PYRIPROXYFEN

715

715/TC/M/-

Method Extension for

Permethrin/Pyriproxyfen LN

Studies for Method Extension of Existing CIPAC Method for Permethrin/Pyriproxyfen LN

by
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1. INTRODUCTION

The CIPAC 715/TC/M/3 was extended to permethrin/pyriproxyfen LN with a few minor modifications.
This report was prepared to demonstrate the validity of the extension of the CIPAC 715/TC/M/3 for permethrin/pyriproxyfen LN.

2. METHOD DESCRIPTION

PYRIPROXYFEN LONG LASTING INSECTICIDAL NET
Extension method of CIPAC 715/TC/M/3

OUTLINE OF METHOD Pyriproxyfen is determined by reversed phase high performance liquid chromatography using UV detection at 254 nm and dicyclohexyl phthalate as internal standard.

REAGENTS
Heptane
1-Propanol
Acetonitrile HPLC grade
Water HPLC grade
Pyriproxyfen standard of known purity. Store refrigerated.
Dicyclohexyl phthalate internal standard. Must not show any peaks with the same retention time as pyriproxyfen and permethrin.
Internal standard solution. Dissolve dicyclohexyl phthalate (5.0 g) in 1-propanol (200 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.
Calibration solution. Homogenise the pyriproxyfen standard. When it is a waxy or partly waxy solid homogenise it by warming it to melting and by stirring. Weigh in duplicate (to the nearest 0.1 mg) 90 to 110 mg (*s* mg) of pyriproxyfen standard into a vial or a stoppered flask (200 ml). Add by pipette to each vial or flask internal standard solution (10.0 ml) and by measuring cylinder acetonitrile (90 ml). Mix well (solutions C_A and C_B).

APPARATUS
High performance liquid chromatograph equipped with a detector suitable for operation at 254 nm, a constant temperature column compartment and an injector capable of delivering 10 µl
Column 250 mm x 4.6 mm (i.d.), stainless steel, packed with Nucleosil C_{18} (5 µm), or equivalent
Electric integrator or data system

Water bath
Rotary evaporator

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column	stainless steel, 250 x 4.6 mm (i.d.), packed with Nucleosil C_{18} (5 µm), or equivalent.
Mobile phase	acetonitrile – water, 700 + 350 (v/v)
Column temperature	40°C
Flow rate	1.0 ml/min
Detector wavelength	254 nm
Injection volume	10 µl
Retention times	pyriproxyfen: about 17 min dicyclohexyl phthalate: about 25 min

(b) Linearity check. Before conducting the analysis check the linearity of the detector response by injecting 10 µl portions of solutions with pyriproxyfen concentrations 0.5, 1 and 2 times that of the calibration solution.

(c) System equilibration. Inject 10 µl portions of calibration solution C_A until the response factors obtained for two consecutive injections differ by less than 1.0%. Then inject a 10 µl portion of calibration solution C_B. The response factor for this solution should not deviate by more than 1.0% from that for the first calibration solution, otherwise prepare new calibration solutions.

(d) Preparation of sample solution. Clean a pair of scissors with acetone before use. Cut the sample with the scissors into 5 – 10 mm squares. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 18 to 22 mg (*w* mg) of pyriproxyfen into a vial or stoppered flask (100 ml). Add by pipette internal standard solution (2.0 ml) and by measuring cylinder heptane (48 ml). Place the vial or stoppered flask in a water bath (85 – 90°C) for 45 min. Shake the vial or stoppered flask once or twice during the extraction. After extraction, cool it to room temperature. Transfer by pipette the solution (10.0 ml) into a round-bottom flask (50 ml). Evaporate the solution *in vacuo*, add by pipette acetonitrile (4.0 ml) and dissolve completely (solutions S_A and S_B).

