Investigation of Spectrophotometric Method for Determination of Organophosphorus Pesticides Based on Hydrolysis Followed by Molybdenum Blue Method

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Summary: A screening method for determination of pesticides based on hydrolysis is reported which is applicable to the determination of many organophosphorus pesticides. The method involves the hydrolysis of pesticides under alkaline conditions at 60-70°C temperature. The organophosphorus pesticides are hydrolysed to o,o-dimethylphosphate and o,o-diethylthiophosphate. These phosphorus containing hydrolysed products are then determined by spectrophotometric method using molybdenum blue method.

Introduction

Organophosphorus pesticides are increasingly being used domestically and in agriculture and new varieties of these pesticides are becoming available every year. Widespread use of toxic organophosphorus pesticides has created a need for sensitive methods for detecting them as spray residues on plant materials. A spectrophotometric method for the determination of malathion was first introduced by Norris *et al* [1] and subsequently modified by other workers [2-9]. Organophosphorus pesticides have also been analysed based on acid decomposition followed by molybdenum blue method [9]. Apart from malathion most of the organophosphorus pesticides now in use, together with many of their oxidation products, can be hydrolysed to produce dialkylphosphate or dialkylthiophosphates, the method has the potential for organophosphorus pesticides screening. In this paper we have exploited this potential and the extended method is applicable to the screening of food stuffs for residues of many organophosphorus pesticides and their metabolites.

Results and Discussion

For the alkaline hydrolysis of organophosphorus pesticides screening conditions including

temperature, incubation time at optimum temperature and volume of sodium ethoxide were optimized.

Table-1 shows that the optimum temperature for the hydrolysis of most of the pesticides was in the range of 60-70°C with the only exception of sumithion having 50°C as the optimum temperature. The incubation time at the optimum temperature was investigated and is given in Table-2. It was observed that the optimum incubation time for the hydrolysis of all pesticides investigated was in the range of 10-15 minutes. Each pesticide hydrolyse at a different rate and the reaction effectively stopes at the dialkylphosphate or thiophosphate stage because further hydrolysis proceeds at very slow rate.

Similarly the volume of sodium ethoxide was optimized for the hydrolysis of organophosphorus pesticides. The results are given in Table-3. It was observed that the optimum volume for the hydrolysis was in the range of 6-8 ml with the exception of dimecron and sumithion where the optimum volume was found to be 4 ml.

Under the conditions used, the products formed indicate that the major site of cleavage for most pesticides occur at the bond linking the phosphorus atom with the oxygen or sulfur atom adjoining the non-alkyl site chain. Thus two products, a dialkylphosphate or dialkylthiophosphate are formed from the four types of organophorus pesticides commonly used.

The method provide a complete screening test to most pesticides which on hydrolysis yield dialkylphosphate or dialkylthiophosphate.

The present method, is better than the one based on, acid decomposition of pesticides followed by molybdenum blue method as this is a non destructive one and other organic compounds are also decomposed in former one. In the hydrolysis method the organic layer could also be used for further investigation.

Experimental

Apparatus

Milton and Roy Spectronic 20D and Thermostated water bath.

Reagents

Ammonium molybdate, sulfuric acid, ferrous sulfate, sodium metal and distilled ethanol.

Solutions

1. *Sulfuric Acid*
   1.75 M sulfuric acid was prepared by diluting 9.5 ml of concentrated sulfuric acid upto 100 ml.
2. *Ammonium Molybdate*
   1.766 g of ammonium molybdate in 1.75 M sulfuric acid and diluted upto 100 ml.

