A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FENTHION WITH DIAZOTISED P-AMINOACETOPHENONE
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SUMMARY

A highly sensitive and simple spectrophotometric method for the determination of sub ppm levels of an organophosphorus pesticide, fenthion is proposed. The method involves alkaline hydrolysis of fenthion to thiophenol and its subsequent coupling with diazotised p-aminoacetophenone. The purple violet colour of the dye has an absorption maxima at 530nm. Beer's law is obeyed in the concentration range of 1.0 - 10.0μg (0.04 - 0.4ppm) fenthion per 25ml of solution. The method is free from the interference of other common pesticides and ions and has been satisfactorily applied for the determination of fenthion in polluted water, vegetables, fruits, foliages and biological samples. The method is compared with other reported methods and found to be superior to them.

INTRODUCTION

Fenthion [o,o-dimethyl-o (3-methyl - 4- (methylthio) phenyl) phosphorothioate], an insecticide and acaricide is also known as Labaycid or Baylex. It has high initial toxicity and prolonged effect and is effective against weevils, aphids, thrips, worms and other pests in fruits, vegetables and stored grains. (1-3). Fenthion is useful for controlling flies, mosquitoes and other flying pests including certain household insects. It is effective against pigeons, starlings and sparrows, inside buildings when used on perches frequented by these pests (4-6).

A colourless oily liquid, fenthion is insoluble in water while it is soluble in most of the organic solvents. Under natural conditions phytolysis and biological degradation are the main pathways for its degradation (7). When reacted with oxidizing agents it gives oxidation products, which are either equally toxic to fenthion or more toxic than it. The structure is as follows:

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{S} \quad \text{CH}_3 \\
\text{P} & \quad \text{O} \quad \text{SCH}_3 \\
\text{CH}_3\text{O} & \quad \\
\end{align*}
\]

Fenthion

Fenthion is moderately toxic to human and warm blooded animals. It has got a higher cumulative as well as acute chronic toxic effect (8,9). Its toxic properties including mutagenic, genotoxic, embryotoxic and inhibitory affects on cholinesterase enzyme have been studied (10-13). The \(LD_{50}\) value for rats is 250mg kg\(^{-1}\) (3). Long exposure leads to drastic mental disorder, and impairment of memory. Other symptoms are anorexia, ataxia, mucus diarrhoea, salivation, inhibition of peripheral and central nervous system (9,14-16). When ingested by a living organism, it accumulates in fat muscles and other internal organs and is secreted with milk. The tolerance limit in grain and meat products are 0.15 and 0.02 mg kg\(^{-1}\) respectively (8,9). Fenthion residues are not tolerated in milk, baby foods
and dietary food products. It persists for a long time in water at concentration of 2mg/l up to 105 days and its residues are not tolerated in water basins(7). Fenthion is dangerous to aquatic life and shows bioaccumulation and other physiological responses (17,18).

Fenthion's toxic nature wide applicability and because of its prolonged protective effect various methods such as gas-chromatography (19-21), thin-layer chromatography (22,23), high-performance liquid chromatography (24), gas liquid chromatography-mass spectrometry (25,26), gas liquid chromatography (5), mass-spectrometry (27), gas chromatography-flame photometric detection (28), liquid chromatography-mass spectrometry(29), etc. are reported for its determination.

A few spectrophotometric methods using reagents like, 4-aminoantipyrine (30), 4-(p-nitrobenzyl) pyridine (31), 3-nitroaniline -4-sulfonic acid (32), benzophenone (33), o-toludene and p-bromophenol (30) have been reported in the literature. These methods suffer from drawbacks such as instability of colour, insufficient sensitivity, absorbance variation of the blank, interference from foreign ions or time required for full colour development.

Here a simple and sensitive spectrophotometric method based on hydrolysis of fenthion (34,35,36) to give thiophenol and its subsequent coupling is described for trace level determination of fenthion. The coupling reagent used in the proposed method is p-aminoacetophenone which on coupling forms purple violet dye having maximum absorbance at 530nm. The method has been applied for the determination of fenthion in various environmental samples and biological fluids.

EXPERIMENTAL

Apparatus -

A Systronics UV-Vis spectrophotometer 108 with 1cm matched silica cell was used for all spectral measurements. A Remi C-854/4 clinical centrifuge having a maximum speed of 3600 rev. min⁻¹ and a maximum centrifugal force of 1850g with fixed swingout
rotors was used. Systronic pH meter model 331 was used for pH measurements.

Reagents -

All the chemicals used were of AnalaR grade or the best available quality. Double distilled demineralised water was used throughout the experiment.

Fenthion (Bayer, India Ltd.) -

A stock solution of 1mg ml⁻¹ was prepared by dissolving 100mg of fenthion in 100ml ethanol. Working standard solutions were prepared by appropriate dilution of the stock standard solution with water.

Sodium hydroxide -

1.0 M aqueous solution.

Sodium nitrite -

2% aqueous solution.

p- Aminoacetophenone (PAAP) (Ferak Berlin) -

A 1% (w/v) solution in 25% alcohol.

