INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again–beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University
Microfilms
International
300 N. Zeeb Road
Ann Arbor, MI 48106

GOODIN, RICHARD REYNOLDS

DETERMINATION OF METHYL AND ETHYL PARATHION RESIDUES ON VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

MIDDLE TENNESSEE STATE UNIVERSITY M.S. 1983

DETERMINATION OF METHYL AND ETHYL PARATHION RESIDUES ON VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Richard Reynolds Goodin

A thesis presented to the
Graduate Faculty of Middle Tennessee State University
in partial fulfillment of the requirements
for the degree Master of Science

May, 1983

DETERMINATION OF METHYL AND ETHYL PARATHION RESIDUES ON VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

APPROVED:

Graduate Committee:

Gale J. Clark
Major Professor

Dan D. Scott
Head of the Department of Chemistry

Mary Martin
Dean of the Graduate School

ABSTRACT

DETERMINATION OF METHYL AND ETHYL PARATHION RESIDUES ON VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Richard Reynolds Goodin

Methyl and ethyl parathion were analyzed on vegetable leaves using reverse phase HPLC with electrochemical detection in the amperometric mode. A sample preparation method was developed which utilized serial solvent extraction of the blended leaf material followed by a partition step and filtration. Parathions were routinely extracted with better than 90% recovery and when subjected to HPLC analysis could be detected to 100 ng/g without sample concentration.

Determinations of ethyl and methyl parathion were performed using vegetables purchased in markets and spiked with a known amount of pesticide. The selectivity of electrochemical detection made it unnecessary to chromatographically resolve the plant peaks from the pesticides and allowed the rapid analysis of leafy green vegetables for parathions.

TO PATRICIA

FOR HER SUPPORT

AND UNDERSTANDING

ACKNOWLEDGEMENTS

The writer would like to thank Dr. Gale Clark for his continued support and advice throughout this project. The writer is also grateful to other members of the Chemistry and Physics staff who have contributed their time, equipment, and laboratory space.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The organophosphorous pesticides which include the parathions, fenitrothion, and malathion are widely used in agriculture because of their broad spectrum insecticidal properties and their rapid decomposition. This latter characteristic has placed these pesticides, particularly the parathions, among the more popular replacements of organochlorine pesticides. The organochlorine compounds have been greatly restricted in their use by the Environmental Protection Agency (EPA) due to their persistance in the environment (1). Although short-lived, ethyl and methyl parathion are more acutely toxic than the organochlorine compounds they replace (2). It is therefore highly desirable to be able to analyze for residues of these pesticides on foodstuffs.

Prior to the introduction of chromatographic methods, ethyl parathion (diethyl p-nitrophenyl phosphorothionate) and methyl parathion (dimethyl p-nitrophenyl phosphorothionate) were determined by colorimetric (3), polarographic (4), and cholinesterase inhibition (5)

methods. These methods could not quantify pesticide residues at the low levels required for trace analysis and also suffered from many interferences.

More recent methods of analysis involve the extraction of pesticides from the sample followed by chromatographic separation and detection. Gas chromatography (GC) is the method recommended by the EPA (6) and is preferred by most workers. GC offers efficient separation, and there is a wide range of detectors which are applicable to organophosphorous pesticides (7,8,9). Two of these detectors are the flame photometric detector (6), which can be made selective for compounds containing sulfur or phosphorous, and the electron capture detector (10). The flame photometric detector is selective for parathions because these compounds contain both sulfur and phosphorous. This detector is linear in the phosphorous mode between 0.4 and 400 nanograms of parathions injected. In the sulfur mode response is not linear and sensitivity is lower than the phosphorous mode. Electron capture detectors are more sensitive than the other detectors reviewed here for many of the organophosphorous pesticides but do not respond to all of these compounds. One disadvantage of GC is the possibility of thermal degredation of parathions at the high temperatures which are necessary to vaporize them, as suggested by Paschal (1). In addition, elaborate sample

preparation is usually required in order to obtain acceptable results.

MacNeil and Frei (11) have reviewed thin layer chromatographic (TLC) methods for the organophosphorous pesticides. They also report their own analysis of lettuce and carrots for organophosphates. The resolution of organophosphate pesticides from vegetable extracts is possible due to the advent of plates with small uniform particles and thin uniform coatings. Drawbacks of TLC include the difficulty of quantifying pesticide spots and the necessity of spotting no more than a few microliters of sample.

Bulk electrochemical analysis, although an old method, is still used to detect parathions in complex mixtures. Smythe and Osteryoung (12) showed that parathions could be detected in solutions as dilute as 2.63 ng/ml by differential pulse polarography. They also reported electrode reactions for the reduction of the nitro group on the ring of the parathion molecule. Figure 1 shows these reactions which give either a 4 or 6 electron reduction. The 6 electron reduction takes place at pH values below five and offers the possibility of greater sensitivity for electrochemical detection. Simultaneous work done in this laboratory by Gordon Woodroof (13) confirmed the sensitivity of electrochemical methods.

