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The analysis of residual monocrotophos in rice plants

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The procedure of Ambrus et al. (2,3) for extraction and clean up was modified to apply specifically to the extraction of monocrotophos in rice plant for determination by gas chromatography using flame photometric detector. An effective clean up system was developed to remove unwanted substances as well as to separate monocrotophos from other organic phosphate pesticides that interfere in the determination. The extraction utilized acetone/methylene chloride with recovery ranging from 41-53% for standards, while clean up recovery averaged 94% for standards or an overall percentage recovery of 41-55%.

Keywords: analysis, monocrotophos, rice plant, clean up procedure, gas chromatography

This study was undertaken to develop an analytical procedure for measurement of residual monocrotophos in rice plant which conforms with the specifications of the Codex Alimentarius Commission (1) for a procedure that can be used for regulatory purposes. Codex described the specifications in selecting an analytical procedure for residue analysis that can be used for regulatory purposes. As prescribed by Codex, the extraction and clean up procedure applicable for multi residue analysis and the determination of the pesticide residue should be by gas chromatography.

Ambrus et al. (2,3) have described a procedure which has been used as the official method for the control of pesticide residues of plant samples, soil and water in 20 laboratories for the Plant Protection and Agrochemistry Organization in Hungary. The scheme of the general method is described in Figure 1. The Ambrus general method for extraction and clean up recommends a set of parameters for the extraction and clean up which depends on the type of sample and the residue to be analyzed. Since this study

EXTRACTION

Analytical Sample	Sample group and extraction condition						
	I	II	III	IV	V	VII	VIII
Homogenization	←—acetone—→			water acetone	CH_2Cl_2	water $CH_3COO^-NH_4^+$ acetone	
Filtration							
Saturation	Na_2SO_4/H_2O			Na_2SO_4/H_2O	Na_2SO_4/H_2O	Na_2SO_4/H_2O	add NaCl soln
Partition	CH_2Cl_2			CH_2Cl_2	acetone/ H_2O	CH_2Cl_2	CH_2Cl_2/ anhyd. Na_2SO_4
Drying							
Evaporation							

CLEAN UP

Sample group and chromatographic conditions

Parameter	Mixed I & II	Alumina N I - VIII	Alumina B I.V & VII	Silica gel I - VIII
Sample Load in benzene	20-50 g/5 ml	5-10 g/ml	5-50 g/5 ml	2-10 g/ml
Fractions and eluants	CH_2Cl_2	1st-hexane 2nd-hexane-ethyl ether (7 + 3)	1st-hexane 2nd-hexane-ethyl ether (2 + 1)	1st-hexane 2nd-hexane-benzene (4 + 1) 3rd-benzene 4th-benzene-ethyl acetate (1 + 1) 5th-ethyl acetate

DETERMINATION

Screening

Gas-Liquid Chromatography
NP Thermionic detection
OV-23 or OV-101 columns

Thin Layer Chromatography
o-Tolidine: Carbamates
Triazine

Organophosphates

Carbamates
Triazines
Other compounds (P,N)

Enzyme inhibition
Organophosphates
Carbamates
Fungicides
Fungispores

ANALYSIS OF CLEANED UP EXTRACTS

Gas-Liquid Chromatography
NP Thermionic detection:
OV-22 or Ov-101 columns:
Same as above
Electron capture detection:
Electron-capturing compounds
Confirmation - Combination of separation column, GLC columns, GLC detectors
Specific methods

Thin Layer Chromatography
Fluoroborate: Carbamates
p-DAB: Ureas
o Tolidine: Same as above
Enzyme Inhibition:
Same as above

♣SAMPLE GROUP

I. Root and bulb vegestables (e.g. carrot, parsley (root), onion, garlic.
II. Fruit and vegestables of low chlorophyll and oil content (pome fruits, stone fruits, citrus, berries, bananas, etc.)
III. Plants and crops of high chlorophyll contents, low oil content (loafy and legume vegetables, plant leaves).
IV. Dried fruits of high sugar content (dates, figs, raisins).
V. Dry crops of low fat (oil) content which can be ground to powder (cereals, grains, maize)
VI. Crops of high oil content (oil seeds, peanuts, cacao, beans, soybeans). - not included in the general scheme because it requires special liquid-liquid partition Steps.
V. Soil
VII. Water and liquid samples

Figure 1. Scheme of the general method for extraction & clean up by Ambrus et al. (1981a)

involves rice plant samples, the extraction procedure for Type V sample was adopted.