Hydrochloric acid -

2 ml of 2 M aqueous solution.

Diazotised p-aminoacetophenone (DPAAP) -

To 10 ml of p-aminoacetophenone solution, 1 ml of 2% sodium nitrite, 2 ml of HCl (2M) was added and the solution was kept in a brown coloured bottle. This was stable for about 4hr. when kept in cold.

Silica gel (BDH) -

100-200 mesh was used for column chromatography.
PROCEDURE

An aliquot of test solution containing 1.0 to 10.0 μg of fenitrothion was taken in a 25 ml graduated tube and to it 2 ml of 1.0 M sodium hydroxide was added. The solution was kept in a hot water bath (90-100°C) for 15 min. and then cooled to room temperature. To it 2 ml of DPAAP reagent was added. The solution was made up to the mark with slightly alkaline solution. The absorbance was measured at 530 nm against distilled water.

RESULTS AND DISCUSSIONS

Spectral characteristics -

The dye formed shows maximum absorption at 530 nm (Fig:1). All the spectral measurements were made against demineralised water as the reagent blank showed negligible absorbance at this wavelength.

Beer's law, molar absorptivity and Sandell's sensitivity -

Beer's law was obeyed over the concentration range of 1.0-10.0 μg of fenitrothion in 25ml of final solution (0.04 - 0.4 ppm) (Fig:2). The molar absorptivity and Sandell's sensitivity of the dye was found to be 5.76 x 10^5 (± 100) l mol^-1 cm^-1 and 0.0004 μg cm^-2 respectively.

Effect of varying reaction conditions -

Effect of reagent concentration -

2 ml of 1.0 M sodium hydroxide was required for hydrolysis of fenitrothion. A minimum of 2 ml of DPAAP was required for maximum colour intensity.

Effect of pH -

The dye was formed under alkaline conditions. The pH range between 12-14 was selected, since constant and maximum absorbance values were obtained at this range.
Effect of temperature -

It was found that complete hydrolysis of fenthion to thiophenol required 15 min. heating at 90-100°C.

Precision -

Precision of the method was checked by determining 5.0 µg of fenthion per 25 ml of final solution over a period of seven days. The standard deviation and relative standard deviation were found to be ±0.0097 and 2.07% respectively.

Effects of foreign species -

To assess the validity of the method, various foreign species and pesticides were added to 5.0 µg per 25 ml of fenthion prior to hydrolysis. The pesticides which liberate phenol after hydrolysis or decomposition, interfere with the method. The tolerance limit for other ions and pesticides are given in Table 1.

APPLICATIONS

To check the validity of this method, the proposed method has been applied for the determination of fenthion in various environmental and biological samples.

a) Determination of fenthion in polluted water -

Run off water from agricultural fields were collected and filtered through a Whatman no. 4 filter paper. Aliquots of water samples were taken in a 25 ml graduated tube and analysed as described above.

b) Determination of fenthion in vegetables, fruits and foliages -

Preparation of column (36) -

Silica gel (100-200 mesh) for column chromatography was used for the preparation of column. A 25 ml glass column (internal diameter 9 mm) was taken in which glass wool pad was kept near stop cock. 5.0 g of silica gel was kept in glass column and
washed with water. For each test a fresh column was used. The column was washed with 10 ml of 50% ethanol, and washings were collected in a 50 ml calibrated flask.

Various samples of vegetables, fruits and foliages were collected from agricultural field, where fenthion had been sprayed as an insecticide. The samples were weighed (each of 50 g), blended and macerated with two 20 ml portions of ethanol water (1+1), filtered and the sample was centrifuged at 1850 g for 10 min. The solution was then passed through a silica gel column to remove chlorophyll present in foliages and other interfering materials. The aliquots were then analysed by the proposed and reported method. (37)

c) Determination of fenthion in biological samples

The presence of fenthion in blood serum and urine has already been reported in detectable concentration (38). The samples were collected from the local pathology laboratory and tested for fenthion. The samples were found to be free of fenthion. So synthetic samples were prepared by adding known amounts of fenthion. Samples were deproteinised by 1 ml of TCA and 1 ml of EDTA was added for masking. The solution of the samples were filtered. Then the aliquots of the samples were analysed by the described method.

To check the applicability and recovery of the proposed method, known amount of fenthion was added to blood and urine samples and later determined by the proposed method with >90% recoveries which is in agreement with the reported method (Table-3).

CONCLUSION

The proposed spectrophotometric method for determination of fenthion is simple, sensitive, selective and is free from interference of various foreign species and pesticides. The method has been compared with other reported methods and found to be superior to them (Table-4).
Fig. - 1: Absorption spectra of the dye and reagent blank.

A. Concentration of fenthion: 9μg / 25ml.
B. Concentration of fenthion: 5μg / 25ml.
C. Reagent blank

Fig. - 2: Calibration data for the determination of fenthion.
### Table 1: Effect of foreign species and pesticides.

