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Flow Injection Spectrophotometric Determination of Cypermethrin Insecticide by Diazotization Method

YASMEEN PERVEZ[1,*], ETESH K. JANGHEL[2] and SANTOSH KUMAR SAR[3]

[1]Department of Applied Chemistry, Chhatrapati Shivaji Institute of Technology, Durg-491 001, India
[2]Department of Applied Chemistry, Ashoka Institute of Technology & Management, Rajnandgaon-491 441, India
[3]Department of Applied Chemistry, Bhilai Institute of Technology, Durg-491 001, India

*Corresponding author: Fax: +91 788 2334808; E-mail: dr.ypervez@gmail.com

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Flow injection spectrophotometric method is developed for the determination of cypermethrin insecticide. The method is based on alkaline hydrolysis of cypermethrin is converted into 2-diphenyl ether cyano ethane and hydrolyzed with HCl and resulting product was diazotized with nitrate and coupled with aniline. The absorption maxima of the azo dye formed is measured at 535 nm in acidic medium. Beer's law is obeyed over the concentration range of 5.5 to 36 µg/25 mL. The molar absorptivity and Sandell's sensitivity were found to be 5.3×10^4 L mol^{-1} cm^{-1} and 0.066 µg cm^{-2}, respectively. The standard deviation and relative standard deviation were found to be ± 0.003 and 0.56 %, respectively. The % recovery for the determination of cypermethrin was found to be 92 %. The sampling frequency was 80 samples per hour for flow injection analysis. 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 is simple, fast and has been satisfactorily applied to the determination of cypermethrin in commercial formation, food and environmental sample.

Keywords: Flow injection, Spectrophotometry, Cypermethrin, Peristaltic pump, Aniline.

INTRODUCTION

Synthetic pyrethroid insecticides are exceptionally active as a contact and stomach poison against lepidoperous larvae. It is used to control a number of insect species on economic crops. Pyrethriod are effective pest control chemical and have low mammalian toxicity. Pyrethroid insecticides containing a nitrile group, *i.e.*, cypermethrin has been identified as highly effective contact insecticides. It has been used in agricultural, veterinary product and home formulation for more than 40 years and account for approximately one-fourth of the world wide insecticide market [1-6].

Cypermethrin (Ambush, Atroban, Biothrin) or (RS)-cyano-3-phenoxybenzyl(1RS,3RS;1RS,3RS)-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropane 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, wines, tobacco and other crops. Cypermethrin is widely used by farmers to control insect pests of vegetables. The acute oral LD_{50} value for rats proposed for cypermethrin is 251 mg kg^{-1}, respectively [7,8].

The general techniques used for the determination of same class of pesticides, are spectrophotometry, TLC, HPLC, HPLC-MS, GC, electron capture method, coupled column liquid chromatography, capillary GC-MS and FT-IR [9-26]. Many chromogenic reagents have been reported, *i.e.* phosphomolybdic acid [27], palladium chloride [28], silver nitrate [29] and copper(II) acetate [30] are selected for pyrethroid insecticides containing a nitrile group. Some analytical method has been reported, *i.e.*, spectrophotometric and thermo analytical study [31,32]. Chromatographic methods are laborious and time consuming and require sophisticated equipment available in only well-equipped centralized laboratories [33,34]. Here, a rapid spectrophotometric method for the determination of the cypermethrin insecticide has been described. The flow injection version of the method provides a useful way to automate the analytical procedures and will prove to be very useful in speeding up the determination of cypermethrin in commercial formation and vegetable samples.

The present work is to develop a rapid, accurate and flow injection method for the determination of cypermethrin at macro level. In this paper, cypermethrin is converted into 2-diphenyl ether cyano ethane and then hydrolyzed with HCl and resulting product was diazotized with nitric acid and coupled with aniline. The absorption maxima of the azo dye formed is measured at 535 nm in acidic medium. The method

is simple, fast and has been satisfactorily applied to the determination of cypermethrin in commercial formation and vegetative samples.

