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COLLABORATIVE INTERNATIONAL PESTICIDES ANALYTICAL COUNCIL LIMITED

Commission Internationale des Méthodes d'Analyse des Pesticides (CIMAP)

Long lasting insecticidal nets (LN) are becoming more and more important in the control and prevention of diseases like malaria. In order to meet an urgent need for methods to characterise LN, CIPAC provides selected methods as download. By downloading these methods you accept the following conditions of use:

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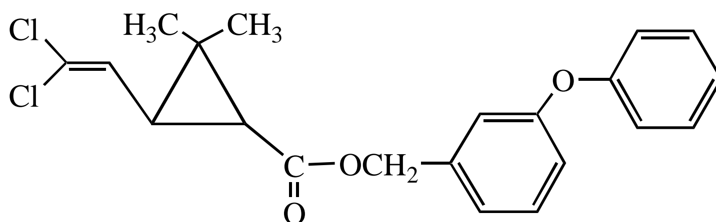
PERMETHRIN
331



ISO common name	Permethrin
Chemical name	3-Phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl) -2,2-dimethylcyclopropanecarboxylate (IUPAC); (3-Phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2 -dimethylcyclopropanecarboxylate (CA)
CAS No.	52645-53-1
Empirical formula	$C_{21}H_{20}Cl_2O_3$
RMM	391.29
v.p.	2.06×10^{-7} Pa (20°C), 6.82×10^{-7} Pa (25°C), 2.24×10^{-6} Pa (30°C), 6.87×10^{-6} Pa (35°C), 1.93×10^{-5} Pa (40°C)
Solubility	In water: 11.1µg/l (20±0.5°C/pH7.0-9.3); soluble in organic solvents
Description	Yellow to yellowish brown oil and solidifies on lowering temperature

PERMETHRIN TECHNICAL
331/TC/m/-

1 Sampling. Take at least 100 g.

2 Identity tests
2.1 GLC. Use the GLC method below. The retention time of permethrin for the sample solution should not deviate by more than 1% from that for the permethrin working standard solution and intensities of the permethrin isomers should give the same pattern as in the working standard solution.
2.2 Infrared. Prepare a film between NaCl plates and scan from 4000 to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard.

3 Permethrin
OUTLINE OF METHOD The content of permethrin in the test samples are determined by capillary GC using flame ionisation detection and triphenyl phosphate as internal standard, and the *trans*-isomer ratio is calculated from the chromatogram obtained.
The content of permethrin is the total content of *cis*- and *trans*-isomers.

REAGENTS
Acetone analytical grade
Permethrin working standard technical product of certified purity. Store refrigerated.
Triphenyl phosphate internal standard. Must not show peaks with the same retention times as *cis*-permethrin and *trans*-permethrin.
Internal standard solution. Dissolve triphenyl phosphate (1.0 g) in acetone (100 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.
Calibration solution. Homogenise the permethrin working standard. When the permethrin working standard is waxy solid or partly waxy solid homogenise it by warming it to melting and by stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 90 to 110 mg (*s* mg) of permethrin working standard into a vial or stoppered flask (100 ml). Add by pipette internal standard solution (5 ml) and dissolve completely. Add by measuring cylinder acetone (45 ml) and mix well (Solutions C_A and C_B).

APPARATUS
Gas chromatograph equipped with a split/splitless injection and a flame ionisation detector.
Capillary column fused silica, 30 m x 0.25 (i.d.) mm, film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane (DB-1 or equivalent)
Electric integrator or data system

PROCEDURE

(a) Gas chromatographic conditions (typical):

Column	fused silica, 30 m x 0.25 (i.d.) mm, film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane (DB-1 or equivalent)
Injection system	
Injector	split injection
Split flow	approximately 100 ml/min
Injection volume	1 µl
Detector	flame ionisation
Temperatures	
Column oven	240°C
Injection port	265°C
Detector	265°C
Carrier gas	helium, 30 cm/sec
Retention times	triphenyl phosphate: about 6.5 min
	permethrin:
	cis-permethrin; about 12.4 min
	trans-permethrin; about 12.9 min

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

(c) System equilibration. Prepare two calibration solutions. Inject 1 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 1.0%. Then inject a 1 µl portion of the second solution. 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. Homogenise the sample. When the sample is waxy solid or partly waxy solid homogenise it by warming it to melting and by stirring. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 90 to 110 mg (*w* mg) of permethrin into a vial or stoppered flask (100 ml). Add by pipette internal standard solution (5 ml) and dissolve completely. Add by measuring cylinder acetone (45 ml) and mix well (Solutions S_A and S_B).

