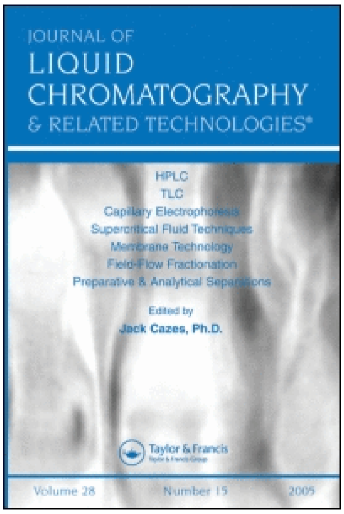

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Separation and Identification of Certain Organophosphorous Pesticides Residues in Vegetable

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SEPARATION AND IDENTIFICATION OF CERTAIN ORGANOPHOSPHOROUS PESTICIDES RESIDUES IN VEGETABLE

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ABSTRACT

A rapid and sensitive method using impregnated thin layer chromatography (TLC) for detection and estimation of organophosphorous pesticides residues from vegetable has been developed. Compared to ordinary thin layer chromatography this method is faster more sensitive and accurate. The correlation with the Impregnant (p-cresol) and the pesticides have been determined by Infrared Spectroscopy.

INTRODUCTION

Until 1982, Separation of organo phosphorous pesticides (1-5) had not been done on impregnated silica gel plates. Reena et.al. have earlier reported that impregnation with metal salts and different phenols led to improved separation (6). It was shown that p-cresol was the most satisfactory impregnent for TLC separation of organophosphorous pesticides.

The isolation and identification of pesticides residues present in food materials in traces is of great national importance, because of the indiscriminate use of pesticides in agriculture by Indian farmers.

Although GLC and HPLC are sensitive and rapid methods, the impregnated TLC method described here is a simple and less expensive technique which can be adopted for application in most routine control laboratories. In this paper authors have studied the identification of pesticides in food materials on impregnated plates and then correlation has been developed between the pesticides and the impregnant by Infrared Spectroscopy.

EXPERIMENTAL

Materials

Analytical grade reagents were obtained from BDH. Recrystallized technical grade pesticides (supplied by City Chemical Corporation, N.Y., Rallis India Limited) were used.

TLC plates (20 x 20 cm) thickness 0.5 mm were coated with a mixture of silica gel g (50 g) and 0.5% (W/V) of p-cresol in water (100 ml) and then activated at 60 ± 1^{o} for 24 hrs.

Extraction

The method has been applied to, Malathion, Phorate, Dimethoate, Methylazinphas.

Macreate 50 g of chopped sample (potato) with 95 ml of ethyl methyl ketone-cyclohexane mixture (3:2) and 30 g. of anhydrous sodium sulphate for 10 minutes, allow the mixture to separate and decant the non-aqueous phase into a kunderna-Danish evaporator fitted with a round bottom flask. Repeat the decanting procedure twice, each time with 95 ml of mixed solvant. Shake the aqueous residue with 100 ml of cyclohexane and spin the mixture in centrifuge. Add the separated cyclohexane phase to non-aqueous phase in the evoporator and reduce the volume to about 10 ml. Transfer the extract to a separating funnel and add 400 ml of 2% sodium sulphate solution. Separate the cyclohexane phase, reextract the aqueous phase with a further 10 ml of cyclohexane. Stir the combined cyclohexane phase with 20 g of anhydrous sodium sulphate and filter the extract through a filter paper (Whatman No.1) into an evoporator fitted with a 100 ml flask. Wash the sodium sulphate with 3 successive 10 ml portions of cyclohexane, add the washings to the evoporator and reduce the volume upto 1 ml. Remove the apparatus, dried with a current of dry air. The residue was dissolved in 1 ml of acetone.

A aliquot (10 µl) of the extracted residue in acetone was spotted on a TLC plate (impregnated with p-cresol 0.5% W/V) together with control organo phosphorous pesticides and the plate was developed in a TLC jar containing the solvent system n-hexane-xylene-ethyl-acetate-acetone (50:15: 5:18). When the solvent front had moved 10 cm, the plate was removed, dried at room temp. and then exposed in iodine vapours. Yellow spots appeared on white back ground.

