DETECTION OF ETHYL PARATHION, METHYL PARATHION AND FENITROTHION IN BIOLOGICAL MATERIAL BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

In this paper a new chromogenic spray reagent has been developed for Ethyl Parathion, Methyl Parathion and Fenitrothion by thin layer chromatography. These insecticides on reduction with stannous chloride in 50 % hydrochloric acid give respective amino derivatives. These amino derivatives further react with 1, 2 napthoquinone-4-sulfonic acid yield red-orange coloured para-quinoidal condensation product. Other organophosphorous, organochloro, carbamate and synthetic pyrithriod insecticides do not reacts with this reagents. Hence it is a simple and selective chromogenic reagent for organophosphorous insecticides containing nitrophenyl group.

KEYWORDS: TLC, Biological tissue, Ethyl Parathion, Methyl Parathion, Fenitrothion, Stannous chloride, 1, 2 napthoquinone-4-sulfonic acid.

1. INTRODUCTION

Among the organophosphorous insecticides nitrophenyl compounds are abundently used in agriculture to protect the crops from insects. As also they are commonly used in houses to kill bugs, ants, cockroaches etc. Being easy availability of these nitrophenyl insecticides they are frequently misused in homicidal and suicidal posoining. However from toxicological point their indentification in biological materials is very essential. Thin layer chromatography (TLC) is the method of choice for characterisation of these insecticides in biological materials.
There are some reagents reported in the literature for the detection of organophosphorous insecticides by TLC. These are palladium chloride\(^{[1,2]}\), 4-(nitrobenzyl) pyridine tetraethylene pentamine\(^{[3]}\), bromine flourescein silver nitrate\(^{[4,5]}\), congo red\(^{[6]}\), mercury (I) nitrate\(^{[7]}\), mercury (II) nitrate-potassium hexacyanoferrate\(^{[8]}\), mercury (II)-diphenyl carbazole. However some of these reagents are less sensitive and other are susceptible to fats and proteins.

It is therefore necessary to have a sensitive reagent to detect these compounds in presence of fats and proteins. In this we report the use of 1,2-naphthoquinone-4-sulfonic acid as a new reagent to detect these compounds in biological material.

2. EXPERIMENTAL

2.1. Chemical and Reagents

All chemical and reagents were of analytical grade. Distilled water was used throughout the analysis.

(a) **Insecticide Standards Solution:** 10 mg of each insecticides (ethyl parathion, methyl parathion and fenitrothion) were dissolved in 10ml of ethanol (1 mg/1ml).

(b) **Sodium hydroxide solution (5%):** dissolve 5g of sodium hydroxide in 100ml of distilled water.

(c) **Stannaous chloride solution (5%):** dissolve 5g of stannaous chloride in 100ml of 1:1 v/v HCl.

(d) **Reagent solution (0.2%):** dissolve 0.2g of 1,2-naphthoquinone-4-sulfonic acid in 100ml of 50% ethyl alcohol and add to it 0.5ml of 5% NaOH solution.

2.2. Extraction of insecticides of biological materials

Ammonium sulphate (10gm) was added to samples of biological tissue (stomach, intestine, liver-spleen and kidney (100gm), containing ethyl parathion, methyl parathion and fenitrothion insecticides. The sample were then minced individually with water and then extracted in separating funnel with diethyl ether (100ml). The ether extract was transferred to an evaporating dish and the aqueous phase was re-extracted with diethyl ether (2 x 50ml). The extracts were combined and the solvent was evaporated at room temperature. The residue was dissolved in ethanol (2ml) and the solution was used for further analysis.
2.3. - Chromatography

Glass plates (20cm x 20cm) were coated with 0.25mm layers of a slurry prepared from silica gel G (Acme) slurry and water (1:27). The plates were dried and then activated at 110°C in an oven for approximately 1 hour. Standard stock solution of ethyl parathion, methyl parathion and fenitrothion (10µl) and extracts from biological tissue material (10µl) were spotted manually, by means of an autoclavable micropipette, volume 0.5-10µl (Nichipet Ex1 Japan) on previously activated TLC plates. The plates were developed to a distance of 10 cm with n-hexane- acetone, 9 + 1 (v/v) as mobile phase in a saturated camag twin trough TLC chamber. After development, the plate was removed, dried in air and sprayed with 5% stannous chloride solution. The plate was then heated for 10min at 100°C. It was cooled and sprayed with freshly prepared slightly alkaline sodium –1,2-,naphthoquinone 4 sulphonic acid reagent. Ethyl parathion, methyl parathion and fenitrothion gave red-orange spots. The Rf values are listed in Table 1.

Table 1: Rf values of organo phosphorous insecticides containing a nitro phenyl group.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Insecticides</th>
<th>Rf</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Ethyl Parathion</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td>Methyl Parathion</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>Fenitrothion</td>
<td>0.55</td>
</tr>
</tbody>
</table>

2.4. Recovery Experiment

Ethyl parathion, methyl parathion and fenitrothion (1mg of each in ethanol) were separately added to the minced visceral tissue (50gm) mixed well and left for 24 hour. The tissue samples were then processed as described in section 2.2 except that the residue from extraction of the tissue were dissolved in 1ml of ethanol. Each solution (10µl) was spotted in separate activated plate with the respective standard solution (10µl) of ethyl parathion, methyl parathion and fenitrothion containing 7,8,9,9.5 and 10 mg in 10ml ethanol. The plates were then developed and processed as described above. The intensity of the spot obtained for the extracts of visceral tissue were compared with those from the standard and found to be most similar to the spot resulting from the 9mg (10ml)\(^{-1}\) std. solution of ethyl parathion, methyl parathion and fenitrothion (average of three experiments) Hence the recovery for each insecticide was 90%.

3. RESULTS AND DISCUSSION

Alkaline solution of 1,2-napthaquinone-4-sulfonic acid reacts with compounds containing two removable hydrogen atoms attached to one carbon or nitrogen atom, gives red-orange
colored para-quinoidal condensation product\textsuperscript{[11]}. Organophosphorous insecticides containing nitro-phenyl group in their structure, on reduction with stannous chloride in (1:1) hydrochloric acid gives respective amino derivative, which has two removable reactive hydrogen atoms attached to nitrogen atom, which further react with the reagent to give red-orange spots. The reagent does not react with other organophosphorous, organochloro, carbamate and synthetic pyrethroid insecticides. The reported reagent is sensitive and selective for Ethyl Parathion, Methyl Parathion and Fenitrothion. Hence reagent can be routinely used in forensic toxicology. The sensitivity of this reagent was 1\textmu g.

REFERENCES