(e) Determination. Inject in duplicate 1 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.

(f) Calculation of permethrin content. Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the permethrin contents of the bracketed sample injections.

$$fi = \frac{Ir \times s \times P}{Hs}$$

$$\text{Content of permethrin} = \frac{f \times Hw}{Iq \times w} \text{ g/kg}$$

where:

fi = individual response factor
f = mean response factor
Hs = total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the calibration solution
Hw = total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the sample solution
Ir = peak area of the internal standard in the calibration solution
Iq = peak area of the internal standard in the sample solution
s = mass of permethrin working standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of permethrin working standard (g/kg)

Repeatability r = 9 g/kg at 953 g/kg active ingredient content
= 9 g/kg at 951 g/kg active ingredient content
Reproducibility R = 23 g/kg at 953 g/kg active ingredient content
= 18 g/kg at 951 g/kg active ingredient content

(g) Calculation of trans-isomer ratio.

$$\textit{trans}\text{-Isomer ratio} = \frac{Hwt}{Hwc + Hwt} \times 100\ \%$$

where:

Hwt = peak area of *trans*-permethrin in the sample solution
Hwc = peak area of *cis*-permethrin in the sample solution

PERMETHRIN LONG LASTING INSECTICIDAL NET
331/LN/m/-

1 Sampling. Take at least 100 g.

2 Identity tests
2.1 GLC. As for **331**/TC/m/2.1
2.2 Infrared. Extract the sample with suitable solvent. Filter and evaporate the solvent. Proceed as for **331**/TC/m/2.2

3 Permethrin As for **331**/TC/m/3 except:
REAGENTS
Heptane analytical grade

Internal standard solution. Dissolve triphenyl phosphate (1.0 g) in heptane (150 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.

Calibration solution. Homogenise the permethrin working standard. When the permethrin working standard is waxy solid or partly waxy solid homogenise it by warming it to melting and by stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 72 to 88 mg (*s* mg) of permethrin working standard into a vial or stoppered flask (200 ml). Add by pipette internal standard solution (10 ml) and dissolve completely. Add by measuring cylinder heptane (90 ml) and mix well (Solutions C_A and C_B).

PROCEDURE

(d) Preparation of sample solution. Clean scissors with acetone before use. Cut the sample with the scissors into 5 – 10 mm square. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 36 to 44 mg (*w* mg) of permethrin into a vial or stoppered flask (100 ml). Add by pipette internal standard solution (5 ml) and by measuring cylinder heptane (45 ml). Place the vial or stoppered flask in a water bath (85 – 90°C) for 45 minutes. Shake the vial or stoppered flask once or twice during extraction. Filter a portion of each sample solution through a filter paper prior to analysis (Solutions S_A and S_B).

(f) Calculation of permethrin content. Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the permethrin contents of the bracketed sample injections.

$$fi = \frac{Ir \times s \times P}{Hs \times 2}$$

$$\text{Content of permethrin} = \frac{f \times Hw}{Iq \times w} \text{ g/kg}$$

where:

- fi = individual response factor
- f = mean response factor
- Hs = total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the calibration solution
- Hw = total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the sample solution
- Ir = peak area of the internal standard in the calibration solution
- Iq = peak area of the internal standard in the sample solution
- s = mass of permethrin working standard in the calibration solution (mg)
- w = mass of sample taken (mg)
- P = purity of permethrin working standard (g/kg)

Repeatability r = 1.6 g/kg at 20.3 g/kg active ingredient content
= 1.3 g/kg at 20.0 g/kg active ingredient content
= 0.9 g/kg at 18.7 g/kg active ingredient content

Reproducibility R = 1.9 g/kg at 20.3 g/kg active ingredient content
= 1.5 g/kg at 20.0 g/kg active ingredient content

= 1.5 g/kg at 18.7 g/kg active ingredient content

4 Surface concentration and release index

REAGENTS As for **331**/TC/m/3 except:

Internal standard solution for calibration solution. Dissolve triphenyl phosphate (0.1 g) in acetone (200 ml) to prepare a stock solution. Transfer by pipette the stock solution (5 ml) to a volumetric flask (50 ml). Make up to volume with acetone and mix well. Ensure that a sufficient quantity of this solution is prepared for all calibration standards to be analysed.

