Determination of Pyrethroidal Insecticides by Titration

Ritsuko FURUTA, Hiroshi NAKAZAWA and Tadashi DOI

*Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,
1-98, Kasugade Naka 3-chome, Konohana-ku, Osaka 554, Japan*

Received October 1, 1990

Assays for typical pyrethroidal insecticides were developed to determine their absolute purity. Resmethrin, furamethrin and phenothrin could be determined by a neutralizing titration after hydrolyzing with potassium hydroxide to achieve relative standard deviations (RSD) of 0.10, 0.19 and 0.35%, respectively. Allethrin, phthalthrin and furamethrin could be determined by a neutralizing titration in a non-aqueous solvent after reacting with ethylenediamine to achieve RSD values of 0.10, 0.14 and 0.09%, and permethrin by a chlorine determination with the oxygen combustion method to achieve RSD of 0.23%. These methods are effective for assays of the reference standards because there is no necessity for another reference standard.

Synthetic pyrethroids are widely used as agricultural and household insecticides owing to their high insecticidal activity and low mammalian toxicity.¹⁾ It is important to control their quality at the time of manufacture for safe and effective use. For this purpose, various methods to determine the contents have been developed, among which, internal standard methods of gas chromatography (GC-IS) are mainly used because of their time-saving and accuracy. These GC methods are based on a comparison between the test sample and a reference standard, whose quality must be precisely verified for an accurate determination of the insecticide preparation. Consequently, it

is necessary to develop so-called absolute assays such as titration, in which determination is carried out without the reference standards. However, there have been few reports about such assays, except for the determination of natural pyrethrins by the mercury reduction method²⁾ or neutralizing titration³⁾ after hydrolyzing the esters, and allethrin (PYN) by reacting with ethylenediamine (ED).^{4,5)} Therefore, we investigated absolute assays for the representative household pyrethroidal insecticides, PYN, phthalthrin (NPY), resmethrin (CHR), furamethrin (PYN·D), phenothrin (SUM) and permethrin (EXM) (Fig. 1), and established their value by using the

Fig. 1. Structures of Pyrethroidal Insecticides.

neutralizing titration after hydrolyzing with potassium hydroxide (KOH) or the reaction with ED, or by the halogen determination with the oxygen flask combustion method.

Materials and Methods

Materials. Manufactured preparations of PYN, NPY, CHR, PYN·D, SUM and EXM were synthesized by Sumitomo Chemical Co., Ltd. (Osaka, Japan). Their reference standards were prepared by Sumitomo Chemical Co., Ltd. by independently recrystallizing manufactured preparations or by synthesis.⁶⁾ The purity of each reference standard was 99.7–100.0% by the GC-peak area percentage method. Authentic samples of hydrolyzed products (chrysanthemic acid and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCPI)) were also synthesized by Sumitomo Chemical Co., Ltd.

Reagents. 2 N, 5 N and other KOH test solutions, 2 N and 5 N hydrochloric acid (HCl), 0.1 N KOH–ethanol (EtOH), 0.1 N sodium methoxide–dioxane, thymolphthalein, and 0.01 N silver nitrate were prepared according to the Pharmacopoeia of Japan, General Tests, Processes and Apparatus.⁷⁾ Curcumin was prepared by dissolving 0.1 g of curcumin (analytical grade) in EtOH to make up 100 ml. ED (anhydrous, special class) was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan), all other reagents being of analytical grade.

Apparatus. A Shimadzu model GC-7A gas chromatograph equipped with a hydrogen flame-ionization detector, and a Hitachi model M-80B mass spectrometer (electron impact ionization, 70 eV ionization voltage, 180°C ion source) were used.

Neutralizing titration after hydrolyzing with KOH (method 1). About 0.3 g of CHR or SUM was accurately weighed, dissolved in 60 ml of EtOH, and 60 ml of 2 N (for CHR) or 5 N (for SUM) KOH was added to the solution. The mixture was refluxed for 2 hr (for CHR) or 3 hr (for SUM) and acidified (pH 3–4) with 2 N or 5 N HCl. After the solution had been cooled, 30 ml of water saturated with sodium chloride was added. The reaction product was extracted with three 60-ml portions of dichloromethane, the combined extracts were washed with two 120-ml portions of water, and the dichloromethane was evaporated under reduced pressure. The residue was transferred to a conical flask with 50 ml of EtOH and titrated with 0.1 N KOH–EtOH (using 2–3 drops of curcumin as the indicator) until the color of the solution changed from yellow to red. A blank determination with 50 ml of EtOH was performed in the same manner, and any necessary correction was made.

