Journal of Chromatography, 324 (1985) 53–63
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 536

POST-COLUMN REACTION DETECTION SYSTEM FOR THE DETERMINATION OF ORGANOPHOSPHORUS COMPOUNDS BY LIQUID CHROMATOGRAPHY

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(Received December 27th, 1984)

SUMMARY

The development and application of a post-column reaction detection system for organophosphorus compounds based upon their photodegradation to orthophosphate followed by the formation of reduced heteropolymolybdate is reported. Photodegradation occurs in a fused-silica tube coiled about a 450-W xenon lamp in the presence of ammonium peroxydisulfate. Photodegradation yields were practically quantitative for the organophosphates, -phosphonates, and -phosphorodithioates tested. Factors affecting the rate of the chromogenic reaction were optimized so that its contribution to the total band-broadening was negligible. The effect of interfering organic modifiers in the mobile phase was studied using the acetate ion as a model. Separations were developed using no organic solvents in the mobile phases. The determination of dialkylphosphates in urine and organophosphorus pesticides in tomatoes illustrate the potential of the methodology.

INTRODUCTION

Interest in organophosphorus (OP) compounds has grown to a high level in recent years as a result of their uses in agriculture, industry and medicine. In some cases involving the determination of polar OP compounds, such as the OP pesticides and their metabolites and degradation products studied in this work, it would be desirable to utilize liquid chromatography (LC) because of sample preparation, chromatographic separation, thermal stability, or volatility considerations. Thus far, the utility of LC in trace-level determinations of OP pesticides and related compounds has been limited by a lack of sufficiently selective and sensitive detection.

The adaptation of the flame photometric detector to the detection of OP compounds separated by microcolumn LC has produced some promising results[1,2]. Szalontai[3] utilized flame ionization detection with a transport wire in his study of the normal-phase separation of OP pesticides. Acetylcholinesterase inhibition has been

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0021-9673/85/$03.30

used to detect OP pesticides by coupling LC with a continuous flow analyzer[4,5]. An automatic phosphorus analyzer has been used as a detector for the LC analysis of phospholipids[6]. Baba *et al.*[7] adapted a flow injection system to LC for the analysis of various inorganic oxo acids of P^{III} and P^{V} by hydrolysis and reaction with molybdate. Conductivity detection has been used in the determination of OP anions by ion chromatography[8,9]. Most reported LC methods for OP pesticide residue analysis rely on UV detection[10,11].

Direct UV absorbance detection is applicable only to those OP pesticides which possess suitable chomophoric substituents and which are present in relatively uncomplicated sample matrices. It thus appeared that a post-column reaction detection system could be used advantageously to increase detection selectivity and sensitivity.

The formation of reduced heteropolymolybdate was chosen as the basis for the post-column reaction detection system. The sensitivity and specificity of this reaction is well-known[12]. The reduced heteropolymolybdate absorbs at long wavelengths (λ_{max} = 885 nm) where other sample matrix components would not be expected to interfere. However, a method for the degradation of eluted OP compounds to orthophosphate was required in order to make use of those desirable properties. In a previous paper[13] we showed that photochemical degradation with UV light in the presence of ammonium peroxydisulfate can be made sufficiently rapid for use in a post-column reaction detection system. In this work, the proposed post-column reaction detection system was constructed and studied. LC separations were developed without the use of organic solvents and some applications of the methodology are demonstrated.

EXPERIMENTAL

Chemicals

All chemicals used in this work were analytical reagent grade and were used without purification, unless otherwise specified below. Triethylphosphate (TEP) and disodium phenylphosphate were obtained from Alfa Products. Dimethyl methylphosphonate (DMMP), ammonium diethyldithiophosphate (DEDTP), and octylamine were obtained from Aldrich. Dimethylphosphoric acid (DMP) and diethylphosphoric acid (DEP) were obtained as mixtures of the mono- and diesters from Pfaltz and Bauer, and were assayed by LC and titrimetry. OP pesticides (dylox, dimethoate, and dichlorvos) were obtained from Chem. Service.