Internal standard solution for sample solution. Transfer by pipette the stock solution (5 ml) to a volumetric flask (500 ml). Make up to volume with acetone and mix well. Ensure that a sufficient quantity of this solution is prepared for all samples to be analysed.

Calibration solution. Homogenise the permethrin working standard. When the permethrin working standard is waxy solid or partly waxy solid homogenise it by warming it to melting and by stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 90 to 110 mg (*s* mg) of permethrin working standard into a volumetric flask (100 ml) and make up to volume with acetone and mix well. Transfer by pipette this solution (1 ml) to a volumetric flask (20 ml), make up to volume with acetone and mix well. Transfer by pipette this solution (5 ml) to a vial (20 ml), add by pipette internal standard solution for calibration solution (5 ml) and mix well (Solutions C_A and C_B).

APPARATUS As for **331**/TC/m/3 except:

Constant temperature oven capable of controlling temperature within the range of ±2°C is recommended.

Rotary evaporator

PROCEDURE As for **331**/TC/m/3 except:

(a) Gas chromatographic conditions (typical):

Injection system

Split flow approximately 10 ml/min

(b) Linearity check. Check the linearity of the detector response by injecting 1 µl of solutions with permethrin concentrations 0.1, 1 and 2.5 times that of the calibration solution before conducting analysis.

(c) System equilibration. Prepare two calibration solutions. Inject 1 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 2.0%. Then inject a 1 µl portion of the second solution. The response factor for this solution should not deviate by more than 2.0% from that for the first calibration solution, otherwise prepare new calibration solutions.

(d) Preparation of sample solution.

Clean scissors and tweezers with acetone before use. Prepare sample solutions in triplicate for each sample *Note 1)*. Cut ca. 5 cm x 5 cm net samples with the scissors. Weigh accurately, to the nearest 0.1 mg, of each sample (*w* mg). Transfer it with the tweezers into a vial (20 ml). Add by pipette internal standard solution for sample solution (10 ml). Cap the vial and shake the

solution by hand for 1 minute [Note 2)]. Take out the netting with the tweezers and discard the solution.
Let the netting dry at room temperature for ca. 10 minutes. Transfer it into a vial (20 ml) with the tweezers. Cap the vial and place it in a temperature-controlled oven set at 70°C. Heat the sample for 2 hours [Note 3)]. After heating, remove the vial from the oven and let it equilibrate to room temperature. Add by pipette internal standard solution for sample solution (10 ml) into the vial. Cap the vial and shake the solution by hand for 1 minute. Take the netting out with the tweezers. Let the netting dry at room temperature for ca. 10 minutes. Transfer the sample solution from the vial into a round-bottom flask. Rinse the vial with acetone (about 1 ml) and transfer it into the same round-bottom flask. Evaporate the solution *in vacuo* to dryness. Add by pipette acetone (2 ml) into the flask to dissolve the residue (Solutions for "surface concentration at post-wash 1", S_{A1}, S_{B1} and S_{C1}).
Transfer the dried netting into a vial (20 ml) with the tweezers. Repeat heating, internal standard solution adding, evaporating and dissolving procedures as above. (Solutions for "surface concentration at post-wash 2", S_{A2}, S_{B2} and S_{C2}).
Again, let the netting dry at room temperature for ca. 10 minutes. Transfer the dried netting into a vial (20 ml) with the tweezers. Repeat heating, internal standard solution adding, evaporating and dissolving procedures as above. (Solutions for "surface concentration at post-wash 3", S_{A3}, S_{B3} and S_{C3}).