(e) Determination. Inject in duplicate 10 µl portions of each sample solution bracketing them by injections of the calibration solutions as

follows; calibration solution C_A, sample solution S_A, sample solution S_A, calibration solution C_B, sample solution S_B, sample solution S_B, calibration solution C_A, and so on. Measure the relevant peak areas. Average the values of the duplicate sample injections. Calculate the mean value of the response factors of the calibration solution bracketing two sample solutions and use this value to calculate the pyriproxyfen concentration of the bracketed samples.

$$f_i = \frac{I_r \times s \times P}{H_s \times 5}$$

$$\text{Pyriproxyfen content} = \frac{f \times H_w}{I_q \times w} \text{ g/kg}$$

where:

f_i = individual response factor
f = mean response factor
H_s = peak area of pyriproxyfen in the calibration solution
H_w = peak area of pyriproxyfen in the sample solution
I_r = peak area of the internal standard in the calibration solution
I_q = peak area of the internal standard in the sample solution
s = mass of pyriproxyfen standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of pyriproxyfen standard (g/kg)

3. METHOD ASSESSMENT

According to the CIPAC method extension guideline, the method extension of the CIPAC 715/TC/M/3 for permethrin/pyriproxyfen LN was investigated. In addition to specificity and repeatability tests, accuracy test was conducted to confirm that pyriproxyfen was determined accurately in the presence of permethrin.

The sample subjected to this assessment was Olyset Duo. The nominal contents of permethrin and pyriproxyfen in the test sample are 20 g/kg and 10 g/kg, respectively.

3.1 Modification of method

In order to apply the CIPAC 715/TC/M/3 to permethrin/pyriproxyfen LN, the extraction procedures and the solvent for the internal standard solution were modified.

In the CIPAC 715/TC/M/3, pyriproxyfen is extracted using acetonitrile at ambient temperature. However, these procedures can not extract pyriproxyfen incorporated in polyethylene nets. Therefore, the thermal extraction step using heptane was added.
In addition, with this modification, the solvent of the internal standard solution was changed from acetonitrile to 1-propanol which is miscible with both acetonitrile and heptane.
These modifications are considered to be minor modifications.

3.2 Check of the acceptability range

Scope of the existing CIPAC method: 982 to 988 g/kg (TC), 126 g/kg (EC), 99.2 g/kg (EW) and 5.05 g/kg (GR).
Acceptability range: above 491 g/kg, 49.6 to 252 g/kg and 2.5 to 10.1 g/kg.
Pyriproxyfen content in permethrin/pyriproxyfen LN: 10 g/kg
The pyriproxyfen content in permethrin/pyriproxyfen LN is within the acceptability content range of the existing CIPAC method.

3.3 Specificity

The sample solution prepared without addition of the internal standard solution and the solutions of the blank formulation treated in the same way as a sample, the pyriproxyfen standard, the permethrin standard and the internal standard were chromatographed. As shown in Figures 1 to 5, there was no significant interference.

3.4 Precision

Six separate sub-samples from a sample of permethrin/pyriproxyfen LN were analyzed in accordance with the modified method.
The repeatability of this method was satisfactory with the relative standard deviation (RSD) of 0.8% as shown in Table 1. The typical chromatogram of the sample solution is shown in Figure 6.

Table 1 Precision Test

No.	Content of pyriproxyfen (g/kg)
1	10.3
2	10.2
3	10.2
4	10.1
5	10.1
6	10.1
Mean	10.2
%RSD	0.8

3.5 Accuracy

The stock solution at appropriate concentrations of pyriproxyfen and permethrin were fortified to the blank formulation so that the fortified concentrations of pyriproxyfen and permethrin were at the levels of each specification. The solutions were analyzed, and the recoveries of pyriproxyfen were calculated by the following equation:

$$R = \frac{C}{C_S} \times 100$$

where, R : recovery (%)
C : observed concentration (g/kg) of pyriproxyfen
C_S : fortified concentration (g/kg) of pyriproxyfen

The recoveries were satisfactory as shown in Table 2.

Table 2 Accuracy Test

No.	Recovery (%)
1	100.9
2	100.6
3	100.5
4	100.8
Mean	100.7
%RSD	0.2

4. CONCLUSION

In order to apply the CIPAC 715/TC/M/2 to permethrin/pyriproxyfen LN, the thermal extraction step using heptane was added and the solvent for the internal standard solution was changed to 1-propanol.
These modifications are considered to be minor modifications.

The shown data demonstrate the validity of the modified method. Therefore, the modified method is considered appropriate for the determination of pyriproxyfen in permethrin/pyriproxyfen LN.
JAPAC proposes to extend the CIPAC 715/TC/M/2 for permethrin/pyriproxyfen LN.