Concentration of fenthion = 5μg/25ml

<table>
<thead>
<tr>
<th>Foreign Species</th>
<th>Tolerance limit (μg/25ml)</th>
<th>Pesticides</th>
<th>Tolerance limit (μg/25ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, Methanol</td>
<td>10,000</td>
<td>DDT, BHC</td>
<td>900</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>870</td>
<td>Parathion, Malathion</td>
<td>600</td>
</tr>
<tr>
<td>Fe²⁺, Fe³⁺</td>
<td>400</td>
<td>Zineb</td>
<td>550</td>
</tr>
<tr>
<td>Ni²⁺, Co²⁺, Cu²⁺</td>
<td>325</td>
<td>Dimethoate, Quinolphos</td>
<td>300</td>
</tr>
<tr>
<td>Pb²⁺, Zn²⁺, SO₄²⁻</td>
<td>250</td>
<td>Phorate, Ethion</td>
<td>250</td>
</tr>
<tr>
<td>Nitrophenol,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene, Xylene</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cresol,</td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amount causing an error of ±2% in absorbance value.

The pesticides which liberated phenol after hydrolysis or decomposition interfere with the method.
Table 2: Application of the method for the determination of fenthion in various environmental samples

<table>
<thead>
<tr>
<th>Sample**</th>
<th>Fenthion originally found (µg)</th>
<th>Fenthion added (µg)</th>
<th>Total fenthion found (µg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Polluted(a)</td>
<td>5.91</td>
<td>5.89</td>
<td>3.0</td>
<td>8.85</td>
</tr>
<tr>
<td>water</td>
<td>3.09</td>
<td>3.10</td>
<td>5.0</td>
<td>8.00</td>
</tr>
<tr>
<td>Tomato (b)</td>
<td>5.02</td>
<td>5.00</td>
<td>3.0</td>
<td>7.90</td>
</tr>
<tr>
<td>foliages</td>
<td>2.18</td>
<td>2.15</td>
<td>5.0</td>
<td>7.05</td>
</tr>
<tr>
<td>Cabbage (c)</td>
<td>4.99</td>
<td>4.93</td>
<td>3.0</td>
<td>7.91</td>
</tr>
<tr>
<td></td>
<td>2.17</td>
<td>2.16</td>
<td>5.0</td>
<td>7.10</td>
</tr>
<tr>
<td>Potato (d)</td>
<td>5.97</td>
<td>5.94</td>
<td>3.0</td>
<td>8.92</td>
</tr>
<tr>
<td></td>
<td>3.92</td>
<td>3.92</td>
<td>5.0</td>
<td>8.86</td>
</tr>
<tr>
<td>Cotton (e)</td>
<td>5.45</td>
<td>5.38</td>
<td>3.0</td>
<td>8.38</td>
</tr>
<tr>
<td>leaves</td>
<td>3.24</td>
<td>3.20</td>
<td>5.0</td>
<td>8.15</td>
</tr>
<tr>
<td>Apple (f)</td>
<td>5.95</td>
<td>5.91</td>
<td>3.0</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>3.87</td>
<td>3.81</td>
<td>5.0</td>
<td>8.81</td>
</tr>
</tbody>
</table>

* Mean of three replicate analysis

** Size of sample a = 50 ml, b, c, d, e and f = 50g

Aliquot of sample a, b, c, d, e and f = 5 ml, after treatment described in procedure section. A = present method B = reported method (37).
### Table - 3: Recovery from biological samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fenthion added (µg)</th>
<th>Fenthion found* (µg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Urine</td>
<td>3.0</td>
<td>2.93</td>
<td>2.93</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.94</td>
<td>4.92</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.91</td>
<td>6.88</td>
</tr>
<tr>
<td>Blood serum</td>
<td>3.0</td>
<td>2.92</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.92</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.87</td>
<td>6.88</td>
</tr>
</tbody>
</table>

*Mean of three replicate analysis.
Size of sample = 2ml, after treatment described in procedure section, sample is analysed. A = present method B = reported method (37).

### Table-4: Comparison with other spectrophotometric method.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Reagents/ Reference</th>
<th>λ max, nm</th>
<th>Detection / Determination limit (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>4-Amino-antipyrene(30)</td>
<td>510</td>
<td>70µg</td>
<td>Reagent unstable metal ions interfere</td>
</tr>
<tr>
<td>2.</td>
<td>4-(p-nitrobenzyl) pyridine (31)</td>
<td>540</td>
<td>0.5-1.0</td>
<td>Non specific, less selective</td>
</tr>
<tr>
<td>3.</td>
<td>3-nitroaniline-4-sulfonic acid (32)</td>
<td>530</td>
<td>0-20</td>
<td>Less sensitive, long waiting time</td>
</tr>
<tr>
<td>4.</td>
<td>Benzophenone (33)</td>
<td>-</td>
<td>35-350</td>
<td>Poor sensitivity</td>
</tr>
<tr>
<td>5.</td>
<td>p-Anisidine (37)</td>
<td>640</td>
<td>0.08 - 0.6</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>6.</td>
<td>p-Amino acetophenone (proposed method)</td>
<td>530</td>
<td>0.04-0.4</td>
<td>Highly sensitive and dye is stable</td>
</tr>
</tbody>
</table>
REFERENCE