Neutralizing titration after reacting with ED (method 2). About 0.3 g of PYN, NPY or PYN·D was accurately weighed into a glass-stoppered flask, dissolved in exactly 10 ml of ED and allowed to stand at 50°C for 1 hr (for PYN), at 25°C for 2 hr (for NPY) or at 70°C for 3 hr (for PYN·D). The stopper was then removed, the walls were washed with 50 ml of pyridine, and the solution was titrated with 0.1 N sodium methoxide–dioxane (using 0.5 ml of thymolphthalein as the indicator) until the color of the solution changed to blue (or from yellow to green with PYN·D). A blank determination was performed in the same manner, and any necessary correction was made.

Chlorine determination by the oxygen flask combustion method (method 3). About 0.01 g of EXM was accurately weighed, and the test was performed according to the Pharmacopoeia of Japan, Oxygen Flask Combustion Method,⁸⁾ 15 ml of water being used as the absorbing liquid. 0.01 N silver nitrate was used as the titrant.

Results and Discussion

Neutralizing titration after hydrolyzing with KOH (method 1)

The effects of KOH concentration and reaction time on the hydrolysis were investigated. The resulting acids after hydrolyzing with KOH were extracted and titrated, the completion of the hydrolysis being determined by GC.^{9–12)} Figure 2 shows that the hydrolysis of CHR was completed in 2 hr with 1 N KOH, in 1 hr with 2 N KOH and in 30 min with 4 N KOH. The reaction for 2 hr with 2 N KOH was selected as the optimum. In a similar manner, the other pyrethroids were determined. Table I shows the optimum conditions and also the analytical results for the reference standards. This method was found to be applicable to CHR, PYN·D and SUM with RSD of 0.10–0.35%. However, the contents of PYN

Fig. 2. Effect of KOH Concentration and Reaction Time on the Hydrolysis of CHR.

○, 1 N KOH TS; ◐, 2 N KOH TS; ●, 4 N KOH TS.

Table I. HYDROLYSIS CONDITIONS AND ANALYTICAL RESULTS FOR THE REFERENCE STANDARDS OF PYRETHROIDS (METHOD 1)

Compound	KOH TS (N)	Reaction time (hr)	Content by titration (%)	RSD ^a (%)	Content by GC ^b (%)
PYN	2	1	132.4	—	98.3
NPY	2	1	128.0	—	128.0
CHR	2	2	100.1	0.10	98.4
PYN·D	1	1	99.3	0.19	57.0
SUM	5	3	100.0	0.35	101.1
EXM	2	3	98.1	2.0	96.0

^a Relative standard deviation: PYN·D, *n*=3; others, *n*=5.

^b The extracted acids by method 1 were determined by GC-MS methods. The GC conditions were as follows. EXM: column, 3% Thermo 3000 (3 mm i.d. × 1 m); column temp., 170°C; internal standard, di-*n*-butyl phthalate. Others: column, 5% FFAP (3 mm i.d. × 2 m); column temp., 155°C; internal standard, diisopropyl phthalate.

Fig. 3. Reaction Mechanism for Method 1.

R₁, see Fig. 1; R₂=CH₃, chrysanthemic acid; R₂=Cl, DCPI.

and NPY were overestimated. The reason is presumed to be that the alcohol moieties of PYN and NPY decomposed to acid products by a side reaction, which might have interfered with the titration. The hydrolysis products proved identical with an independently synthesized authentic sample of chrysanthemic acid or DCPI by judging from GC-mass spectral criteria (GC conditions are shown in Table I). The reaction mechanism is shown in Fig. 3.

To validate the results obtained by the titration method, the extracted acids by method 1 were determined by the GC-IS method under the conditions shown in Table I, authentic samples of the hydrolysis products being used as the reference standards. As shown in Table I, the analytical values for CHR and SUM were found to be the same as those by titration. However, that of PYN·D was lower than that by titration, the reason for which is believed to be that there was a peak of an unknown product overlapping that of the internal standard. On the other hand, the content of NPY was overestimated, as was the case by titration, and overlapping of chrysanthemic acid and a by-product was observed on the gas

chromatogram. The analytical value of PYN by GC shows that the hydrolysis conditions were valid, although the titration was unsuccessful as just described.

For EXM, method 1 was not applicable because the RSD was not acceptable.

*Neutralizing titration after reacting with ED
(method 2)*

This method has been adopted as the determination method for PYN in AOAC.⁽⁴⁾ We therefore tried to apply it to the other pyrethroids. In this method, each pyrethroid was reacted with ED (a milder reagent than KOH) to form an equivalent acid, which was then titrated with sodium methoxide.