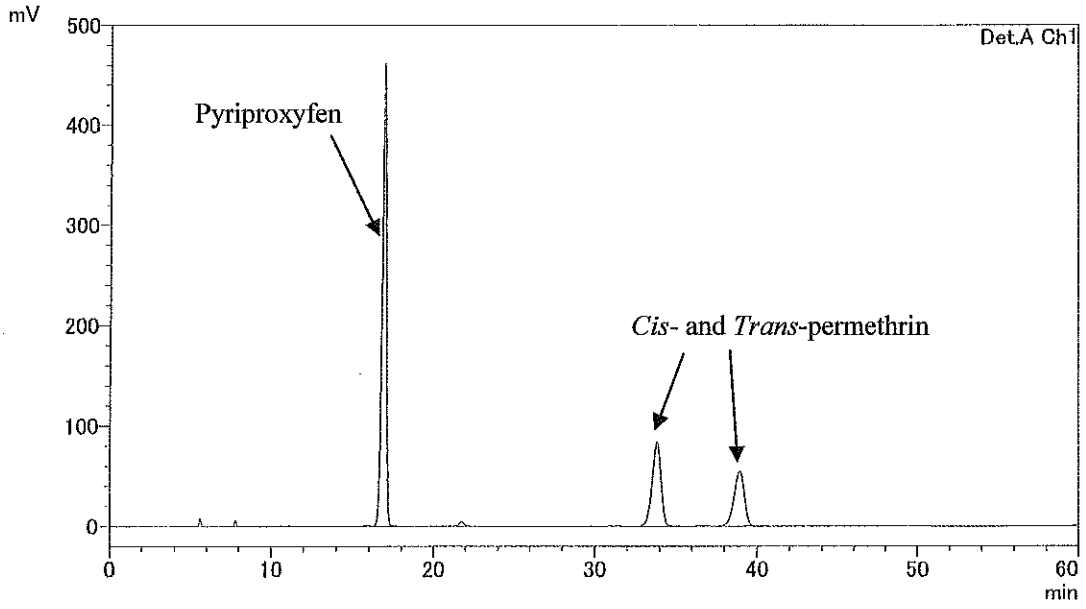

Fig. 1 Liquid chromatogram of permethrin/pyriproxyfen LN, Olyset Duo

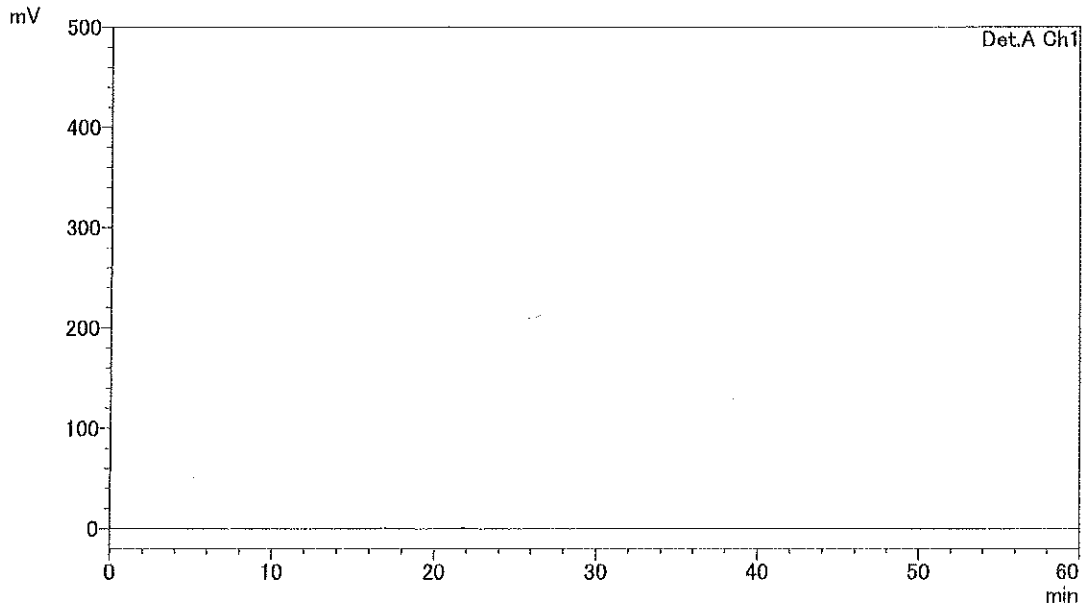

Fig. 2 Liquid chromatogram of blank formulation

