A New Sensitive Spectrophotometric Determination of Cypermethrin Insecticide in Environmental and Biological Samples

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A new and highly sensitive spectrophotometric method was developed for the determination of parts per million levels of widely used cypermethrin insecticide. The method is based on alkaline hydrolysis of cypermethrin to cyanide ion, which further reacts with potassium iodide and leuco crystal violet. The absorption maxima of the crystal violet dye formed was measured at 595 nm in acidic medium. Beer’s law obeys over the concentration range of 3.0 to 17 μg in a final solution volume of 25 mL (0.12-0.68 ppm). The molar absorptivity and Sandell’s sensitivity were found to be 3.3×10⁵ L mol⁻¹ cm⁻¹ and 0.054 μg cm⁻², respectively. The method is simple, sensitive and free from interferences of other pesticides and diverse ions. Other pyrethroid insecticides do not interfere in the proposed method. The method has been satisfactorily applied to the determination of cypermethrin in environmental and biological samples.

Keywords: spectrophotometry, cypermethrin, environmental, biological samples

Introduction

Pyrethroid insecticides are used to control a number of insect species on economic crops. Pyrethroid are effective pest control chemicals and have low mammalian toxicity. The pyrethroid insecticides containing a nitrile group, viz. Cypermethrin have been identified as highly effective contact insecticides. Owing to its availability, insecticides are misused in homicidal/suicidal poisoning cases. Consequently, detection and determination of these insecticides is necessary in forensic toxicology.¹⁶

Cypermethrin (Ambush, Atroban, Biothrin) or (RS)–cyano–3–phenoxybenzyl(1RS, 3RS; 1RS, 3RS)–(2,2–dichloro–vinyl)–2,2–dimethylcyclopropanecarboxylate (IUPAC name cyano–(3–phenoxypyphenyl)methyl] 3–(2,2–dichloroethenyl)–2,2–dimethyl–cyclopropane–1–carboxylate) is a digestive and contact insecticide effective against a wide range of pests, particularly leaf- and fruit-eating Lepidoptera and Coleoptera in cotton, fruit, vegetables, vines, tobacco and other crops. Cypermethrin is widely used by farmers to control insect pests of vegetables. The acute oral LD₅₀ for rats proposed for cypermethrin is 251 mg kg⁻¹.⁷,⁸

Several modern instrumental techniques such as LC-MS-MS,⁹ GC-MS,¹⁰ GC-ECD¹¹ and a large number of
chromatographic methods have been reported for residue analyses of synthetic pyrethroids. In autoradiographic thin layer chromatography (TLC), using 14C labeled compounds, particularly in metabolic studies where the unlabelled compounds were detected by visualization on silica gel 60 F254 chromatographic plates under ultraviolet (UV) light,12-14 chromogenic reagents have been reported. For example, phosphomolybdic acid,15 palladium chloride,16 silver nitrate17 and copper(II) acetate,6 are selective for pyrethroid insecticides containing a nitrile group. Other analytical methods have been reported, like spectrophotometric and thermoanalytical study18 and spectrophotometric method with MBTH19 (3–methyl–2–benzothiazolinone hydrochloride).

The aim of the present work is to develop a rapid, low cost, accurate and simple analytical method for the determination of cypermethrin at trace levels. In this paper, a simple and sensitive spectrophotometric method is described for the determination of cypermethrin, where cypermethrin is hydrolysed to give cyanide ion, which further reacts with potassium iodide and leuco crystal violet to produce a crystal violet dye with maximum absorbance at 595 nm in acidic medium. The reagent is selective for cypermethrin, amongst the pyrethroid group. The colour system obeys Beer’s law in the range of 0.12-0.68 ppm of cypermethrin. The method has been applied to the determination of cypermethrin in various samples of water, vegetables, fruits, foliages and biological samples.

Experimental

Apparatus

A Systronics UV-Vis spectrophotometric model 104 with matched silica cells was used for all spectral measurements. A Systronic pH meter model 335 was used for pH measurements. A Remi C-854/4 clinical centrifuge force of 1850 g with fixed swing out rotors was used for centrifugation.