Apparatus

A Varian Model 5000 liquid chromatograph, Varichrom detector (equipped with a Hamamatsu R928 photomultiplier tube for enhanced sensitivity at longer wavelengths), and CDS 111L integrator comprised the basic LC system. Varian Micropak CH-10 and CN-10 30 cm × 4 mm I.D. columns were used. A 4 cm × 4 mm I.D. guard column filled with Vydac 40 μm C_{18} pellicular packing was used in conjunction with the CH-10 analytical column.

The post-column reaction detection system is depicted schematically in Fig. 1. A Rainin Rabbit 4-channel peristaltic pump was used for the addition of reagents. The chromogenic reaction coil, connecting tubing, tees, and fittings were assembled from the Cheminert product line (LDC Division of Milton Roy, Inc.). The tubing

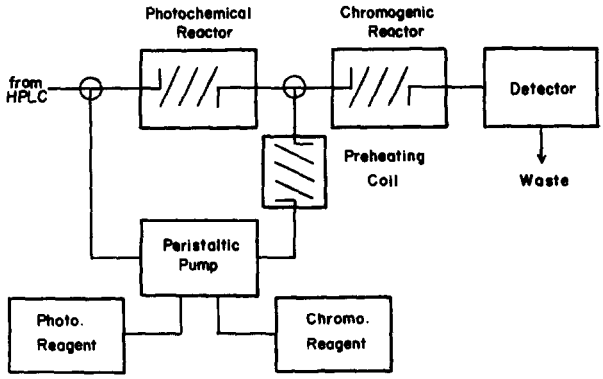

Fig. 1. Diagram of post-column reaction detection system.

was 0.5 mm I.D. throughout, except for the 1-m preheating coil for the chromogenic reagent which was 0.76 mm I.D. The chromogenic reactor was 1 m in length and formed into a coil of 3.5 turns about a 5-cm coil diameter. The temperature of the preheating coil and the chromogenic reaction coil was controlled by the use of a water bath equipped with a temperature regulator and stirrer motor. The sample flow cell of the detector was thermostatted by connection to the water bath through the peristaltic pump.

The photochemical reaction coil was constructed of fused-silica tubing (6 mm O.D. and nominally 0.5 mm I.D.) by Reliance Glassworks (Bensonville, IL, U.S.A.). It was 580 cm in length and formed into a spiral having 16 turns about an 11-cm coil diameter. The photochemical reaction coil was centered about the arc of the lamp and supported by a tripod. Connection was made to the Cheminert system by means of Swagelok SS-400-1ZV fittings. The photochemical reactor housing was 18 × 19 × 38 cm and was constructed of sheet metal (3 sides, bottom) and asbestos (electrode side) with no efforts made to increase internal reflectivity. A Dayton Model 2C781 blower was used for cooling and ozone removal. A Harrison Model 6269A power supply was used to operate the Osram XBO 450W/4 xenon lamp at 25 A. The lamp was allowed to warm up for at least one hour prior to the collection of data.

Conditions for the study of the detection system

To facilitate the study of the post-column reactions, no column was used. The operating conditions for these experiments were as follows: the carrier stream was 0.1 ppm (P) orthophosphate pumped at a flow-rate of 1.0 ml/min, the flow-rate of the photodegradation reagent was 0.5 ml/min, the flow-rate of the chromogenic reagent was 0.16 ml/min, the sample was 10 μl of 10 ppm (P) orthophosphate solution, and the detection wavelength was 885 nm. For the study of the chromogenic reaction 0.1 *M* ammonium peroxydisulfate was used as the photodegradation reagent, but with the lamp off. The chromogenic reagent composition used as the starting point for variations was prepared by dissolving 1.403 g $Na_2MoO_4 \cdot 2H_2O$ in water, followed by the addition of 13.4 ml conc. sulfuric acid, 1.02 g ascorbic acid, and 10 mg K(SbO)tartrate · 0.5 H_2O and dilution to 100 ml. This results in approximately the same concentrations in the final stream as used by Murphy and Riley[12] in their batch procedure.

