

Separation and Speciation of Organophosphorus Pesticides Based on Hydrolysis Using Reverse Phase HPLC

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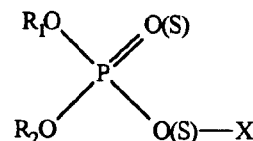
Summary: A new method for separation and speciation of organophosphorus pesticides have been investigated using reverse phase HPLC. Pesticides are hydrolysed by the addition of sodium ethoxide, the organic layer is separated from the aqueous phase which contain the acidic hydrolysed portion of the respective pesticides. Different pesticides give different acidic products on hydrolysis leading to variation in the retention time. The hydrolysed products are injected and separated on reverse phase HPLC. Identification and quantification is done by running the respective standards.

Introduction

Organophosphorus pesticides vary in toxicity and half lives. This necessitate the determination of total pesticides on one hand and identification of specific organophosphorus pesticides on the other. Organophosphorus pesticides can be determined as a whole molecule by HPLC, gas chromatographic and thin layer chromatographic methods [1-8], or phosphorus can be determined after decomposition or hydrolysis by spectrophotometric method [9-13] or non-phosphorus group after hydrolysis can be determined spectrophotometrically be a reaction depending on some properties of the non-phosphorus group [14-17]. Non-phosphorus methods can often be made specific for a single pesticide, or at least for a group of related pesticides. Such specific methods are of advantage if one of several pesticides in a sample needs to be determined. In this paper a new HPLC method capable of sepiciation and determination of organophosphorus pesticides based on hydrolysis is presented.

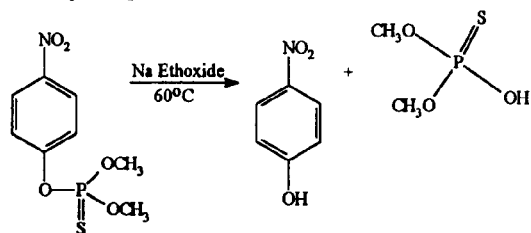
Results and Discussion

Speciation of pesticides is one of the key requirements for assessing the toxicity of a particular pesticide in the environments. Total organophosphorus pesticides can be determined by molybdenum blue method, while sulphur containing pesticides could be determined by methylene blue method. In the present work HPLC method was exploited for the speciation and determination of organophosphorus pesticides. The general structure of the organophosphate pesticides may be represented as

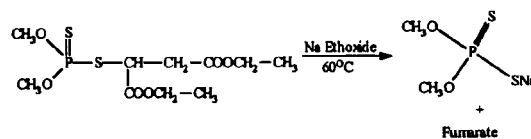


where R_1 and R_2 are methyl or ethyl groups and X is an organic radicle. The R_1 and R_2 substituent groups are less easily hydrolysed from the phosphorus than is the X group. In the hydrolysis methods the pesticide is hydrolysed with alkali to break the P-O(S)-X link, and then the liberated organic substituent is isolated and determined.

On hydrolysis methyl parathion was hydrolysed into the following products O,O-dimethylthiophosphate and p-nitrophenol.



Similarly malathion was hydrolysed into O,O-dimethylthiophosphate and fumarate as given below:



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Conditions were optimized for the separation and identification of p-nitrophenol and fumaric acid using reverse phase HPLC with U.V. detector (254 nm) on a pecosphere C18 cartridge column. Water:Metahnol (1:1) was chosen as the optimum mobile phase with a flow rate of 1.0 ml/min. The identification of the components was made both by the retention time as well as spiking method. As different pesticides leads to different hydrolysed products leading to variation and this property was exploited for speciation in mixture. A representative chromatogram for the hydrolysed product of malathion (fumaric acid) is given in Fig. 1, while chromatograms for standard fumaric acids given in Fig. 2 The retention time of fumaric acid and p-nitrophenol was found to be 0.2 and 0.6 minutes respectively. Working standards of Fumaric acid and p-nitrophenol were injected in the range of 10-50 ppm and calibration graphs of peak height vs concentration were plotted and as shown in Figures 3 and 4. The detection limit for p-nitrophenol and fumaric acid was investigated by this method and was found to be 3.0 ppm for both with 10 μ l injection volume standard reference pesticides were hydrolyzed at optimum conditions and linearity was checked at optimum conditions by injection of the organic phase of the hydrolysed product. The method was applied for active ingredient determination in the commercially available formulations using their respective calibration plot of nitrophenol and fumaric acid. The results are given in Table-1. As can be seen from the Table that the concentration of pesticides in these commercially available pesticides are lower than the label value.

Table-1: Determination of active ingredient in pesticides using reverse phase HPLC.

Pesticide	Active ingredient (g/ml)	
	Found	Label
Methyl parathion	0.156	0.5
Malathion	0.196	0.5

Experimental

Instrument

HPLC system consisted of a Bacharach coleman Tri Det. with a pecosphere 3x3 C18 cartridge column, Perkin Elmer 100 series pump and recorder and thermostated water bath were used during this investigation.

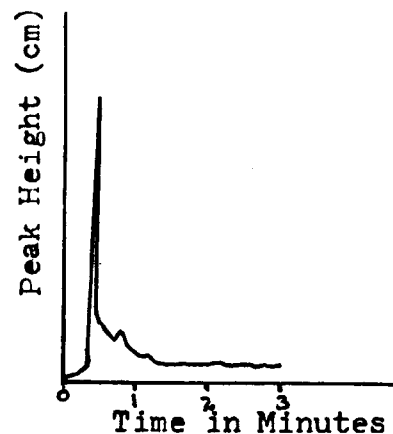


Fig. 1: Chromatogram of a hydrolysed malathion.