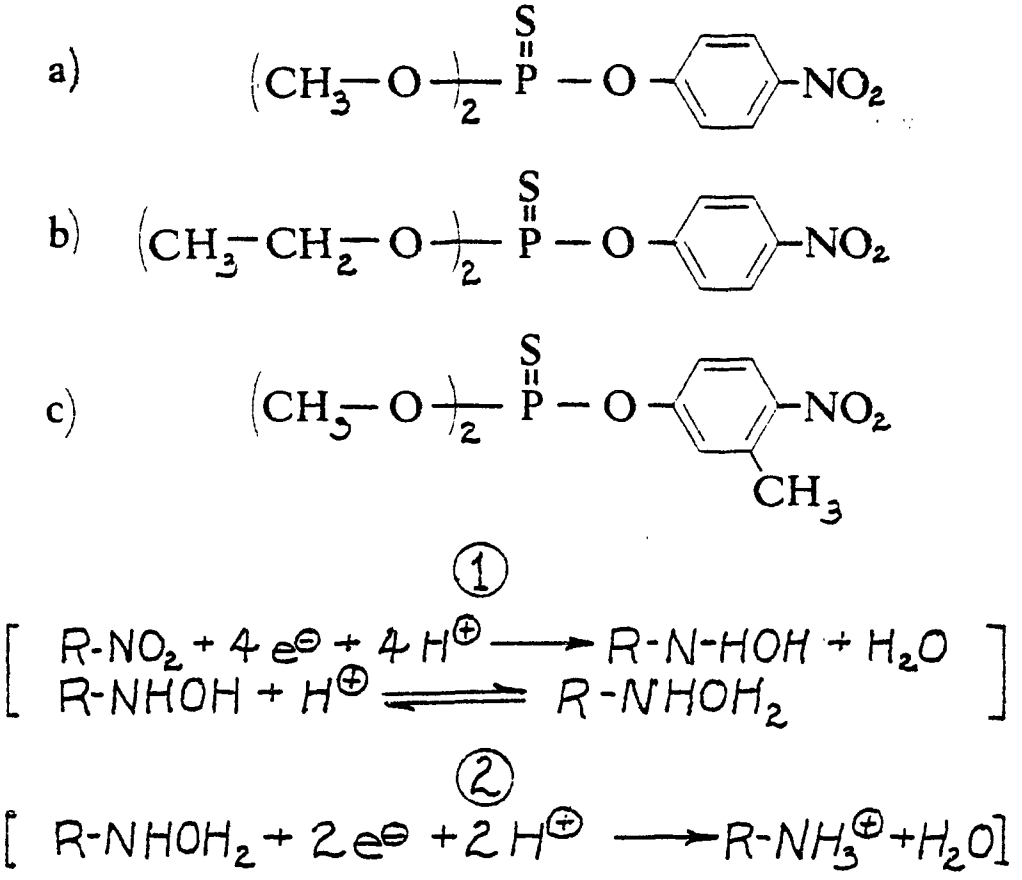

FIGURE 1. Electrode reactions for the reduction of parathions: (a) methyl parathion; (b) ethyl parathion; and (c) fenitrothion; (1) takes place at pH values above 5; (2) at pH values below 5 this reaction occurs in addition to reaction 1.

High performance liquid chromatography (HPLC) offers a gentle method for the separation and detection of organophosphorous pesticides in samples at ambiant temperatures. New bonded reverse phase columns can tolerate samples with little cleanup if care is taken to remove solids and proteins, and as much as 50 microliters of sample can be injected without degradation of column performance (14). When compared to GC where 5 microliters is a large injection volume, this column capacity is an advantage for HPLC methods.

Paschal (1) reported the analysis of runoff water for parathion residues using HPLC with UV detection. Injections containing approximately 3 nanograms of ethyl or methyl parathion gave measurable chromatographic peaks. In general, UV detectors are dependable but lack the sensitivity of GC detectors. The UV detector is also comparatively unselective and many interferences are likely with complex matrices. Despite the limitations of UV detection, several workers have reported its use in this analysis of pesticides (14,15,16,9).

If a selection of detectors with detection limits in the picogram range were available, HPLC would be a more generally accepted method for the separation and detection of pesticides. Electrochemical detection (EC) has been used in a number of analyses to provide improved detection limits over UV and other HPLC detectors. Excellent selectivity can

also be obtained by using EC detection. Using the oxidative mode, Fenn and co-workers (17) demonstrated detection limits for catecholamines between 2 and 3 picograms per milliliter. Detection limits in the 40 nanogram per milliliter or better range have been demonstrated for acetominophen (18), chlorinated analines (19), morphine metabolites (20), electroactive carbamate pesticides (21,22) and phenols (23), to mention just a few.

Most electrochemical detectors operate by controlling an applied voltage between working and auxiliary electrodes of a small volume flow cell. The resulting chromatogram is a record of the changes in faradiac current with respect to time. This current is proportional to the concentration or reducible or oxidizable compounds at the working electrode for the operating voltage selected. Techniques using AC (24,25) differential pulse (26,27) and constant unmodulated voltage have been compared by MacCrehan (28), Swartzfager (26), and Mayer (29). They report the greatest sensitivity and the lowest instrumental noise in the amperometric mode and the greatest selectivity in the differential pulse mode.

The appropriate potential of the working electrode can be established by referring to classic current voltage polarization curves or by the repeated injection of the compounds of interest at increasing voltages to construct a response curve from the chromatograms. The selection of

operating voltage is a major factor in obtaining optimum sensitivity and selectivity.

In order to use EC detection, the mobile phase must be to some extent conductive. This usually requires that reverse phase columns be used with relatively polar mobile phases. Solvent programming requires the correction of large changes in background current. In addition, the solid electrodes which are commonly used are subject to the adsorption of compounds to the electrode surface (30,31).

The evaluation of flow cell designs constitute a large part of work published on HPLC with electrochemical detection (HPLC-EC). Figure 2 shows a typical flow cell design which was used by MacCrehan (28). Other researchers utilize polarographic (32,33,34,35,36), coulometric (37,38, 39,40), dual working electrode (41,42), and potentiometric (43) designs. Beauchamp and Boinay (44) recently reviewed flow cell design parameters. They designed a cell which is capable of being configured as a wall-jet or laminar flow. Carbon or mercury electrodes can be used and cell volume can be adjusted. An in-depth comparison of electrodes for HPLC electrochemical detectors can be found in work published by Lund (45), Stulik (46), and MacCrehan (47).

EC detection is a sensitive method, but perhaps as important is the selectivity advantage of EC. Detection can be made selective by choosing a working voltage slightly more negative than the halfwave potential of a reducible

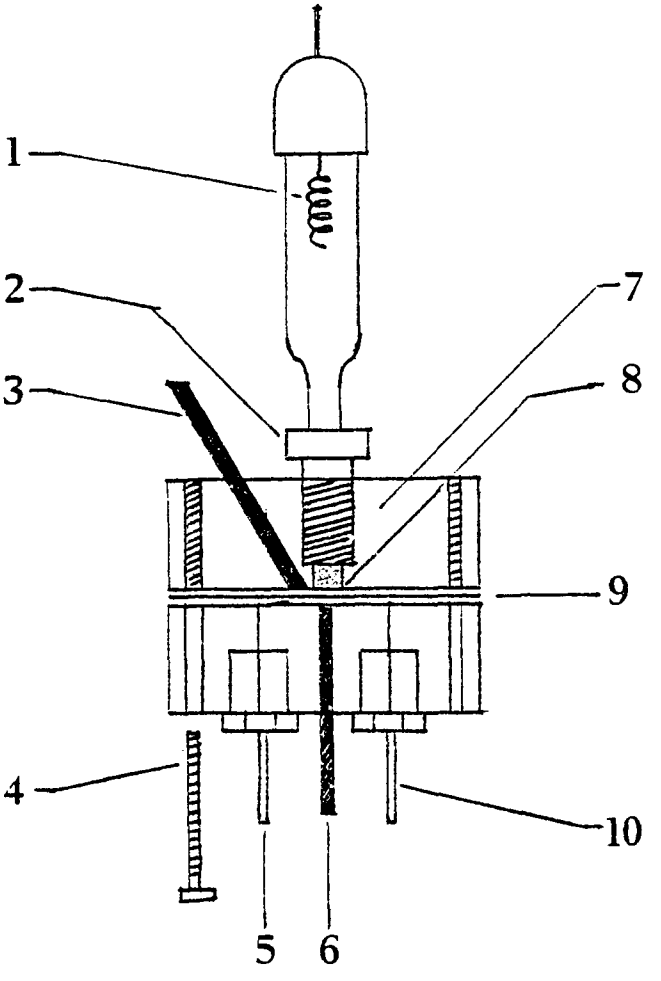

FIGURE 2. Typical electrochemical flow cell design: laminar flow type. (1) reference electrode, Ag/AgCl; (2) threaded Teflon holder; (3) auxiliary electrode, platinum; (4) assembly screw; (5) solution outlet; (6) working electrode, gold amalgamated with mercury; (7) Nylon body; (8) porous Vycor frit; (9) polyethylene cell gasket; (10) solution inlet.