Analysis procedure for urine samples

Urine samples were collected around midday on the day of the analysis and allowed to cool to room temperature. A 50-ml portion was pipetted into a beaker and sufficient water added to bring the volume to about 80 ml. DMP and/or DEP additions were made at this point. The sample was stirred magnetically at a rapid rate and 5.0 ml of 1.0 *M* ferric perchlorate was added. The pH was adjusted to 4.75 with dilute ammonia. The sample was stirred for 30 min during which time the pH was readjusted to 4.75 as necessary. The entire sample, which at this point contained brown precipitate, was quantitatively transferred to a 100-ml volumetric flask, diluted to volume, and mixed thoroughly. About 20 ml of this was filtered through a Millipore Type HA 0.45-μm filter. The filtrate was analyzed by LC using the CH-10 column at a temperature of 30°C, a 100-μl injection loop, and mobile phase consisting of 0.002 *M* octylamine, 0.01 *M* sodium perchlorate, and 0.001 *M* sodium acetate (adjusted to a pH of 4.75 with perchloric acid) pumped at a flow-rate of 1.0 ml/min. The photodegradation reagent was 0.8 *M* ammonium peroxydisulfate delivered at a flow-rate of 0.5 ml/min and the chromogenic reagent was the "optimized" composition (see Results and discussion section) delivered at a flow-rate of 0.16 ml/min.

Analysis procedure for tomato samples

Ripe tomatoes were acquired from private gardens or local stores. One tomato, or a portion of very large tomatoes, was taken for analysis (typical sample weight was 250 g). The tomato was sliced into quarters, homogenized in a Waring Blender for 15 min, and cooled to room temperature. Pesticide spikes were made at this point using a 100 ppm (P) solution in acetonitrile. A volume of acetonitrile equal to 10% of the sample weight was then added. This volume was adjusted to take into account the acetonitrile added with the pesticide spikes. The sample was homogenized for an additional 10 min and then quantitatively transferred to a 500-ml volumetric flask, using water to rinse the blender. The homogenate was degassed and diluted to volume. Magnetic stirring was used to maintain homogeneity. An aliquot (normally 10 ml) of the homogenate was filtered through a 0.45-μm Type HA Millipore filter and the solid residue washed with water. A Waters Assoc. C_{18} Sep-Pak cartridge was prepared by rinsing it with 20 ml of 50% acetonitrile, followed by 20 ml of water. The entire filtrate and washings were applied to the Sep-Pak, which was then washed with 2 ml of water. The desired components were eluted from the Sep-Pak with 4 ml of 50% acetonitrile into a 10-ml volumetric flask and diluted to volume with water. This was analyzed by LC using the CN-10 column, a 100-μl injection loop, and a mobile phase consisting of 0.05 ppm (P) orthophosphate pumped at a flow-rate of 1.0 ml/min. The photodegradation reagent was 0.01 *M* ammonium peroxydisulfate delivered at a flow-rate of 0.5 ml/min and the chromogenic reagent was the "optimized" composition (see Results and discussion section) delivered at a flow-rate of 0.16 ml/min.

RESULTS AND DISCUSSION

Study of the detection system

In the early study of the post-column reaction detection system it was found that the use of low concentrations of orthophosphate in the carrier stream resulted

in substantial improvement of peak symmetry and peak height for injections of orthophosphate solution. This suggests that adsorptive sites are present on the tubing used. For this reason, 0.05 or 0.1 ppm (P) orthophosphate was maintained in the carrier stream or mobile phase in subsequent work, where possible.

