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Method For The Determination of Some Pyrethroid Insecticides In 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 cynogen bromide, the pyridine rings are spilt by Konig reaction and the reaction products are coupled with diazotised p-aminobenzoic acid. The colour 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 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 for determination of nitrile containing insecticides in environmental and biological samples.

Keywords: Nitrile pesticides (Cypermethrin, Fenvalerate, Deltamethrin), Environmental & 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 These have been widely used for over two decades, are now being superseded by synthetic pyrethroids because of their greater photo stability and enhanced insecticidal activity.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 LD_{50} value for rats proposed for pyrethroids group is 251- 4150 mg kg^{-1} [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 methods [8-10] for residue analysis of synthetic pyrethroids have been reported, as has Autoradiographic thin layer chromatography (TLC), using ^{14}C – labeled compounds, particularly in metabolic studies where the unlabelled compounds were detected by visualization on silica gel 60 F_{254} chromatographic plates under ultraviolet (UV) light[11-13], chromogenic reagents have been reported, for example, phosphomolybdic acid [14], palladium chloride [15], silver nitrate[16] and copper (II) acetate [6] are selective for pyrethroid insecticides containing a nitrile group. Some instrumental method i. e., gas chromatography-high resolution mass spectrometry [17], direct high-performance liquid chromatographic method [18] and leaching of pesticides in herbal decoction [19] 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 diazotised p-aminobenzoic acid. 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 has been applied to the determination of pyrethroids in various samples of water, vegetables, fruits, foliages and biological samples.

MATERIALS AND METHODS

Apparatus and reagents: 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.
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- aminobenzoic acid, [PABA] (E. Merck, Germany): 0.1% (m/v) solution of the reagent was prepared by dissolving 500 mg of p-aminobenzoic acid in 50 ml ethanol.

Diazotized p- aminobenzoic acid [DPABA]: To 10 mL of p- aminobenzoic acid, 1 ml of 1% sodium nitrite in hydrochloric acid was added and the solution was kept in a brown bottle. This was stable for 4 h when kept in cold.

Preparation of calibration graph: An aliquot of test solution containing 5.0 to 25 µg of pyrethroids was taken in a 35mL calibrated midget impinger, 4.0 mL of 20% sodium hydroxide was added and kept in hot water bath (~ 60^0C). The impinger was then connected to an air sampling train carrying one impinger containing brown water and a rotameter connected in series [20]. Air was sampled in the beginning at flow rate of less than 0.5 L min^{-1} for 5 min and the flow rate was then increased to ~ 1 L min^{-1} for next 10 min. After sampling the absorbing solution was quantitatively transferred to a 25mL 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 (40-50^0C) for 30 min. A pink color solution was obtained. The tube was taken out, cooled and, to it, 1 ml diazotized p-aminobenzoic acid 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 410 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 1**).

Table 1. Optical characteristics, precision and accuracy of the method with the coupling agent.

Effect	Cypermethrin	Fenvalerate	Deltamethrin
Beer 's Law(ppm)	0.13-0.93	0.27-2.0	0.2-1.33
Molar absorptivity (liter mol^{-1} cm^{-1})	8.1x 10^5(± 100)	6.1x10^5 (±100)	2.9x10^5(±100)
Sandell's Sensitivity (µg cm^{-2})	0.022	0.035	0.060
Relative standard deviation (%)	0.33	0.41	0.81
Stability of colour (h)	7	5	7
Correlation coefficient	0.9819	0.9985	0.9997

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 hydrolysis [6] was observed with 20% sodium hydroxide at temperature range of 50 - 70^oC as it gave maximum absorbance values, good stability and quantitative results. It was observed that 1 ml of diazotized p- aminobenzoic acid 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 than 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^{-1} 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 2).

Table 2. Effect of foreign species for pyrethroids group (5 ppm)

Foreigen species	Tolerance limit* µg in 25ml	Foreigen species	Tolerance limit* µg in 25 ml
Benzene	3500	Al^{3+}, Mg^{2+}, Co^{2+}	1200
Phenol, Ethanol	2200	Zn^{2+}, Cu^{2+} , Mn^{2+}	1000
Benzaldehyde, Acetone	1900	Fe^{3+} Fe^{2+} , Sb^{3+}	900
Toluene, Xylene	1500	Ni^{2+} , Pb^{2+} , Ca^{2+}	600
Aniline, Formaldehyde	800	Br^- , CO_3^{2-}, Cl^-	430
Parathion, Malathion, Cresol	500	NO_2^-	100

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

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 1).

APPLICATIONS

Determination of pyrethroids in vegetables, fruits and foliages: Various samples of vegetables, fruits and foliages each of 25 g, 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 midget impinger, 4.0 mL of 20% sodium hydroxide was added and kept in hot water bath (~ 60^0C). The impinger was then connected to an air sampling train carrying one impinger containing brown water and a rotameter connected in series [20]. Air was sampled in the beginning at flow rate of less than 0.5 liter min^{-1} for 5 min and the flow rate was then increased to ~ 1 liter min^{-1} 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 (40-50^0C) for 30 min. A pink color solution was obtained. The tube was taken out, cooled and, to it, 1 ml diazotized p-aminobenzoic acid 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 runoff 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 analyzed as described above (Table 3).