compound of interest or slightly more positive than the halfwave potential of an oxidizable compound of interest. When the working voltage is selected in this way, the maximum sensitivity is attained, but compounds requiring a higher voltage to be reduced or oxidized will not be seen. Few electroactive compounds can be detected by both the oxidative and the reductive modes so reducible compounds will not be seen in the oxidative mode and oxidizable compounds are not seen in the reductive mode. Many compounds are not electroactive at all within the voltage ranges of available electrodes and electrolytes. Therefore, complex samples can give simple chromatograms and co-eluting compounds can be resolved by selectively detecting only one. Figure 3 illustrates the selectivity of electrochemical detection. In Figure 3, a hypothetical mixture of four compounds was separated by HPLC and detected by UV absorption and by an electrochemical detector. Current versus applied voltage curves for compounds A, B, and C are shown in Figure 3a. Compound D is not electro-active within the voltage range shown but is a good UV absorber. Compounds A and B can be oxidized at potentials E_1 and E_2 to give an anodic current signal. Compound C can be reduced at potential E_3 to give a cathodic current. Figure 3b shows chromatograms of the hypothetical mixture by UV detection and EC detection at the three different working potentials.

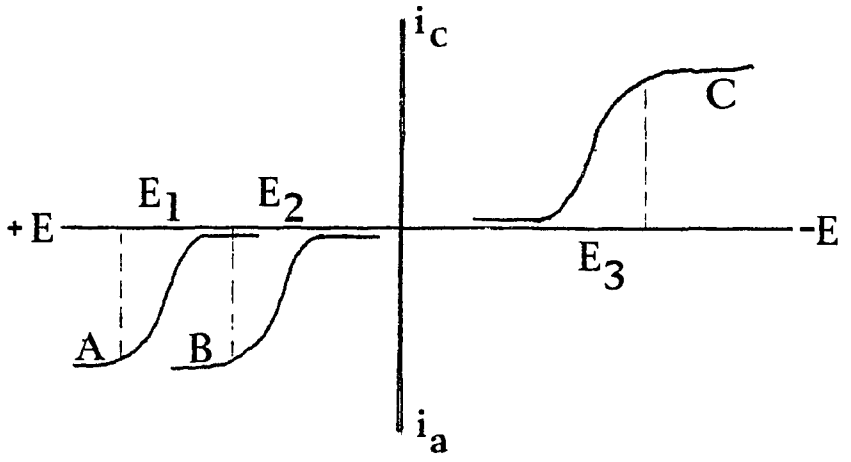

FIGURE 3a. Current-potential curves of compounds A, B, and C.

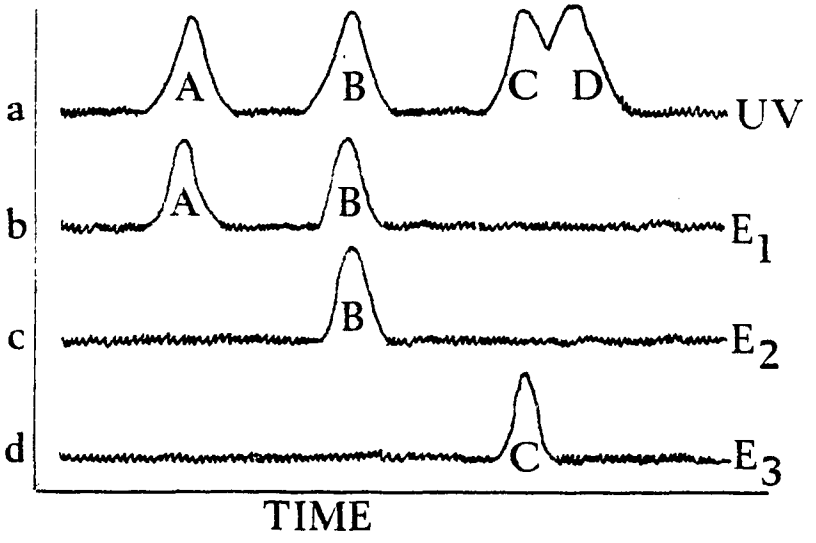

FIGURE 3b. Chromatographic detection of a sample mixture containing A, B, C, and D. (a) by UV detection; (b) by EC detection at potential E_1; (c) by EC detection at potential E_2; (d) by EC detection at potential E_3.

Techniques which make use of electrochemical oxidation have been much more widely used than reduction and generally show lower detection limits, but reductive electrochemical detection has been demonstrated to be a sensitive method. Injections of 2 nanograms or less of N-nitrosamines (48,34), organometallics (49,28,27), and reductible pesticides (47,50,51) can give measurable chromatographic peaks.

In separations where reduction is utilized, oxygen must be removed from the mobile phase to avoid high background current. MacCrehan (52) reports the placement of the entire HPLC instrument in a nitrogen purged box. He also reduces the mobile phase against a mercury pool prior to outgassing in order to remove all reducible contaminants.

In a technique more suitable for routine use, Smiley (53) demonstrated that careful outgassing could lower background current to the extent that ethyl and methyl parathion could be detected upon injecting approximately 2.0 nanograms. He also suggested that this could be further lowered by reducing the background current which was due to the presence of reducible species other than oxygen. In the determination of methyl and ethyl parathion in runoff water reported by Smiley, EC detection was shown to be feasible at water concentrations as low as 10 ng/ml. In order to achieve determinations at these low levels, samples were preconcentrated by trapping the pesticides on a

macroreticular resin and elution with a small volume of acetonitrile. The linear working range of the amperometric detector was shown to be between 5 and 1000 nanograms of parathions injected. In an interference study, only six out of fourteen pesticides commonly used in the middle Tennessee area were electroactive, and several of these had halfwave potentials which were dissimilar enough for the selective detection of only one or two of the fourteen. No interfering peaks were found in the water samples with either UV or EC detection, probably indicating that the water samples were relatively clean.

Because of the selectivity of EC detection, it was decided to evaluate the use of this detector in the analysis of pesticide residues from plant material. For this work the samples chosen for analysis were leafy green vegetable crops. These crops are often treated with methyl and ethyl parathion and other organophosphate pesticides to control aphids. Extraction of the pesticides from the plant material was expected to give a large number of potentially interfering compounds in addition to the pesticide. It was hoped that the selectivity of EC detection would make the complete chromatographic separation of these many compounds unnecessary and would therefore allow a quick determination of methyl and ethyl parathion.

CHAPTER II

EXPERIMENTAL

(A) Materials

Instrumentation. Chromatography was accomplished with a Varian Series 4100 liquid chromatograph for all work using EC detection. This chromatograph, shown in Figure 4, was modified to facilitate nitrogen purging of oxygen in the mobile phase. All Teflon tubing was replaced with 316 stainless steel tubing to prevent reentry of oxygen into the mobile phase. All metal parts not made of 316 stainless were removed or replaced to prevent corrosion.

All samples were introduced with a Rheodyne Model 7105 variable volume injector. A reversible Regis octadecyl column (0.46 X 25 cm.) with 5 - micrometer support particles was used to accomplish all separations.

