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Trace Analysis for Organothiophosphate Agricultural Chemicals by High-Performance Liquid Chromatography–Photolysis–Electrochemical Detection

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Organic thiophosphate agricultural chemicals, such as malathion, parathion, and others, can be satisfactorily analyzed by the newer method of high-performance liquid chromatography (HPLC) with on-line photolysis (hv), followed by electrochemical detection (EC) using single- or dual-electrode approaches for the species generated. This approach, HPLC–hv–EC, has been applied to about 20 different thiophosphates, most of which are widely used agriculturally and for which trace residue levels are routinely monitored. Dual-electrode response ratios have been determined for all of these analytes, along with minimum detection limits (MDLs) in many cases. These approaches can also be used for the quality control evaluation of commercial formulations by flow injection analysis (FIA) with hv–EC and no HPLC separations. Wheat middling extracts have been analyzed by the commonly used gas chromatography (GC) flame photometric detection (FPD) method of residue analysis, as well as by HPLC–hv–EC. These comparative studies indicate that the newer method is reproducible, accurate, precise, and entirely reliable. Standard additions have been applied to wheat middling extracts, and the quantitative results are compared with the external standard method.

Many government regulatory or private service laboratories still monitor trace residue levels of various agricultural chemicals (Das, 1981). Although many routine-type analyses still utilize thin-layer chromatography (TLC) or gas chromatography (GC), several pesticide-, herbicide-, or fungicide-type analyses are now using high-performance liquid chromatography (HPLC) (Moye, 1975; Lawrence et al., 1980; Lawrence, 1984; Papadopoulou-Mourkidou et al., 1981; Harvey and Zweig, 1980). Nevertheless, most government agencies today still routinely utilize involved sample preparation and workup together with GC-selective detection. Organic thiophosphates represent a very large class of agricultural chemicals in widespread use, and there remains a need for improved, specific HPLC approaches for their detection. Although many of these thiophosphates are aromatic derived and chromophoric, a large number of them are not. Thus, HPLC with either ultraviolet (UV) or fluorescence (FL) detectors is not a satisfactory detection method. Derivatization for improved HPLC–UV/FL detection of pesticides is always a real possibility, though this requires additional sample handling, treatment, and workup and provides additional room for error (Frei and Lawrence, 1981). Ideally, derivatization should be continuous, on-line, in real-time, postcolumn, and very selective for the particular analyte of interest (Xie et al., 1983). For those organic thiophosphates that are not UV or FL active, there remains electrochemical detection (EC), assuming that such materials respond oxidatively and/or reductively. Several organic thiophosphate agricultural chemicals can be analyzed by reductive LCEC (liquid chromatography–electrochemical detection) (Shoup, 1982). Reductive LCEC, however, has its own operational difficulties, and it generally requires more expertise and experience than oxidative LCEC (Krull et al., 1983). EC detection approaches, especially with the dual-electrode transducers (series or parallel), can provide improved analyte specificity over single-electrode methods. Dual-electrode LCEC can also provide minimum detection limits (MDLs) equal to or better than single-electrode LCEC, especially in the series (upstream–downstream) mode.

It occurred to us that oxidative dual-electrode LCEC could provide an easy-to-use, sensitive, and highly selective approach to all organic thiophosphates, but only if the original analytes could be easily and reproducibly converted into derivatives, on-line, pre- or postcolumn, that were then suitable for oxidative EC detection. This particular class of thiophosphates is not suitable for direct oxidative EC. Most derivatization approaches in LCEC have used off-line, precolumn techniques (Shoup, 1982; Krull et al., 1983). Though continuous, postcolumn, on-line photochemistry has been used in HPLC–UV and HPLC–FL, very little has yet been described in LCEC (Krull and

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Lankmayr, 1982; Lefevre et al., 1982; Snider and Johnson, 1979; Sherwood and Johnson, 1981; Scholten et al., 1980; Green et al., 1977; Shuker and Tannenbaum, 1983). Walters (1983) has reported on the use of a commercial photoconductivity detector for organic thiophosphates, in a study analogous to ours. His method used an on-line, postcolumn photohydrolysis of the thiophosphates leading to an ionic derivative that was detected by the built-in conductivity detector. Even the work of Snider and Johnson (1979) did not utilize continuous, on-line, real-time photolysis of the HPLC eluants prior to the final EC detection. All of the remaining uses of photochemistry or photolysis, on-line, postcolumn, in HPLC have used UV/FL detection. At times, chemical visualization reactions of the photolytically generated products derived from the HPLC analytes have been used (Shuker and Tannenbaum, 1983).