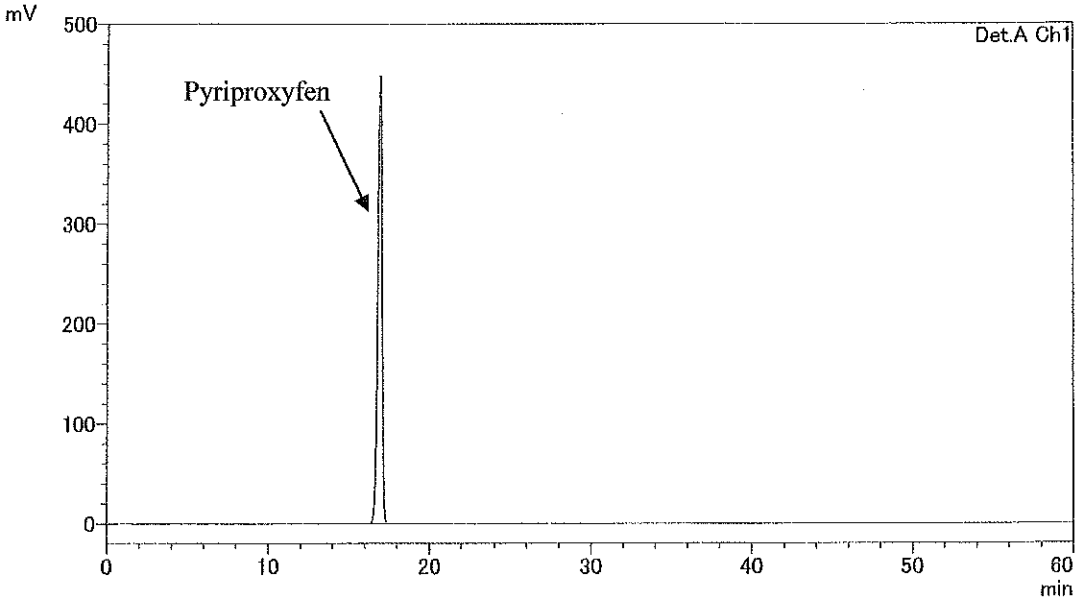

Fig. 3 Liquid chromatogram of pyriproxyfen standard

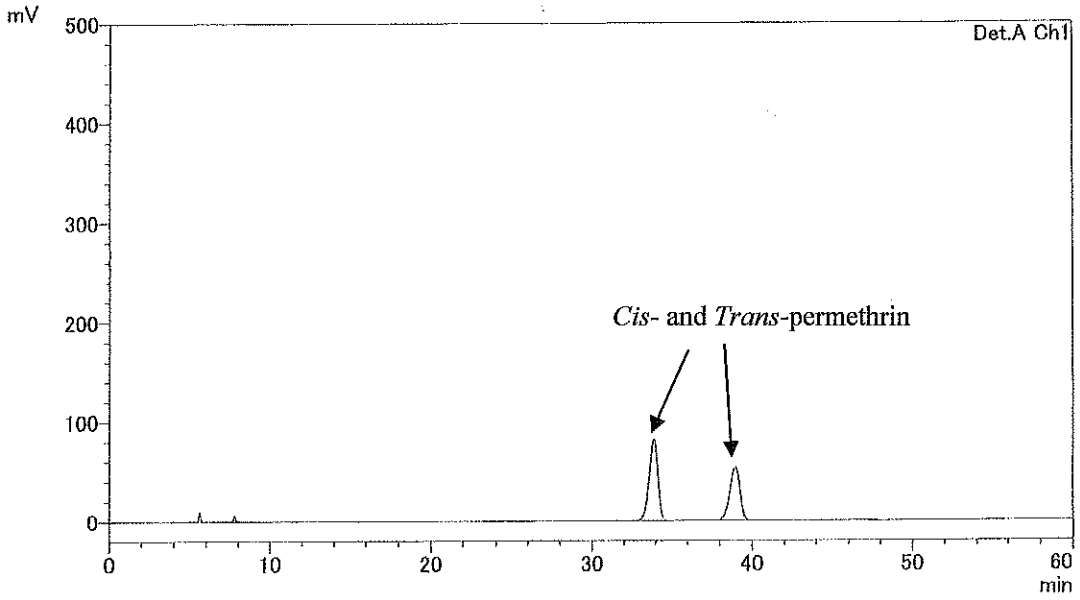

Fig. 4 Liquid chromatogram of permethrin standard

