

A Highly Sensitive Method for the Determination of Some Pyrethroid Insecticides in Different Environmental and Biological Samples

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ABSTRACT

A modified and sensitive spectrophotometric method for the determination of nitrile insecticides i. e., cypermethrin, fenvalerate, deltamethrin, in sub-microgram levels is described. During alkaline hydrolysis of pyrethroids to produce cyanide ion, which react with bromine-water to form cyanogen bromide, the pyridine rings are spilt by Konig reaction and the reaction products are coupled with p-anisidine. The colour system obeys Beer's law in the following working range in ppm : cypermethrin, 0.11-0.89; fenvalerate, 0.21-1.76 ; and deltamethrin, 0.2-1.24 respectively. The Molar absorptivity, Sandell's sensitivity, Correlation coefficient have been determined. Other pyrethroids not containing a hydrolysable nitrile group (permethrin, resmethrin, allenthin, etc), should not interfere; moreover organochlorine, organophosphorous and carbamate insecticides do not give colour spot. The method is highly reproducible and have been successfully applied to determination of nitrile containing insecticides in environmental and biological samples.

Keywords: Nitrile pesticides (Cypermethrin, Fenvalerate, Deltamethrin), Environmental and Biological samples.

INTRODUCTION

Pyrethroids are widely used insecticides both in agriculture and households. These have been widely used for over two decades, are now being superseded by synthetic pyrethroids because of their greater photo stability and enhanced insecticidal activity. The

acute oral LD₅₀ value for rats proposed for pyrethroids group is 251- 4150 mg/kg.¹⁻³

Pyrethroid insecticides are used to control a number of insect species on economic crops. These pyrethroid are effective pest control chemical and have low mammalian toxicity.⁴⁻⁵ Three major pyrethroid insecticides containing a nitrile group, viz., cypermethin fenvalerate and deltamethrin, have been identified as highly effective contact insecticides. Their use is increasing for the control of insects. Owing of their availability, insecticides are misused in homicidal suicidal poisoning cases. Consequently, characterization of these insecticides is necessary in forensic toxicology.⁶

Pyrethroids are 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. These are widely used by farmers to control insect pests of vegetables. There is currently an increasing concern and awareness on the hazards of pesticides to consumers. Even with the adoption of integrated pest management, farmers still believe in the control of pests using pesticide because of its quick effect.⁷

A large number of gas – liquid chromatographic methods⁸⁻¹⁰ for residue analysis of synthetic pyrethroids have been reported, as has 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,¹¹⁻¹³ Gas Chromatography- Flame ionization detection,^{14,15} silver nitrate¹⁶ and copper (II) acetate⁶ are selective for pyrethroid insecticides containing a nitrile group. Some instrumental method i.e., gas chromatography-high resolution mass spectrometry¹⁷, direct high-performance liquid chromatographic method¹⁸ and leaching of pesticides in herbal decoction¹⁹ have been reported.

In the present paper a new simple and sensitive spectrophotometric method is described for the determination of pyrethroids, where pyrethroids is hydrolysed to give cyanide ion, which further react with bromine-water to form cyanogen bromide, the pyridine rings are spilt by Konig reaction and the reaction products are coupled with p-anisidine. The reagent is selective for pyrethroids group. The color system obeys Beer's law in the following working range in ppm: cypermethrin, 0.13-0.93; fenvalerate, 0.27-2.0; and deltamethrin, 0.2-1.33 respectively. The method have been applied to the determination of pyrethroids in various samples of water, vegetables, fruits, foliages and biological samples.

EXPERIMENTAL

Apparatus and reagents

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.

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), Deltamethrin (Bayer Crop Science, India) and Fenvalerate (Rallts, Tata Enterprise): 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.

Sodium hydroxide: A 20% aqueous solution was used.⁶

Absorbing solution (bromine water): Saturated bromine water was used.

Sodium nitrite: A 1% m/v solution was prepared in 10 v/v hydrochloric acid.

Pyridine: Pyridine was used.

p-anisidine: 0.1% (m/v) solution of the reagent was prepared by dissolving 500 mg of p-anisidine in 50 ml ethanol.