We have applied photolytic derivatization in LCEC, or HPLC–hv–EC, for the determination of a number of organic thiophosphate agricultural chemicals. These same approaches were previously used for organic nitro compound analyses, including explosives, drugs, and environmental pollutants (Krull et al., 1984). For organic nitro derivatives, we believe that inorganic nitrite and/or nitrate are formed photolytically, and these are then detected by oxidative and/or reductive EC. In the case of organic thiophosphates, we are not certain what specie(s) is (are) being formed photolytically, though this may be inorganic sulfide, which is EC active at these oxidative working potentials. Studies are under way using photolysis–cyclic voltammetry (hv–CV) to demonstrate the nature of the specie(s) being generated.

We report the instrumentation, methods, maximization, and application of HPLC–hv–EC approaches for about 20 organic thiophosphates. These results include minimum detection limits (MDLs), linearity of calibration plots, optimal oxidative potentials, dual-electrode response ratios, analyses of spiked samples, analyses of crop extracts for residues of malathion, and standard additions applied to these crop extracts. These results strongly suggest that this newer method of residue analysis for agricultural organothiophosphates provides suitable MDLs together with unusually selective analyte specificity. It is hoped that these methods of trace analysis will soon be adopted and applied by others interested in agricultural chemical residue analysis.

EXPERIMENTAL SECTION

Reagents, Chemicals, and Agricultural Chemicals. Standard agricultural chemicals were obtained from various sources: (1) Analabs, Inc. (North Haven, CT); (2) Boston District Office of the U.S. Food and Drug Administration (FDA); (3) Pesticide and Industrial Chemical Research Center, U.S. FDA, Detroit, MI. Inorganic salts added to the mobile phase were from J. T. Baker Chemical Co. (Phillipsburg, NJ), Fisher Scientific Co., Aldrich Chemical Co. (Milwaukee, WI), and others. HPLC solvents were from Waters Associates (Milford, MA) or MCB Chemicals Co. (Cincinnati, OH), the latter as the Omnisolv brand.

Instrumentation and Equipment. Figure 1 illustrates the HPLC–hv–EC instrumentation and arrangement of the parts used. The HPLC portion utilized a Rheodyne Model 7125 syringe loading injection valve (Rheodyne Corp., Cotati, CA), a Laboratory Data Control (LDC) Constametric II solvent delivery system (Laboratory Data Control, Riviera Beach, FL), a LiChromaDamp II pulse dampener (Alltech Associates, Inc., Deerfield, IL), a Bioanalytical Systems pulse dampening column (Bioanalytical Systems, Inc., West Lafayette, IN), a Photronix Model 816 HPLC batch irradiator (Photronix Corp., Medway, MA), a BAS Model LC-4A single-electrode amperometric controller or a BAS Model LC-4B dual-electrode system for LCEC, a BAS glassy carbon single or dual working electrode with an Ag/AgCl reference electrode, and a Linear Instruments Model 585 dual-pen strip chart recorder (Linear Instruments, Inc., Reno, NV). At times, a Honeywell dual-pen strip chart recorder was used (Honeywell Instruments, Inc., Minneapolis, MN). HPLC injections were made with either a 25- or 250-μL flat-tipped Hamilton HPLC syringe (Hamilton Corp., Reno, NV). HPLC mobile phases were degassed and filtered prior to use with a 0.45-μm solvent filtration kit (Millipore Corp., Bedford, MA). Samples for HPLC injection were filtered with a sample filtration kit using a 0.45-μm filter (Millipore Corp.). The irradiation finger was maintained at 0–5 °C with a constant-temperature water bath (Forma Scientific, Model 2095, VWR Scientific Co., Boston, MA) or with an ice-water bath. Irradiation of the HPLC effluents took place within a 10–12 ft, $^1/_{16}$-in. o.d., 0.8-mm i.d., Teflon FEP tubing, catalog no. HGC-024 (Rainin Instruments Co., Woburn, MA). Swagelok stainless steel fittings and ferrules were used for all connections, except where the EC cell required its own fittings (Cambridge Valve & Fittings Co., Billerica, MA). The dual-electrode HPLC–hv–EC system used equipment similar to that already described but replacing the single electrode with the dual cell and two LC-4B controllers (BAS). HPLC columns were obtained from various sources: (1) a Biophase C-18, 10 μm, 25 cm × 4.6 mm i.d. (Bioanalytical Systems, Inc.); (2) a Perkin-Elmer Fast-LC C-18, 3 μm, 10 cm × 4.6 mm i.d. (Perkin-Elmer Corp., Norwalk, CT); (3) a Waters μBondapak C-18, 10 μm, 25 cm × 4.6 mm i.d. (Waters Associates); (4) in-house slurry packed C-8 or C-18 reversed-phase columns.

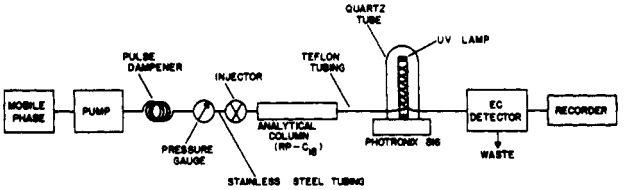

Figure 1. Schematic diagram of the HPLC–photolysis–electrochemical detection system in operation.