RESULTS AND DISCUSSION

The hR_f values of the spots are given in Table 1 along with the hR_f values on plain silica gel plates. It indicates that the solvent system used provides a clear separation of all 4 pesticides on impregnated plates.(Plate A). The detection limit for different pesticides is in the range of 1-2.1 µg on impregnated plate. On impregnated plate the separation has greatly improved the spots are compact, tailing has been eliminated. Further on 0.5% p-cresol impregnated plate the hR_f values are generally less as compared with plain silica plates. This suggests that there is rather weak intraction between the pesticides and the impregnant.

The organo phosphorous pesticides under this study are characterized by having lone pair of electrons on P and S atoms. Therefore, the separation

TABLE I

hR_f values of organophosphorous pesticides on impregnated silica plate solvent system - n-hexane:xylene:ethylacetate:acetone (50:15:5:18)

Pesticides	hR_f		Detecting Limit (ug)
	On plain silica gel G	0.5% W/V p-cresol as impregnant	
Malathinon	76 mT	72	2.1
Phorate	98	85	1.0
Dimethoate	53 ST	45	2.1
Methyl azinphos	15 ST	14	2.1

mT = medium tailing

ST = slight tailing

On plain silica gel - 10 cm run in 35 mints.

On p-cresol impregnated plate - 10 cm run in 28 mints.

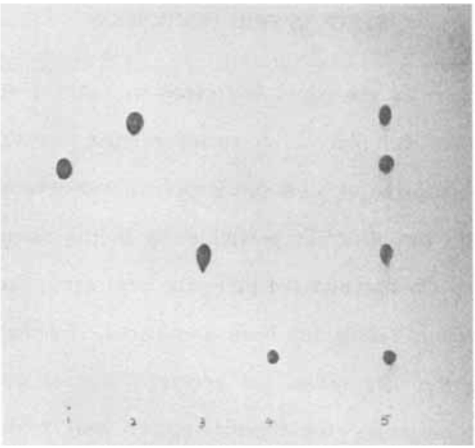

Plate A

1. Malathion
2. Phorate
3. Dimethoate
4. Methyl azinphes
5. A mixture of organophosphorous pesticides extract from vegetable.

of organo-phosphorous pesticides on p-cresol impregnated plates may be due to hydrogen bond formation between the hydrogen atom of the OH group of the p-cresol and P and S atoms of the organo-phosphorous pesticides.

Infrared spectroscopy offers a suitable method for distinguishing between intramolecular hydrogen bonding and chelation (very strong intramolecular hydrogen bonding). Generally, when a compound is examined in gas phase or in every dilute solution in a non polar solvent, absolutely free O-H stretching absorption bands appear in the spectrum. Practical differentiation between the various types of hydrogen bonding is possible using infrared spectrascopy. Intramolecular hydrogen bonded and chelated O-H stretching absorption band frequencies are unaffected by dilution. In contradiction, dilution of an intermolecularly hydrogen bonded hydroxylic compound in a non-polar solvent causes a reduction of the intermolecular bonding (7). Further a change from intramolecular to intermolecular hydrogen bond should result in an upward shift of the O-H band, the magnitude of shift will be dependent on the strength of the resulting band.

The infrared spectra for two of the pesticides (namely malathion and Phorate) with p-cresol mixtures in different concentration were recorded on Beckman IR 20 spectrophotometer on chloride plates.