All chromatographic analysis utilizing electrochemical detection was done with a Varian Verichrome UV detector installed ahead of the electrochemical detector, and the absorbance signal was recorded simultaneously with the amperometric signal. Amperometric detection was

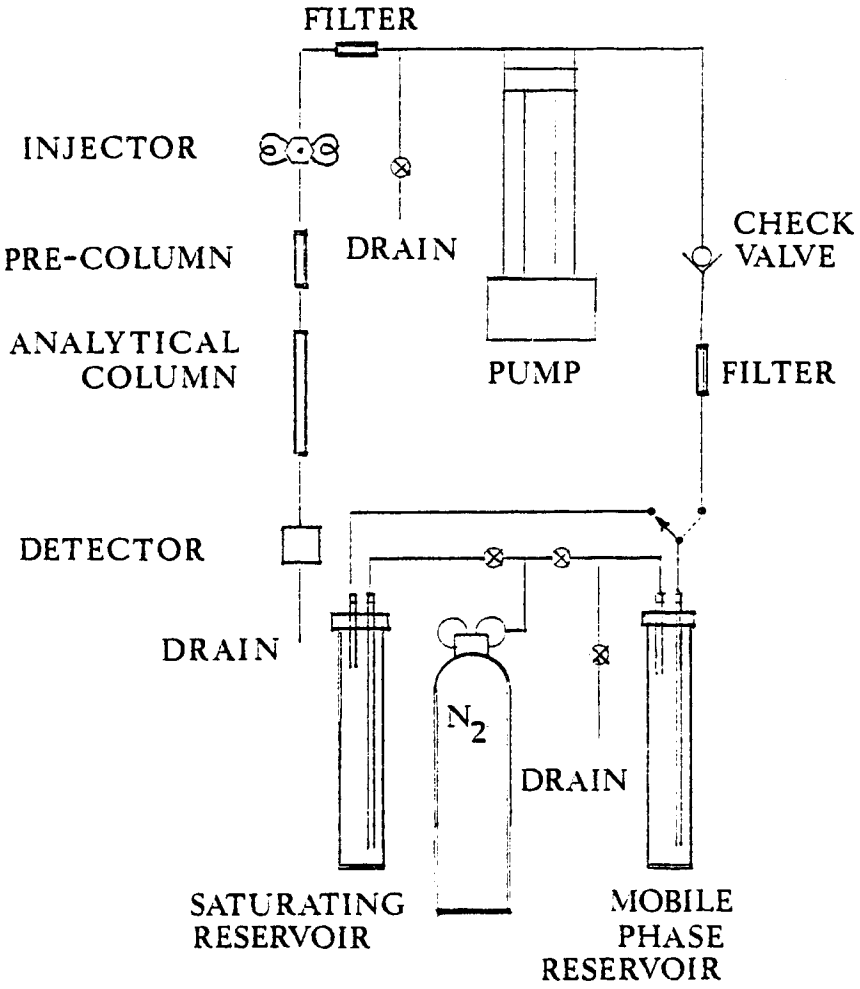

FIGURE 4. Diagram of the liquid chromatograph after modification to accommodate bubbling of the mobile phase with nitrogen.

accomplished with a Metrohm electrochemical cell supplied by Brinkmann. This cell, as shown in Figure 5, utilizes glassy carbon working and auxiliary electrodes and a silver/silver chloride reference electrode. Electronics for this cell were supplied by a Princeton Applied Research Model 170 instrument.

Polarographic detection was done with an EG&G Model 310 detector shown in Figure 6. This cell utilized a dropping mercury electrode, a platinum wire auxiliary, and a silver/silver chloride reference electrode. The electronic analyzer made for this cell by EG&G was a Model 364.

Chromatograms utilizing solvent programming and UV detection alone were obtained with a Perkin-Elmer 3B Series pump and a LC-75 UV detector with an autocontroller.

Reagents

All water was distilled and then deionized and filtered by a Milli-Q water system made by Millipore. Acetonitrile was Burdick & Jackson distilled in glass or Fisher HPLC grade.

The ultra-high purity nitrogen used for oxygen purging was supplied by Matheson or Airco. All pesticides were supplied by Chem Services. CDTA was purchased from Aldrich. The 0.05M ammonium acetate mobile phase electrolyte was prepared with high purity ammonium hydroxide and

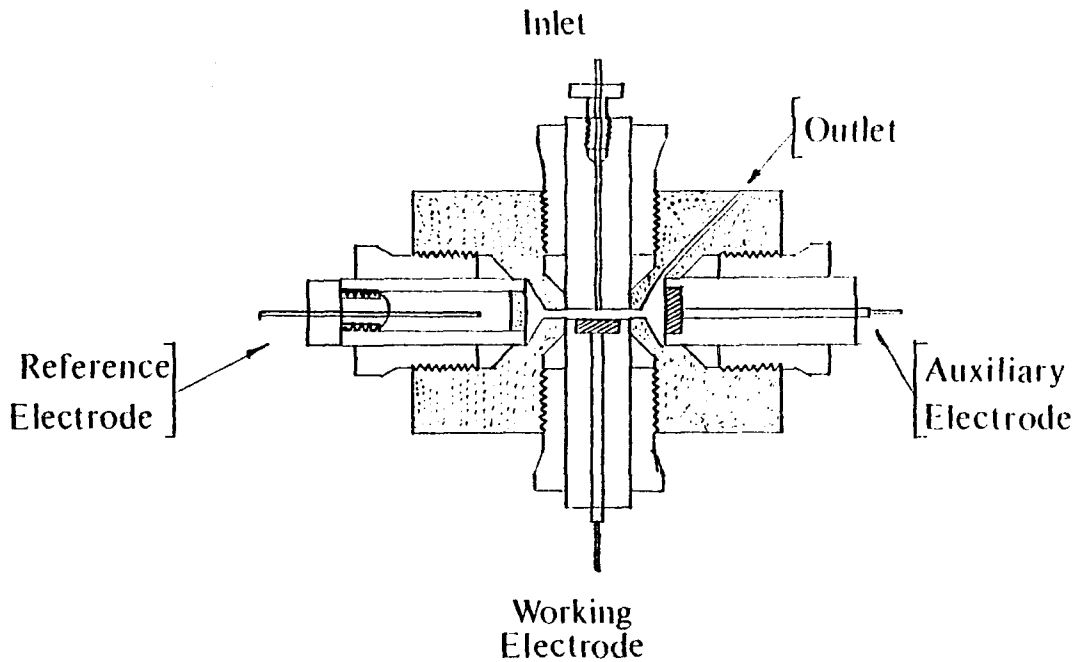

FIGURE 5. Electrochemical detector cell: wall-jet electrode, turbulent flow. Glassy carbon working and auxiliary electrodes and a silver/silver chloride reference are installed.