The gas chromatographic procedure in screening organophosphate residues using Flame Photometric Detector recommended by the U.S. EPA was adopted for the determination of monocrotophos in the plant extracts. Due to the non-availability of the specified length of column in the EPA procedure, a shorter column was tried in the gas chromatographic determinations. Preliminary investigations of the efficiency of the gas chromatographic system adopted indicated that the system is sensitive enough to detect the minimum residue limit (MRL) for monocrotophos in rice. However, it was also shown that the column system adopted was not efficient in separating monocrotophos from malathion and methyl parathion, two other common organophosphate pesticides used in the farms. To be able to use the gas chromatographic system that is readily available in the laboratory for the determination of monocrotophos, the other interfering pesticides must be effectively separated from monocrotophos.

The silica gel clean up described by Ambrus et al. (2) in Figure 1 was tested to fractionate different organophosphorus pesticides. Preliminary tests showed that it was effective in separating malathion and methyl parathion but the solvent elution systems described failed to elute monocrotophos.

This study describes the modification of the silica gel clean up of Ambrus et al. (2) to become an effective preliminary separation technique for the determination of monocrotophos using the EPA-recommended gas chromatographic system with a shorter column. This study also describes the validation of a procedure for the extraction, clean up and gas chromatographic determination of monocrotophos in rice plant adopted from the published multiresidue analytical procedure for extraction and clean up for pesticides by Ambrus et al.(2,3) and the gas chromatographic determination of the USEPA (4).

Experimental

1. *Reagents*

All reagents were analytical grade.

Solvents: all solvents were obtained from Merck and redistilled.

Silica Gel (Merck, activity 1) : Add 5 ml water to 95 grams silica gel and mix thoroughly in a jar. Keep closed for two hours before use.

Pesticide Standards: monocrotophos, diazinon, methyl parathion, malathion, and ethyl parathion with purity between 98-99.9% were supplied by the US Environmental Protection Agency, Cincinnati, Ohio, USA; pesticide solutions were prepared in redistilled acetone.

2. *Apparatus*

Chromatographic columns: glass, 1 cm i.d. and 20 cm long fitted with a teflon stopcock.

Gas Chromatograph: Varian Aerograph 3700 equipped with Flame Photometric Detector and Phosphorus filter with transmission at 530 nm.

Operating conditions:

Temperature: injector, 220°C; column, 200°C; Detector: 220°C.
Flow Rates: carrier gas, Helium -- 75 ml/min, 60 psig.; Air #1 - 80 ml/min; Air #2 - 170 ml/min, 60 psig
Hydrogen - 140 ml/min, 40 psig.
Chromatographic column: Pyrex glass, 2 mm i.d. x 400 cm long packed with 4% SE- 30+ 6% OV-210 on Gas Chrom. Q. (Applied Science Laboratories, Inc.)

3. *Preparation of sample*

The whole plant including the roots is cut, mixed well, and a 50 g aliquot is taken. The aliquots are placed in a plastic bag and kept in the freezer until analysis time.

4. *Procedures*

a) Extraction (2)

Fifty grams of the preserved analytical sample is transferred into a blender. The sample is blended with 150 ml acetone for two minutes at high speed. The extract is then filtered with suction through a Buchner funnel. The blender is rinsed and the residue is washed consecutively with 30 and 20 ml portions of acetone. Then the extract and rinsings are transferred into a one liter separtory funnel containing 450 ml of 4% Na_2SO_4 solution. Extractions with 100, 50, and 50 ml portions of methylene chloride were done

and the extracts were filtered through 30 g anhydrous Na_2SO_4. The sodium sulfate layer was rinsed with 20 ml methylene chloride. The volume of combined mathylene chloride extracts was reduced to about 2 ml with a vacuum rotary evaporator at room temperature (30°C). Ten ml acetone was added to the extract and evaporated to 2-3 ml. This step was done two times. The concentrated extracts were transferred with a Pasteur pipet to a conical glass tube and rinsed with 2 ml portions of acetone. The excess solvent was evaporated and the final volume was adjusted to 5 ml. Three ml acetone extract was transferred to another conical tube. The solution is evaporated to 0.5-0.8 ml and the final volume is adjusted to 3 ml with benzene. This benzene solution is used for chromatographic clean up. The acetone extract is used for direct GC determination.

