Deltamethrin + Chlorfenapyr

333 + 570

in Technical material (TC)
and Long-Lasting Insecticide-treated Net (LN or ITN)

CIPAC 5297/m

Full scale collaborative trial

HPLC-DAD method

ANALYTICAL METHOD FOR
LONG-LASTING INSECTICIDE-TREATED NETS CONTAINING
DELTAMETHRIN AND CHLORFENAPYR

SCOPE

This method is intended for determining deltamethrin and chlorfenapyr content in long-lasting insecticidal net (LN/ITN).

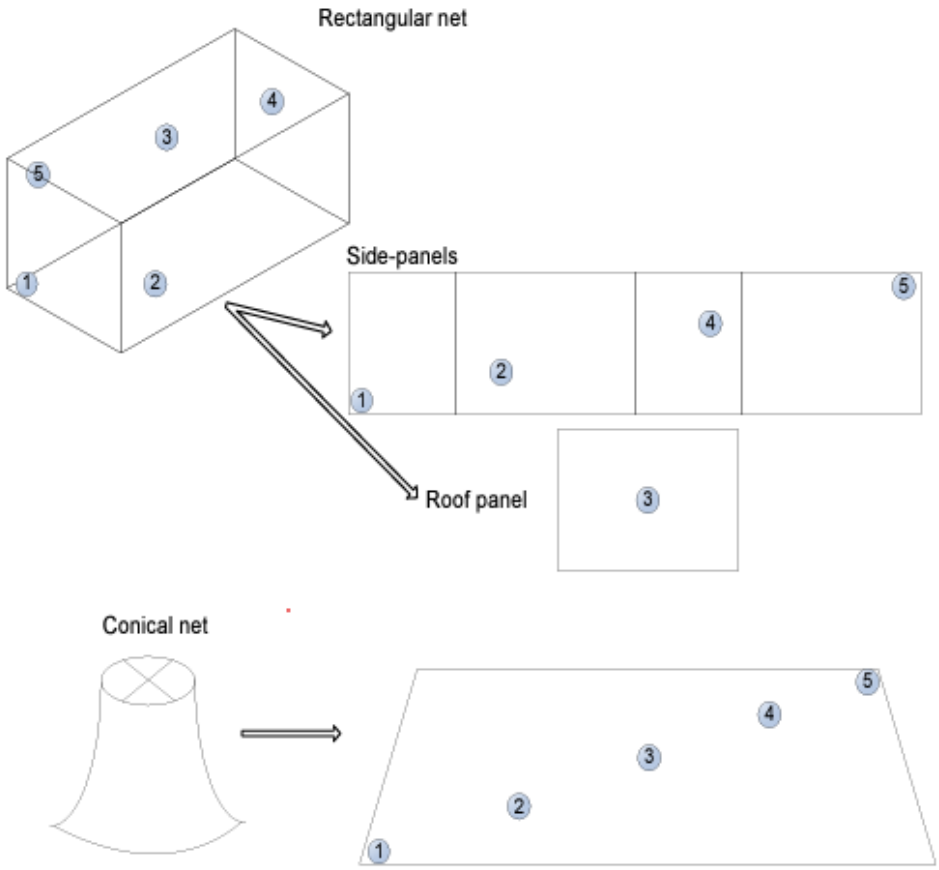
Determination of deltamethrin or chlorfenapyr in Technical material (TC) can be also performed by this method.

1. Sampling:

This sampling procedure is suitable for net samples taken from either new or used LN/ITN.

Samples of at least 25 x 25 cm from LN/ITN are taken following the sampling method described in the specification template for insecticide treated nets or netting (LN/ITN) of the Manual on the development and use of FAO and WHO specifications for chemical pesticides, second edition, Rome and Geneva, 2022.

Fig. 1: General method for sampling rectangular and conical nets



In total 5 net pieces are taken.

The net pieces are cut in small pieces (max. 5 x 5 mm) and mixed. The net pieces can be pooled together before analytical determination or analyzed individually.

When the small pieces are pooled, they have to be carefully mixed to get a homogenous aggregated sample. The analysis of this one gives only information about the average content of active ingredient(s) in net. However, the analysis of each net pieces allows getting information about the spatial distribution of the active ingredient(s) besides the mean of content of active ingredient(s) in net.