Cyclic voltammograms (CVs) were obtained on a BAS Model CV-1B unit, with a separate Linear Instruments X-Y recorder. Distilled water was obtained from a Corning Mega-Pure distillation apparatus (Corning Corp., Corning, NY).

Procedures. CVs were performed by using a supporting electrolyte of 50/50 (v/v) methanol (MeOH)/0.1 M sodium chloride (NaCl), with a scan rate of 150 mV/s, with an Ag/AgCl reference electrode and a glassy carbon working electrode surface. The CVs were obtained by plotting applied working potentials vs. current generated in the usual manner. Linear hydrodynamic voltammograms (LHVs) were derived by using flow injection analysis (FIA)–hv–EC methods, varying the applied potential and measuring current generated. LHVs were obtained on the FIA–hv–EC instrumentation used for FIA work on HPLC–hv–EC. Final LHVs were derived by plotting applied working potentials against current generated.

The HPLC–hv–EC and FIA–hv–EC systems had to be optimized, and this was crucial for the photolytic portion. This was done by varying the internal diameter of the Teflon tubing and its total length. By measuring the peak heights on the EC as a function of either the inner diam-

eter or total length, it was possible to determine the optimum tubing parameters that would give maximum anion formation (nitrite, sulfide) with minimum concomitant destruction. Final optimal tubing parameters depend on the particular wrapping configuration. The final HPLC flow rate was determined by flow injection methods. These experiments gave the ideal flow rates and analyte residence times for HPLC–hv–EC. The FIA–hv–EC system also had to be optimized for salt concentration, nature of the electrolyte salt, and its compatibility with photolysis and EC detector conditions. A number of inorganic salts were studied, but only NaCl appeared to be inert to the photolysis and EC. It was also free of responding impurities. The final pH had to be optimized for FIA–hv–EC approaches by determining maximum current generated at optimal potential, as a function of pH of the mobile phase.

MDLs were determined by standard HPLC techniques, together with the MDL definition of a signal-to-noise (S/N) ratio of at least 3/1. These were physically determined by injecting lower and lower concentrations of the analytes at the maximum sensitivity settings possible on the EC. MDLs have been determined for both 20- and 200-μL injections of standard solutions of thiophosphates.

Wheat Middling Extraction and Cleanup. These methods are from the "Pesticide Analytic Manual" (1977, 1982). For products containing 2 g or less of fat/20 g of sample, the sample was ground to pass a 20-mesh sieve, and 20 g of this prepared sample was put into a high-speed blender jar. Then, 350 mL of 35% HOH/acetonitrile (ACN) was added, and this was blended 5 min at high speed and filtered with suction through a 12-cm Büchner funnel fitted with sharkskin paper into a 500-mL suction flask. A measured volume (250 mL) of this filtrate was transferred to a 1-L separatory funnel, 100 mL of petroleum ether was added in the same 250-mL cylinder used to measure the volume of the extract, and this was poured into the 1-L separatory funnel containing the extract. This was shaken vigorously for 1–2 min, and 10 mL of saturated NaCl solution and 600 mL of HOH were added. The separatory funnel was held in a horizontal position and mixed vigorously for 30–45 s. The two phases were then allowed to separate, the aqueous layer was discarded, and the organic layer was gently washed with 2 × 100 ml portions of HOH. Washings were discarded, the organic layer was transferred to a 100-mL glass-stoppered graduate, and final volume was recorded. About 15 g of anhydrous sodium sulfate (Na_2SO_4) was added and the solution was shaken. Within 1 h the organic extract was transferred to a Florisil column ("Pesticide Analytical Manual", 1982, Section 211.14d), and successively eluted with 200 mL volumes (v/v) of the following solvents: (1) 6% diethyl ether in petroleum ether (DE/PE); (2) 15% DE/PE; (3) 50% DE/PE. The final organic solution was concentrated in a Kuderna-Danish concentrator to a volume of 5–10 mL. This solution (petroleum ether) was then used for both the GC analyses at the Boston FDA and all HPLC–hv–EC analyses in The Barnett Institute.

The GC analyses of these wheat middling extracts used a packed glass column of 3% SP2100 plus 4.5% SP2401 on Chromosorb W/HP (80–100 mesh), 6 ft × 4 mm i.d., operated isothermally at 200 °C. A nitrogen carrier gas flow rate of about 100 mL/min was used throughout, with an injector temperature of 210 °C and the FPD temperature of 225 °C. The FPD was operated in the phosphorus mode at 750 V with 1×10^{-7} A full-scale deflection (FSD).

