Determination of deltamethrin and/or piperonyl butoxide in Long-Lasting (incorporated into polyethylene) Insecticidal Mosquito Nets

Analytical method by GC-FID

REPORT TO CIPAC

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1. Scope

This method is concerned with the determination of deltamethrin (and deltamethrin R-isomer) and/or piperonyl butoxide in Long-Lasting (incorporated into polyethylene) Insecticidal Mosquito Nets (incorporated LNs). This method is applicable and was validated for products such as PermaNet 3.0® (roof) and NetProtect®. This method is also applicable for Long-Lasting (coated on polyester) Insecticidal Mosquito Nets (coated LNs), but for these products the method recommended is the CIPAC method 333/LN/M3.

2. Outline of method

Deltamethrin and/or piperonyl butoxide are extracted from net samples by heating under reflux for 60 minutes with xylene and determined by Gas Chromatography with Flame Ionisation Detection (GC-FID) using the internal standard calibration.

3. Security measures

Each analyst has to be informed about eventual risks and hazards due to products, reagents and solvents used in this method before starting the analysis and take any necessary precaution measure (work under fume hood, wearing of laboratory coats, gloves, masks …).
Safety information are written in the Master Safety Data Sheets (MSDS) or labels distributed with these products, reagents or solvents, in the literature or are provided by providers or sponsors.

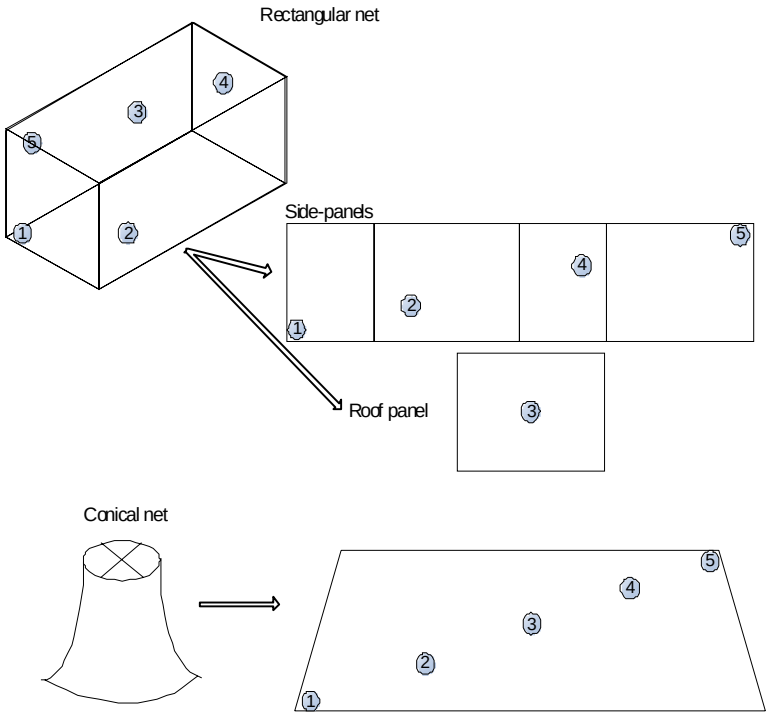
4. Sampling

(a) Sampling for the analysis of a piece of LN

Cut the piece of LN with scissors in small pieces of 5-10 mm square, mix thoroughly and weigh analytical portions of 300 mg for determination of deltamethrin and/or piperonyl butoxide content.

(b) <u>Sampling for the analysis of an entire LN of the same composition</u>

Take from the finished net 5 pieces of 25 cm x 25 cm according to the recommendations of the FAO/WHO Specifications Manual (figure hereafter).