Note 1: Analytical error is larger than average content determinations. Triplicate determinations, therefore, are recommended.
Note 2: The shaking speed is about 30 times per 10 seconds.
Note 3: Put vials in a covered cardboard box while heating so that the vials are not exposed to direct stream of warm air.

(e) Determination. Inject in duplicate 1 μl portions of each sample solution bracketing them by injections of the calibration solutions as follows; calibration solution C_A, sample solution S_{A1}, sample solution S_{A1}, calibration solution C_B, sample solution S_{B1}, sample solution S_{B1}, calibration solution C_A, and so on. Measure the relevant peak areas.

(f) Calculation of surface concentration. Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the surface concentrations of the bracketed sample injections.

$$fi = \frac{Ir \times s \times P}{Hs}$$

$$\text{Surface concentration} = \frac{f \times Hw}{Iq \times w \times 2} \ \mu g/g$$

where:

fi = individual response factor
f = mean response factor
Hs = total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the calibration solution

Hw= total peak area of permethrin (*cis*-permethrin + *trans*-permethrin) in the sample solution
Ir = peak area of the internal standard in the calibration solution
Iq = peak area of the internal standard in the sample solution
s = mass of permethrin working standard taken (mg)
w = mass of sample taken (mg)
P = purity of permethrin working standard (g/kg)

(g) Calculation of release index. Calculate the mean value of the two injections of sample solutions S_{A3}, S_{B3} and S_{C3} by the equations described in *(f)*, and the release index for each piece of the netting.

$$\text{Release index} = \frac{C}{B}$$

where:
B = mean value of surface concentration at post-wash 2 (μg/g)
C = mean value of surface concentration at post-wash 3 (μg/g)