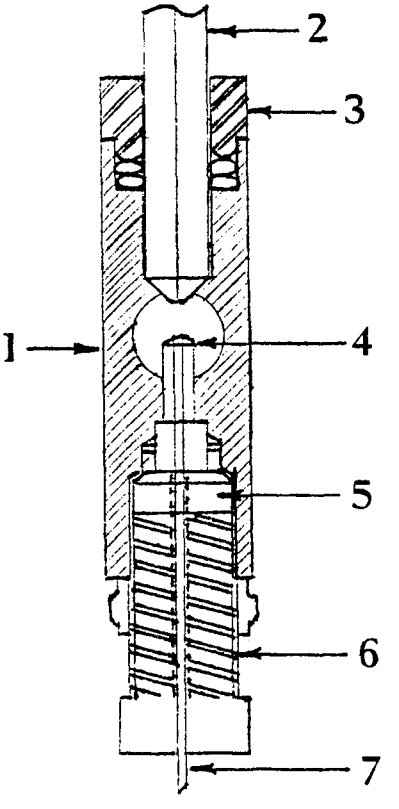

FIGURE 6. Polarographic detector cell: (1) Nylon body; (2) mercury working electrode; (3) Nylon fitting with rubber seal; (4) solution outlet which impinges upon the mercury drop; (5) inlet tube fitting; (6) adjustable inlet barrel; (7) inlet tube.

glacial acetic acid. All other reagents were ACS grade or better.

(B) Sample Preparation

Vegetables were bought in the surrounding markets. The leaves were washed and 40 grams of leaf were weighed out for each sample. Each sample was spiked with one milliliter of a pesticide solution made up in acetonitrile. The pesticide mixture was allowed to dry on the leaves before proceeding. After the acetonitrile had evaporated from the leaves, they were homogenized in 60 milliliters of acetonitrile, and the glass dishes in which the leaves were spiked were rinsed with solvent to recover pesticide not on the leaves. This homogenate was transferred to a Millipore vacuum filtration apparatus and filtered through a Whatman GF/A glass filter. The crude plant material left behind was extracted again by removing the vacuum and adding 12 milliliters of acetonitrile. After 20 minutes the vacuum was restored and the extract was drawn through the filter to be combined with the first extract in the collection flask. This extraction procedure was repeated two more times. The combined extracts were transferred to a 250-milliliter separatory funnel. Two grams of anhydrous calcium chloride were added to each sample to separate the extracts into an aqueous layer and an organic layer. Thirty milligrams of trans-1,2-diaminocyclohexane N,N,N',N'-tetraacetic acid

monohydrate (CDTA) were also added to the extract to facilitate the removal of metals from the organic layer which contains the pesticide. Each funnel was shaken well and allowed to equilibrate. The aqueous layer was removed and washed with 10 milliliters of fresh acetonitrile. The pesticide layer and the wash were then filtered through a 0.45 micrometer Rainin Nylon 66 filter and the fenitrothion internal standard was added.

All samples were brought to 100 milliliters with acetonitrile. An overnight refrigeration at $4^{o}C$ was found to precipitate unidentified components of the vegetable extracts but had no deleterious effects upon pesticide recoveries. This precipitate was removed by a repeated filtration through a 0.45 micron Nylon 66 filter. The samples were then brought to room temperature and were analyzed.

(C) Chromatographic Analysis

The mobile phase was prepared by mixing the 0.05M ammonium acetate with acetonitrile to the proportions desired for an isocratic run. The mobile phase was bubbled with ultrapure nitrogen for one hour or more to assure sufficient removal of any oxygen. A slight vacuum was applied after nitrogen bubbling to remove enough nitrogen so that small gas bubbles would not form due to the pressure drop in the analytical column. Gas bubbles, if formed,

would collect in the electrochemical flow cell and would adversely affect detector performance. The mobile phase was loaded onto the pump without exposure to oxygen by proper positioning of the valves on the liquid chromatograph.

After initiation of liquid phase flow, the electrochemical detector was attached and cyclic voltammograms were taken between +1 V and -1 V to assess the electrochemical purity of the mobile phase and the degree to which oxygen had been removed. The mobile phase was rejected if the current at -1 V was above 6 microamperes using a sweep rate of 50 mV/sec. Reducible contaminants would often result in a current peak in the voltage sweep from 0 V to -1 V. The presence of reducible contaminants producing peaks larger than 1 microampere was grounds for rejection even if the current at -1 V was acceptable. This criteria was adopted due to the adsorption effects which can often result from reducible contaminants in the mobile phase (30,31).

The electrochemical detector was polarized at the working potential and allowed to equilibrate before any solution was injected. Small aliquots of samples were bubbled with nitrogen for one minute before injecting 20 or 50 microliters of sample.

After every two injections of vegetable sample the glassy carbon electrodes would require electrochemical cleaning which was achieved by 10 cycles between +1 V and

-1 V ending at -1 V. A standard would be reinjected after each cleaning to compensate for any changes in electrode activity.

Chromatographic peaks were analyzed using routine internal standard procedures. The use of an internal standard reduced errors associated with sample outgassing and injection. Calibration standards were used to compute pesticide recoveries.

CHAPTER III

RESULTS AND DISCUSSION

Using the method of sample preparation described earlier, spinach, endive, lettuce, kale, turnip greens, and mustard greens were examined to evaluate the selectivity of EC detection for the determination of parathion residues on vegetables. In all vegetables surveyed, methyl and ethyl parathion and fenitrothion were easily resolved in electrochemical chromatograms. Simultaneous UV detection suffered from multiple interferences in all vegetables surveyed. Figure 7 shows representative chromatograms of a turnip green sample which were obtained using simultaneous UV and amperometric detection. In order to achieve baseline resolution of the pesticides and other compounds which give a UV signal, solvent programming and analysis times in excess of forty minutes were required. Figure 8 shows such a UV chromatogram. Due to large changes in background current which are induced by solvent programming, this technique was not used for HPLC with EC detection.

A detection limit study was done to find the minimum detectable quantities of methyl and ethyl parathion on