Procedure

Preparation of calibration graph

An aliquot of test solution containing 5.0 to 25 μg of pyrethroids were taken in a 35-ml calibrated midget impinger, 4.0 ml of 20% sodium hydroxide was added and kept in hot water bath ($\sim 60^\circ\text{C}$). The impinger was then connected to an air sampling train carrying one impinger containing brown water and a rotameter connected in series²⁰. Air was sampled in the beginning at flow rate of less than 0.5 liter/min for 5 min and the flow rate was then increased to ~ 1 liter/min for next 10 min. After sampling the absorbing solution was quantitatively transferred to a 25-ml graduated tube. The excess of bromine was decolorized by drop wise addition of 1% formaldehyde solution. 3 ml pyridine and 1 ml of 1 molar sodium hydroxide were added and kept in a hot water bath ($40\text{-}50^\circ\text{C}$) for 30 min. A pink color solution was obtained. The tube was taken out, cooled and, to it, 1 ml p-anisidine was added, shaken thoroughly and kept for 15 min for full color development. The solution was then diluted to the mark with water and absorbance was measured against a reagent blank.

RESULTS AND DISCUSSION

Spectral Characteristics

The color system shows maximum absorption at 610 nm for cypermethrin, fenvalerate and deltamethrin, respectively. All spectral measurements were carried out against demineralized water as the reagent blank showed negligible absorption at this wavelength. (Table 2)

Conditions for the Color Development

Hydrolysis of pyrethroids to cyanide ion was studied at different temperatures and alkalinity. It was observed that alkaline conditions were required for the hydrolysis. Maximum hydrolysis⁶ was observed with 20% sodium hydroxide at temperature range of $45\text{-}60^\circ\text{C}$ as it gave maximum absorbance values, good stability and quantitative results.

It was observed that 1 ml of p- anisidine was sufficient for complete color reaction.

Effect of pH

The effect of pH on the color reaction was studied and it was found that constant absorbance values were obtained at pH range of ~ 9.5 – 11.0 and no buffer solution was required to stabilize the color. The colored species remain stable for more then 7 hr. under optimum conditions.

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 containing 5µg/ml of pyrethroids group 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)

Precision

Precision of the method was checked by the replicate analysis of working standard solution containing 6µg of cypermethrin, fenvalerate and deltamethrin in 25 ml final solution over a period of seven days, respectively. The standard deviation, relative standard deviation and correlation coefficient have been calculated, respectively. (Table 2)

APPLICATION

Determination of pyrethroids in vegetables, fruits and foliages

Various samples of vegetables, fruits and foliages each of 25 gm, were taken, collected from agricultural field, where pyrethroids 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 calibrated flask and made up to the mark with 50% ethanol.²¹ An aliquot of supernatant were taken in a 35-ml calibrated midjet impinger, 4.0 ml of 20% sodium hydroxide was added and kept in hot water bath (~60°C). The impinger was then connected to an air sampling train carrying one impinger containing brown water and a rotameter connected in series²⁰. Air was sampled in the beginning at flow rate of less than 0.5 liter/min for 5 min and the flow rate was then increased to ~ 1 liter/min for next 10 min. After sampling the absorbing solution was quantitatively transferred to a 25-ml graduated tube. The excess of bromine was decolorized by drop wise addition of 1% formaldehyde solution. 3 ml pyridine and 1ml of 1 molar sodium hydroxide were added and kept in a hot water bath (40-50°C) for 30 min. A pink color solution was

obtained. The tube was taken out, cooled and, to it, 1 ml p-anisidine was added, shaken thoroughly and kept for 15 min for full color development. The solution was then diluted to the mark with water and absorbance was measured against a reagent blank.

In case of foliages, the filtrate was passed through a silica gel column (10x1cm) filled with 5 gm silica gel,²⁰ 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 35-midget impinger and aliquots were analysed as recommended above (Table 3).

Determination of pyrethroids in water

River water samples, which received run off water from agricultural field, were collected.²⁰ These samples were filtered through a Whatman No. 40 filter paper. Aliquots of water samples were taken in a 35-midget impinger, to it sodium hydroxide was added and analysed as described above (Table 3).