2. Identity test

Deltamethrin	**HPLC.**	Use the HPLC method below. The retention time of deltamethrin in the sample solution should not deviate by more than 3 % from that of the calibration solution.
Chlorfenapyr	**HPLC.**	Use the HPLC method below. The retention time of chlorfenapyr in the sample solution should not deviate by more than 3 % from that of the calibration solution.

3. Deltamethrin and chlorfenapyr content

OUTLINE OF METHOD

The sample is extracted with heptane using dicyclohexyl phthalate as internal standard. Deltamethrin and chlorfenapyr contents are determined by normal phase liquid chromatography with ultraviolet detection (HPLC-DAD).

REAGENTS

Deltamethrin (DM), certified analytical standard of known purity

Chlorfenapyr (CFP), certified analytical standard of known purity

Dicyclohexyl phthalate, internal standard (ISTD) of known purity

n-Heptane, analytical reagent and HPLC grade

iso-propanol, analytical reagent and HPLC grade

Mobile phase : *n*-heptane/*iso*-propanol 98:2, v/v

Internal standard stock solution

Weigh, accurately to the nearest 0.1 mg, about 250 mg of dicyclohexyl phthalate into a 100 ml volumetric flask. Add heptane and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane (solution $ISTD_{stock}$). Mix thoroughly.

Ensure sufficient quantity of this solution is prepared for all the samples and calibration solutions to be analyzed.

Deltamethrin and chlorfenapyr calibration stock solutions

Weigh in duplicate, accurately to the nearest 0.1 mg, about 25 mg of deltamethrin and about 50 mg of chlorfenapyr analytical standards (*s* mg) into two separate 100 ml volumetric flasks, each flask containing both analytical standards. Add heptane and place the flasks in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane (Solutions C_{DM+CFP} and C^*_{DM+CFP}). Mix thoroughly.

Deltamethrin and chlorfenapyr calibration working solutions

Prepare the following calibration solutions into conical flasks at room temperature, using the calibration stock solution C_{DM+CFP} as described in the below table (= calibration solutions C_1, C_2, C_3, C_4 and C_5).

Internal standard and deltamethrin + chlorfenapyr solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Code	$ISTD_{stock}$	C_{DM+CFP}	Deltamethrin (µg/mL), approx.	Chlorfenapyr (µg/mL), approx	Heptane	Final volume
C_1	**1 ml**	**0.8 mL**	8	16	Up to volume	25 ml
C_2	**1 ml**	**2 mL**	20	40	Up to volume	25 ml
C_3	**1 ml**	**4 mL**	40	80	Up to volume	25 ml
C_4	**1 ml**	**6 mL**	60	120	Up to volume	25 ml
C_5	**1 ml**	**8 mL**	80	160	Up to volume	25 ml

C^*_{DM+CFP} is used to control the weighing of C_{DM+CFP} : for this, prepare a C^*_3 using the calibration stock solution C^*_{DM+CFP} as described in the below table (= calibration solution C^*_3).

Internal standard and deltamethrin + chlorfenapyr solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Code	IS	C^*_{DM+CFP}	Deltamethrin (µg/mL), approx.	Chlorfenapyr (µg/mL), approx	Heptane	Final volume
C^*_3	**1 ml**	**4 ml**	40	80	Up to volume	25 ml

Stock and working calibration solutions should be stored out of direct sunlight and in a refrigerated (<10°C) zone.

APPARATUS

High performance liquid chromatograph (HPLC), equipped with a constant flow pump, an auto-sampler capable of delivering 10 µl, a column oven and an UV detector capable of measuring at 230 nm.

Electronic integrator or data system

HPLC column, stainless steel, 150*3 mm, packed with CN phase (3 µm), or equivalent material with same selectivity.

PTFE or Nylon filter, with maximum 0.45 µm pore size.

Usual laboratory equipment, e.g. analytical balance, standard laboratory glassware, water bath, ultrasonic bath and volumetric pipette of suitable volume.