Table 3. 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	2.3	3.5	3.1	10	10	10	11.5	12.6	12.8	9.2	9.1	9.7	92	91	97
	Y	4.7	4.3	3.9	20	20	20	23.4	24	23.6	18.7	19.7	19.7	93.5	98.5	98.5
Tomoto***	X	2.6	1.5	1.9	10	10	10	11.9	10.6	11.2	9.3	9.1	9.3	93	91	93
	Y	4.3	2.9	2.8	20	20	20	23.6	21.9	22.1	19.3	19	19.3	96.5	95	96.5
Apple***	X	1.3	0.98	ND	10	10	10	11.1	10.6	9.6	9.8	9.62	9.6	93	96.2	96
	Y	2.7	1.6	ND	20	20	20	22.3	21.2	19.1	19.6	19.6	19.1	98	98	95.5
Cauliflower***	X	0.63	ND	ND	10	10	10	10.3	9.6	9.5	9.67	9.6	9.5	96.7	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

Recovery of cypermethrin 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 then analysed after deproteination with trichloroacetic acid [24-25] as described above (Table 4).

Table 4. Recovery from biological samples.

Samples		Pyrethroids added (µg)	Pyrethroids found*(µg)			% Recovery		
			A	B	C	A	B	C
Blood**	X	2.0	1.69	1.38	1.29	84.5	69.0	64.5
	Y	4.0	3.82	3.56	3.49	95.5	89.0	87.2
Urine**	X	2.0	1.85	1.87	1.77	92.5	93.5	88.5
	Y	4.0	3.89	3.98	3.58	97.2	99.5	89.5
Cystein**	X	2.0	1.69	1.65	1.43	84.5	82.5	71.5
	Y	4.0	3.54	3.33	3.31	88.5	83.2	82.7

* = Mean of three replicate analyses.
** = Amount of biological sample = 1ml.
A = Cypermethrin, B = Fenvalerate, C = Deltamethrin

CONCLUSIONS

The proposed method is rapid, simple, sensitive and reagent described here is sensitive and selective for pyrethroids insecticides containing a nitrile group. The proposed method has been applied satisfactorily to the determination of pyrethroids in various samples of water, vegetables, fruits, foliages and biological samples.

To check the recoveries, known amount of pyrethroids were added to various samples of vegetables, fruits, foliages and biological samples and then analyzed by the proposed method (Table 3, 4).

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REFERENCES

[1] " Pesticide Residues in Food 1981, Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues," FAO Plant Production and Protection Paper, No. 37, FAO, Rome, **1982**.
[2] B.C.Fine, *Pyrethrum Post*, **1963**, 7, 18.
[3] A.W.Farnham, *Pestic. Sci,* **1973**, 4, 513.
[4] M. Elliott, N.F. Janes, C. Potter, *Annu. Rev. Entomol*, **1978**, 23, 443.

[5] M. Elliott, *Pestic. Sci*, **1980**, 11, 119.
[6] B.P. Vitthal, T.S. Murlidhar, V.P. Sudhakar, *Analyst*, **1992**, 117, 75.
[7] M.Abdullah, O. Sarnthoy, S. Jiwajinda, J. *Kasetsart, Nat. Sci*, **2001**, 35, 115.
[8] R.A.Chapman, H.S. Simmons, *J. Assoc. Off. Anal. Chem,* **1977**, 60, 977.
[9] N.S. Talekar, *J. Assoc. Off. Anal. Chem,* **1977**, 60, 908.
[10] Y.W. Lee, N.D. Westcott, R.A. Reichle, *J. Assoc. Off. Anal. Chem,* **1978**, 61, 869.
[11] T.R.Roberts, M.E. Standen, *Pestic. Sci*, **1977**, 8, 305.
[12] L.O. Ruzo, R.L. Holmstead, J.E. Casida, *J. Agric, Food Chem*, **1977**, 25, 1385.
[13] L.C. Gaughan, M.E. Ackerman, T. Unai, J.E. Casida, *J. Agric. Food Chem,* **1978**, 26, 613.
[14] T.Shono, K. Ohsawa, J.E. Casida, *J. Agric. Food Chem,* **1979**, 27, 316.
[15] L.O.Ruzo, J.E. Engel, J.E. Casida, *J. Agric. Food Chem,* **1979**, 27, 725.
[16] R.Sundararajan, R.P. Chawla, *J. Assoc. Off. Anal. Chem,* **1983**, 66**,** 1009.
[17] G.Leng, W. Gries, *Journal of Chromatography B,* **2005**, 814, 285.
[18] W.Bicker, M. Lammerhofer, W. Lindner, *Journal of Chromatography A,* **2004**, 1035, 37.
[19] D. Tewary, V. Kumar, A. Shanker, *Chemical Health and Safety*, **2004**, 11, 25.
[20] J.V. Das, K.N. Ramachandran, V.K. Gupta, *Microchemical Journal*, **1994**, 50, 51.
[21] D. Bose, P. Shivhare, V.K. Gupta, *Journal of Planar Chromatography*, **1994**, **7**, 415.
[22] S.Upadhyay, V.K. Gupta, *Analyst*, **1984**, 109, 1619.
[23] J. Raju, V.K. Gupta, *Microchem J,* **1989**, 39, 25.
[24] W.N. Aldridge, *Analyst*, **1994**, 69, 262.
[25] J.V. Das, V.K. Gupta, *Chem. Anal, (*Warsaw), **1994**, 39, 693.

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