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Determination of Organophosphorus Pesticides in Rice by TLC

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Multi-residue analysis of organophosphorus pesticides (OPs) in rice was studied. Semi-automatic gel permeation chromatography was used to clean-up the extracts. The organophosphorus pesticides were analyzed by TLC-cholinesterase inhibition method and GC-FPD methods. The minimum detectable quantities (MDQ), R_f and RR_f values of pesticides by TLC were determined. The average recoveries of organophosphorus pesticides from fortified rice samples at three different levels were 75-88 % by TLC and 82-93 % by GC. Comparison of the results with GC showed that TLC method was less sensitive than GC, but it could be used by monitoring laboratories to carry out the preliminary screening of samples in order to supplement instrumental analyses.

Key Words: Organophosphorus pesticides, Gel permeation chromatography, TLC, GC-FPD.

INTRODUCTION

Organophosphorus pesticides (OPs) are widely used in plant protection in China. The residues of these pesticides in grains used for food are of great public concern. In this study the determination of organophosphorus pesticides in rice by thin layer chromatograph (TLC)[1] and verification by gas chromatography (GC)[2] were carried out. The principle of TLC detection method is based on the inhibition of cholinesterase enzyme by organophosphorus pesticides[3]. This method is specific for phosphoric and thio-phosphoric acid esters and carbamate pesticides, plant extracts usually do not interfere. The extraction and cleanup of rice samples were carried out with ethyl acetate and gel permeation chromatography (GPC)[4].

EXPERIMENTAL

Analytical standards (purity ≥ 98 %) of dichlorvos, monocrotophos, parathion-methyl, fenitrothion, pirimiphos-methyl, malathion and parathion were, obtained from the Institute of Control of Agrochemicals of Ministry of Agriculture. Stock solutions (1 mg/mL) for each pesticide were prepared in 50 mL volumetric flasks with ethyl acetate. Portions of stock solutions were diluted to 25 mL with ethyl acetate to make an intermediate mixed standard solution.

Cow liver extract was used as enzyme source. The reagents were prepared and applied according to the procedures described by Ambrus *et al.*[5].

Sample extraction and GPC cleanup: Weigh 20 g of ground rice sample into the Erlenmeyer flask. Add 5 mL ethyl acetate and 20 mL distilled water and stir vigorously. Then add 95 mL ethyl acetate, 10 g $NaHCO_3$ and 70 g anhydrous Na_2SO_4 into the flask. Homogenize the mixture with waring blender. Filter and decant 50 mL aliquot (equal to 10 g sample). Evaporate the extract to dryness. Dissolve the residue in 0.5-1.0 mL mixture of cyclohexane and ethyl acetate (1:1) for GPC cleanup. Fill the 10 mm × 200 mm glass column with 7-8 g swelled bio-bead SX-3 gel. Inject 0.5 mL concentrated sample extract into the column and elute the sample with cyclohexane and ethyl acetate (1:1). Discard the first 8 mL eluate. Collect fraction of 9-25 mL. Evaporate it nearly to dryness and take it up in 1 mL acetone for determination of pesticide residues.

Thin layer chromatograph separation and detection: Use freshly activated 20 cm × 20 cm TLC plate. Prepare a spotting plan for the plate in advance and spot 10-20 μL of the pesticide solution in order of spots numbered 1-11 from the left edge of the plate. Apply the same volume from the plant extracts as well as from the standard solutions on the plate. Fill the tank with acetyl acetate to obtain 1 cm immersion depth for the plate. Place filter paper into developing tanks for 0.5 h before eluting the plates to obtain saturated vapour phase in the tank. Place the developing tanks into water basin and keep the temperature within 25 ± 2 ºC for improving reproducibility of retention values. Elute plates up to 10-12 cm from the original points of spotting. Adjust the volume of the mobile phase after each elution. Air-dry the commercially available silica gel plate and treat the silica layer with bromine vapour before detection.

Spray the plate with enzyme solution until it gets thoroughly wet and place it into an incubator or oven saturated with water vapour at 37 ºC for 0.5 h. Make sure that the plate does not get dry during incubation. Spray the plate with the substrate solution. The white spots occur in pink (bluish-red) background. Evaluate the plates immediately after the full colour development. Mark the position (boundary) of the spots and measure the R_f values.

Detection method: Multi-residue analysis of organophosphorus pesticides (OPs) in rice were determination by Agilent 6890N GC with FPD(P), attached with Agilent 7683 autoinjector and Agilent Enhanced ChemStation for data acquisition. A DB-17 column (30 m × 0.32 mm i.d. × 0.25 µm) was used. The injector and detector were operated at 210 and 250 ºC, respectively. Volume of injection was 1 µL in the splitless mode and the oven temperature was programmed as follows: 100 ºC for 1 min, rising to 220 ºC (15 ºC min^{-1}) for 10 min. The carrier gas was nitrogen with the flow rate of 1.5 mL min^{-1}. The pressures of hydrogen and air were 1.0 and 0.6 kg/cm^2.