RESULTS AND DISCUSSION

Figure 1 illustrates the overall HPLC–hv–EC analytical system and the broad-spectrum UV irradiation finger in place. Though this apparatus was intended for batch or flow-through-type HPLC water irradiations to remove organics, it appears useful for the present on-line photolysis purposes in LCEC. There is dead volume present within the Teflon tubing taking the HPLC analytes to the EC system. We have compared this band broadening with a direct HPLC–UV system and have estimated the loss in peak height as about 1–2 orders of magnitude. This adversely affects the MDLs and chromatographic resolutions possible, but even with this first approach, MDLs are more than adequate for environmental crop samples. Efforts are under way to improve the design of the Teflon tubing, so as to reduce the effective dead volume and overall variance (Nondek et al. 1983; Scholten et al., 1982). This could provide MDLs that are at least 1 order of magnitude lower than those achieved here.

Analyte specificity and qualitative information is possible to achieve in HPLC–hv–EC by three parameters: (1) capacity factors (k') as a function of the HPLC stationary phase and mobile phase composition and flow rate; (2) EC active species present as a function of having the lamp on or off; (3) parallel dual electrode response ratios as a function of oxidative and/or reductive potentials. All of this work has involved parallel dual-electrode orientations rather than the series mode, because we believe that improved analyte specificity is possible. Though some workers have shown that analyte detectability can be improved by going to the series dual EC approach, in general, dual-electrode response ratio measurements are more reliable and meaningful in the parallel situation (Krull et al., 1983). The success of these overall methods in HPLC–hv–EC or FIA–hv–EC for analyte identification depends on the photolytic generation of an EC-active species, perhaps an anion, from an appropriate precursor (Krull et al., 1984). These requirements are similar to those for photoconductivity detection in HPLC (Walters, 1983). It is possible that inorganic anions, EC inactive in the absence of photolysis, might be converted to EC-active ions photolytically. The reverse scenario is just as possible. It is further possible that impurities in the mobile phase might be EC active in the absence of irradiation or that EC-inactive impurities could be photolytically converted into EC-active derivatives. These situations could lead to elevated background noise levels, which would adversely affect MDLs and signal-to-noise ratios. Thus, system optimization with regard to mobile-phase constituents is important. This information can be derived by FIA–hv–EC or using CV in the absence and presence of photolysis. The approach of using photolysis–CV (hv–CV) has proven of use also for screening purposes. It requires about 5–10 min/candidate to determine if it is a suitable analyte for HPLC–hv–EC or FIA–hv–EC using hv–CV. There are no other reports on the use of hv–CV as a screening method for LCEC.

Figure 2 summarizes the structures for all of the thiophosphates that have been studied here. This group represents some of the more widely used and studied thiophosphates, especially with regard to crop residue, environmental water, or soil contamination levels.

Qualitative FIA–hv–EC Responses for Agricultural Chemicals Using Lamp On/Off. Table I lists the agricultural thiophosphates studied now by FIA–hv–EC methods, wherein we have compared dual glassy carbon electrode oxidative responses with the lamp off and lamp on at the same potentials. Methanol has been included to demonstrate that with or without the lamp on there is no EC response (control). These analytes are not amenable to oxidative EC detection with the lamp off, but they all

Table I. Qualitative Responses and Dual Electrode Response Ratios[a]

compound name	lamp off (+0.9 V/ +0.8 V)	lamp-on, dual-electrode ratios[b] (+0.9 V/+0.8 V)
methanol	NR[d]	NR
guthion	NR	1.45 ± 0.034
o,p'-kelthane	NR	response[c]
malathion	NR	1.88 ± 0.16
lorox	NR	response[c]
famphur	NR	1.70 ± 0.078
EPN	NR	1.43 ± 0.016
imidan	NR	3.40 ± 0.21
ethyl guthion	NR	2.43 ± 0.20
coumaphos	NR	response[c]
leptophos	NR	response[c]
supracide	NR	3.69 ± 0.121
pirimiphos ethyl	NR	1.68 ± 0.078
parathion	NR	2.10 ± 0.23
ethion	NR	1.53 ± 0.098
abate	NR	1.21 ± 0.027
phosalone	NR	1.34 ± 0.052
thimet	NR	1.44 ± 0.046
dioxathion	NR	1.60 ± 0.22
dasanit	NR	1.18 ± 0.053
mocap	NR	1.48 ± 0.043

[a] These results were obtained by using the flow injection approach, FIA-hv-EC; mobile-phase conditions identical with lamp off/lamp on. EC used a parallel dual-electrode glassy carbon cell with an LC-4B amperometric detector. [b] Dual-electrode response ratios were determined as the average ± standard deviation of three or more replicate injections under FIA-hv-EC conditions. [c] Only a qualitative response was determined for these compounds, but a quantitative response ratio could be experimentally determined at will. [d] No response.

become so with photolytic derivatization. FIA-hv-EC could therefore be used to quickly and efficiently evaluate a large number of potential HPLC-hv-EC analytes. Together with dual-electrode response ratios, FIA-hv EC may also be used in quality control studies to determine the qualitative purity or even the quantitative levels of agricultural chemicals. Such methods could also serve as rapid screens for environmental or crop samples, where some preliminary information was available to indicate which agricultural chemicals might be present.