The main objective in the study of the post-column reactions was to maximize the rates so as to minimize band-broadening. In the case of the chromogenic reaction, the effects of varying the reaction temperature and the composition of the reagent were examined using a 1-m reactor and an initial reagent composition derived from the work of Murphy and Riley[12]. The response obtained for injections of 0.1 μg (P) orthophosphate was found to increase with reactor temperature over the range 25–50°C. At 35°C, the extent of reaction was sufficiently low so as to permit increases in rate due to variations in the reagent composition to be readily apparent. The concentration of each component of the chromogenic reagent was varied individually, while the others were held constant. The results of these studies are depicted in Figs. 2–5 in terms of the concentration of the component in the final stream (*i.e.* after mixing with the effluent from the photochemical reactor). Decreasing the concentration of sulfuric acid led to large increases in response for the orthophosphate injections (Fig. 2). However, combinations of lower acidities and higher concentrations of molybdate were found to produce reagents which were unstable to direct reduction of molybdate to form a blue solution which precluded their use. For this reason, the original acidity (0.46 equiv./l) was used for the study of the effect of increasing the concentration of molybdate, while a lower acidity (0.2 equiv./l) was used in the study of the effects of varying the concentrations of ascorbic acid and antimony. Although increases in the concentration of molybdate also caused considerable increases in response (Fig. 3), the original concentration was retained so as to permit the use of the lower acidity, which had a greater effect on the rate. Concentrations of antimony greater than those shown in Fig. 4 could not be used because of turbidity in the reagent. Increasing the concentration of ascorbic acid led to a levelling-off of the response (Fig. 5), which suggested that the reaction was reaching completion. This was confirmed by the preparation and testing of a reagent formulated to produce 0.2 equiv./l sulfuric acid, 0.027 mg/ml antimony, 0.0055 *M* molybdate, and 0.0165 *M* ascorbic acid in the final stream (*i.e.* elevated concentrations of both ascorbic acid and antimony). This reagent produced an equivalent response and was adopted as the "optimized" composition used throughout the remainder of this work. The reaction temperature was increased to 40°C to further ensure a high degree of completion. Further increases in rate probably could be achieved and counterbalanced by

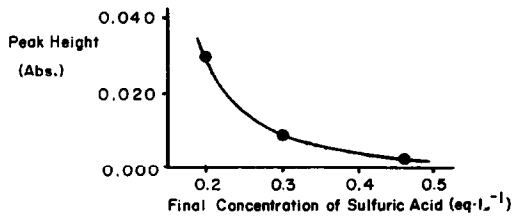

Fig. 2. Effect of acidity on response obtained for injection of 0.1 μg (P) orthophosphate. Conditions: carrier stream, 0.1 ppm (P) orthophosphate, 1.0 ml/min; photodegradation reagent, 0.1 *M* ammonium peroxydisulfate, 0.5 ml/min; chromogenic reagent flow-rate, 0.16 ml/min; detection wavelength, 885 nm.

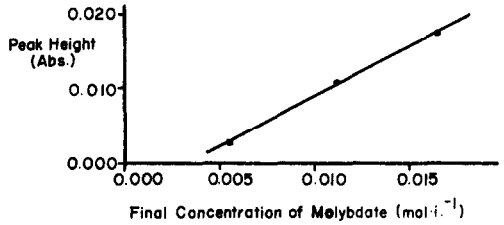

Fig. 3. Effect of molybdate concentration on response. For conditions see Fig. 2.

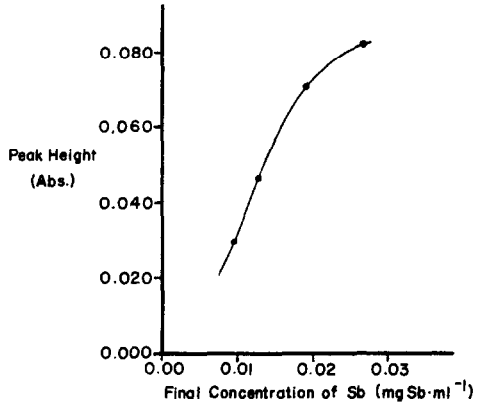

Fig. 4. Effect of antimony concentration on response. For conditions see Fig. 2.