b) Column Chromatographic Clean up (2)

Five grams of deactivated adsorbent was placed in a 15 mm. i.d. x 35 cm column with gentle vibration. The adsorbent was pre-wetted with 15 ml n-hexane. One ml extract was pipetted onto the top of the adsorbent. The pesticides were eluted using the following solvents: 40 ml n-hexane (1st fraction), 16 ml n-hexane/benzene (4:6) (2nd fraction), 16 ml benzene (3rd fraction), 20 ml benzene/ethyl acetate (1:1) (4th fraction), and 50 ml ethyl acetate (5th fraction). The solvent in each fraction was evaporated in the rotary evaporator, the extract transferred in a calibrated test tube and dissolved to 2 ml with acetone. This solution was used for gas chromatographic determinations.

c) Modifications Adopted in Extraction and Clean up Procedures

1. In the process of homogenizing the sample, the 50 g sample is divided into two parts and each part is blended with 200 ml acetone. A total of 550 ml acetone is used including the rinsing.

2. Before extraction with methylene chloride, the volume of acetone extract is first reduced to about 200 ml in a rotary evaporator at room temperature (30°C).

3. Acetone and methylene chloride extracts are concentrated in a rotary evaporator with a stream of nitrogen gas under normal atmospheric pressure.

4. The repeated addition of acetone and evaporation to 2-3 ml during concentration of extract are omitted because the analysis employs a flame photometric detector which is not affected by methylene chloride.

5. In eluting the fifth fraction, 50 ml of ethyl alcohol/ethyl acetate (2:1) is used instead of 50 ml ethyl acetate.

The scheme of the modified procedure is summarized in Figure 2.

d) Validation of Modified Extraction and Clean up Procedures

1. Recovery values were determined in the extraction step. Column profile and recovery values were determined in the clean up step.

2. Analytical rice plant samples fortified with monocrotophos were analyzed. Recovery values were determined.

3. Analytical rice plant samples without fortification with monocrotophos were analyzed.

Results

Gas Chromatographic Analysis

Injection of 1 μl of a mixture of standard ethyl parathion (0.50 ng), methyl parathion (0.38 ng), diazinon (0.17 ng) and malathion (0.51 ng) gave the chromatogram shown in Figure 3.

Table 1 shows that peak height (greater than 20% full scale deflection) of unresolved methyl parathion and malathion is almost twice the peak height of ethyl parathion. While the column maybe considered efficient in detecting the specified weight of the standard organophosphate pesticides, it was not able to resolve the peaks of malathion and methyl parathon which maybe due to the shorter column used in the experiment. The USEPA specified a six foot column.

The relative retention times observed compare favorable with the values given by the USEPA (Table 1).

The temperature conditions and the carrier gas flow rate recommended by the USEPA (4) were found to be optimum for the

THE ANALYSIS OF RESIDUAL MONOCROTOPHOS

EXTRACTION

	Sample Group and Extraction Conditions
Analytical sample (50 grams)	III
Homogenization	Acetone (550 ml)
Filtration	
Evaporation	Rotary evaporation of thin film
Saturation/Partition	Na_2SO_4 /H_2O/CH_2Cl_2
Drying	Anhydrous Na_2SO_4
Evaporation	Rotary Evap by evaporation of thin film

CLEAN UP

Parameter	Sample Group & Chromatographic Conditions
2-10 g/ml sample load in Benzene	Silica Gel (activated)
Fractions	Eluants
1st	hexane
2nd	hexane/benzene (4:1)
3rd	benzene
4th	benzene/ethyl acetate (1:1)
5th	ethyl alcohol/ethyl acetate (2:1)

DETERMINATION

Direct Analysis of Extract	Analysis of Cleaned up Extracts
Gas Liquid Chromatography	Gas Liquid Chromatography
Flame Photometric Detector	Flame Photometric Detector
4 part SE 30 6 part OV 210	4 part SE 30 6 part OV 210
3 ft glass column	3 ft glass column
monocrotophos	monocrotophos fractionated in Silica Gel

Figure 2. Scheme of the modified extraction and clean up procedure of Ambrus et al. (2,3) and gas chromatographic determination by the USEPA (1980).

