Pergamon

0039-9140(93)E0042-C

Talanta, Vol. 41, No. 5, pp. 761–764, 1994
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0039-9140/94 $7.00 + 0.00

SPECTROPHOTOMETRIC DETERMINATION OF FLUCYTHRINATE (SYNTHETIC PYRETHROID) USING PHENYLHYDRAZINES

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(*Received* 11 *January* 1993. *Revised* 28 *June* 1993. *Accepted* 24 *November* 1993)

Summary—Three spectrophotometric methods are described for determining flucythrinate, based on formation of coloured compounds when one of the hydrolysed products of flucythrinate is condensed with 2,4-dinitrophenylhydrazine (2,4-DNPH), 4-nitrophenylhydrazine (4-NPH) or 2,4,6-trinitrophenylhydrazine (2,4,6-TNPH) in the presence of methanolic potassium hydroxide. These coloured compounds absorb strongly at 465 nm (2,4-DNPH), 540 nm (4-NPH) and 485 nm (2,4,6-TNPH). The methods are applicable over the range 0.1–8.0 μg/ml for 2,4-DNPH, 0.5–6.5 μg/ml for 4-NPH and 0.1–6 μg/ml for 2,4,6-TNPH. The methods are rapid, sensitive and selective and can be used for microdetermination of flucythrinate in a commercial formulation, water, grains and crop samples.

Flucythrinate, (*RS*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-difluoromethoxyphenyl)-3-methylbutyrate, is a new synthetic pyrethroid and exhibits high insecticidal activity. It is a broad spectrum, contact and stomach action insecticide with non-systemic activity. It is used to control Lepidoptera, Homoptera and Coleoptera in a wide range of field crops, fruits, vegetables, ornamentals and flowers.[1] Several methods such as thin layer chromatography[2,3] and gas chromatography[4] are cited in the literature for its determination.

We have now developed a simple, sensitive, reproducible and accurate method for the determination of flucythrinate, based on our observation that when 2,4-dinitrophenylhydrazine (2,4-DNPH), 4-nitrophenylhydrazine (4-NPH) and 2,4,6-trinitrophenylhydrazine (2,4,6-TNPH) couple with hydrolysis products of the pesticide in the presence of methanolic potassium hydroxide they yield red (465 nm for 2,4-DNPH and 485 nm for 2,4,6-TNPH) and red-purple (540 nm for 4-NPH) compounds. The sensitivities of these reactions were determined[5] and limits of identification and dilution were 0.05 μg and 1:10^6 respectively.

EXPERIMENTAL

Equipment

A digital Elico LI-120A pH-meter and Shimadzu UV-240 recording spectrophotometer were used.

Reagents

All solvents and reagents were of analytical reagent grade. Standard solutions of flucythrinate, 1 mg/ml: 25 mg of the insecticide was dissolved and made up to volume in a 25 ml standard flask with methanol. Working solutions of the insecticide were prepared by dilution with methanol as required.

2,4-Dinitrophenylhydrazine (2,4-DNPH) solution, 1 mg/ml: 2,4-DNPH (0.1 g) was dissolved in 100 ml methanol containing 0.5 ml conc HCl. 4-Nitrophenylhydrazine (4-NPH) solution, 1 mg/ml: 4-NPH (0.1 g) was dissolved in 100 ml of methanol containing 0.5 ml conc HCl. 2,4,6-Trinitrophenylhydrazine (2,4,6-TNPH) solution, 1 mg/ml: 2,4,6-TNPH (0.1 g) was dissolved in 100 ml of methanol containing 0.5 ml conc HCl. Potassium hydroxide solutions, 2 and 4%, were freshly prepared in methanol. Hydrochloric acid, 0.2*N*, was prepared in distilled water.