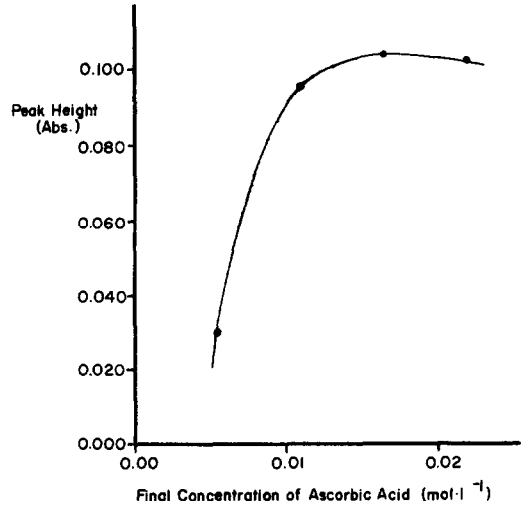

Fig. 5. Effect of ascorbic acid concentration on response. For conditions see Fig. 2.

reduction in the length of the reactor; however, the gain in reduced band-broadening would be slight as a consequence of the much greater residence time of the analyte in the photochemical reactor (90 sec *vs.* 7 sec). In addition, the reagent composition adopted was sufficiently stable so as to cause no significant drift in response over an 8-h period.

The approach taken in the study of the photodegradation reaction was necessarily different from that taken for the optimization of the chromogenic reaction. Because of the cost of the custom-made photochemical reactor, its length could not be varied and, therefore, the only factor which remained to manipulate the residence time was the flow-rate. However, the flow-rate is dictated to a large extent by the chromatographic separation and by the need to avoid excessive dilution of the analyte. The length and bore of the photochemical reactor were specified to the manufacturer so as to provide reasonable photodegradation yields at flow-rates of 1–2 ml/min on the basis of previous studies[13].

The responses obtained for 10-μl injections of 10 ppm (P) solutions of three test OP compounds relative to that obtained for an equivalent amount of orthophosphate, on a phosphorus basis, are given in Table I. The yields obtained using only water as the photodegradation reagent are surprisingly high in relation to what was expected from the previous batch irradiation studies[13]. The factors believed to be responsible for this are: the use of a higher intensity lamp, the irradiation of a much thinner layer of solution, and dilution of the analyte by the carrier stream. The yields obtained using 0.01 *M* ammonium peroxydisulfate as the photodegradation reagent are practically quantitative. A more rigorous evaluation of the yields could be undertaken to determine the feasibility of a single calibration graph based on orthophosphate peak areas for the quantitation of various OP compounds.

The effect of the presence of sodium acetate in the carrier stream on the photodegradation was of interest because of the desirability of utilizing an acetic acid–acetate pH buffer in the mobile phases used for the separation of ionizable OP compounds. This also provides a model for the behavior of the reaction detection system when other interfering species such as ion-pairing reagents and organic solvents are present in the mobile phase. The presence of 0.005 *M* sodium acetate (adjusted to pH 5.0 with sulfuric acid) in the carrier stream was found to prevent any response from the injection of 10 μl of 10 ppm (P) DMMP solution. This interference could be partially overcome by increasing the concentration of ammonium peroxydisulfate in the photodegradation reagent as is shown in Fig. 6 along with analogous data for

TABLE I

PHOTODEGRADATION YIELDS FOR TEST OP COMPOUNDS

Conditions: no column; 0.1 μg (P) injected; reaction time, 90 sec.

Photodegradation reagent	*Yield (%)**		
	TEP	*DMMP*	*DEDTP*
Water	94 ± 3	91 ± 4	90 ± 3
0.01 *M* ammonium peroxydisulfate	100 ± 2	97 ± 2	96 ± 3

* The uncertainties listed are the standard deviations of three replicate determinations.