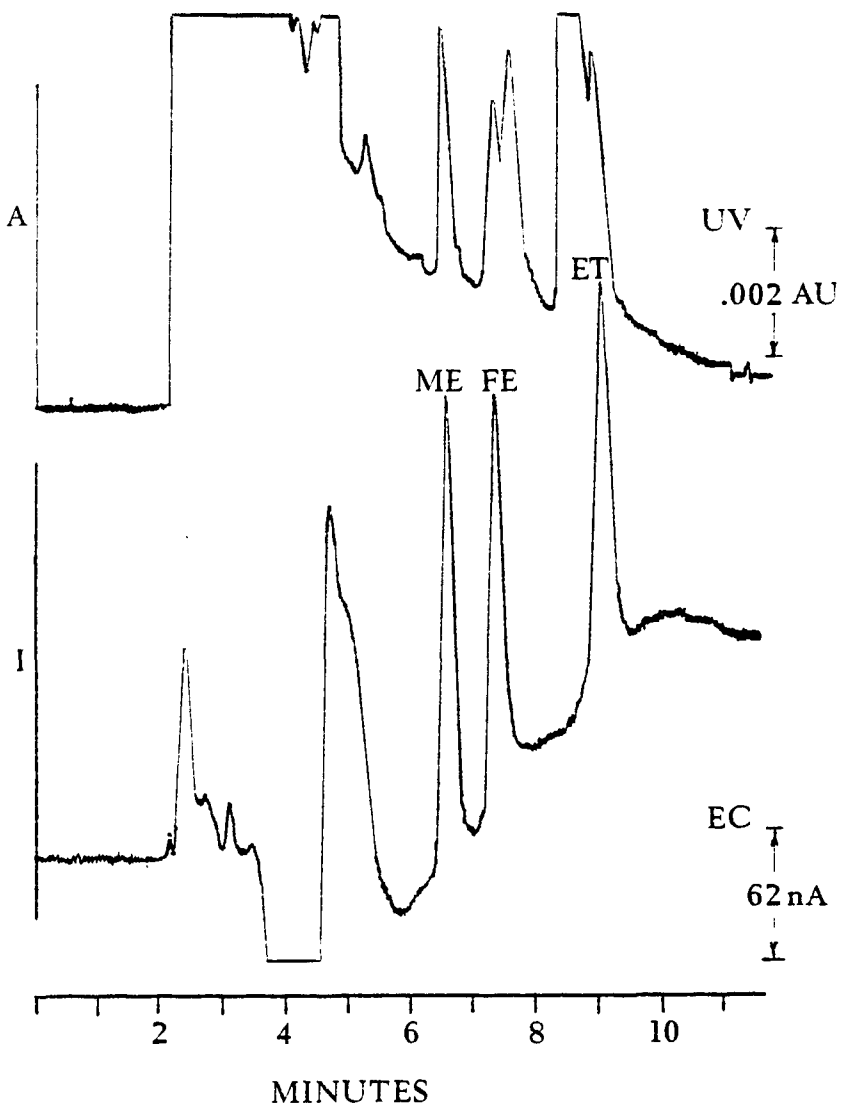

FIGURE 7. Chromatograms of a turnip green sample taken by simultaneous UV and EC detection. 2.02 and 2.26 nanograms per microliter of methyl (ME) and ethyl (ET) parathion, respectively, were present. 20 microliters was the injection size. UV detection was done at 270 nm with a 16 nm bandpass. EC detection was done at -0.97 V. The mobile phase was 64% acetonitrile and 36% 0.05 M ammonium acetate at pH 5. Flow rate was 1 ml/minute. Fenitrothion (FE) was added as an internal standard.

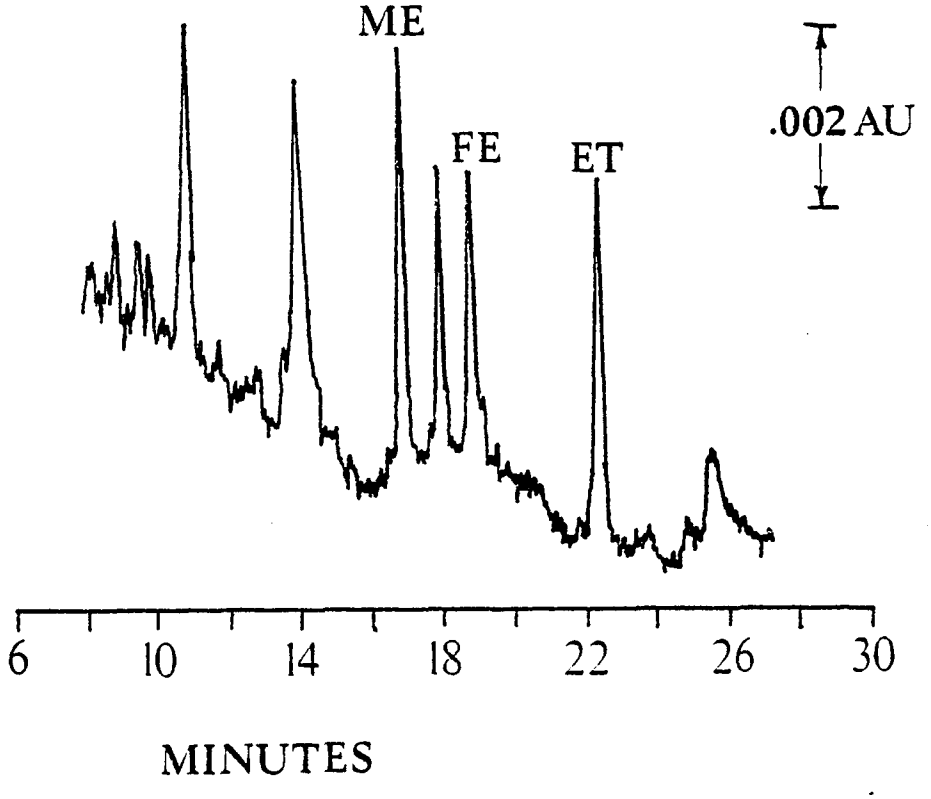

FIGURE 8. UV chromatogram of a turnip green sample taken utilizing solvent programming. This sample contained 1.8 and 1.84 ng/µl of methyl and ethyl parathion, respectively. Flow rate was 1 ml/minute and UV detection was done at 270 nm. Fenitrothion was added as an internal standard. (ME) methyl parathion; (FE) fenitrothion; (ET) ethyl parathion.

vegetables for HPLC with the Metrohm amperometric detector. The detection limits found are shown in Table 1 and are the quantity of each pesticide required to give a S/N ratio of 2:1. Figure 9 shows simultaneous UV and EC chromatograms of a pesticide standard made to the detection limits reported in Table 1. In this chromatogram it can be seen that the UV detector gives no response at the retention times of methyl and ethyl parathion or fenitrothion.

The detection limits for the UV detector used in this work were 3 and 4 nanograms injected for methyl and ethyl parathion, respectively. These limits are in agreement with previous work done in this laboratory by Smiley (53) and with work reported by Paschal (1).

These detection limits for parathions on plant material were acquired using a 20 microliter sample injection size. The injection of larger volumes was found to allow the detection of lower concentrations than reported here. However, contrary to Moye (14), who reported the injection of 500 microliters (2μg/ml solution of carbaryl) without damage to column performance (0.4 X 30 cm. ODS), it was found during this work that injections larger than 50 microliters produced significant peak broadening.