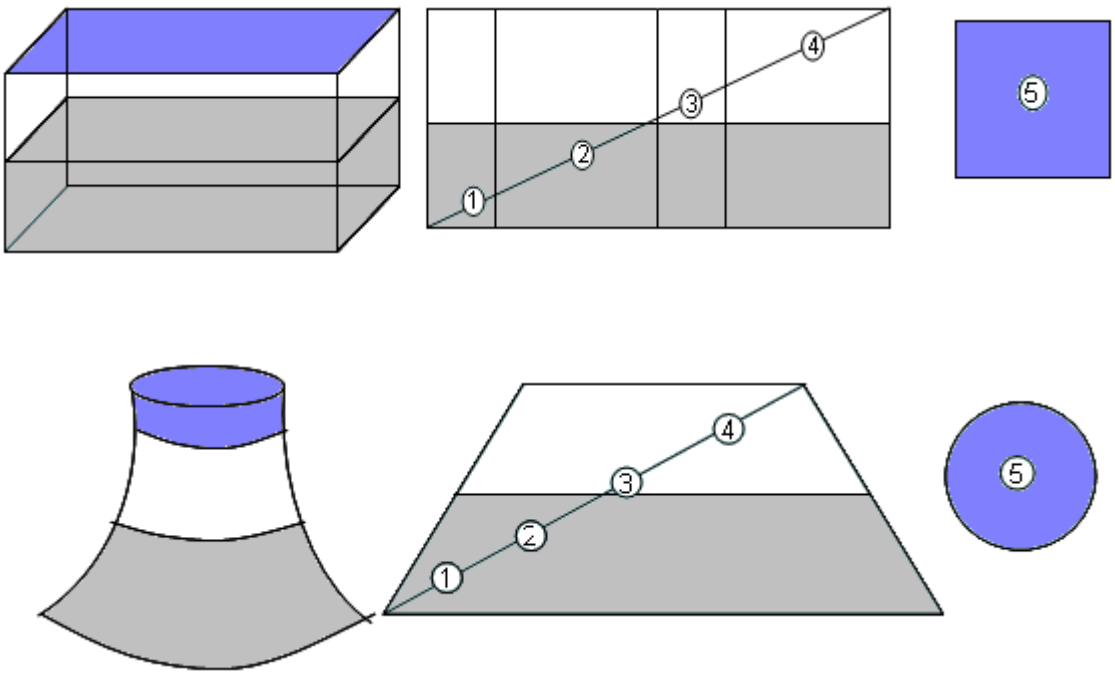


For the average content, combine these 5 pieces and cut them with scissors in small pieces of 5-10 mm square, mix thoroughly and weigh analytical portions of 300 mg for determination of deltamethrin content.

(c) <u>Sampling for the analysis of an entire combination LN</u>

Take from the finished net 5 pieces of 25 cm x 25 cm according to the figure hereafter.

For the determination of the average deltamethrin content in the side netting, combine the pieces 1, 2, 3 and 4 and cut them with scissors in small pieces of 5-10 mm square. Mix thoroughly and weigh analytical portions of 300 mg for determination of deltamethrin content. For the determination of the average deltamethrin and piperonyl butoxide content in the roof netting, cut the piece 5 with scissors in small pieces of 5-10 mm square. Mix thoroughly and weigh analytical portions of 300 mg for determination of deltamethrin and piperonyl butoxide content.



5. Reagents

- Deltamethrin, certified analytical standard of known purity.
- Deltamethrin R-isomer, certified analytical standard of known purity.
- Piperonyl butoxide, certified analytical standard of known purity.
- Dipropyl phthalate, analytical reagent grade.
- Xylene, analytical reagent grade.

Internal standard solution

Weigh (to the nearest 0.1 mg) about 100 mg of dipropyl phthalate into a 200 mL volumetric flask. Add xylene (150 mL) and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to ambient temperature and fill to the mark with xylene. Mix thoroughly (= **solution IS**).

Deltamethrin and piperonyl butoxide calibration solutions

Weigh (to the nearest 0.1 mg) about 30 mg of deltamethrin analytical standard into a 50 mL volumetric flask. Add xylene (40 mL) and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to ambient temperature and fill to the mark (at 20°C) with xylene. Mix thoroughly (= **solution DS**).

Weigh (to the nearest 0.1 mg) about 150 mg of piperonyl butoxide (PBO) analytical standard into a 50 mL volumetric flask. Add xylene (40 mL) and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to ambient temperature and fill to the mark (at 20°C) with xylene. Mix thoroughly (= **solution PS**).

Prepare the following calibration solutions into 25 mL volumetric flasks (at 20°C) (= **calibration solutions C_1, C_2, C_3, C_4 and C_5**).

Code	IS	DS + PS	Xylene	Deltamethrin (mg)	PBO (mg)	Total volume
C_1	1 mL	0.2 mL	Up to volume	0.12	0.60	25 mL
C_2	1 mL	0.5 mL	Up to volume	0.30	1.50	25 mL
C_3	1 mL	1.0 mL	Up to volume	0.60	3.00	25 mL
C_4	1 mL	1.5 mL	Up to volume	0.90	4.50	25 mL
C_5	1 mL	2.0 mL	Up to volume	1.20	6.00	25 mL

Deltamethrin R-isomer calibration solution

Weigh (to the nearest 0.1 mg) about 10 mg of deltamethrin R-isomer analytical standard into a 50 mL volumetric flask. Add xylene (40 mL) and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to ambient temperature and fill to the mark with xylene. Mix thoroughly. Transfer 1 mL of this solution into a 50 mL volumetric flask and fill to the mark with xylene. Mix thoroughly. This solution is used to check the good chromatographic separation between deltamethrin and deltamethrin R-isomer.

6. Apparatus

- Analytical balance (to 0.1 mg).
- Analytical balance (to 0.01 g).
- Ultrasonic bath.
- Solvent extraction apparatus: 100 mL conical flask, reflux condenser and sand heating plate.
- Laboratory glassware.
- Gas chromatograph, equipped with a flame ionisation detector (GC-FID) and a pulsed splitless injection system.
- Capillary column, fused silica, 30 m x 0.25 mm (i.d.), 0.25 µm film thickness, coated with 5 % phenyl methyl siloxane (DB-5) (or equivalent material with the same selectivity).

7. Procedure

(a) Operating chromatographic conditions

Chromatographic determination by GC-FID (typical)

APPARATUS

- Gas chromatograph : Agilent Technologies 6890 N.
- Autosampler : Agilent Technologies 7683 Series.
- Injection system : pulsed splitless.
- Liner : splitless, double taper, no glass wool, deactivated (Agilent ref. 5181-3315).
- Detector : Flame Ionisation Detector (FID).
- Software of integration : Agilent Technologies ChemStation.