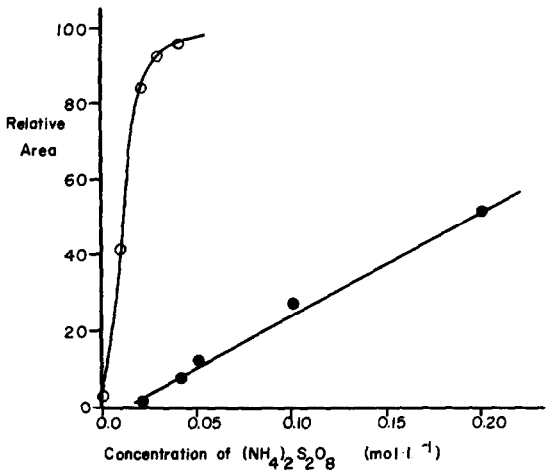

Fig. 6. Effect of increasing the concentration of ammonium peroxydisulfate on the photodegradation yields of DMMP (●) and TEP (○) in the presence of 0.005 *M* sodium acetate in the carrier stream.

TEP. From this, it is clear that the concentration of ammonium peroxydisulfate needed to achieve high photodegradation yields will be dependent upon the presence of interfering species in the mobile phase. The strategy that was adopted was to use the highest concentration of ammonium peroxydisulfate which did not produce bubbles in the detector cell for each set of conditions and to use minimum amounts of organic modifiers. DMMP is a good test compound because of its high resistance to degradation as a result of its phosphorus–carbon bond.

The above studies, conducted without a chromatographic column, show that high yields can be obtained in a reasonable reaction time. However, the 97-sec residence time causes significant band-broadening. Studies of the band-broadening as a function of flow-rate were carried out[14], but only a practical example is reported here. A 10-μl aliquot of a solution containing 20 ppm (P) phenylphosphate was injected onto the CH-10 column and detected using the post-column detection system developed in this work and by simple UV absorbance, in which case the column was connected directly to the detector. Peak width doubled in terms of volume, but the increase was only 20% in time units. Phenylphosphate was not appreciably retained under the conditions used and thus represents a "worst case" example of the band-broadening. Some possibilities for the improvement of the band-broadening performance include the use of segmentation and some novel reactor designs[15].

Determination of dialkylphosphates in urine

The determination of DMP and DEP was selected for consideration in the present study because these compounds are urinary metabolites of many OP pesticides in humans and because there has been some interest in developing screening procedures based on these compounds to indicate exposure[16]. The determination of these metabolites in urine by gas chromatography methods requires derivatization[17,18]. It was apparent from some preliminary injections of urine onto the CH-10

column using an ion-pairing separation mode that the large amount of orthophosphate present in urine is the only serious interference to be dealt with. A sample pretreatment procedure based on the precipitation of orthophosphate by iron(III) at a pH of 4.75 was developed. This pretreatment reduces the concentration of orthophosphate to a level where the injection of 100-μl samples and operation at high sensitivities is possible. Additional features include its speed, simplicity, and removal of monoalkylphosphates. The recoveries of DMP and DEP from urine spiked at levels ranging from 2 to 11 ppm were determined. The mean recovery of DMP was 101% (S.D. = 6.5, n = 4) and that of DEP was 102% (S.D. = 4.4, n = 3). A chromatogram of urine spiked with 2 ppm DMP and 5 ppm DEP is depicted in Fig. 7. Detection limits [signal-to-noise ratio (S/N) = 2] were about 0.3 ppm for DMP and 0.8 ppm for DEP. Some improvement in detection limits and precision can be expected from the monitoring of the absorbance at 850 nm, rather than 885 nm (see below). Levels which could be associated with potentially toxic exposure[16] to OP pesticides could be detected with this method.

