Moate [9] and briefly summarized below. Airborne residue samples were collected in an apple orchard 1992–93. Guthion 35 WP was applied at the typical active ingredient use rate of 1.12 kg/hectare. Samples were collected during mid-day at regular intervals from 0 to 21 days post application. Air samplers were configured with 10.2-cm diameter cartridges containing approximately 140 ml of XAD-4 resin. Flow-rates for the air samplers were approximately 1 m^3/min. XAD-4 samples were transferred to storage bottles with excess solution of acetone–hexane (1:1) and placed in freezer storage at <−15°C until analysis.

Resin purification and sample extraction methods are based on modifications of the procedures described by Wehner et al. [5]. Samples were removed from freezer storage and quantitatively transferred to 500-ml Erlenmeyer flasks with 150 ml ethyl acetate. The flasks, with aluminum foil affixed to the top, were then placed on a wrist-action shaker. Samples were extracted for 1 h with agitation sufficient to completely wet the resin bed. Excess solvent was decanted and gravity filtered through Whatman No. 1 filter papers into Turbovap flasks. Extractions were repeated two additional times for 15 min with 75 ml ethyl acetate. Next, samples were concentrated on a Turbovap at 60°C to 1 ml. One-ml extracts were then solvent exchanged two times with 5 ml of 2,2,4-trimethylpentane (isooctane) and taken down to a final volume of 1.0 ml. Finally, the extracts were prepared for GC analysis by passing through a 0.45-μm syringe filter into 1.5-ml autosampler vials.

GC analyses were completed using a Varian 3600 Series GC equipped with an 8200 series autosampler and a septum equipped programmable injector (SPI) with cryofocusing (Varian Analytical, Sugarland, TX, USA). A 26-gauge syringe needle with a Varian megabore on-column glass insert were employed for sample introduction. A 1 m×0.53 mm I.D. deactivated fused-silica guard column (J&W Scientific, Folsom, CA, USA) was installed between the glass inlet liner and the analytical column. In the SPI injector, on-column injection was achieved with an inlet liner that is an extension of the guard column.

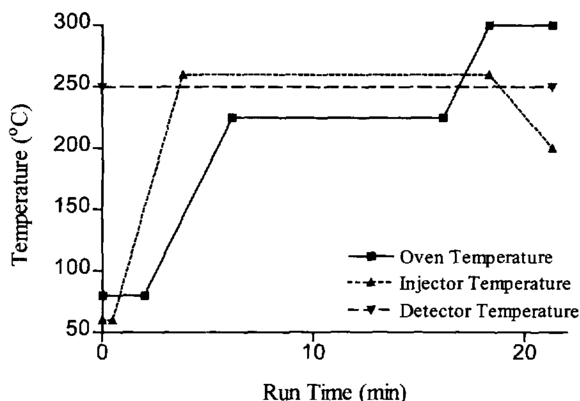

Fig. 2. Gas chromatograph temperature programming by zone. Cool, on-column injection with retention gap. Solvent focusing achieved on the analytical column.

The guard column was connected to a DB-5, 15 m×0.32 mm I.D., 1.5-μm film analytical column (J&W Scientific) with a capillary press-fit column union. Analyte response was determined with flame photometric detection (FPD) in phosphorous mode. A sapphire column cutting tool was used to make neat, uniform column cuts (Supelco, Bellefonte, PA, USA).

Grade 5.0 helium was used for the carrier gas at an initial flow-rate of 4.5 ml/min. This flow-rate corresponds to a linear velocity of 60 cm/s during peak elution. Flow-rates of hydrogen and medical grade air for the FPD were 150 and 160 ml/min, respectively. In addition, the detector required prepurified nitrogen as a sweep gas at 25 ml/min.

GC zone temperature programming is depicted in Fig. 2. The injector temperature ramp initiates before the oven temperature ramp, as shown. This technique refocuses the solvent at the head of the analytical column in order to reduce analyte band broadening. Sample injection volume was 5 μl, introduced at a rate of 1 μl/s.