CHROMATOGRAPHIC PARAMETERS

- Column : capillary fused silica, 30 m x 0.25 mm (i.d.), 0.25 µm film thickness, coated with 5 % phenyl methyl siloxane (DB-5) (or equivalent material with the same selectivity).
- Carrier gas : helium - 1 mL / minute (constant flow).
  Alternative carrier gas : helium - 2 mL / minute (constant flow).
- Pulse pressure : 60 psi.
- Pulse time : 1 minute.
- Purge flow to split vent : helium - 60 mL / minute.
- Purge time : 45 seconds.
- Make-up gas : helium - 30 mL / minute.
- Hydrogen flow to detector : 30 mL / minute.
- Air flow to detector : 400 mL / minute.
- Inlet temperature : 280°C.
- Oven temperature : 130°C for 1 minute
  30°C / minute to 280°C
  280°C for 12 minutes.

Retention times with these conditions : dipropyl phthalate : 5.8 minutes
piperonyl butoxide : 8.4 minutes
deltamethrin R-isomer : 15.1 minutes.
deltamethrin : 15.8 minutes.

Alternative oven temperature : 130°C for 1 minute
30°C / minute to 250°C
2°C / minute to 275°C
30°C / minute to 300°C
300°C for 7 minutes.

- Detector temperature : 300°C.
- Injection volume : 1 µL.

These chromatographic conditions may be adapted in order to achieve a good chromatographic separation.

(b) System suitability check

Inject the calibration solutions until the deltamethrin or piperonyl butoxide to internal standard peak area ratio obtained for two consecutive injections differ by less than 2 % (for concentrations in the calibration range). Moreover the peak area for deltamethrin R-isomer has to be not less than 2 % of the peak area for deltamethrin. The retention time of the deltamethrin, deltamethrin R-isomer or piperonyl butoxide peak in the sample solution should not deviate by more than 0.5 % from that of the calibration solution.

(c) Calibration curve and linearity check

Check the linearity of the detector response by injecting the calibration solutions. Calculate the deltamethrin or piperonyl butoxide to internal standard peak area ratio in function of the deltamethrin or piperonyl butoxide concentration. The determination coefficient of the calibration curve has to be not less than 0.99.

(d) Preparation of sample

Weigh (to the nearest 0.1 mg) 300 mg of sample (w mg) into a 100 mL conical flask. Add 1 mL of the internal standard solution (at 20°C) and 24 mL xylene. Connect the flask to a reflux condenser and heat to reflux for 60 minutes (polyester nets are not dissolved, polyethylene nets are completely dissolved). Allow the solution to cool to ambient temperature. Filter an aliquot of the solution through a 0.2 µm nylon filter and put into an injection vial (= **sample solutions S_1, S_2, S_3** …).

(e) Determination

Inject in duplicate two sample solution (S_1, S_2 …) bracketing them by duplicate injections of calibration solutions (C_1, C_2 …) using the following sequence:

$$C_1, C_1, S_1, S_1, S_2, S_2, C_2, C_2 \ldots$$

Determine the peak area of deltamethrin, deltamethrin R-isomer, piperonyl butoxide and dipropyl phthalate.

(f) Calculation

For the calibration solutions, plot the deltamethrin or piperonyl butoxide to internal standard peak area ratio versus the deltamethrin or piperonyl butoxide concentration (µg/mL).

Construct the calibration curve using the method of least square linear regression and calculate the equation of the curve.

$$x = \frac{y - b}{a} \quad \text{corresponding to} \quad Cs = \frac{Rs - b}{a}$$

where:

Cs = deltamethrin or piperonyl butoxide concentration in the calibration solution (µg/mL) corrected for the purity of the analytical standard

Rs = deltamethrin or piperonyl butoxide to internal standard peak area ratio for the calibration solution

a = slope of the calibration curve

b = origin intercept of the calibration curve

Using the calibration curve, calculate the deltamethrin or piperonyl butoxide concentration (µg/mL) in the sample solution using the following equation.

$$Cw = \frac{Rw - b}{a}$$

where:

Cw = deltamethrin or piperonyl butoxide concentration in the sample solution (µg/mL)

Rw = deltamethrin or piperonyl butoxide to internal standard peak area ratio for the sample solution

a = slope of the calibration curve

b = origin intercept of the calibration curve

Calculate the deltamethrin or piperonyl butoxide content (g/kg) using the following equation.

$$\text{a.i. content} = \frac{Cw \times 25}{w} \quad \text{(g/kg)}$$

where:

Cw = deltamethrin or piperonyl butoxide concentration in the sample solution (µg/mL)

w = mass of sample taken (mg)

Calculate the deltamethrin R-isomer content (g/kg) using the following equation.

$$\text{Deltamethrin R - isomer content} = \frac{\text{Deltamethrin content} \times Hri \times 100}{Hd} \quad \text{(g/kg)}$$

where:

Hri = peak area for deltamethrin R-isomer

Hd = peak area for deltamethrin