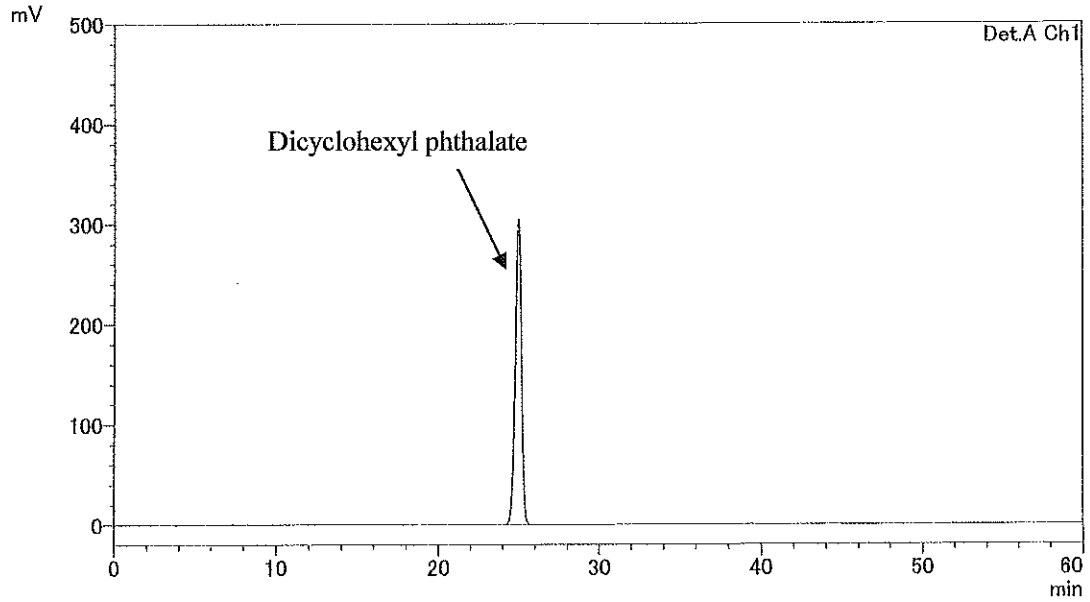

Fig. 5 Liquid chromatogram of internal standard

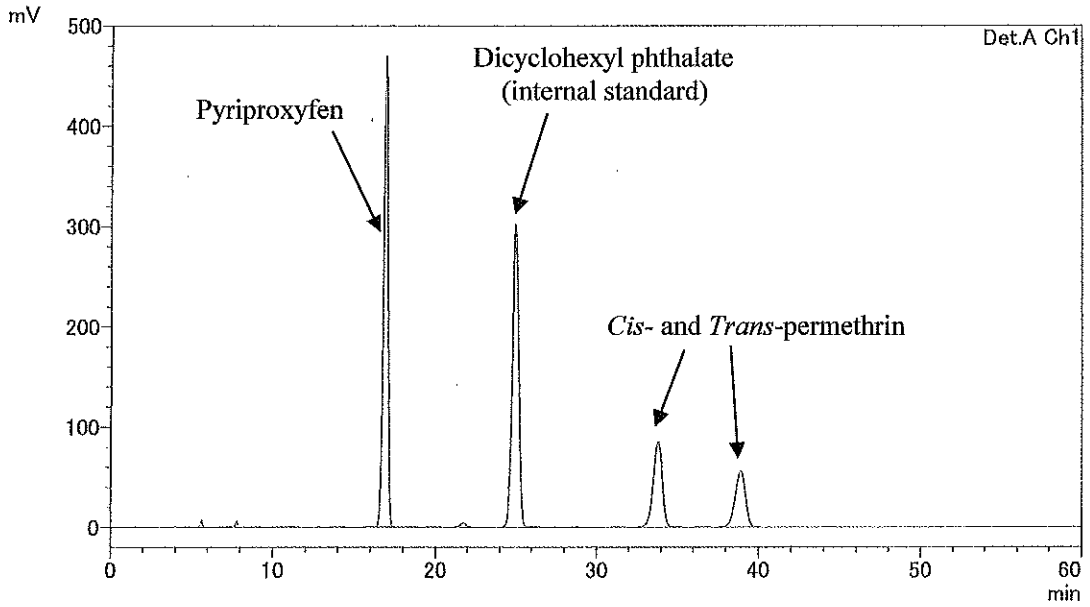

Fig. 6 Liquid chromatogram of sample solution, Olyset Duo