Figure 4 shows the effect of temperature on the reaction rate, the reaction proceeding more rapidly as the temperature increased, and being completed within 3 hr at 70°C in the case of PYN·D. The optimum conditions for the other pyrethroids were determined by the same procedure. Table II shows these optimum conditions and the analytical results for the reference standards. Although method 1 was not applicable to PYN and NPY, method 2

Fig. 4. Effect of Temperature on the Reaction Rate of PYN·D.

○, 50°C; ◐, 60°C; ●, 70°C.

Table II. REACTION CONDITIONS AND ANALYTICAL RESULTS FOR THE REFERENCE STANDARDS OF PYRETHROIDS (METHOD 2)

Compound	Reaction temp. (°C)	Reaction time (hr)	Content (%)	RSD ^a (%)
PYN	50	1	99.9	0.10
NPY	25	2	99.8	0.14
CHR	70	1	1.5	—
PYN·D	70	3	100.0	0.09
SUM	70	1	1.6	—
EXM	70	1	2.8	—

^a Relative standard deviation: $n=5$.

was applicable to these compounds and also to PYN·D with RSD values of 0.09–0.14%. It is considered that KOH is too strong as a reagent to break the ester bonds of PYN and NPY.

We also confirmed that the reaction product was identical with an independently synthesized authentic sample of chrysanthemic acid by judging from GC-mass spectral criteria (GC conditions are shown in Table I). Chrysanthemic acid produced by the reaction with ED was determined by the same GC-IS method as described in the foregoing section, although the analytical values varied, probably because of interference from excessive ED.

Owing to the low reactivity of CHR, SUM and EXM with ED, the analytical values were about 2% at 70°C for 1 hr, and therefore method 2 was not applicable to these compounds.

This method was simpler than method 1 because extraction was unnecessary.

Table III. EFFECTS OF THE AMOUNT OF SAMPLE AND ABSORBING LIQUID ON THE EXM CONTENT (METHOD 3)

Amount of sample (mg)	Absorbing liquid ^a	EXM content (%)
100	10% H ₂ O ₂	99.5 ^b
50	10% H ₂ O ₂	99.9 ^b
50	Water	100.8 ^b
30	10% H ₂ O ₂	100.2 ^c
30	Water	99.8 ^c
10	10% H ₂ O ₂	100.3 ^c
10	Water	100.5 ^c

^a Volume: 15 ml.

^b 0.05 N silver nitrate was used as the titrant.

^c 0.01 N silver nitrate was used as the titrant.

Table IV. ANALYTICAL RESULTS FOR MANUFACTURED PREPARATIONS OF PYRETHROIDS

Compound	Methods 1–3 (%)	GC-IS methods ^{12–15} (%)	Difference ^a (%)
PYN	96.5 ^c	91.7	4.8
NPY	97.5 ^c	95.0	2.5
CHR	94.8 ^b	93.1	1.7
PYN·D	93.2 ^c	91.1	2.1
SUM	96.6 ^b	92.7	3.9
EXM	98.3 ^d	92.5	5.8

^a Difference = (methods 1–3) – (GC-IS methods).

^b Method 1 was used.

^c Method 2 was used.

^d Method 3 was used.

Chlorine determination by the oxygen flask combustion method (method 3)

The oxygen flask combustion method is a general method for identifying or determining halogens or the sulfur produced by combusting organic compounds containing chlorine, bromine, iodine, fluorine or sulfur.⁸⁾ Permethrin (EXM), which contains chlorine, was determined by this method. The amount of the sample and the absorbing liquid had no effect on the content of EXM as shown in Table III. The conditions described in the Materials and Methods, that is, a 10 mg sample and water as the absorbing liquid, were adopted for easy operation. The average content and the RSD of five determinations of the EXM reference

standard by the adopted methods were 100.2% and 0.23%, respectively.

Determination of manufactured preparations

We applied methods 1–3 to manufactured preparations of the pyrethroids. Their contents, however, were overestimated by 2 to 6% in comparison with the contents by GC-IS methods^{9–12)} (Table IV) owing to related substances which were present in the manufactured preparations and which reacted in addition to the active ingredients.

Although GC-IS methods were found to be more appropriate for determining the manufactured preparations than the titration methods from the foregoing results, these GC-IS methods require verified reference standards. Therefore, absolute assays for the reference standards established by titration are necessary, and they should be more effective by combining with purity tests such as the GC-peak area percentage in order to support GC-IS methods.

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