Reagents

All reagents used were of Anala R grade or of the best available quality. Double distilled demineralized water was used throughout. Cypermethrin (Syngenta Crop Protection Private Limited, India): A stock solution of 1 mg mL−1 was prepared in ethanol. Working standard solutions were prepared by appropriate dilution of the stock standard solution with water. A 3% hydrochloric acid aqueous solution was used. A 20% sodium hydroxide aqueous solution was used. A 0.1% potassium iodide aqueous solution was used. Leuco crystal violet (Eastman Kodak Co.) [LCV] was prepared adding to a 1 liter volumetric flask 200 mL of water, 3 mL of 85% phosphoric acid and 250 mg of leuco crystal, (4,4′,4′′-methylidinetrin(2,2′,2′′-dimethylamine) (CH2(CH3)2)3). It was shaken gently until the dye gets dissolved. The content of the flask was then diluted to 1 liter with water.20

General procedure: preparation of calibration curve

An aliquot of test solution containing 3.0 to 17 μg of cypermethrin was taken in a 25 mL graduated cylinder and to it 1.0 mL of 20% sodium hydroxide was added. The solution was kept for 10 min at room temperature for complete hydrolysis. Then, 1 mL of 0.1% potassium iodide was added in acidic medium to liberate iodine and then 1 mL leuco crystal violet was added and shaken thoroughly and kept for 15 min for full colour development. The crystal violet dye was produced. The solution was then diluted to the mark with water and absorbance was measured at 595 nm against a reagent blank.

Results and Discussion

Spectral characteristics

The crystal violet dye formed in the proposed reaction shows maximum absorption at 595 nm (Figure 1). All spectral measurements were carried out against demineralized water as the reagent blank which showed negligible absorption at this wavelength. The colour system obeys Beer’s law in the range of 3.0 to 17 μg of cypermethrin per 25 mL of final solution at 595 nm (Figure 2). The molar absorptivity and Sandell’s sensitivity were found to be 3.3×105 L mol−1 cm−1 and 0.054 μg cm−2, respectively.

Optimization of conditions

Hydrolysis of cypermethrin to cyanide ion was studied at different temperatures and alkalinity. It was observed that...
alkaline conditions were required for the hydrolysis (Figure 3). Maximum hydrolysis was observed with 20% sodium hydroxide at temperature range of 30-35 °C as it gave maximum absorbance values, good stability and quantitative results. It was observed that 1 mL of leuco crystal violet was sufficient for complete colour reaction (Figure 4).

The effect of pH on the colour reaction was studied and it was found that constant absorbance values were obtained at pH range of ~ 4.5-5.5 by hydrochloric acid and no buffer solution was required to stabilize the colour. The coloured species remain stable for more then 7 days under optimum conditions.

Precision of the method was checked by the replicate analysis of working standard solution containing 4 μg of cypermethrin in 25 mL final solution over a period of 7 days. The standard deviation and relative standard deviation were found to be ± 0.001 and 0.22% respectively.

**Effect of foreign species**

The effect of common foreign species and pesticides were studied to assess the validity of the method. Known amounts of foreign species and pesticides were added to the standard solution contained 10 μg of cypermethrin prior to hydrolysis and the solution was analysed by the proposed method. The method was found to be free from interferences of most of the foreign species and pesticides (Table 1).

**Application**

**Determination of cypermethrin in vegetables, fruits and foliages**

Various samples of vegetables, fruits and foliages each of 25 mg, were taken, collected from agricultural fields, where cypermethrin had been sprayed as an insecticide. The samples were macerated with two 20 mL portions of ethanol-demineralized water (1+1), filtered through a thin cotton cloth and filtrate was centrifuged at 1850 g for 10 min. In case of vegetables and fruits the filtrate was quantitatively transferred into a 50 mL volumetric flask and made up to the mark with 50% ethanol. Aliquots of supernatant were taken in a 25 mL graduated cylinder and then 1.0 mL of 20% sodium hydroxide was added and kept for 10 min at room temperature for complete hydrolysis. Then 1 mL of potassium iodide and leuco crystal violet was added in acidic medium and shaken thoroughly and kept for 15 min for full colour development. The solution

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit/μg in 25 mL</th>
<th>Foreign species</th>
<th>Tolerance limit/μg in 25 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>5000</td>
<td>Al³⁺, Mg²⁺, Co³⁺</td>
<td>2200</td>
</tr>
<tr>
<td>Phenol, Ethanol</td>
<td>2500</td>
<td>Zn²⁺, Cu²⁺, Mn²⁺</td>
<td>1600</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>2100</td>
<td>Fe³⁺, Fe²⁺, Sb⁵⁺</td>
<td>1300</td>
</tr>
<tr>
<td>Toluene, Xylene</td>
<td>1500</td>
<td>Ni²⁺, Pb²⁺, Ca²⁺</td>
<td>750</td>
</tr>
<tr>
<td>Aniline, Formaldehyde</td>
<td>900</td>
<td>Br⁻, CO₃²⁻, Cl⁻</td>
<td>450</td>
</tr>
<tr>
<td>Parathion, Malathion, Cresol</td>
<td>500</td>
<td>NO₃⁻</td>
<td>150</td>
</tr>
<tr>
<td>Fenvalerate, Deltamethrin</td>
<td>2⁻</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The amount causing an error of ±2% in absorbance value. *Tolerance limit without its removal from the sample.
was then diluted to the mark with water and absorbance was measured at 595 nm against a reagent blank.