Recovery of Pyrethroids in biological samples

Since the presence of pyrethroids in blood, urine and cystein has been reported in detectable concentration.²²⁻²³ The method has been applied for the determination of pyrethroids in biological samples. Synthetic samples were prepared by adding known amounts of pyrethroids to these samples and than analysed after deproteination with trichloroacetic acid²⁴⁻²⁵ as described above. (Table 4)

Table 1. Effect of foreign species for pyrethroids group (5 ppm)

Foreign species	Tolerance limit* µg in 25ml	Foreign species	Tolerance limit* µg in 25 ml
Benzene	3300	Al ³⁺ , Mg ²⁺ , Co ²⁺	1300
Phenol, Ethanol	2100	Zn ²⁺ , Cu ²⁺ , Mn ²⁺	1200
Benzaldehyde, Acetone	1700	Fe ³⁺ Fe ²⁺ , Sb ³⁺	800
Toluene, Xylene	1400	Ni ²⁺ , Pb ²⁺ , Ca ²⁺	700
Aniline, Formaldehyde	700	Br ⁻ , CO ₃ ²⁻ , Cl ⁻	550
Parathion, Malathion, Cresol	400	NO ₂ ⁻	170

* = The amount causing an error of ±2% in absorbance value.

Table 2. Optical characteristics, precision and accuracy of the method with the coupling agent.

Effect	Cypermethrin	Fenvalerate	Deltamethrin
1. Beer's Law(ppm)	0.11-0.89	0.21-1.76	0.2-1.33
2. Molar absorptivity (liter mol ⁻¹ cm ⁻¹)	8.3x 10 ⁵ (± 100)	6.5x10 ⁵ (±100)	3.5x10 ⁵ (±100)
3. Sendell's Sensitivity (µg cm ⁻²)	0.021	0.034	0.061
4. Relative standard deviation (%)	0.34	0.42	0.85
5. Stability of colour (h)	7	5	7
6. Correlation coefficient	0.9816	0.9981	0.9992

Table 3. Determination of pyrethroids in environmental and agricultural samples.

Table 2. Determination of pyrethroids in environmental and agricultural samples.

Samples	Pyrethroids originally found* (µg) (a)			Amount added (µg) (b)			Total Pyrethroids found (µg) (c)			Difference (c-a)			Recovery % (c-a) / b x100			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Water**	X	2.1	3.5	3.2	10	10	10	11.5	12.6	12.7	9.4	9.1	9.5	94	91	95
	Y	4.7	4.1	3.9	20	20	20	23.4	24	23.6	18.7	19.9	19.7	93.5	99.5	98.5
Tomoto***	X	2.3	1.4	1.9	10	10	10	11.6	10.2	11.2	9.3	8.8	9.3	93	88	93
	Y	4.3	2.7	2.8	20	20	20	23.6	22.2	22.1	19.3	19.5	19.3	96.5	97.5	96.5
Apple****	X	1.1	0.98	ND	10	10	10	10.4	10.6	9.6	9.3	9.62	9.6	93	96.2	96
	Y	2.7	1.5	ND	20	20	20	22.3	21.1	19.1	19.6	19.6	19.1	98	98	95.5
Cauliflower***	X	0.61	ND	ND	10	10	10	10.2	9.6	9.5	9.59	9.6	9.5	95.9	96	95
	Y	0.23	ND	ND	20	20	20	19.6	19.4	18.9	19.3	19.4	18.9	96.5	97	94.5
Cotton Foliages***	X	ND	ND	ND	10	10	10	9.7	9.6	8.9	9.7	9.6	8.9	97	96	89
	Y	ND	ND	ND	20	20	20	18.8	19.6	18.6	18.8	19.6	18.6	94	98	93

* = Mean of three replicate analyses.

** = Water sample 25 ml.

*** = Sample 25 gm (taken from a field where pyrethroids have been sprayed)

A = Cypermethrin, B = Fenvalerate, C = Deltamethri, ND = Not Detected,

X and Y = Different sample from different site