EXPERIMENTAL

Digital analytical balance, peristaltic pump, PTFE tubing (i.d. 1.19 mm, Becton Dickson, USA), Silicon Tube (i.d. 1.71 mm), V-450 6 port injection valve, Systronics UV-visible spectrophotometer 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. A layout of the system is given in Fig. 1.

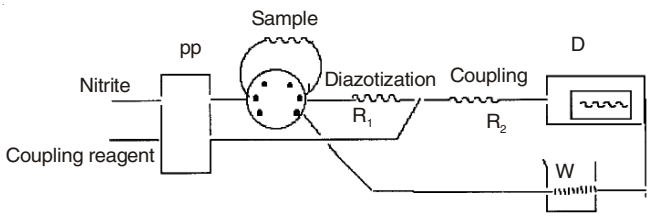


Fig. 1. FI manifolds used in the determination of insecticide cypermethrin. PP, peristaltic pump; R_1, diazotization reactor; R_2, coupling reactor; D-detector; W-waste

All reagents used were of AnalaR grade. 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.

Hydrochloric acid: 3 % aqueous solution was used. Sodium hydroxide: A 20 % aqueous solution was used. Nitrate solution: A nitrate solution (0.15 %) was prepared by dissolving 0.225 g of sodium nitrate in water and diluted up to 100 mL with distilled water. Aniline solution: An aniline solution (2 %) was prepared by dissolving 2 mL of aniline in 70 mL of ethanol and diluting up to 100 mL with water.

Procedure

Spectral characteristics: The azo dye formed in the proposed reaction shows maximum absorption at 535 nm. All spectral measurements were carried out against demineralized water as the reagent blank showed negligible absorption at this wavelength (Fig. 2).

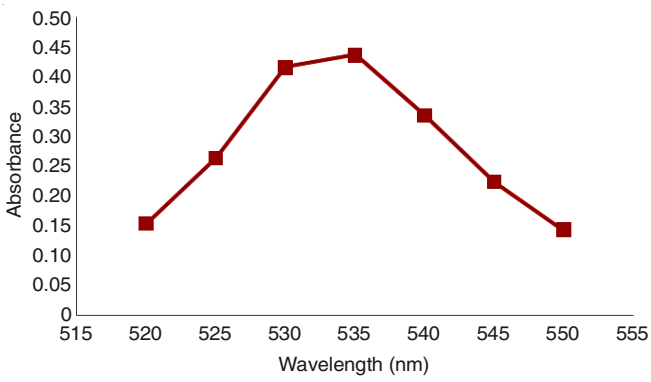


Fig. 2. Absorption spectra of the azo dye formed

Preparation of calibration graph: An aliquot of test solution containing 5.5 to 36 µg of cypermethrin was taken in a 25 mL graduated tube and, 1 mL of 20 % sodium hydroxide was added to it. The solution was kept for 15 min at room temperature for complete hydrolysis. Cypermethrin is converted to 2-diphenyl ether cyano ethane and hydrolyzed with HCl and resulting product was diazotized with nitric acid and coupled with aniline. The azo dye was produced (**Scheme-I**). The solution was then diluted to the mark with water and absorbance was measured at 535 nm against a reagent blank (Fig. 3).