RESULTS AND DISCUSSION

GC Separation: Retention time (R_t), calibrated range of standard curve and limit of detection (LOD) of 7 OP compounds by GC method are listed in Table-1. The results showed, that 7 pesticides could be separated under the experimental conditions described according to their retention times. The correlation coefficients of the linear range were higher than 0.9845 and the MDQs were in the range of 1-4 × 10^{-10} g (0.1-0.4 ng). The LOQs of these pesticides in rice samples were in the range of 0.005-0.02 mg/kg.

Elution pattern of OPs on GPC: The amount of OPs was determined by GC method described above. The results from the eluting OPs showed, the pesticides were not eluted at the first 8 mL, but all of them were eluted between the 9th and 19th mL. When sample extracts were eluted the first 8 mL was discarded and 9-25 mL fraction was collected for further determination. The results are shown in Table-2.

Recovery of OPs from fortified rice samples by GC method: The rice samples were fortified with OPs at 3 different levels, 0.05, 0.10 and 0.5 mg/kg, in three replicates. The samples were extracted, cleaned up and determined by GC with the methods described above. The results (Table-3) showed that the average recoveries of 7 OPs were in the range of 82.3-93.1 % with CV 1.6-8.9 %.

Thin layer chromatograph determination: The results of TLC determination are listed in Table-4. The R_f values of parathion methyl, fenitrothion and parathion in ethyl acetate system were very close to each other and their RR_f values were 1, 1.006 and 0.996. Thus, they could not be separated on one plate after development. In thin layer chromatograph experiments these three pesticides had to be detected separately. The correlation coefficients of the linear range were higher than 0.9561, which are not as good as GC. The MDQs of 7 organophosphorus pesticide compounds were in the range of 2 × 10^{-9} g × 10^{-7} g, which were 10-100 times higher than those obtained with GC. The LOQs of these pesticides in rice samples were 0.043-0.214 mg/kg.

Recovery of OPs from fortified rice samples with TLC method: The rice samples were fortified with four OPs at 3 different levels, 1.5 LOQ, MRL and 2 MRL, respectively with five replicates. The samples were extracted, cleaned up and determined by TLC with the method described above. The fortification levels [mg/kg] and the recovery values are shown in Table-5. The average recoveries of 4 OPs were in the range of 75.4-88.1 % with CV 3.1-15 %.

Comparison of the results of GC and TLC methods: Seven OPs could be separated under the GC conditions described, but in the TLC experiments, especially by using only ethyl acetate as developing solvent, several OPs, such as

TABLE-1
RETENTION TIME, LINEARITY, LIMIT OF DETECTION AND LIMIT OF QUANTITATION OF SEVEN ORGANOPHOSPHORUS PESTICIDES IN RICE BY GC METHOD

Pesticide	R_t (min)	Calibrated range (ng)	Linear equation	γ^2	MDQ (g)	LOQ (mg/kg)
Dichlorvos	2.202	0.1-6	Y = 12218x - 2343.8	0.9858	1 × 10^{-10}	0.005
Monocrotophos	7.317	1.0-6	Y = 4476.2x - 354.5	0.9845	4 × 10^{-10}	0.020
Parathion-methyl	12.312	0.1-6	Y = 9008x - 135.2	0.9922	2 × 10^{-10}	0.005
Fenitrothion	14.833	0.1-6	Y = 9193.7x - 413.7	0.9885	2 × 10^{-10}	0.005
Pirimiphos-methyl	16.353	0.1-6	Y = 11092x1 - 130	0.9959	2 × 10^{-10}	0.005
Malathion	17.075	0.4-6	Y = 12648x1 - 199.8	0.9977	2× 10^{-10}	0.005
Parathion	17.762	0.1-6	Y = 46328x - 1298.6	0.9990	2 × 10^{-10}	0.005

TABLE-2
ELUTION PATTERN OF SEVEN ORGANOPHOSPHORUS PESTICIDES BY GPC

Fraction No.	GPC elution volume range (mL)	Dichlorvos (%)	Monocrotophos (%)	Parathion-methyl (%)	Fenitrothion (%)	Pirimiphos-methyl (%)	Malathion (%)	Parathion (%)
1	1-7	–	–	–	–	–	–	–
2	7-8	–	–	–	–	–	–	–
3	8-9	7.0	36.9	–	–	–	–	–
4	9-11	40.7	63.1	15.8	14.1	17.9	16.0	5.8
5	11-13	41.2	–	31.0	31.2	30.8	31.0	45.5
6	13-15	10.1	–	30.0	31.0	30.1	30.2	47.6
7	15-17	1.0	–	22.7	0.6	21.2	22.1	1.1
8	17-19	–	–	0.5	–	–	0.7	–
9	19-21	–	–	–	–	–	–	–