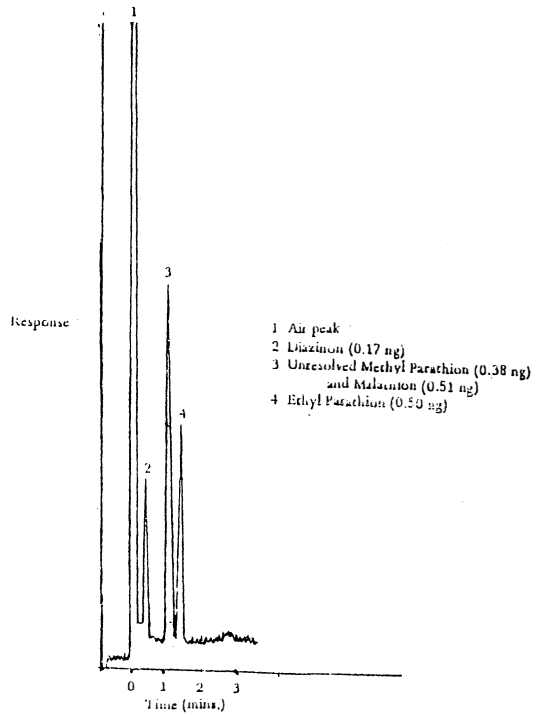

Figure 3. Chromatogram of mixed standards diazinon, methyl parathion, malathion and ethyl parathion

Table 1. Flame photometric detector response to mixed standards diazinon, methyl parathion, malathion and ethyl parathion

	PESTICIDE STANDARD			
	Diazinon (0.17 ng)	Methyl Parathion (0.38 ng)	Malathion (0.51 ng)	Ethyl parathion (0.50 ng)
Retention Time (min)	0.55	1.3	1.3	1.7
Peak Height (mm)	50	124	124	70
% FSD	22	55	55	31
RRt Observed	0.32	0.76	0.76	1.0
RRt USEPA (USEPA,1980)	0.31	0.76	0.78	1.0

analysis of monocrotophos. With a retention time of barely one minute, these conditions gave the sharpest and the largest peaks for the standard monocrotophos solutions tested.

Mixed standards including monocrotophos gave the chromatograms in Figures 4 and 5. From these chromatograms, it appears that the 400 centimeter column cannot efficiently separate monocrotophos form methyl parathion and malathion. With this column, fractionation of the pesticides prior to gas chromatography is necessary.

The minimum detectable quantity (MDQ), defined as 2x noise was determined to be 0.10 ng. This value should be much lower than the maximum residue limit (MRL) of monocrotophos in rice, for the determination to be applicable in monocrotophos residue analysis. Since no information could be obtained on the MRL of monocrotophos in rice, the MRL value of 0.2 mg/kg for corn (5) was taken as reference in evaluating the sensitivity of the gas chromatograph for the determination of residue.