3. Results

Sample extract concentrations were calculated using external standard calibration. A maximum of six calibration levels were used with composite standards of both azm and azm-o from 0.5 to 50 μg/ml in isooctane. External standards were injected

Table 1
Summary of quality control recovery data

Fortification (μg)	Azinphosmethyl	Azinphosmethyl-oxon
25 (n=13)	88±12	78±12
5.0 (n=9)	93±15	93±13
2.0 (n=10)	93±18	104±16
Overall	91±15	90±17

Values are given as mean percentage recovery±percentage relative standard deviation. Number of observations appear in parentheses.

in increasing order at the beginning of the run sequence. In addition, selected standards were reinjected at regular intervals during the sample run sequence to verify uniform detector response. Calibration curve linearity was >0.995 r^2. The airborne residue analytical procedures were validated using fortifications of purified XAD-4 resin in acetone–hexane (1:1). These quality control fortifications were prepared at three levels for both compounds. A summary of the quality control recovery data appears in Table 1.

Limits of quantitation (LOQs) for the airborne residue analytical method were determined at an approximate FPD signal-to-noise ratio of 10:1. Calculated LOQs for the analytical method are 8.1 ng/m^3 for azm and 10.2 ng/m^3 for azm-o. These values correspond to a 5-μl injection of a 2.0 μg/ml matrix fortification with a final sample volume of 1 ml. Precision at this fortification level, determined by the average percentage recovery and percentage relative standard deviation for 10 replicates, was 93±18% for azm and 104±16% for azm-o (see Table 1). Limits of detection were determined to be LOQ/5.

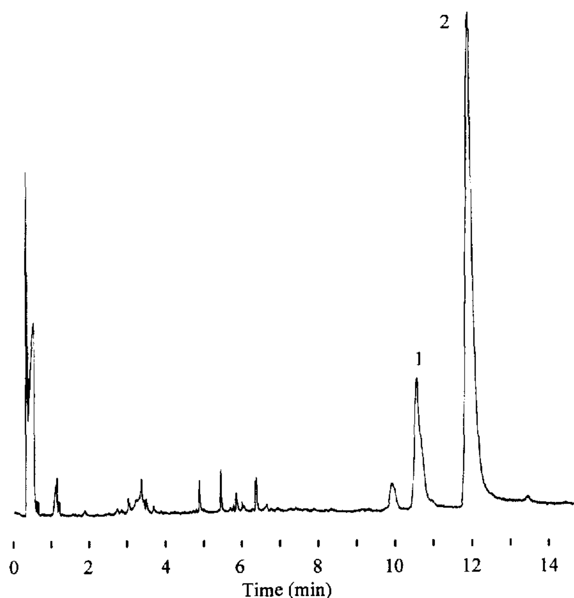

Fig. 3. Chromatogram of an XAD-4 resin extract, 3 days post application, 5-m orchard height. Azinphosmethyl-oxon and azinphosmethyl determined at 2.5 and 4.1 μg, respectively. Peaks: 1=azinphosmethyl-oxon; 2=azinphosmethyl.

A chromatogram for an XAD-4 resin air sample 3 days following application appears in Fig. 3. Peaks were identified by the relative retention times (t_R) of external standards. t_R peak identification windows were set to 5%. Azm-o is identified at t_R 10.48–10.56 and azm at 11.87–11.88 min. Azm and azm-o were determined at 4.1 and 2.5 μg, respectively. These values correspond to air concentrations of 34 and 21 ng/m^3.

4. Discussion

Quantitative external standard determination of azm, a thermally labile organophosphate insecticide, can be achieved with GC. GC methodology permits the use of sensitive, element-specific detection methods such as FPD. The selectivity of FPD for phosphorous-containing compounds minimizes interferences from environmental matrices. This rapid method of analysis requires a minimum of time and sample preparation.

Maintaining sharp, reproducible peaks required periodic maintenance of the GC injection port. Injection port maintenance was performed on an as-needed basis, usually between 75–100 injections. Maintenance included removing the guard column from the inlet liner at regular intervals. Cutting approximately 10 cm from the injector end of the guard column restored performance to original levels. The importance of a clean-cut is twofold. A tight seal between the glass inlet liner and the head of the guard column prevents carrier gas leakage. Also, the cut needs to be free of barbs, cracks and rough edges to maintain a minimum of active sites in the injection port. Eventual replacement of the guard

column was necessary when the total length was reduced to approximately 30 cm. No maintenance of the analytical column was necessary. Fig. 4 shows chromatograms for a 2.5 μg/ml composite standard in isooctane before and after injection port maintenance. Azm is identified at t_R 11.83 and 11.81 min, and azm-o is identified at t_R 10.36 and 10.27 min in Fig. 4a,b, respectively. Fig. 4a depicts peak splitting, band broadening and a reduction in peak height. Fig. 4b illustrates the improvement achieved in chromatography after maintenance of the guard column.