PROCEDURE

(a) Operating chromatographic conditions (typical)

Column	stainless steel, 150*3 mm, packed with CN phase (3 µm), or equivalent material with same selectivity
Column temperature	25°C
Flow rate	isocratic, 0.6 ml/min
Injection volume	10 µl
Detection mode	UV
Measuring wavelength	at 230 nm
Run time	about 8 min. Run time may be increased for column clean-up to avoid interferences of co-formulants.
Retention times	dicyclohexyl phthalate (internal standard) : about 2.9 min deltamethrin : about 4.1 min chlorfenapyr : about 5.1 min Note : These retention times are those obtained with the HPLC column mentioned above and may vary with its size and its particles size.

(b) System equilibration

Pump sufficient mobile phase through the column to equilibrate the system.

Inject 10 µl portions of the 2 calibration working solutions C_3 and C^*_3 before analysis to ensure that the relative response factors for C^*_3 ($f_{i\ DM}$ *vs* $f^*_{i\ DM}$ and $f_{i\ CFP}$ *vs* $f^*_{i\ CFP}$) does not deviate by more than 2.0 % from that of solution C_3, for both active ingredients. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula :

$$f_{i\ DM\ or\ CFP} = \frac{I_r \times s_{DM\ or\ CFP} \times P_{DM\ or\ CFP} \times V_{DM+\ CFP\ transferred}}{H_{s\ DM\ or\ CFP} \times V_{stock\ DM+\ CFP} \times V_{working\ cal\ DM+\ CFP}}$$

Where :

$f_{i\ DM\ or\ CFP}$ = individual response factor, for deltamethrin or chlorfenapyr

$H_{s\ DM\ or\ CFP}$ = peak area of deltamethrin or chlorfenapyr in the calibration solution (C_3 or C^*_3)

I_r = peak area of internal standard in the calibration solution (C_3 or C^*_3)

$S_{DM\ or\ CFP}$ = mass of deltamethrin or chlorfenapyr reference standard in the calibration stock solution C_{DM+CFP} and C^*_{DM+CFP} , in mg

$P_{DM\ or\ CFP}$ = purity of deltamethrin or chlorfenapyr reference standard used to prepare the calibration stock solution C_{DM+CFP} and $C^*_{DM+CFP,}$ in g/kg

$V_{DM+CFP\ transferred}$ = volume of the calibration stock solution ($C_{DM\ +\ CFP}$ or $C^*_{DM\ +\ CFP}$) transferred to prepare the working calibration solution (C_3 or C^*_3), in mL (= 4 mL)

$V_{stock\ DM\ +\ CFP}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+CFP} or C^*_{DM+CFP}), in mL (= 100 mL)

$V_{working\ cal\ DM\ +\ CFP}$ = total volume of the calibration working solution (C_3 or C^*_3), in mL (= 25 mL)

If the peak retention times differ significantly from the values given, then adjust the flow rate accordingly.

(c) Preparation of samples solutions for deltamethrin TC :

Weigh in duplicate, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add heptane and place the flasks in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane. Mix thoroughly.

Transfer precisely with a volumetric pipette 1 mL of this solution, at 20°C ± 1°C, into a cap glass bottle/flask or a 50 mL disposable tube. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and 23 mL of heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial. (Note 1).

Blank solution should be prepared following the previously described conditions, but without adding any TC sample (= Solution “blank ISTD”).

(d) ***Preparation of samples solutions for chlorfenapyr TC :***

Weigh in duplicate, accurately to the nearest 0.1 mg, about 50 mg of TC sample into a 25 mL volumetric flask. Add heptane and place the flasks in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20°C ± 1°C with heptane. Mix thoroughly.

Transfer precisely with a volumetric pipette 1 mL of this solution, at 20°C ± 1°C, into a cap glass bottle/flask or a 50 mL disposable tube. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and 23 mL of heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial (Note 1).

Blank solution should be prepared following the previously described conditions, but without adding any TC sample (= Solution “blank ISTD”).

(e) Preparation of samples solutions for LN/ITN :

Weigh in duplicate, accurately to the nearest 0.1 mg, about 500 mg of ITN/LN sample cut in small pieces into a 100 mL cap glass bottle/flask or into a 50 mL disposable tube. Add precisely at 20°C ± 1°C and by volumetric pipette 1 mL of internal standard stock solution and 24 mL of heptane. Put the flask in an ultrasonic bath for 5 minutes. Note that the net sample is not dissolved. Allow the solution to cool to room temperature and mix thoroughly. Filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial (Note 1).