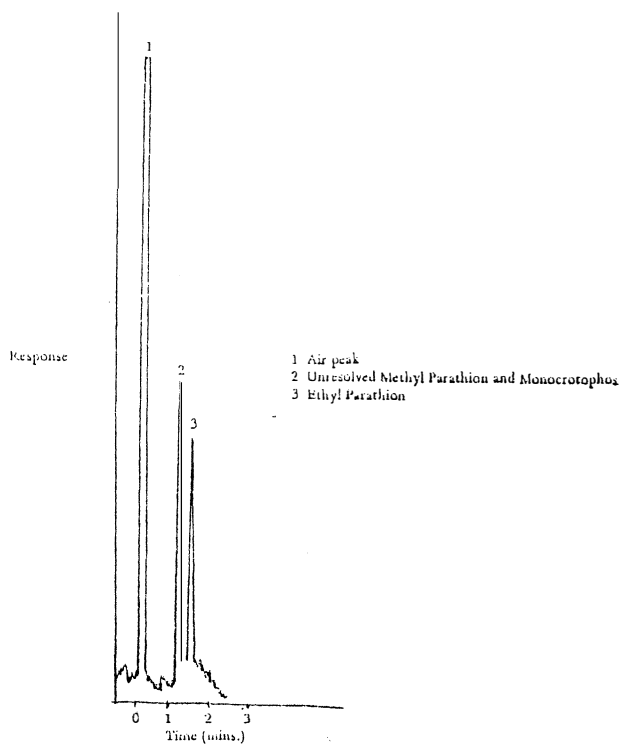

Figure 4. Chromatogram of mixed standards ethyl parathion, and monocrotophos

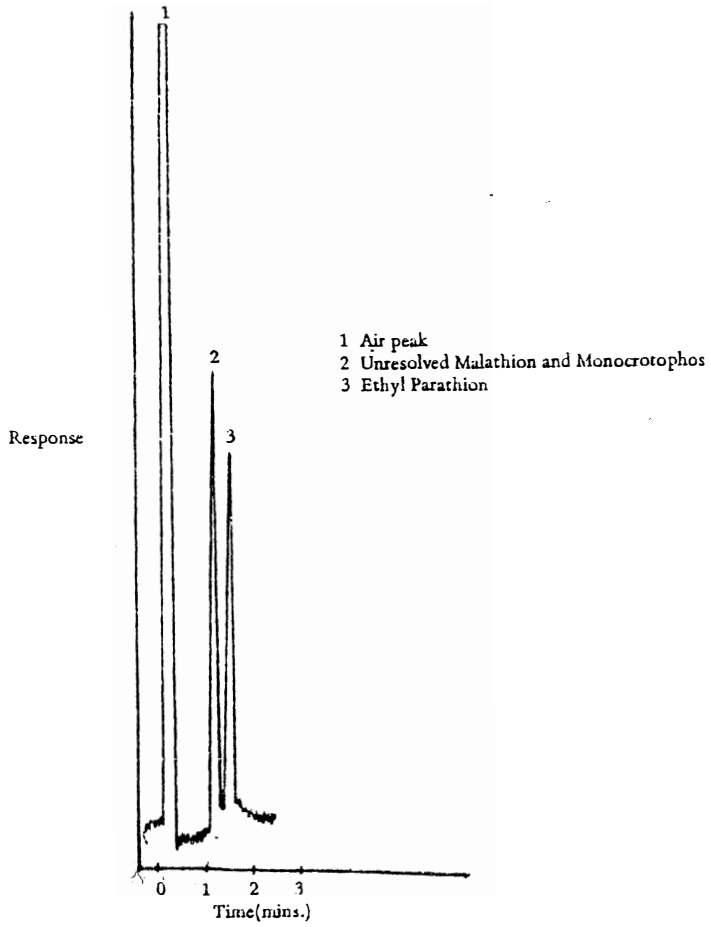

Figure 5. Chromatogram of mixed standards ethyl parathion, malathion, and monocrophotos

At this level, the minimum concentration of the solution to be injected should be 2 μg/ml in the acetone extract extract and 1 μg/ml in the fifth fraction.

Since the gas chromatographic system employed has a minimum detectable limit of 0.1 ng/μl, it can be used for monocrotophos residue determination.

Table 2 shows that the sensitivity of the FPD detector at the most sensitive setting using one microliter injection of standard is 18.55 nA/ng/sec.