Determination of flucythrinate

General procedure. Portions (0.0, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml) of standard

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flucythrinate (100 μg/ml) solution were transferred into 10 ml graduated test tubes. Methanolic KOH (1 ml) was added to each test tube and shaken well, then put on a water bath at 45°C for 30 min. The test tubes were removed and stood for 10 min at room temperature and excess alkali neutralized by adding 0.2*N* HCl. 2,4-DNPH (1 ml) was added to each test tube followed by one drop of conc HCl, shaken well and heated on a water bath at 55°C for 30 min. It was cooled to room temperature, 2 ml of 4% methanolic KOH added, diluted with methanol and shaken well. The absorbance at 465 nm (Fig. 1) was measured against the corresponding reagent.

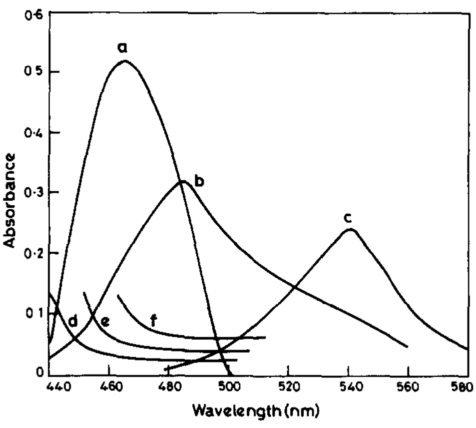

Fig. 1.

This procedure was repeated for 4-NPH (1.5 ml) and 2,4,6-TNPH (2.0 ml) and colours developed with 3.5 and 2.0 ml methanolic KOH, respectively. The absorbances were read at 540 and 485 nm (Fig. 1) for 4-NPH and 2,4,6-TNPH, respectively.

Formulation. Formulation (0.25 ml) equivalent to 25 mg of the insecticide was transferred in a porcelain dish, 10 ml of methanol added, stirred well and evaporated. This was repeated five times, the residue dissolved in methanol and diluted in a 25 ml standard flask and the solution analysed using the above procedure.

Water samples (*cattle dip water*). Water samples were filtered through Whatman No. 42 filter paper and 1 l. fortified with 25 mg of pesticide in 15 ml methanol and the pH adjusted to 3–4 with 50% sulphuric acid. Sodium sulphate (10 g) was added to each sample. The pesticide was extracted thrice using about 50 ml chloroform for each extraction with shaking for 10 min, the mixed extracts were washed with three 10 ml portions of 0.1*M* K_2CO_3 solution. The chloroform solution was dried by passing it through 15 g anhydrous sodium sulphate, washing the sodium sulphate thrice with 20 ml portions of chloroform, collecting the washings with the same solution and then evaporating the chloroform in a current of dry air at room temperature. The residue was dissolved in methanol and diluted to 250 ml in a volumetric flask. The solution was analysed by the above procedure.

Grains (rice and wheat). Grain (50 g) was transferred into separate conical flasks, spiked with 25 mg of pesticide in 15 ml of methanol, shaken well, kept for 2 hr and then shaken thoroughly with 200 ml of chloroform and transferred into a 250 ml separating funnel through Whatman No. 1 filter paper. The samples were extracted with three 10 ml portions of chloroform. The solvent was evaporated from the combined extracts in a current of dry air at room temperature and the residue dissolved in methanol and dilute in a 250 ml volumetric flask. The solution was analysed using the above procedure.

Crop sample (tobacco). Finely chopped dry tobacco sample (25 g) was transferred into a conical flask, treated with 15 ml distilled water, spiked with 25 mg of pesticide in 15 ml methanol, shaken well, allowed to stand for 2 hr and then the solvent evaporated. The sample was transferred with 100 ml of chloroform into a 250 ml separating funnel, shaken thoroughly and filtered through Whatman No. 1 filter paper. The operations were repeated thrice with 25 ml portions of chloroform and finally the solvent evaporated in a current of dry air at room temperature. The residue was dissolved in methanol and diluted in a 250 ml standard flask. The solution was analysed using the above procedure.