A detection limit study was also conducted for the polarographic detector made by EG&G. Using pesticide standards in acetonitrile, detection limits of 202, 226, and 382 nanograms per milliliter were found for methyl and ethyl

TABLE 1

AMPEROMETRIC DETECTION LIMITS

PESTICIDE	DETECTION LIMITS		
	pg/µl	ng/g on plant	total weight injected
METHYL PARATHION	40.4	100	808 pg
ETHYL PARATHION	45.2	113	904 pg
FENITROTHION	76.4	191	1.52 ng

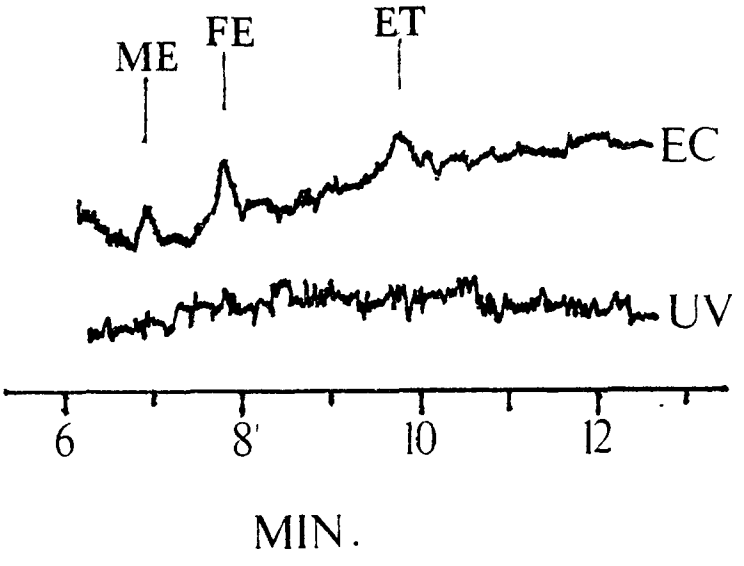

FIGURE 9. Simultaneous EC and UV chromatograms of a standard solution at the detection limits. Sensitivity for EC is 10 nA/cm and for UV is 0.001 AU/in. All other conditions were the same as Figure 7. (ME) methyl parathion; (FE) fenitrothion; (ET) ethyl parathion.

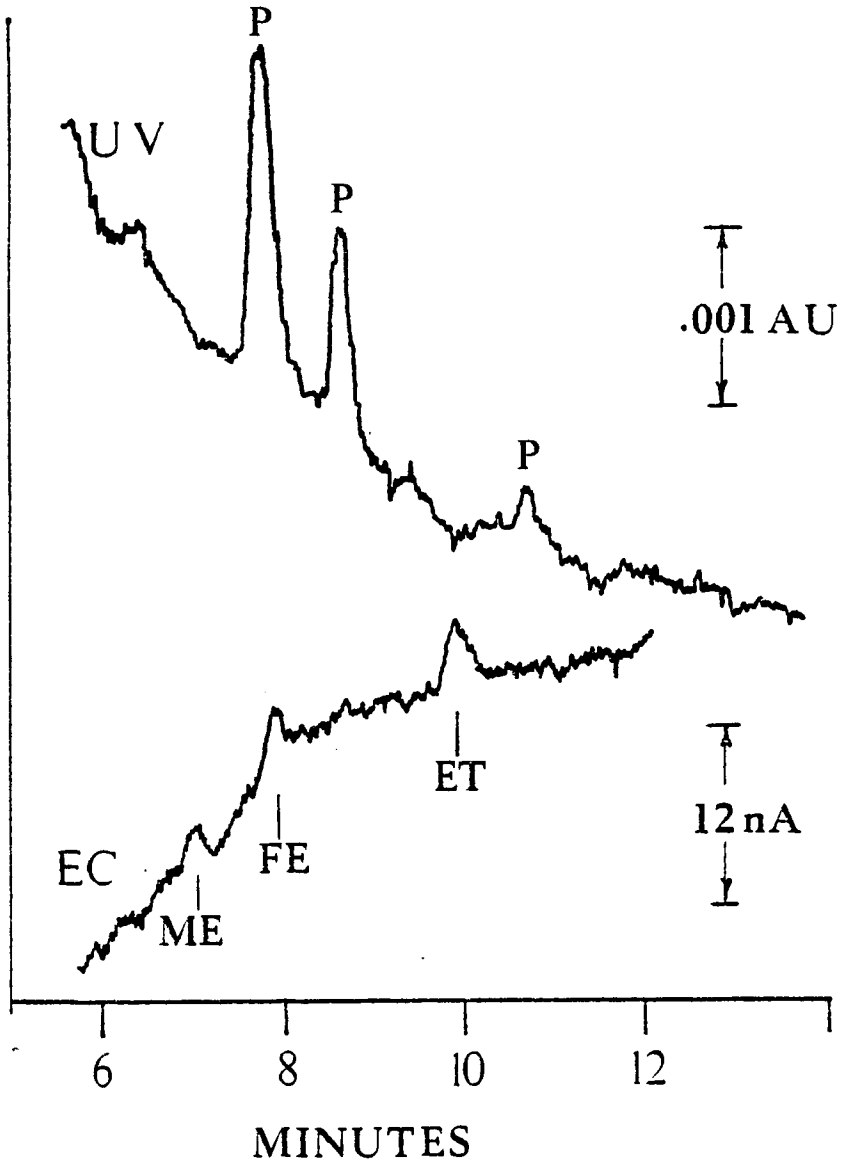

FIGURE 10. Simultaneous UV and EC detection of a mustard green sample spiked to the detection limits. (ME) methyl parathion; (FE) fenitrothion; (ET) ethyl parathion; (P) plant peaks. All conditions were the same as Figure 9.

parathion and fenitrothion, respectively. When vegetables spiked to these levels were analyzed, interfering peaks in the plant chromatogram could not be resolved from the pesticides without analysis times in excess of one hour. This detector did not exhibit the selectivity of the Metrohm amperometric detector. This may be attributed to the different catalytic properties of the mercury and glassy carbon working electrodes. In addition, large disturbances in background current which were associated with the vibrational effects of the drop knocker and with the mobile phase creeping up the mercury capillary (Barker Effect) were continuing problems. These problems with detector stability are consistent with polarographic detection work reported by Kissinger (51).

The sample preparation method developed during this work was designed to involve no more work than was necessary to protect the analytical column and to quantitatively recover the pesticide residues. Although the sample cleanup was minimal with this method, more than 50 20-microliter injections were made without significant damage to column performance. Plate counts during this period went from 10,000 to 9,312 for methyl parathion. This loss was acceptable because of the workload which was not related to this work. A guard column was used throughout this work and it was repacked frequently.

The efficiency of parathion recovery from turnip greens and mustard greens is shown in Table 2. Three different amounts of each pesticide were applied to the vegetable leaves to check for concentration dependent recovery. Each experiment involved the application of a mixed pesticide solution of methyl and ethyl parathion to three or more samples. Control samples were used to check for parathions already on the purchased vegetables. Several samples were rejected from this study because of the discovery of methyl parathion in the 3 to 4 microgram/gram range in the control.

The stability and sensitivity of EC detection was greatly enhanced by the elimination of reducible metals from the mobile phase and samples. The use of CDTA during the partition step of the sample cleanup greatly enhanced the baseline stability of the vegetable chromatograms. The contamination of the mobile phase with metals from the pump was lowered by weekly nitric acid passivation of the stainless steel in the interior of the pump syringe and in the solvent resevoirs. The use of halide salts in the mobile phase was avoided due to their known ability to promote metal corrosion. These measures required only a few minutes of time and were well worth the effort.