Table 2. Flame photometric response at most sensitive setting (attenuation setting: 16 x 10^{10})

Concentration of monocrotophos (ng)	Peak Height (mm)	Sensitivity* (A/ngP/sec)
0.12	10	27.32
0.29	15	16.65
0.59	25	13.59
0.88	47	17.12
1.17	66	18.09
		Ave. = 18.55
		%RSD = 27.90

*Sensitivity of Flame Photometric Detector $= \frac{(16 \times 10^{10}/100)\ (\text{peak ht.mm}/225 \times 100)}{(\text{wt of monocrotophos.ng} \times 0.13856)/6.25\ \text{sec}}$

The slope of the plot of log (nA) vs. log (ng P/sec) for the most sensitive setting is 1.14 versus a value of 1.0 if the detector response were linear.

Table 3 gives the detector response at different days of operation. Sensitivity averaged at 20.1 nA/ngP/sec with a standard deviation of 22. The analysis of variance for the mean sensitivity based on pooled standard deviation showed that there is a significant change in the detector response at different days of operation. The slopes of the plot of log (nA) vs. log (ng P/sec) averaged at 1.0178 with % relative standard deviation (% RSD) of 2.35. This suggests that although sensitivity of detector varies, linearity of detector response could be retained. This is also indicated in Figure 6 which shows the plots of the average peak heights versus the average concentration of monocrotophos for the different days of observation in Table 3.

Table 3. Detector response at different days of operation

	Day 1		Day 2		Day 3	
	Ave. conc of Stds (ng)	Ave. Pk Ht.(mm)	Ave. conc of Stds (ng)	Ave. Pk Ht.(mm)	Ave. conc of Stds (ng)	Ave. Pk Ht.(mm)
	1.46	31.5	1.44	17.0		
	2.93	57.3	2.91	31.0	2.88	41.5
	4.28	94.0	4.31	48.0	4.32	60.0
	5.72	122.0	5.81	69.5	5.74	84.0
Sensitivity (nA/ngP/sec)	27.13		14.80		18.35	
Linearity (slope)	1.0081		1.0003		1.0451	

Clean up step

Monocrotophos was eluted in the fifth fraction with ethanol/ethyl acetate (2:1). Table 4 shows the column profile for the mixture of malathion, methyl parathion, ronnel and monocrotophos.

Table 4. Silica gel column chromatography profile of mixed standards ronnel, methyl parathion, malathion and monocrotophos

Pesticide Standard	Fraction Eluted	Rt* (min)
ronnel	2nd	0.7
methyl parathion	2nd	0.95
malathion	4th	1.0
monocrotophos	5th	0.7

*Rt measured at column T = 210°C

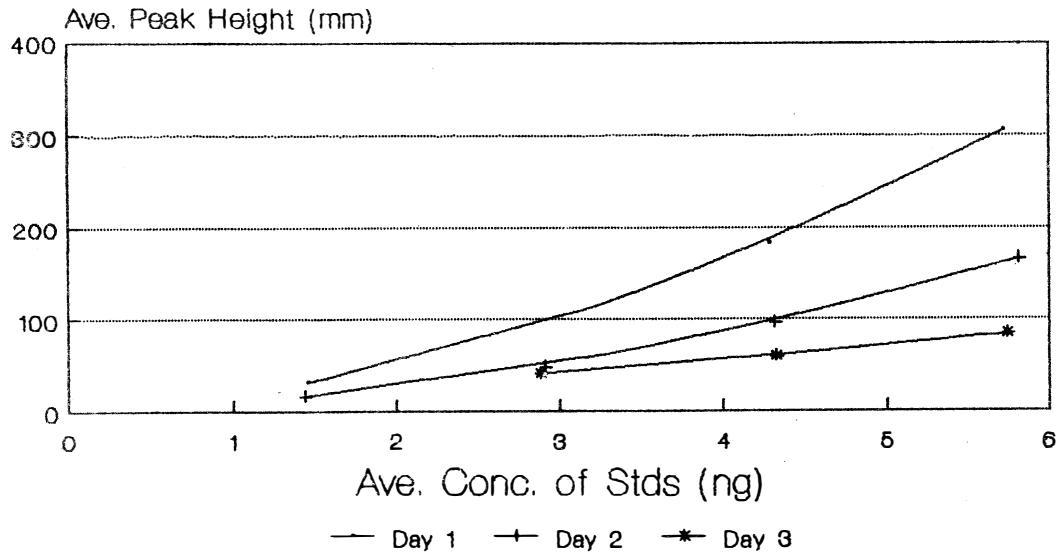

Figure 6. Detector response at different days of operation

Recovery tests

Table 5a indicates that % recovery values for extraction of standards obtained range from 36.8% to 54.5% . The Q test conducted at the 90% confidence level did not reject any of the results obtained. The percentage recovery for the extraction step is 47% with % RSD of 17.