Interference from aldehydes. The water samples (1 l.) were fortified with known amounts of pesticide in 15 ml methanol, known amounts of benzaldehyde in 10 ml of methanol added, and the pH of each solution adjusted to 3–4 with 50% sulphuric acid. Sodium sulphate (10 g) was added to each sample, shaken well, and the pesticide extracted along with benzaldehyde thrice with chloroform using 50 ml for each extraction. The combined extracts were placed in a 500 ml round-bottomed flask and 100 mg of metachloroperoxobenzoic acid[6,7] added to it. The resulting solution was refluxed on a hot water bath for about 15 min to convert the aldehyde into an acid, cooled, washed thrice

Table 1. Optical characteristics, precision and accuracy of the methods

Pesticide	Reagent	pH range	Stability period (*hr*)	Molar absorptivity × 10^4 ($l/mol^{-1}/cm^{-1}$)	Sandell's sensitivity ($\mu g/cm^2$)	Relative standard deviation (%)	Confidence limit (p 0.05)	Standard error
Flucythrinate	2,4-DNPH	11–12	48	5.43	0.009	0.54	± 0.0019	0.0008
	4-NPH	11–12	48	4.06	0.011	0.23	± 0.0012	0.0005
	2,4,6-TNPH	11–12.5	45	5.00	0.009	0.56	± 0.0026	0.0010

with 0.2*M* sodium hydrogen carbonate solution employing 25 ml for each washing to remove the acid and unreacted metachloroperoxobenzoic acid, and finally washed four times with distilled water using 50 ml for each washing to remove the excess carbonate. Finally, the chloroform layer was separated from the aqueous solution and dried by filtering over 15 g anhydrous sodium sulphate, washing the sodium sulphate thrice with 20 ml portions of chloroform, collecting the washings into the same solution and then evaporating the solvent in dry air. The residue was dissolved in methanol and diluted to 250 ml in a calibrated flask. Known amounts of this solution (0.1–0.5 ml) were transferred into 10 ml graduated test tubes and determination of flucythrinate with reagents 2,4-DNPH, 4-NPH and 2,4,6-TNPH determined according to the general procedure described above.

RESULTS AND DISCUSSION

The optimum conditions were established in the usual way by altering one variable at a time. The period of stability, pH range, molar absorptivity, Sandell's sensitivity, relative standard deviation, confidence limit and standard error of the coloured products are given in Table 1. Results of recoveries of flucythrinate from formulation, spiked water, grains and crop samples are presented in Table 2. The data obtained for the determination of flucythrinate from water samples containing an aldehyde are reported in Table 3.

Mechanism

The alkaline hydrolysis of flucythrinate was found to proceed solely via ester cleavage to yield 2-(4-difluoromethoxyphenyl)-3-methylbutyric acid and 3-phenoxybenzaldehyde. This is a pseudo-first order reaction. This aldehyde will react with 2,4-DNPH to give the corresponding phenylhydrazone which, in the presence of methanolic potassium hydroxide, forms red coloured compound. The course of reactions with 4-NPH and 2,4,6-TNPH is similar and they yield red-purple and red coloured compounds, respectively. The coloured compounds are formed due to conversion of phenylhydrazones into quinonoid ions in the presence of alkali (Fig. 2).

Smaller amounts of phenylhydrazines and potassium hydroxide gave incomplete colour formation, but larger amounts did not increase the absorbance. Interference due to various inorganic ions and their compounds is eliminated at the stage of extraction of the pesticide with chloroform from the samples. Except for aldehydes with other organic compounds functional groups do not interfere. Among the pesticides only those with the pyrethroid group will yield an aldehyde on alkaline hydrolysis and hence the other group of pesticides will not

Table 2. Determination of flucythrinate in 10% formulation, water, grains and crop