Dual Electrode Response Ratios for Agricultural Chemicals. Table I also summarizes the dual glassy carbon electrode response ratios obtained in the parallel (side-by-side) mode for these thiophosphates, at potentials of +0.9 and +0.8 V. These numbers represent the average ± standard deviation for at least three replicate analyses, with the standard deviations less than ±5% of the average value. The fact that these ratios are experimentally different suggests that the starting analytes are not photolytically leading to the same single EC-active species. We had thought that the main species might be inorganic sulfide (S^{2-}), which seemed likely given the starting structures. The observed results suggest that more than a single EC-active entity is being formed, perhaps in different ratios when the same mixture of species is formed. None of these species, as yet, have been isolated or identified. The dual-electrtode ratios for these chemicals, especially by HPLC-hv-EC, provide an important quantitative approach for analyte identification.

Minimum Detection Limits for Agricultural Chemicals by HPLC-hv-EC. Table II lists minimum detection limits for these agricultural chemicals, in some instances for both 20- and 200-μL injections. Using the larger volumes has not appreciably sacrificed peak shape, peak height, or overall resolutions. However, the approach

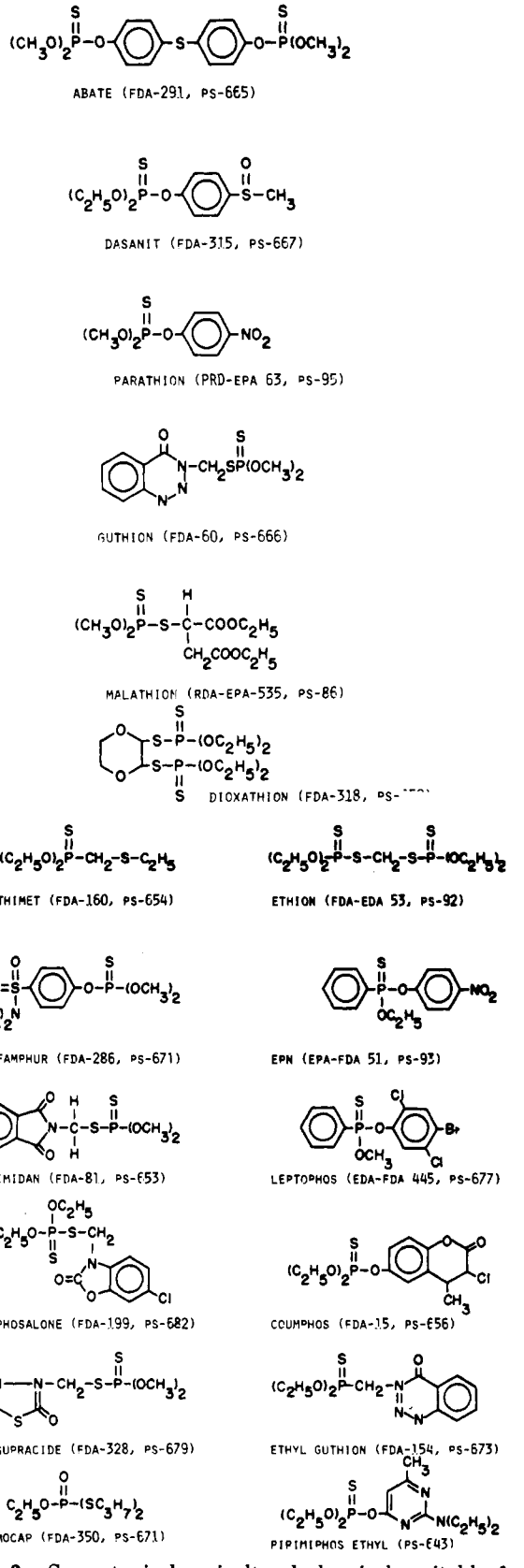

Figure 2. Some typical agricultural chemicals suitable for HPLC-hv-EC.

does significantly lower MDLs, often by almost 1 order of magnitude. In almost all cases, MDLs are below 100 ppb (parts per billion). MDLs are, in part, a function of dead volume effects, due to the lengths and dimensions, as well as configuration, of the Teflon tubing. We have estimated that dead volume effects could be reduced by about 1–2 orders of magnitude. There are certain alternative winding configurations for the tubing that could provide substan-