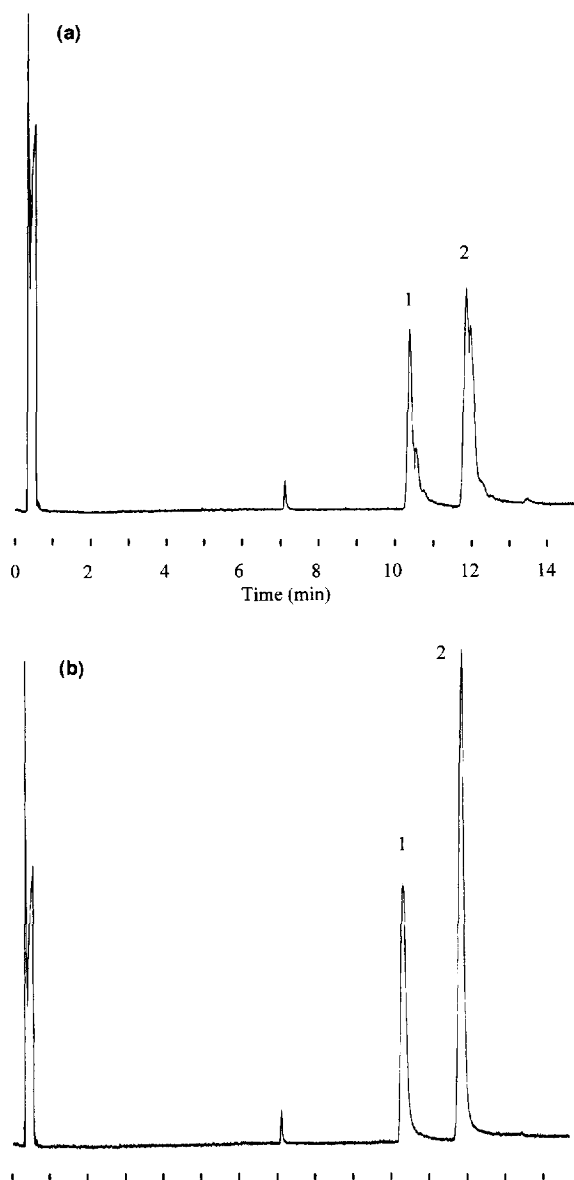

Fig. 4. Comparison of 2.5 μg/ml composie standards in isooctane with a dirty guard column after approximately 100 injections (a) and after injection port maintenance on the guard column (b). Peaks: 1=azinphosmethyl-oxon; 2=azinphosmethyl.

Isooctane proved to superior to hexane for use as a GC injection solvent. The higher boiling point of isooctane lends itself to on-column injection. In on-column injection, the injector temperature must be kept lower than the boiling point of the solvent, usually by at least 10°C. Higher injection port temperatures can be maintained with a higher boiling point solvent, which reduces the range of injector temperature programming. This can reduce overall analysis time.

Also, it is important to consider the coefficient for volumetric expansion of the GC solvent from the liquid phase to the gaseous phase. The coefficient for volumetric expansion (dV) is a function of density divided by molecular mass and multiplied over a temperature range. Considering the same temperature, $dV_{isooctane} < dV_{hexane}$. Less volumetric expansion of the solvent is favorable for on-column chromatography due to the limited space available in the injection port. The time required for the vaporized solvent to be swept from the retention gap in the injection port and carried to the analytical column is minimized. This reduces the opportunity for breakdown of the analytes and can minimize band broadening. On-column injection with isooctane can also improve detection limits since larger injection volumes can be used than with typical splitless techniques.

Ethyl acetate proved to be a superior extraction solvent when compared to acetone–hexane mixtures. Improved quantitative yields were obtained with ethyl acetate in the liquid extractions. Also, ethyl acetates boiling point, 77.2°C, makes it more favorable than acetone or hexane for extract evaporation and subsequent solvent exchange to isooctane. This is because approximately 1 ml of water present in the sample extracts can be efficiently removed via codistillation with ethyl acetate on the Turbovap. Residual water cannot be codistilled with acetone–hexane mixtures because of the physical properties of the binary azeotrope formed. In the sample extracts, residual water proved to be problematic for

azm-o recovery and is best removed before solvent exchange to isooctane.

This method was developed specifically for the analyses of airborne residues of azm and azm-o collected with XAD-4 macroreticular resin. Specific matrix effects may be encountered in other applications.

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