Table 5a also shows that % recovery values for extraction of fortified samples range from 30.0 to 64.3%. The average value is 44.9% with % RSD of 39.

Table 5b shows that average % recovery for the clean up step using standard solutions only is 94%, while % recovery for the clean up step using the extracted fortified samples averaged at 104% with % RSD of 5.7.

Three sets of data, each with 4-6 replications were obtained to determined % recovery for the entire procedure (extraction and clean up). The average % recovery from sets I-III is 48.5% with %RSD OF 5.7 (Table 5c).

Table 5. Data on percent recovery

a. Extraction Step

	Trial	Final conc of monocrotophos in acetone, ppm	Conc. of monocrotophos extracted ppm	% Recovery
Using standard solutions only	I.	2.34	1.18	50.2
	II.	2.34	0.886	36.8
	III.	2.34	1.12	47.6
	IV.	2.34	1.28	54.5
				Ave. 47.0
				%RSD 17.2
Using fortified samples	I.	2.92	1.18	40.3
	II.	2.13	0.639	30.0
	III.	2.02	1.3	64.3
				Ave. 44.9
				%RSD 34.3

b. Clean up step

	Conc. of std. ppm	Ave. peak height, mm		% Recovery
		w/o col chrom. 5th fraction	w/ col chrom 5th fraction	
Using standard soln only	5	156	145	92.6
	1.17	41.8	40.3	96.4

	Conc. of monocrotophos in 5th fraction assuming 100% recovery (ppm)	Conc. of recovered monocrotophos in 5th fraction (ppm)	% Recovery
Using extracted samples	0.32	0.35	109
	0.65	0.63	97
	0.59	0.62	105
			Ave. = 104
			%RSD = 5.76

c. Entire procedure using 1.17 ppm fortified samples in three sets of determination

	Set	Average % Recovery
	I	45.9
	II	51.4
	III	48.2
		Ave. = 48.5
		% RSD = 5.7

THE ANALYSIS OF RESIDUAL MONOCROTOPHOS

Discussion

The silica gel column and the chromatographic conditions for the clean up suitable for all types of samples recommended by Ambrus et al. (2) did not elute monocrotophos in any of the five fractions recovered. The 400 centimeter glass column packed with the adsorbent recommended by the USEPA (4% SE-30 + 6% OV-210) was not able to separate monocrotophos from malathion, methyl parathion and ronnel (organophosphate pesticides with close relative retention times with respect to ethyl parathion under the same gas chromatographic conditions). The acetone extraction and the 400 centimeter column for the GC determination proved adequate in the analysis when the clean up step was improved.

The modified clean up procedure using (2:1) ethyl alcohol/ethyl acetate for the eluting solvent in the fifth fraction recovered 94% monocrotophos (5th fraction) from ronnel (2nd fraction), methyl parathion (2nd fraction) and malathion (4th fraction).

Percent recovery of monocrotophos in the entire procedure (41-55%) compares well with the percent recovery of monocrotophos in the extraction step (41-55%). There is a need to improve the extraction efficiency of the method. It appears that the pesticide favors the aqueous phase. Varying the ratio of methylene chloride to aqueous phase during extraction may result in a better extraction recovery. The use of some buffers to vary the ionic strength of the aqueous phase during extraction could be explored to improve extraction recovery.

The poor repeatability of the method could be attributed to the high variability of the detector responses to monocrotophos. A calibration curve for the detector response has to be obtained at the time of analysis. Since monocrotophos is thermally labile, it maybe sensitive to small changes in the temperature of the hydrogen flame of the detector.

This method may be used in the analysis of monocrotophos in the rice plant provided that the recovery efficiency of the method is determined. The concentration of monocrotophos can be obtained by correcting values obtained by % recovery.

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