Table II. Minimum Detection Limits and Capacity Factors by HPLC-hv-EC[a]

compound name	MDL (20 μL) (200 μL),[b] ppm	capacity factor (k')
famphur	1.25	0.22
EPN	0.63 (80 ppb)	1.64
imidan	0.31 (40 ppb)	0.43
ethyl guthion	0.31	0.78
supracide	0.16	0.33
pirimiphos ethyl	1.25	2.41
guthion	0.20	0.52
malathion	0.5 (50 ppb)	0.47
parathion	0.2 (20 ppb)	1.11
ethion	1.25 (40 ppb)	3.12
abate	0.33 (80 ppb)	3.84
phosalone	0.75	1.16
thimet	0.63 (50 ppb)	1.43
dioxathion	0.63	1.62
dasanit	0.63	0.32
mocap	1.4	0.80

[a] HPLC conditions used an Alltech C-18 reversed-phase column, 10 μm, 25 cm × 4.6 mm i.d., with a mobile phase of MeOH/0.2 M NaCl (70/30), at a total flow rate of 1.2 mL/min. Parallel dual glassy carbon electrodes at +0.9 V/+0.8 V. [b] Indicates volume of HPLC injections as either 20 or 200 μL. MDLs in terms of mass rather than concentration can be obtained by multiplying volume injected times concentrations determined at that level injected.

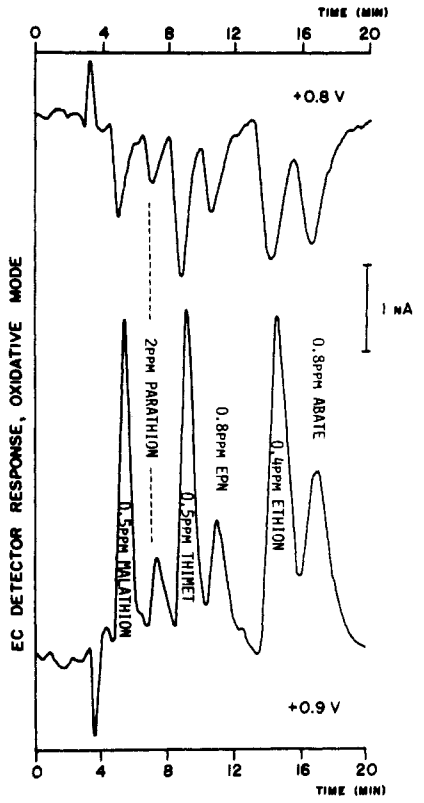

Figure 3. HPLC-hv-EC dual detector chromatograms for a mixture of standard thiophosphate ag chemicals. HPLC used an RP C-18 10-μm column with MeOH–0.2 M NaCl (70:30) mobile phase at a 1.2 mL/min flow rate and BAS GC dual electrodes.

tially lower MDLs. Current efforts are aimed at this goal.

Dual-Electrode HPLC–hv–EC Chromatogram of Standard Agricultural Chemicals and Wheat Middling (Animal Feed) Extracts for Malathion. Calibration plots for these agricultural chemicals have been determined from the MDLs to the ppm levels (40–50 ppm) by using 200-μL injections at all times. Each calibration plot was prepared by using four to five individual concentration points, and such plots have coefficients of linearity of at least 0.999. These were all determined at two oxidative potentials, +0.9 and +0.8 V with the dual parallel glassy carbon electrode. Capacity factors (k') have also been determined, Table II. In most cases, these chemicals can be adequately resolved from each other, but it would be unusual to have an environmental sample or commercial formulation that contained two or more of these analytes. HPLC retention times should serve as strong evidence for a suspected pesticide residue, especially when this is combined with the above hv-EC approaches.