TABLE-3
RECOVERY OF THE ORGANOPHOSPHORUS PESTICIDES IN FORTIFIED RICE BY GC METHOD

Pesticide	Fortification (mg/kg)	Recovery (%) 1	2	3	Average recovery (%)	CV (%)
Dichlorvos	0.05	80	81	86	82.3	3.9
	0.1	82	85	88	85.3	3.6
	0.5	91	87	79	85.5	7.4
Monocrotophos	0.05	93	95	89	92.6	3.4
	0.1	85	91	84	86.9	4.3
	0.5	84	94	79	85.9	8.9
Parathion-methyl	0.05	92	89	92	91.2	1.9
	0.1	87	95	90	90.7	4.1
	0.5	86	88	83	85.5	2.5
Fenitrothion	0.05	90	86	92	89.3	3.6
	0.1	96	90	87	91.1	4.9
	0.5	92	87	92	90.4	2.9
Pirimiphos-methyl	0.05	83	87	85	85.3	2.3
	0.1	88	84	86	86.2	2.3
	0.5	94	90	86	89.8	4.5
Malathion	0.05	85	91	85	86.8	3.8
	0.1	91	85	95	90.5	5.3
	0.5	88	84	92	88.0	4.6
Parathion	0.05	89	87	91	89.2	2.6
	0.1	93	95	92	93.1	1.6
	0.5	84	91	87	87.0	4.2

TABLE-4
R_f, LINEARITY, MDQ AND LOQ OF SEVEN ORGANOPHOSPHORUS PESTICIDES BY TLC METHOD

Pesticide	R_f	RR_f	Linear range (ng)	Linear equation	γ^2	MDQ (ng)	LOQ (mg/kg)
Dichlorvos	0.438	0.653	20-100	Y = 7.9115x - 6.121	0.9803	20	0.2
Monocrotophos	0.110	0.164	120-600	Y = 3.2789x - 2.4022	0.9848	200	–
Parathion-methyl	0.671	1.000	2-40	Y = 2.5338x + 3.3983	0.9831	2	0.04
Fenitrothion	0.675	1.006	10-150	Y = 7.0343x + 2.196	0.9703	10	0.2
Pirimiphos-methyl	0.615	0.917	3-40	Y = 5.4579x + 2.202	0.9857	3	–
Malathion	0.645	0.961	200-600	Y = 14.752x - 28.964	0.9561	200	–
Parathion	0.668	0.996	2-40	Y = 0.5672x + 1.5699	0.9708	2	0.04

TABLE-5
RRECOVERIES OF TESTED PESTICIDES IN FORTIFIED RICE SAMPLES BY TLC METHOD

Pesticide	Spike level (mg/kg)	Recovery (%) 1	2	3	4	5	Average recovery (%)	CV (%)
Parathion-methyl	0.043	70	95	70	77	71	76.6	14.0
	0.100	79	89	88	75	68	79.8	11.0
	0.200	74	73	79	76	75	75.4	3.1
Dichlorvos	0.430	94	90	85	78	77	84.7	8.7
	0.100	ND	ND	ND	ND	ND	–	–
	0.200	88	97	86	81	84	87.3	6.9
Fenitrothion	0.214	90	85	91	80	75	84.1	7.8
	0.500	87	102	92	82	77	88.1	11.0
	1.000	80	84	82	71	68	76.8	9.0
Parathion	0.043	76	78	86	73	76	77.7	6.2
	0.100	87	92	93	69	68	82.0	15.0
	0.200	86	82	76	78	75	79.2	6.0

parathion methyl, fenitrothion, parathion, malathion and pirimiphos-methyl, could not be separated on one plate. The correlation coefficients of the linear range were higher than 0.9845 and 0.9561 for GC and TLC, respectively. The MDQs were in the range of 1-4 × 10^{-10} g for GC and the 2 × 10^{-9} g-10^{-7} g for TLC. The LOQs of these pesticides in rice samples were 0.005-0.02 and 0.04-0.2 mg/kg for GC and TLC, respectively. The average recoveries of OPs were in the range of 82.3-93.1 % with CVs of 1.6-8.9 % and 75.4-88.1 % with CVs of 3.1-15 % by GC and TLC, respectively. In the TLC study the LOQ of dichlorvos was 0.2 mg/kg, this method could not detect the maximum residue limit (0.1 mg/kg) of dichlorvos in grain, but the sensitivity of parathion methyl and parathion was very high, their MDQs were 2 ng and LOQs were 0.04 mg/kg. In general TLC methods can be used for specified compounds to carry out the preliminary screening of samples in order to supplement instrumental analyses.

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