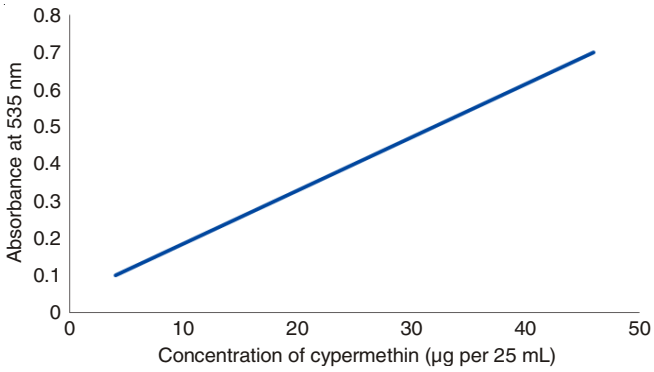
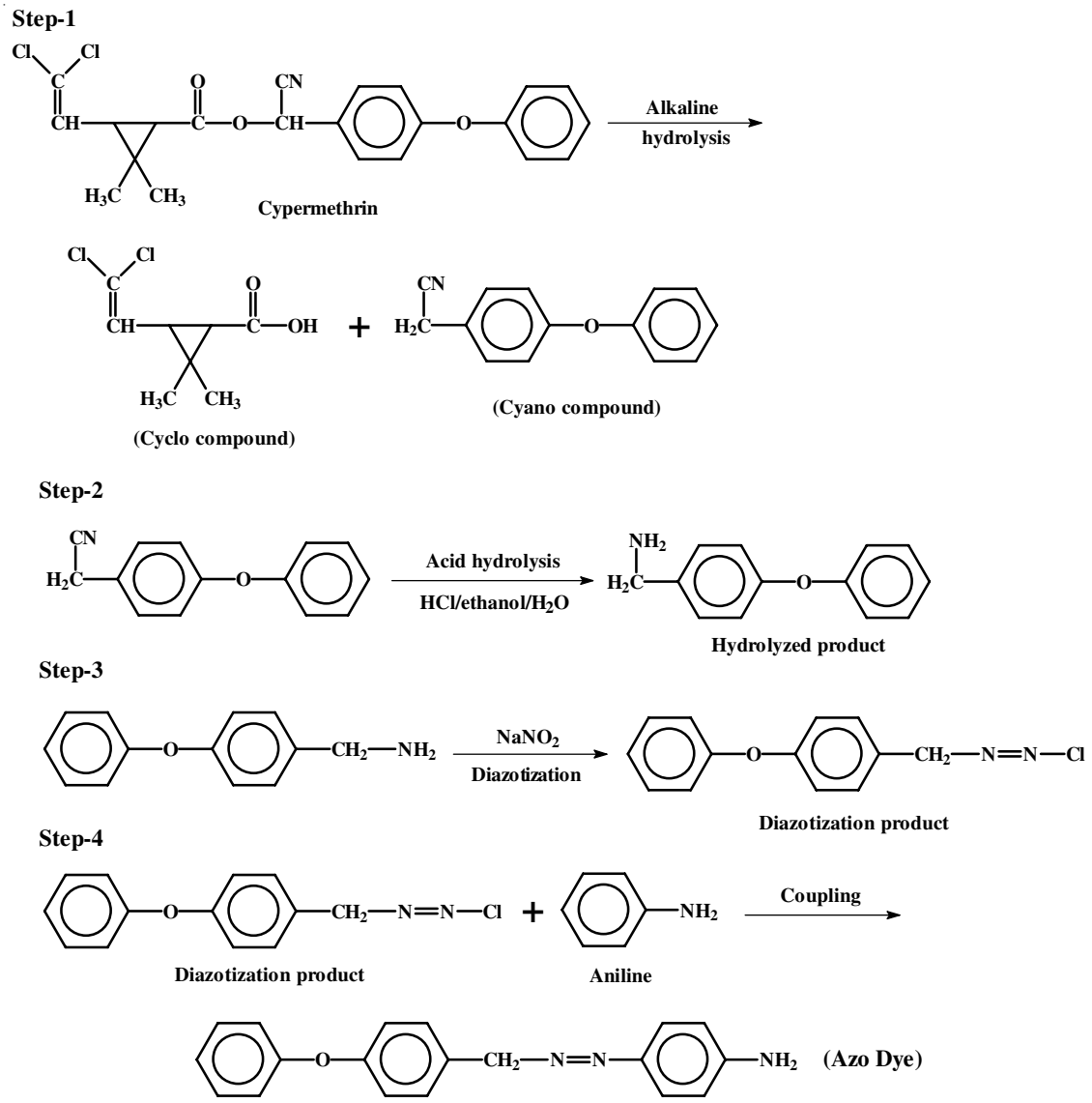


Fig. 3. Beer's law of cypermethrin

Method of batch and FIA system: In case of the batch analysis from a standard stock solution of cypermethrin (1 ppm), 1 to 5 mL, portions were taken in 25 mL graduated tube and to it, 1 mL of 20 % sodium hydroxide was added. The solution was kept for 15 min at room temperature for complete hydrolysis, followed by adding the optimum volume of 0.15 % nitrite solution (1 mL) and shaken well. To these solutions the optimum volume of aniline 2 % (1 mL) was added and diluted to 25 mL with distilled water. The absorbance of the resulting azo dyes was measured at 535 nm after 45 min of equilibration using a spectrophotometer.