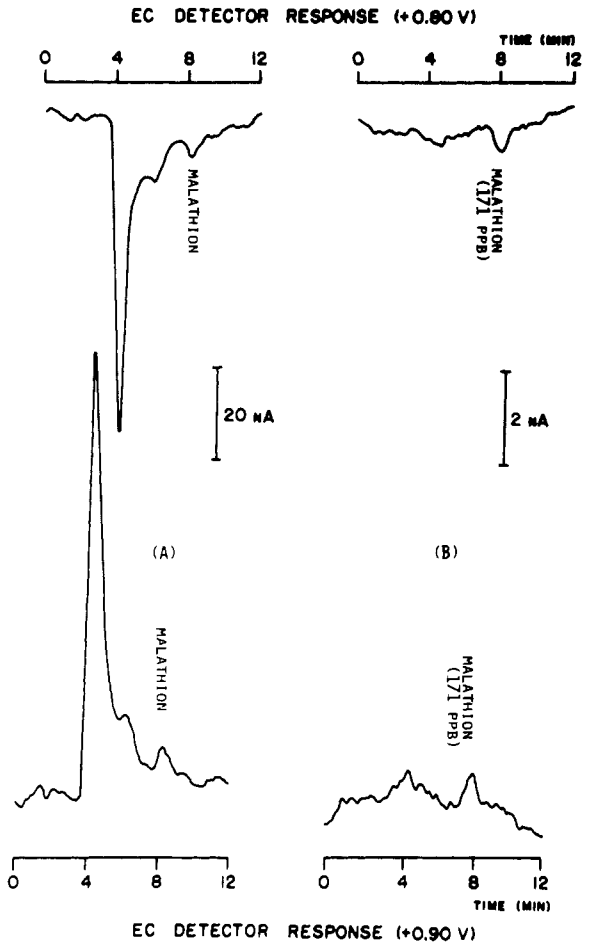

Figure 4. HPLC-hv-EC analysis of the FDA sample extract of wheat middlings (animal feed) using lamp on conditions for photolysis. HPLC used an RP C-18 column, 10 μm, 25 cm × 4.5 mm i.d., a mobile phase of MeOH (60)–0.2 M NaCl (40) at 1.2 mL/min, and 200-μL injections. (A) FDA sample extract containing malathion; (B) malathion standard at a 171-ppb level. BAS dual GC electrode cell at +0.8 and +0.9 V.

Figure 3 illustrates typical HPLC–hv–EC chromatograms for a mixture of six standard thiophosphates at the 0.4–2.0-ppm levels. These are dual EC chromatograms obtained from a single injection with the EC in the parallel mode. The total analysis time here is less than 20 min. These responses were obtained with the photolysis lamp turned on, and when this is switched off, no responses at all are observed with the same injection. Thus, these chromatograms are due to the generation, photolytically, of new ionic species that are EC active. A large number of analogous HPLC–hv–EC chromatograms have now been generated for the other thiophosphates.

Figure 4 consists of a set of two dual-electrode chromatograms: (A) chromatograms derived from a wheat middling extract prepared by the Boston District Office of the U.S. FDA. Figure 4B is the same set of chromatograms obtained under identical conditions but now when

Table III. Analysis of Wheat Middling Samples for Residues of Malathion Pesticide[a,b]

sample no.	HPLC-hv-EC no. 1,[c] ppb	HPLC-hv-EC no. 2,[c] ppb	BDO-FDA results no. 1,[d] ppb
BLK-50	0.0	0.0	0.0
853-50	131	134.2	130
856-50	96	104.2	90
857-50	74.6	107.4	90

[a] HPLC-hv-EC conditions used an analytical column, 10 μm, 25 cm × 4.6 mm i.d., RP C-18 with a mobile phase of MeOH (60)/0.2 M NaCl (40) at a 1.2 mL/min flow rate. BAS glassy carbon dual electrodes operated at +0.9 and +0.8 V oxidatively. All HPLC-hv-EC injections were 200 μL. [b] Samples were derived from wheat middlings used as animal feed in the United States. [c] HPLC-hv-EC results were obtained by using the above conditions with freshly prepared external standard of malathion. Confirmation of direct analysis results was done by method of standard additions with four to five spikings of organic extracts with malathion. Direct results and standard additions results agreed within experimental error on two samples. [d] These results obtained within the BDO-FDA using the accepted (PAM) method for malathion in crop analysis using GC-FPD specific for phosphorus. GC conditions used a 1 + 1 column isothermally.

a standard sample of malathion is analyzed. The wheat middling extract in Figure 4A appears to contain a low level of malathion, and this peak has the same capacity factor as the standard. Also, the dual-electrode response ratio for this suspected malathion peak in Figure 4A is identical, within experimental error, to that obtained from the standard malathion (Figure 4B). Thus, both HPLC retention time data and dual-electrode response ratio data support a malathion residue present in this particular sample.

We have analyzed three samples of wheat middling extracts and have repeated the extraction and HPLC-hv-EC analyses on these samples on two separate occasions. Each individual analysis of each sample extract was repeated at least in duplicate or triplicate, depending on the total volume of sample present. Analyses of these same sample extracts were also performed, at about the same time, in the Boston FDA laboratories. The FDA analyses used the now accepted pesticide analytical method ("Pesticide Analytical Manual", 1982, 1977) using GC with electron capture (ECD) and flame photometric detection (FPD) simultaneously. All of these wheat middling extract results are summarized in Table III. Reproducibility is good from day to day for the HPLC-hv-EC results, and agreement between these and the FDA results is also acceptable. The blank extract did not show any malathion present.