Intermolecular hydrogen bond appears between 3200 - 3400 cm^{-1} as a broad band and intramolecular hydrogen bond appears at 3500 cm^{-1}. Both p-cresol and a mixture of p-cresol and organo phosphorous pesticides show intermolecular hydrogen bonding. The intermolecular hydrogen bonding in p-cresol is much stronger as compared to one expected in the mixture. In the case of p-cresol the intermolecular hydrogen bond appears at 3200 cm^{-1} and any other weak intermolecular hydrogen bond should appear at higher frequencies. It is assumed that there is a weaker hydrogen bond between

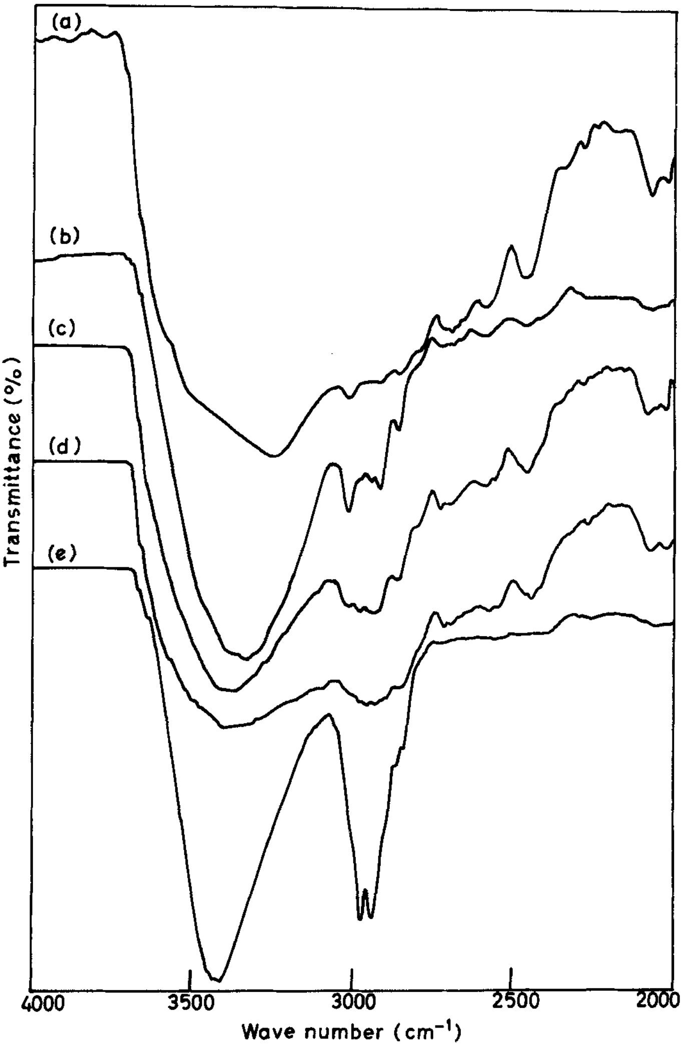

Fig.1. I.R. Spectra of :

(a) p-cresol.

(b) 16.67 % Malathion and 83.33% p-cresol.

(c) 33.3% Malathion and 66.7% p-cresol.

(d) 41.1% Malathion and 58.9% p-cresol.

(e) 60.0% Malathion and 40.0% p-cresol.