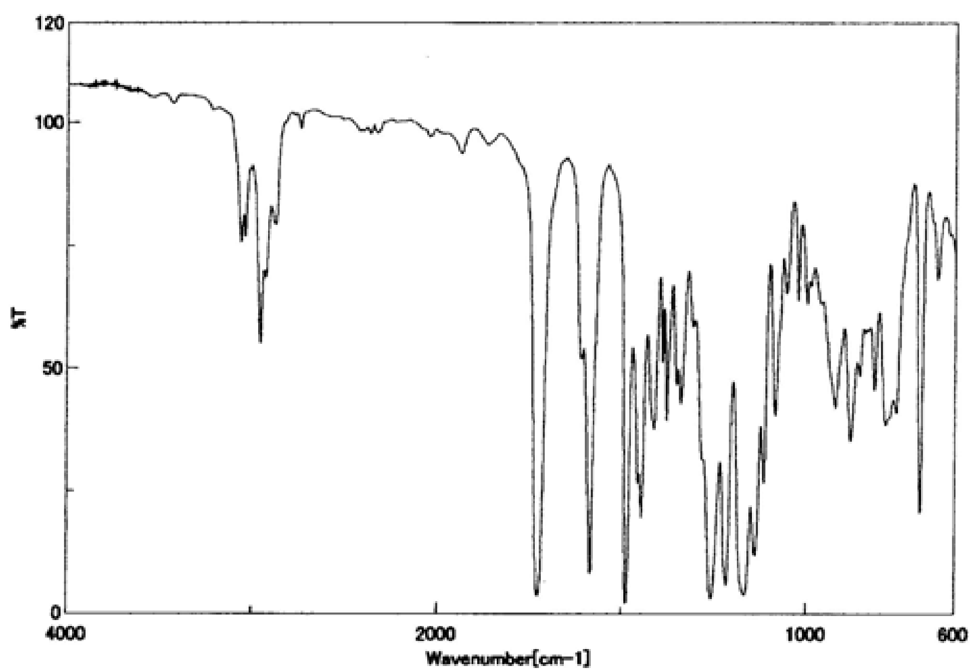


Fig. 1 Infrared Spectrum of Permethrin

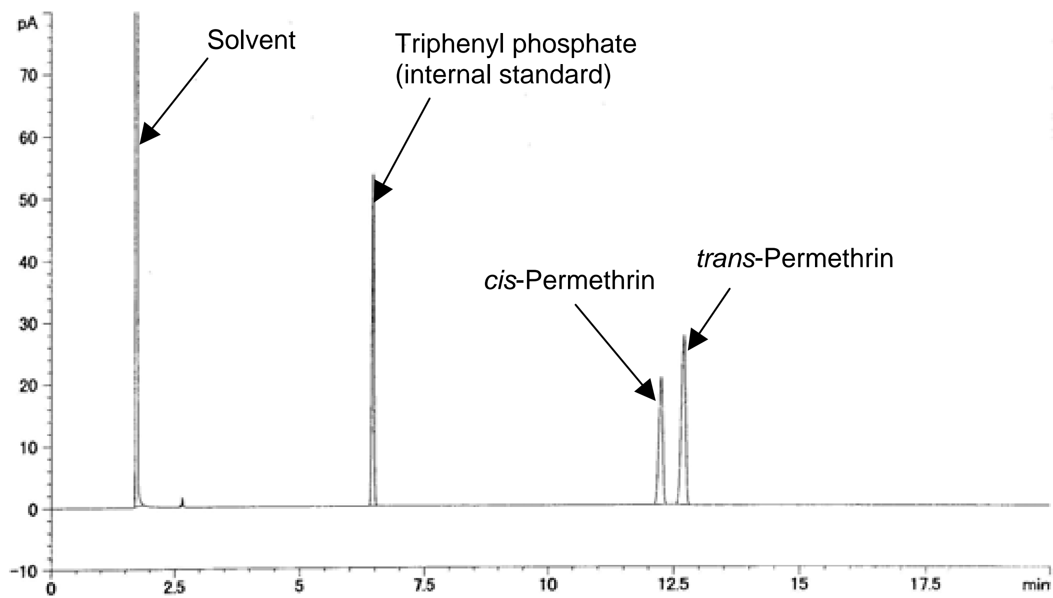


Fig. 2 Example of gas chromatogram of Permethrin TC

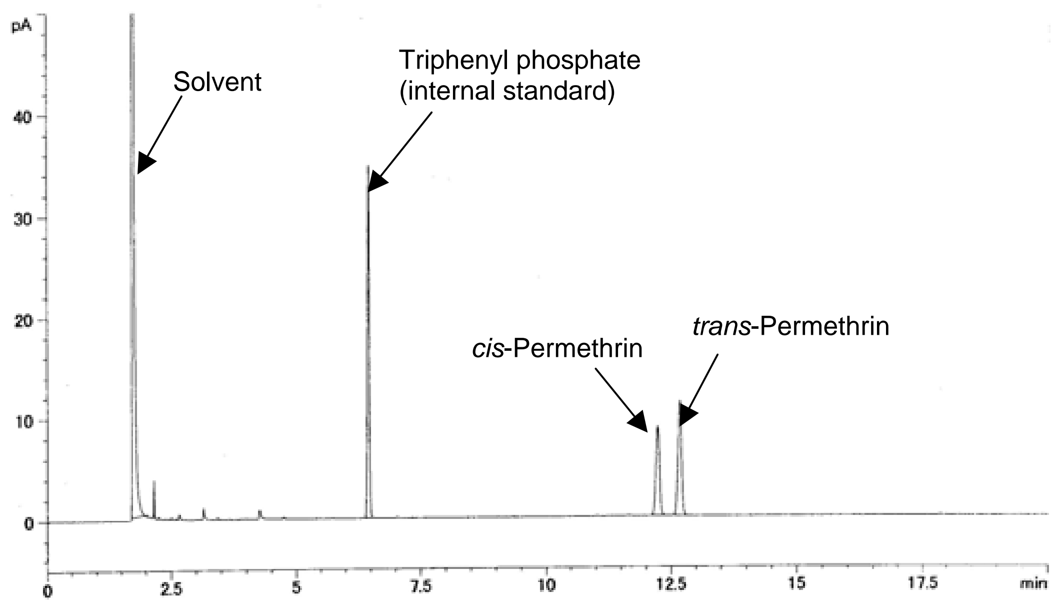


Fig. 3 Example of gas chromatogram of Permethrin LN