In order to further validate these newer HPLC-hv-EC methods of analysis for agricultural chemical residues, we have performed two standard addition studies, one of which is presented, Table IV. This was performed by combining the three sample extracts from Table III and diluting with hexane to give the final malathion level (32 ppb). This could not be accurately quantitated since it was now below the MDL for malathion, but it could be assessed knowing the volumes of the original solutions and concentration levels. This has been termed the "blank", because it was the level of malathion present before any standard additions. Table IV indicates five serial additions of malathion to the blank solution. Also indicated are the concentrations of malathion then determined using external standards, the levels obtained when the background level of malathion was substracted, and a final column of

Table IV. Method of Standard Additions Applied to Wheat Middling Extracts for Confirmation of Analysis[a]

sample no.	[malathion] spiked, ppb	[malathion] determined, ppb	background subtracted, ppb	% recovery[c]
blank[b]	0.0	32		
1	880	870	838	95.2
2	440	470	438	99.5
3	220	245	213	96.8
4	110	120	88	80.0
5	55	95	63	114.0

[a] All standard addition analyses and original sample were determined for malathion by HPLC-hv-EC methods (200-μL injections). [b] Blank was the original FDA sample diluted with a known volume of hexane to a final malathion concentration level of 32 ppb. Known concentrations and volumes of malathion standard were then added in steps to this original diluted sample and analyzed at each stage of addition. [c] Average percent recovery = 97.1% and standard deviation = 10.8% for all five percent recoveries here.

percent recovery. A plot of five serial standard additions in terms of concentration determined vs. peak height on the EC detector provided a standard addition straight line that could be extrapolated back to the original concentration that must have been present. This came out to be about 40 ppb, whereas the determination of the blank solution at the start was about 32 ppb. An identical study was done on the second batch of three samples, again combined, with an initial blank malathion level of 110 ppb. The standard additions method on this sample gave a concentration of about 123 ppb. For the data in Table IV, the average percent recovery was 97.1 ± 10.8% (SD) (n = 5). Duplicate or triplicate injections were made at each level of standard addition for both samples. For the second study, the average percent recovery was 98.7 ± 8.6% (SD) (n = 4). Thus, the direct method of analysis using external standards injected alongside the samples provides quantitative results that are accurate and precise. Reproducibility of these analyses within a given day or day to day is also good.

CONCLUSIONS

We have demonstrated the interfacing of HPLC with reversed-phase conditions, with on-line, continuous photolytic derivatizations of HPLC analytes, followed by single- or dual-electrode EC detection. These HPLC-hv-EC approaches have been evaluated with regard to their possible usefulness in the analysis of a variety of environmentally useful organic thiophosphates. Minimum detection limits are sufficiently low that the methods can be applied to crop extracts at levels routinely encountered. Comparative studies have been performed using the accepted GC method of analysis for malathion with this newer HPLC-hv-EC approach, with excellent agreement between the methods. These methods have been further validated in two separate, but related, studies using repetitive standard addition methods, again with a high degree of agreement vs. the external standard method. Reproducibility, accuracy, and precisison have all been acceptable. It is hoped that these newer methods, using HPLC or flow injection analysis, will soon find widespread acceptance and application.

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Registry No. Guthion, 86-50-0; *o,p'*-kelthane, 10606-46-9; malathion, 121-75-5; lorox, 330-55-2; famphur, 52-85-7; EPN, 2104-64-5; imidan, 732-11-6; ethyl guthion, 2642-71-9; coumaphos, 56-72-4; leptophos, 21609-90-5; supracide, 950-37-8; pirimiphos ethyl, 23505-41-1; parathion, 56-38-2; ethion, 563-12-2; abate, 3383-96-8; phosalone, 2310-17-0; thimet, 298-02-2; dioxathion, 78-34-2; dasanit, 115-90-2; mocap, 13194-48-4.

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Determination of Oxamyl Residues in Peppermint Hay and Oil Using a Radioisotope Dilution Technique

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Procedures are described for the determination of the nematocide oxamyl [methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate] in peppermint hay and oil. After extraction of the hay with ethyl acetate, oxamyl residues are cleaned up on an alumina column and hydrolyzed with alkali to the oxime (methyl *N',N'*-dimethyl-*N*-hydroxy-1-thiooxamimidate), followed by additional cleanup on silica gel, formation of a trimethylsilyl ether derivative, and quantitation by sulfur specific flame photometric gas chromatography. Peppermint oil is diluted with toluene and extracted with water, followed by hydrolysis, silica gel cleanup, derivative formation, and gas chromatography as described for hay. A radioisotope dilution method was used to compensate for low recoveries. The method is sensitive to 0.05 ppm in peppermint hay and to 0.1 ppm in peppermint oil. Although low residues of oxamyl were found in fresh peppermint hay at harvest, no residues were detected in peppermint oil.

The nematocide oxamyl [methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate] has shown considerable promise for the control of the nematode *Longidorus elongatus* in mint fields of Oregon. Registration of oxamyl for the control of this pest depends in part on the availability of a sensitive and specific method for residue determinations in this crop.

The first analytical method for oxamyl was described by Holt and Pease (1976), who hydrolyzed oxamyl to its oxime and determined this more stable and volatile derivative by sulfur-specific flame photometric gas chromatography. A spectrophotometric method was presented by Singhal et al. (1977) and HPLC was employed by Thean et al. (1978), Davis et al. (1978), and Chiba et al. (1983).

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