For the determination of cypermethrin by flow injection analysis, nitrite solution (0.15 %) and aniline (2 %) solution was pumped continuously in the flow injection manifolds. A hydrolyzed solution of cypermethrin was injected in a nitrite stream to make the diazonium ion. The diazotized reagent was allowed to mix with a coupling reagent and the dye was passed through a flow cell of the spectrophotometer and the absorbance was continuously measured at 535 nm.

For the determination of cypermethrin in a commercial formulation, cypermethrin was separated from 2-methyl-1,4-chlorophenoxy acetic acid, which is in a 1:1 ratio in the commercial products using cyclohexane. For this, 0.5 mL of the sample was diluted to 25 mL and extracted twice with 10 mL of cyclohexane using separatory funnel. The extract was then evaporated on a boiling water bath and the remaining residue was dissolved in ethanol, than diluted with distilled water, made up to the volume and hydrolyzed with 3 % hydrochloric acid. The resulting solution was diazotized and the colour was developed in the same way as mentioned before. The absorbance of the coloured solution was measured at 535 nm. Six replicate readings were taken for each sample. The amount of cypermethrin present in the preparation was determined from the calibration plot using standard curve.



Scheme-I: Proposed reaction for formation of azo dye

Sample preparation: 5 g amount of a vegetable samples was taken in a beaker. 25 mL of a solvent [petroleum ether and acetone in (1:1)] was added to it for extracting insecticides. This was kept for 1.30 h with shaking at intervals and then filtered and evaporated on a rotary evaporator up to a volume of 3 mL. This was further diluted up to 25 mL with ethanol. In the case of FIA, 1 mL from this diluted extracted solution was injected with the reagent stream for diazotization followed by coupling with aniline; the absorbance was measured at 535 nm. For a batch analysis 3 mL was taken from this diluted extracted solution and colour was developed under the optimum conditions and the absorbance was measured at the optimum wavelength.

In case of foliages, the filtrate was passed through a silica gel column (10 × 1 cm) filled with 5 mg silica gel [35], 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 analyzed.

Recovery (%): Recovery (%) tests were carried out on control samples fortified with 1 ppm insecticide. Extraction and determination was done using the same procedure as mentioned above.

RESULTS AND DISCUSSION

Optimization studies: The proposed chemical reaction for the hydrolysis of cypermethrin diazotization and the coupling reaction of the hydrolyzed products are shown in **Scheme-I**. Various parameters for standard cypermethrin like the amount of acid for the hydrolysis of cypermethrin and the nitrite concentration for the diazotization of the hydrolyzed cypermethrin were optimized in the batch method.

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. 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 aniline was sufficient

TABLE-1
EFFECT OF FOREIGN SPECIES FOR CYPERMETHRIN (CONCENTRATION OF 15 µg/mL)

Foreign species	Tolerance limit[a] (µg in 25 mL)	Foreign species	Tolerance limit[a] (µg in 25 mL)
Benzene	6500	Al^{3+}, Mg^{2+}, Co^{2+}	2500
Phenol, Ethanol	2700	Zn^{2+}, Cu^{2+}, Mn^{2+}	1400
Benzaldehyde	1800	Fe^{3+}, Fe^{2+}, Sb^{3+}	1100
Toluene, Xylene	1300	Ni^{2+}, Pb^{2+}, Ca^{2+}	750
Aniline, Formaldehyde	800	Br^{-}, CO_3^{2-}, Cl^{-}	450
Parathion, Malathion, Cresol	200	NO_2^{-}	100
Fenvalerate, Deltamethrin	2[b]		