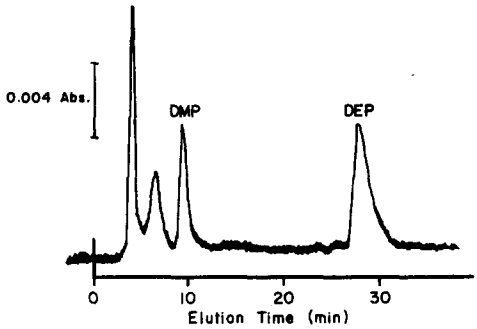

Fig. 7. Separation and detection of DMP and DEP in urine sample. Conditions given in Experimental section.

Determination of OP pesticides in tomatoes

It was found that tomatoes also contain an intolerable amount of orthophosphate and possibly other phosphorus-containing interferences as well. The iron(III) pretreatment procedure was not as effective in this case as it was in the analysis of urine samples and, therefore, a solid-phase extraction procedure was developed. The recovery of dimethoate from tomatoes using this procedure was 69% (S.D. = 2, n = 4) at a spike level of 3 ppm. A cyano-phase column provided suitable retention for the OP pesticides studied using no organic solvents in the mobile phase. A chromatogram illustrating the detection of dimethoate, along with dylox and dichlorvos, is shown in Fig. 8. These pesticides are representative of the phosphate, phosphonate, and phosphorodithioate classes of OP pesticides and have poor UV absorbance characteristics. A two-fold preconcentration factor could be achieved by taking a 20-ml aliquot of tomato homogenate at the expense of additional time required for filtration. The detection limit for dimethoate of 0.09 ppm (S/N = 2), obtained using the two-fold preconcentration technique, compares favorably with the legal tolerance of 2.0 ppm[19].

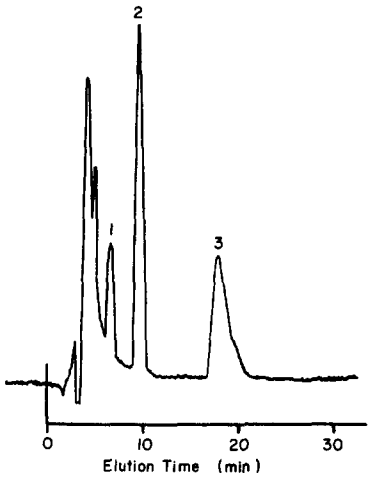

Fig. 8. Separation and detection of OP pesticides in tomato sample. Peaks: 1 = dylox, 4.7 ppm; 2 = dimethoate, 4.0 ppm; 3 = dichlorvos, 4.7 ppm. Conditions given in Experimental section.

Although λ_{max} for the reduced heteropoly is 885 nm, it was found near the end of this work that detection at 850 nm provided the best signal-to-noise ratio as a result of the spectral response characteristics of the R928 photomultiplier tube as is shown in Fig. 9.

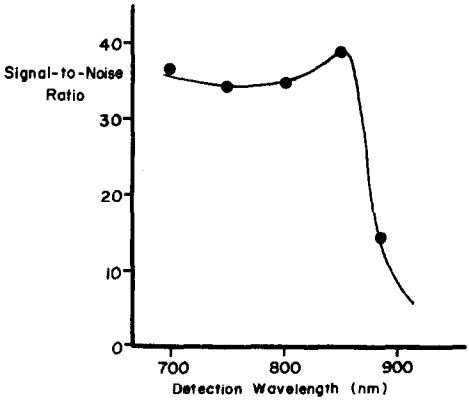

Fig. 9. Effect of varying the detection wavelength on the signal-to-noise ratio for injection of 4 ppm dimethoate in tomato sample. Conditions given in Experimental section.

CONCLUSIONS

The above applications serve to demonstrate the potential of the detection system developed in this work. The sensitive, phosphorus-selective detection permits sample preparation procedures to be reduced to a minimum. The limitation on the presence of organic solvents in the mobile phase restricts this methodology to wa-

ter-soluble OP compounds, but it is in this area that supplementary methods are most needed.

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