Blank solution should be prepared following the previously described conditions, but without adding any LN/ITN sample (= Solution "blank ISTD").

(f) Determination.

Inject blank solutions and calibration working solutions (C_3 and C^*_3) first. The calibration working solution C^*_3 is used to check the accuracy of the weighing of the calibration solution C_{DM+CFP}. The following sequence is advised: solvent, blank ISTD, C_3 in duplicate and C^*_3 in duplicate. Then, inject the sample extracts in duplicate. Each 2 to 4 sample extracts are bracketed with a calibration solution (C_1 to C_5), as follows: calibration solution C_1, sample solution $S1_A$, sample solution $S1_B$, sample solution $S2_A$, sample solution $S2_B$, calibration solution C_2, sample solution $S3_A$, sample solution $S3_B$, sample solution $S4_A$, sample solution $S4_B$, calibration solution C_3 and so on for further samples. Measure the relevant peak areas.

(g) ***Calculation.***

Quantitative determination of deltamethrin and chlorfenapyr in the sample solutions is carried out by comparing the ratio of peaks area of deltamethrin or chlorfenapyr to the peak area of dicyclohexyl phthalate in the sample solutions with that of the standard solutions, on basis of a calibration curve calculated with standard solutions (C_1 to C_5) bracketing the sample solutions.

The calibration curves for deltamethrin and chlorfenapyr are obtained by the internal standard calibration method from the injection of deltamethrin and chlorfenapyr standard solutions containing dicyclohexyl phthalate and plotting the ratio of peaks areas (peak area DM or CFP / peak area ITSD) versus the deltamethrin or chlorfenapyr concentration (in µg/mL). Calculate the equation of the linear regression obtained.

- y- axis = $\frac{H_{w\ DM\ or\ CFP}}{I_q}$
- x- axis = $\frac{S_{DM\ or\ CFP} \times P_{DM\ or\ CFP} \times V_{DM+\ CFP\ transferred}}{V_{stock\ DM+\ CFP} \times V_{working\ cal\ DM+\ CFP}}$

Where :

$H_{w\ DM\ or\ CFP}$ = peak area of deltamethrin or chlorfenapyr in the sample solution

I_q = peak area of internal standard in the sample solution

$S_{DM\ or\ CFP}$ = mass of deltamethrin or chlorfenapyr reference standard in the calibration stock solution C_{DM+CFP}, in mg

$P_{DM\ or\ CFP}$ = purity of deltamethrin or chlorfenapyr reference standard used to prepare the calibration stock solution $C_{DM+CFP,}$ in g/kg

$V_{DM+CFP\ transferred}$ = volume of the calibration stock solution (C_{DM+CFP}) transferred to prepare the working calibration solutions (C_1 to C_5), in mL (= 0.8, 2, 4, 6 and 8 mL, respectively)

$V_{stock\ DM+CFP}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+CFP}), in mL (= 100 mL)

$V_{working\ cal\ DM+CFP}$ = total volume of the calibration working solution (C_1 to C_5), in mL (= 25 mL)

The amount of deltamethrin and chlorfenapyr in the samples is expressed in g of deltamethrin and in g of chlorfenapyr per kg of sample; taking into account of dilution factor and sample weight.

Content of deltamethrin or chlorfenapyr in the samples :

$$= \frac{C_{DM\ or\ CFP} \times D}{W}\ g/kg$$

Where :

$C_{DM\ or\ CFP}$ = concentration of deltamethrin or chlorfenapyr in the sample solution, in µg/mL, found using the equation of the calibration curve

D = dilution factor of the sample solution (= 25 for LN, = 625 for TC)

W = weight of the sample, in g.

deltamethrin:

Repeatability r = g/kg at g/kg active ingredient content (ITN/LN)

Reproducibility R = g/kg at g/kg active ingredient content (ITN/LN)

chlorfenapyr:

Repeatability r = g/kg g/kg active ingredient content (ITN/LN)

Reproducibility R = g/kg at g/kg active ingredient content (ITN/LN)

Note 1: Do not keep the heptane extract more than 24h in disposable conical tube before filing the injection vials, as the polymer becomes porous in contact with heptane. Heptane could evaporate. If you do not put an aliquot of the sample solutions into a vial within 24h after adding heptane, prepare the sample solutions in glassware with glass stopper.