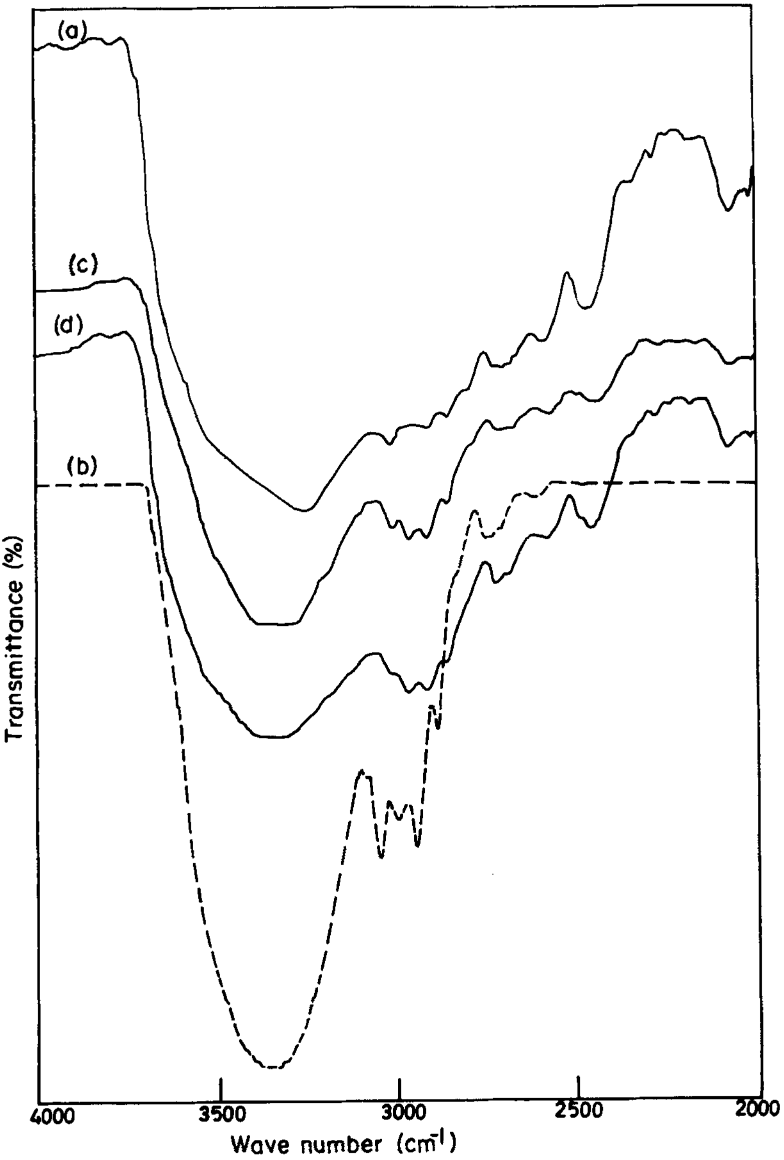

Fig.2. I.R. Spectra of :

(a) p-cresol
(b) 16.67 Phorate and 83.33% p-cresol
(c) 33.3% Phorate and 66.7% p-cresol
(d) 41.1% Phorate and 58.9% p-cresol

TABLE 2
Systematic shift to higher Frequencies on increasing the concentration of Malathion in mixture

*Percentage of malathion	*Presentage of p-cresol	Hydrogen bonded - OH band cm^{-1}
0	100	3200
16.67	83.33	3340
33.3	66.7	3380
41.1	58.9	3400
60	40	3420

* The percentage are in W/W ratio.

TABLE 3
Systematic shift to higher frequencies on increasing the concentration of Phorate in mixture

*Percentage of Phorate	*Presentage of p-cresol	Hydrogen bonded - OH band cm^{-1}
0	100	3200
16.67	83.33	3350
33.3	66.7	3360
41.1	58.9	3360

* The percentage are in W/W ratio.

p-cresol and pesticide. The 3200 cm^{-1} band should, therefore, shows a systematic shift to higher frequencies on increasing the concentration of the pesticide in the mixture. This is in fact observed as is evident from the data presented in Tables 2 and 3 (I.R. spectra presented in Figs. 1 and 2).

On the basis of infrared spectroscopic evidence obtained for the hydrogen bond formation between p-cresol and organophosphorous pesticide, it can, therefore be concluded that the predominant factor responsible for adsorption of organo phosphorous pesticides on the p-cresol impregnated layer should be hydrogen bond formation between the two.

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REFERENCES

1. Kirchner, J.G., Editor, "Thin-Layer Chromatography", Second Edition, A Wiley-Interscience, John Wiley and Sons, New York, 1978.
2. Tesvetkova, T.S., Khristodorva, V. and Sergeeva, D., Vet-Med.Nauki, 1974, 11(6), 50.
3. Ligeti, G., Kalona, A., Gyogyszereszet., 1974, 18(1), 11.
4. Leshchev, V.V., Talanov, G.A., Khim.Sel'sk.khoz., 1977, 15(7), 46.
5. Mendoza, C.E., Residue Rev., 1972, 43, 105.
6. Srivastava, S.P., Reena; Anal.Letters; 1982, 15(A1), 39.
7. Cross, A.D., Editor, "An Introduction to Practical Infra Red Spectroscopy", Butterworths, London.