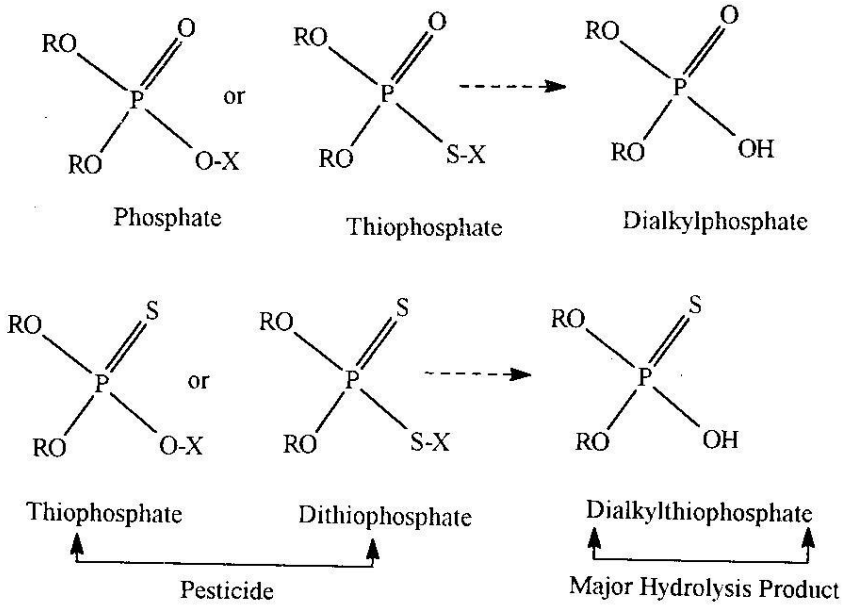

Table-1: Optimization of Temperature for the Hydrolysis of Pesticides

Temp °C	Absorbance									
	Actelic	Anthio	Bidrin	Diazinon	Dimecran	Malathion	Methyl Parathion	Monitor	Nogos	Sumithion
40	No. Decomp Osition	0.247 ±0.001	0.410 ±0.002	0.120 ± 0.0006	0.185 ± 0.005	0.120 ± 0.002		0.034 ± 0.002	0.084 ± 0.003	0.194 ± 0.002
50	0.053 ± 0.002	0.354 ± 0.003	0.658 ± 0.002	0.152 ± 0.002	0.201 ± 0.009	0.158 ±0.002	0.052 ± 0.002	0.054 ± 0.003	0.150 ± 0.008	0.201 ± 0.001
60	0.068 ± 0.0006	0.596 ± 0.002	0.601 ± 0.002	0.203 ± 0.002	0.240 ± 0.038	0.210 ± 0.003	0.138 ± 0.008	0.074 ± 0.002	0.214 ± 0.004	0.155 ±0.004
70	0.074 ± 0.004	0.420 ± 0.0006	0.340 ±0.001	0.252 ± 0.001	0.279 ± 0.0006	0.291 ± 0.003	0.084 ± 0.0006	0.064 ± 0.005	0.175 ± 0.0006	0.125 ± 0.004
80	0.048 ± 0.001	0.232 ± 0.001	0.208 ± 0.025	0.105 ± 0.001	0.169 ± 0.003	0.164 ± 0.004	0.054 ± 0.002	0.040± 0.002	0.073 ± 0.002	0.010 ± 0.004

Table-2: Optimization of Incubation time for the Hydrolysis of Pesticides

Time (min)	Absorbance									
	Actelic	Anthio	Bidrin	Diazinon	Dimecran	Malathion	Methyl Parathion	Monitor	Nogos	Sumithion
5	0.061 ± 0.001	0.410 ± 0.002	0.162 ± 0.002	0.175 ± 0.002	0.184 ± 0.004	0.208 ± 0.003	0.085 ± 0.001	0.075 ± 0.001	0.141 ± 0.0006	0.166 ± 0.001
10	0.088 ± 0.0007	0.599 ± 0.001	0.450 ± 0.001	0.253 ± 0.002	0.284 ± 0.001	0.293 ± 0.004	0.139 ± 0.004	0.084 ± 0.003	0.190 ± 0.0006	0.245 ± 0.002
15	0.098 ± 0	0.501 ± 0.0006	0.732 ± 0.003	0.207 ± 0.001	0.359 ± 0.0006	0.265 ± 0.004	0.173 ± 0.001	0.196 ± 0.001	0.242 ± 0.001	0.225 ± 0.005
20	0.071 ± 0.001	0.412 ± 0.002	0.601 ± 0.004	0.151 ± 0.0006	0.211 ± 0.0006	0.232 ± 0.003	0.143 ± 0.004	0.102 ± 0	0.200 ± 0.01	0.187 ± 0.002
25	0.057 ± 0.001	0.350 ± 0.0006	0.443 ± 0.004	0.105 ± 0.002	0.162 ± 0.0006	0.215 ± 0.001	0.107 ± 0.004	0.067 ± 0.001	0.127 ±0.002	0.114 ± 0.0006