In case of foliages, the filtrate was passed through a silica gel column (10×1 cm) filled with 5 mg silica gel, which was found to be sufficient for removal of chlorophyll and other interfering materials present in the extracted sample. The column was washed with 10 mL of 50% ethanol, washings were collected in a 25 mL volumetric flask and aliquots were analysed as recommended above (Table 2).

**Determination of cypermethrin in water**

River water samples, which received run off water from agricultural field, were collected. These samples were filtered through a Whatman No. 40 filter paper. Aliquots of water samples were taken in a 25 mL graduated cylinder, to it sodium hydroxide was added and analysed as described above (Table 2).

**Recovery of cypermethrin in biological samples**

The presence of cypermethrin in blood and urine has been reported in detectable concentrations. Thus the method has been applied for the determination of cypermethrin in biological samples. Synthetic samples were prepared by adding known amounts of cypermethrin to these samples and then analysed after deproteination with trichloroacetic acid as described above (Table 3).

**Conclusions**

The proposed method is rapid, simple and sensitive and the reagent described here is sensitive and selective for cypermethrin insecticides containing a nitrile group. The lower limit of detection of the method is about 0.003 μg.

The proposed method has been applied to the determination of cypermethrin in various samples of water, vegetables, fruits, foliages and biological samples.

To check the recoveries, known amount of cypermethrin were added to various samples of vegetables, fruits, foliages and biological samples and then analysed by the proposed method (Tables 2 and 3).

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**Table 2. Determination of cypermethrin in environmental and agricultural samples**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cypermethrin originally found(^a) (μg)</th>
<th>Cypermethrin Added (μg)</th>
<th>Total Cypermethrin Found (μg)</th>
<th>Difference (μg)</th>
<th>Recovery (◦)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water(^b)</td>
<td>1.36</td>
<td>5.0</td>
<td>6.31</td>
<td>4.95</td>
<td>99</td>
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<td></td>
<td>4.56</td>
<td>15.0</td>
<td>19.13</td>
<td>14.57</td>
<td>97</td>
</tr>
<tr>
<td>Tomato(^c)</td>
<td>2.03</td>
<td>5.0</td>
<td>6.84</td>
<td>4.81</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>4.81</td>
<td>15.0</td>
<td>19.36</td>
<td>14.55</td>
<td>97</td>
</tr>
<tr>
<td>Apple(^d)</td>
<td>1.03</td>
<td>5.0</td>
<td>5.88</td>
<td>4.85</td>
<td>97</td>
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<tr>
<td></td>
<td>3.24</td>
<td>15.0</td>
<td>17.67</td>
<td>14.43</td>
<td>96</td>
</tr>
<tr>
<td>Cauliflower(^e)</td>
<td>3.42</td>
<td>5.0</td>
<td>7.56</td>
<td>4.14</td>
<td>83</td>
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<td></td>
<td>5.35</td>
<td>15.0</td>
<td>19.65</td>
<td>14.3</td>
<td>95</td>
</tr>
<tr>
<td>Cotton foliages(^e)</td>
<td>2.96</td>
<td>5.0</td>
<td>7.86</td>
<td>4.90</td>
<td>98</td>
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<tr>
<td></td>
<td>4.56</td>
<td>15.0</td>
<td>18.96</td>
<td>14.4</td>
<td>96</td>
</tr>
</tbody>
</table>

\(^a\) Mean of three replicate analyses; \(^b\) Water sample 25 mL, 1 mL aliquot of sample was analyzed, after treatment as described in procedure; \(^c\) Sample 25 g (sample taken from a field where cypermethrin had been sprayed).

**Table 3. Recovery of cypermethrin in biological samples**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Amount added (μg)</th>
<th>Amount found (μg)</th>
<th>Recovery (◦)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.0</td>
<td>3.69</td>
<td>92</td>
</tr>
<tr>
<td>B</td>
<td>8.0</td>
<td>7.76</td>
<td>97</td>
</tr>
<tr>
<td>Urine(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.0</td>
<td>3.89</td>
<td>97</td>
</tr>
<tr>
<td>B</td>
<td>8.0</td>
<td>7.81</td>
<td>98</td>
</tr>
<tr>
<td>Cystein(^b)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>4.0</td>
<td>3.92</td>
<td>98</td>
</tr>
<tr>
<td>B</td>
<td>8.0</td>
<td>7.86</td>
<td>98</td>
</tr>
</tbody>
</table>

\(^a\) Mean of three replicate analyses. \(^b\) Amount of biological sample = 1 mL.
References


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