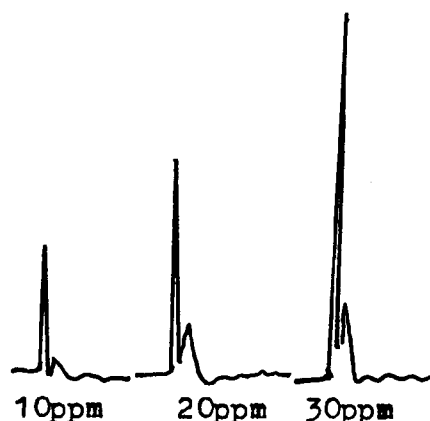


Fig. 2: Chromatograms of standard fumaric acid.

Reagents

p-nitrophenol, fumaric acid, carbontetra chloride, sodium metal, ethanol and methanol of A.R. grade and commercially available pesticides were used without further purification.

Solutions

1. p-nitrophenol (1000 ppm) solution

Stock solution of p-nitrophenol was prepared by dissolving 0.1 g of the reagent in distilled water and diluted upto 100 ml in volumetric flask.

2. Fumaric acid (1000 ppm) solution

Stock solution of fumaric acid was prepared by dissolving 0.1 g of fumaric acid in distilled water and diluted upto 100 ml in a volumetric flask.

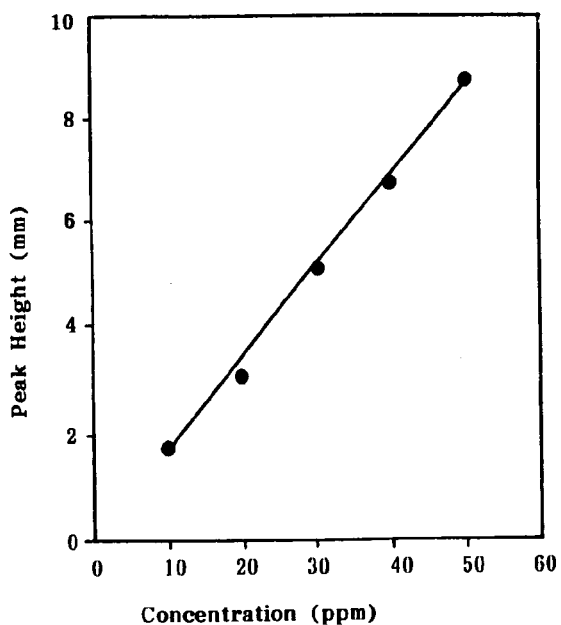


Fig. 3: The effect of concentration on peak height of fumaric acid using reverse phase HPLC.

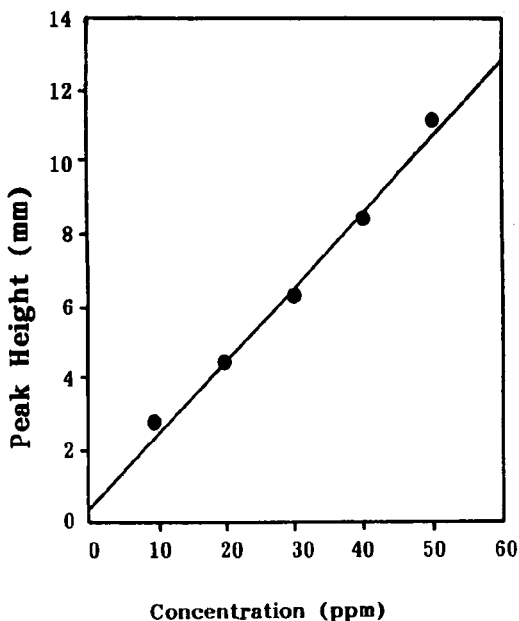


Fig. 4: The effect of concentration on peak height of P-nitrophenol using reverse phase HPLC.

3. Sodium ethoxide (1%) solution

1% solution of sodium ethoxide was prepared by dissolving 1 g of sodium metal in ethanol and diluted upto 100 ml.

4. Mobile phase

Water:methanol mobile phase was prepared in 1:1 ratio and degassed by bubbling N gas through the solution for about 10 minutes.

Procedure

Pesticide sample (0.1 ml) was taken in three separate separatory funnels, 6 ml of 1% sodium ethoxide, 5 ml water and 5 ml carbon tetrachloride was added to each separatory funnel. The resulting solutions were shaken for two minutes and transferred to beakers.

The solutions containing beakers were heated on water bath for 15 minutes at 60°C optimum conditions investigated for complete hydrolysis [18]. The aqueous phase was separated from organic phase using separatory funnel. The organic phase was used for analysis by HPLC using reverse phase chromatography.

Conclusion

Different pesticides lead to different products after hydrolysis. Pesticides could be identified as well as quantified based on the retention time and peak height of the hydrolysed product of the specific pesticide. Speciation studies for malathion and methyl parathion were conducted exploiting this technique using reverse phase HPLC using water:methanol as mobile phase with UV detection. After optimization of mobile phase and retention time these pesticides were determined in pesticide formulations based on hydrolysis products. The method is fairly simple, economical and compare well in term of detection limit (30 ng) with the standard established gas chromatographic method.

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