Table-3: Optimization of Sodium Ethoxide for the Hydrolysis of Pesticides

Vol. of Nathoxide (ml)	Absorbance									
	Actelic	Anthio	Bidrin	Diazinon	Dimecran	Malathion	Methyl Parathion	Monitor	Nogos	Sumithion
2	0.0044 ± 0.001	0.211 ± 0.004	0.162 ± 0.002	0.0153 ± 0.002	0.310 ± 0.0006	0.208 ±0.003	-	0.151 ± 0.0006	0.113 ± 0.003	0.112 ± 0.003
4	0.071 ± 0.001	0.405 ± 0.008	0.450 ± 0.003	0.203± 0.002	0.397 ± 0.0006	0.293 ± 0.004	.120 ±0.002	0.040 ± 0.002	0.205 ±0.004	0.224 ±0.003
6	0.099 ± 0.008	0.600 ± 0.0006	0.732 ± 0.005	0.253 ± 0.004	0.361 ± 0.0006	0.254 ± 0.004	0.173 ± 0.002	0.205 ± 0.003	0.242 ± 0.009	0.191 ± 0.002
8	0.113 ± 0.002	0.551 ± 0.02	0.601 ± 0.004	0.278 ± 0.008	0.309 ± 0.003	0.232 ± 0.003	0.141 ± 0.001	0.172 ± 0.002	0.209 ± 0.009	0.155 ± 0.02
10	0.087 ± 0.003	0.422 ± 0.003	0.443± 0.001	0.225 ± 0.004	0.241 ± 0.002	0.215 ± 0.001	0.123 ± 0.004	0.125 ± 0.001	0.175 ± 0.0006	0.150 ± 0.01
12	-	-	0.130 ±0.001	-	-	-	0.080 ± 0.004	-	-	-

3. *Sodium Ethoxide*
   1% sodium ethoxide solution was prepared by dissolving 1 g of sodium metal in ethanol and diluted upto 100 ml.
4. *Ferrous Sulfate*
   10% ferrous sulfate solution was prepared by dissolving 10 g of ferrous sulfate in water and diluted upto 100 ml.

Procedure

Pesticides (0.1 ml) was taken in a beaker and optimum volume (6-8 ml) of 1% sodium ethoxide was added to it. This was followed by the addition of 5 ml water and 5 ml carbontetrachloride. The resulting solution was shaken for two minutes using separatory funnel. The aqueous phase was transferred to a beaker and heated on water bath for 10-15 minutes at 60-70°C. To this 10 ml of ammonium molybdate was added and again heated on boiling water bath for 12 minutes. Then 8 ml of freshly prepared 10% ferrous sulfate was added.

Each solution was transferred to 100 ml flask and diluted upto mark and equlibrated for 15 minutes. Blank solution was prepared in the same way except the addition of pesticide. The absorbance was measured at 825 nm using blank as a reference. Replicate readings were taken in each case.

Conclusion

The conditions of existing method for the hydrolysis of malathion were reinvestigated and optimized. The method was applied to all organophosphorus pesticides. It was observed that the method could be equally applied to all organophosphorus pesticides. The method is an improvement over the one based on acid decomposition followed by molybdenum blue method.

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