	Flucythrinate, 10%			Cattle dip water	Rice	Wheat	Tobacco
Reagent	Taken (*µg*)	Formulation* (%)	Flucythrinate (*µg*)	Recovery* (%)	Recovery* (%)	Recovery* (%)	Recovery* (%)
2,4-DNPH	1.0	9.65 ± 0.15	1.0	96.39 ± 1.27	96.89 ± 1.02	96.55 ± 1.50	97.05 ± 1.87
	3.0	9.52 ± 0.13	3.0	96.79 ± 1.15	95.89 ± 0.84	97.27 ± 0.68	97.27 ± 0.68
	5.0	9.55 ± 0.04	5.0	98.09 ± 0.80	96.62 ± 0.90	97.47 ± 0.32	98.09 ± 0.80
4-NPH	1.0	9.53 ± 0.21	1.0	95.55 ± 0.96	96.72 ± 0.75	96.22 ± 0.38	96.39 ± 1.27
	3.0	9.76 ± 0.14	3.0	96.44 ± 1.70	97.28 ± 1.18	97.16 ± 1.17	97.63 ± 1.42
	5.0	9.72 ± 0.20	5.0	97.53 ± 1.18	97.26 ± 2.05	97.76 ± 0.25	97.68 ± 1.38
2,4,6-TNPH	1.0	9.63 ± 0.26	1.0	96.72 ± 0.75	96.94 ± 0.48	97.22 ± 0.48	96.79 ± 1.32
	3.0	9.79 ± 0.04	3.0	97.56 ± 1.24	97.18 ± 1.34	96.12 ± 0.49	97.94 ± 0.42
	5.0	9.70 ± 0.15	5.0	98.14 ± 1.28	97.53 ± 1.60	97.80 ± 1.06	97.04 ± 1.56

*Mean ± standard deviation of three determinations.

Table 3. Interference from benzaldehyde in water samples

Flucythrinate added (μg)	Benzaldehyde added (μg)	Flucythrinate Found (μg)	Flucythrinate Recovery (%)
1	40	0.96	96.00
2	80	1.96	98.00
3	120	2.93	97.66
4	160	3.96	99.00
5	200	4.95	99.00
Mean			97.93
S.D.			1.23
R.S.D			1.26%
S.E.			0.61

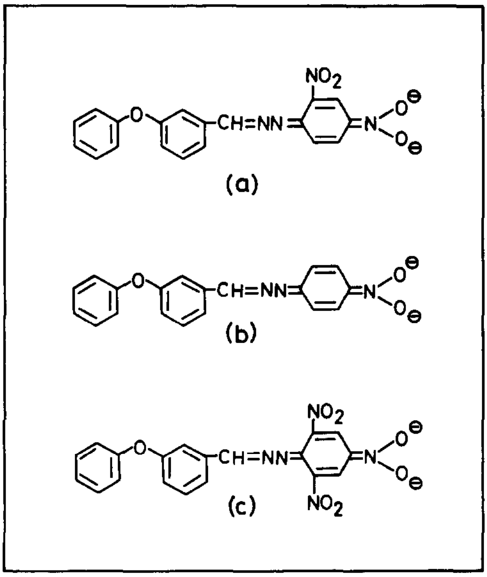

Fig. 2.

cause interference. Furthermore, interference by other substances is also eliminated by measuring the absorbance against a similarly prepared blank. In these methods rapid colour development is a notable advantage. The reagent has minimum absorption at the maximum absorption of the coloured compound.

The recoveries of flucythrinate from formulation are in the range 95.2–97.9% with variability of ⩽0.27% and the flucythrinate content in the formulation is in close agreement with that labelled by the manufacturer. The recoveries from cattle dip water, wheat and tabacco range from 95.55 to 98.14% with variability of ⩽2.1%. Recovery (97.93%) of flucythrinate from a water sample containing an aldehyde are quite satisfactory. For cattle dip water, rice, wheat and tobacco only a simple extraction method was used. Hence, by the present methods it is possible to determine an amount ⩾0.5 μg/ml of flucythrinate in contaminated cattle dip water, rice, wheat and tobacco samples.

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