[a]The amount causing an error of ± 2 % in absorbance value; [b]Tolerance limit without its removal from the sample.

for complete colour reaction. The effect of pH on the colour reaction was studied and it was found that constant absorbance values were obtained at pH range of about 4-5 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 10 µg of cypermethrin in 25 mL final solution over a period of 7 days. Similarly the concentration of aniline as a coupling reagent was investigated in the range of 1 to 5 %. The maximum coupling was observed using 2 % aniline. The standard deviation and relative standard deviation were found to be ± 0.003 and 0.56 %, respectively.

The physical parameters for the FIA like reactor lengths (R_1 and R_2) the injected volume and the flow rate was optimized. The flow rate system was 3.5 mL min^{-1}. The volume of injected sample was 1 mL. The optimum lengths of the diazotization coil (R_1) and the coupling coil (R_2) were found to be 120 and 165 cm, respectively. Using these tube lengths it was observed that the diazotization and coupling reaction was fast and reproducible under these conditions. The sampling rate was 80 samples per hour. The advantages of the developed FI procedure are of a higher sampling rate and low wastage of reagents.

The applicability of Beer's law by the FIA system was checked and linear relationship was observed for the concentration range 0.22-1.4 ppm. The relative standard deviation (RSD) for six runs of 0.22 of was 0.56 %. The molar absorptivity and Sandell's sensitivity were found to be 5.3×10^4 L mol^{-1} cm^{-1} and 0.066 µg cm^{-2}, 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 containing 15 µg of cypermethrin prior to hydrolysis and the solution was analyzed by the proposed method. This method was found to be free from the interferences of most of the foreign species and pesticides which are given in Table-1.

Application to real sample: This method was applied to the determination of cypermethrin in vegetable samples like tomoto, apple, cauliflower and Cotton foliages. The results are given in Table-2.

Present recovery tests are performed for vegetable samples and formulations. The results are given in Table-3. The recovery was good and the result of the batch and FIA methods were comparable. The method was successfully applied to determine the concentration of active ingredients in commercial formulations of cypermethrin. The results indicate the applicability of the method for both real samples of complex nature and formulation.

TABLE-2
DETERMINATION OF CYPERMETHRIN
VEGETATIVE SAMPLES

Vegetative samples	Batch analysis (ppm)	FIA (ppm)
Tomato	94.5 ± 0.0056	88.3 ± 0.006
	86.93 ± 0.0044	84.69 ± 0.0036
Apple	70.98 ± 0.058	73.12 ± 0.045
	85.5 ± 0.05	94.5 ± 0.036
Cauliflower	98.5 ± 0.049	94.5 ± 0.088
	91.5 ± 0.089	94.5 ± 0.079
Cotton foliages	69.5 ± 0.049	68.5 ± 0.036
	55.5 ± 0.067	48.3 ± 0.087

TABLE-3
RECOVERY (%) OF CYPERMETHRIN FROM REAL SAMPLES

Vegetative samples	Batch analysis (ppm)	FIA (ppm)
Cypermethin	94.6 ± 0.003	95.7 ± 0.022
Tomoto	94.5 ± 0.0056	88.3 ± 0.006
	86.93 ± 0.0044	87.69 ± 0.003
Apple	74.12 ± 0.003	75.12 ± 0.045
	85.50 ± 0.05	94.5 ± 0.036
Cauliflower	98.5 ± 0.049	99.5 ± 0.077
	91.5 ± 0.089	94.5 ± 0.079
Cotton foliages	68.5 ± 0.049	70.5 ± 0.036
	55.50 ± 0.05	58.3 ± 0.08

Conclusion

A new spectrophotometric method was investigated for cypermethrin insecticide containing a nitrile group. The method was successfully applied to vegetable samples. The result of the batch and FIA method was compared. The method is simple, inexpensive and compatible in sensitivity. The low RSD indicates good reproducibility of the method. The method has an additional advantage of being environment friendly because no clean up procedure is required.

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