The amperometric detector used in this work demonstrated an advantage in selectivity over UV detection for the detection of methyl and ethyl parathion in vegetable

TABLE 2

RESULTS OF THE PESTICIDE RECOVERY STUDY

EXP. NO.	VEGETABLE SAMPLE	PARATHION PESTICIDE	(µg/ml) CONCENTRATION		AVERAGE PERCENT RECOVERY	S.D. µg/ml	N
			EXPECTED	AVERAGE FOUND			
1	MUSTARD GREENS	METHYL	2.02	1.95	96.9	.018	3
		ETHYL	2.26	2.23	99.1	0	
2	MUSTARD GREENS	METHYL	0.504	0.512	101.0	.029	5
		ETHYL	0.565	0.561	99.4	.037	
3	MUSTARD GREENS	METHYL	0.252	0.244	97.0	.007	3
		ETHYL	0.282	0.277	98.6	.016	
4	TURNIP GREENS	METHYL	2.02	1.83	90.6	.242	5
		ETHYL	2.26	2.31	101.0	.028	
5	TURNIP G.	ETHYL	0.565	0.561	99.3	.018	5
6	TURNIP GREENS	METHYL	0.252	0.221	88.7	.006	5
		ETHYL	0.282	0.267	96.0	.019	

samples. Additional advantage was found in terms of sensitivity and detection limits. However, it should be mentioned here that there are definite disadvantages to EC detection. EC detection requires considerable operator skill unlike UV detection which requires almost no operator attention. The selective advantage of EC detection allowed a savings in time spent in sample cleanup but there was considerable time spent to remove oxygen from the mobile phase. However, the purging of oxygen requires little operator attention.

The sample cleanup developed during this work showed quantitative recoveries and was sufficient to prevent damage to the analytical column. However, this sample cleanup takes no advantage of sample concentration. The addition of a concentration step would allow the detection of lower levels of pesticides on vegetables than reported here.

LITERATURE CITED

LITERATURE CITED

1. Paschal, D. C., Bicknell, R., and Dresbach, D., Anal. Chem., 49, 1551 (1977).

2. Windholz, Martha, ed., The Merck Index, 9th ed., Rahway, N.J.: Merck & Co., Inc., 1976.

3. Averell, P. R., and Morris, M. V., Anal. Chem., 20, 753 (1948).

4. Bowen, C. V., and Edwards, F. I., Anal. Chem., 22, 706 (1950).

5. American Public Health Association, Standard Methods for the Examination of Mater and Wastewater, 14th ed., New York: American Public Health Association, 1970, p. 570.

6. Sherma, Joseph, Manual of Analytical Quality Control for Pesticides and Related Compounds, U.S. Environmental Protection Agency, Environmental Toxicology Division, Health Effects Research Laboratory, Research Triangle Park, N.C., EPA-600/1-7-008 (1979).

7. Cahill, W. P., Estensen, B., and Ware, G. W. Bull. Envir. Cont. Tox., 13, 334 (1975).

8. McLean, H. R., Futagaki, S., and Leffingwell, W. Bull. Envir. Con. Tox., 18, 247 (1977).

9. Weinberger, P., and Moody, R., Environ. Sci. Technoc., 16, 470 (1982).

10. Archer, T. E., Bull. Envir. Cont. Tox., 15, 488 (1976).

11. MacNeil, J., and Frei, R., J. Chrom. Sci., 13, 279 (1975).

12. Smith, M. R., and Osteryoung, J., Anal. Chim. Acta, 96, 335 (1978).

13. Woodroof, G. L., Master's Thesis, Middle Tennessee State University, Murfreesboro, Tenn., 1977.

14. Moye, H. A., J. Chrom. Sci., 13, 268 (1975).

15. Szalontai, G., J. Chromatog., 124, 9 (1976).

16. Lawrence, J., and Turton, D., J. Chromatog., 159, 207 (1978).

17. Fenn, R. J., Sigga, S., and Curran, D. J., Anal. Chem., 50, 1067 (1978).

18. Riggin, R. M., J. Pharm. Sci., 64, 680 (1975).

19. Lores, E. M., Meekins, and Moseman, J. Chromatog., 188, 412 (1980).

20. White, M. W., J. Chromatog., 178, 229 (1979).

21. Mayer, W., and Greenberg, J. Chromatog., 208, 295 (1981).

22. Anderson, J., and Chesney, D. J., Anal. Chem., 52, 2156 (1980).

23. Weisshaar, D., and Tallman, D., Anal. Chem., 53, 1809 (1981).

24. Kemula, W., and Kuntner, W., J. Chromatog., 204, 131 (1981).

25. Hanekamp, H., Voogt, Frei, and Bos, Anal. Chem., 53, 1362 (1981).

26. Swartzfager, D., Anal. Chem., 48, 2189 (1976).

27. MacCrehan, W. A., Anal. Chem., 50, 2108 (1978).

28. MacCrehan, W. A., Anal. Chem., 53, 74 (1981).

29. Mayer, W. J., and Greenberg, M. S., J. Chrom. Sci., 17, 614 (1979).

30. Van Der Linden, D., Anal. Chem. Acta, 119, 1 (1980).

31. VanRooijen, H. W., and Poppe, H., Anal. Chim. Acta, 130, 9 (1981).

32. Kutner, W., J. Chromatog., 191, 47 (1980).

33. Baltensperger, U., Anal. Chim. Acta, 123, 107 (1981).

34. Vohra, S., and Harrington, G., J. Chrom. Sci., 18, 379 (1980).

35. Hanekamp, H. B., Bos, P., and Frei, R. W., J. Chromatog., 186, 489 (1979).

36. Buchta, R. C., J. Chrom. Sci., 14, 213 (1976).

37. Takata, Y., Anal. Chem., 45, 1864 (1973).

38. Kissinger, P. T., Clin. Chem., 20, 992 (1974).

39. Tjaden, U. R., J. Chromatog., 125, 275 (1976).

40. Lankelma, J., and Poppe, H., J. Chromatog., 125, 375 (1976).

41. MacCrehan, W., and Durst, R. A., Anal. Chem., 53, 1700 (1981).

42. Blank, C. L., J. Chromatog., 117, 35 (1976).

43. Alexander, P. W., Haddad, P. R., Low, K. C., and Maitra, C., J. Chromatog., 209, 29 (1981).

44. Beauchamp, R., and Boinay, P., J. Chromatog., 204, 123 (1981).

45. Lund, W., J. Chromatog., 173, 249 (1979).

46. Stulik, K., and Pacakova, V., J. Chromatog., 208, 269 (1981).

47. MacCrehan, W., submitted for publication in Anal. Chem.; presented in part at the joint International Meeting of the American Chemical Society and the Chemical Society of Japan, Honolulu, 1979.

48. EG&G. Princeton Applied Research Application Note C-4, Analytical Instrument Division, Princeton, N.J.

49. MacCrehan, W. A., and Durst, R. A., Anal. Chem., 50, 2108 (1978).

50. Kohen, J. G., Huber, J. F. K., Poppe, H., and Boef, G., J. Chrom. Sci., 8, 192 (1970).

51. Kissinger, P. T., Pesticide Analytical Methodology, John Harvey Gen. ed., A.C.S. Symposium Series 136, Washington: American Chemical Society, 1980, p. 57.

52. MacCrehan, W., Durst, R., and Bellama, J., Anal. Lett., 10, 1175 (1977).

53. Smiley, J., Master's Thesis, Middle Tennessee State University, Murfreesboro, Tenn., 1981.