Analytical method:

LONG-LASTING INSECTICIDAL NETS CONTAINING ALPHA-CYPERMETHRIN AND / OR CHLORFENAPYR

SCOPE

This method is intended for determining alpha-cypermethrin and/or chlorfenapyr content in technical materials (TC) and on long-lasting insecticidal nets (LNs).

OUTLINE OF METHOD

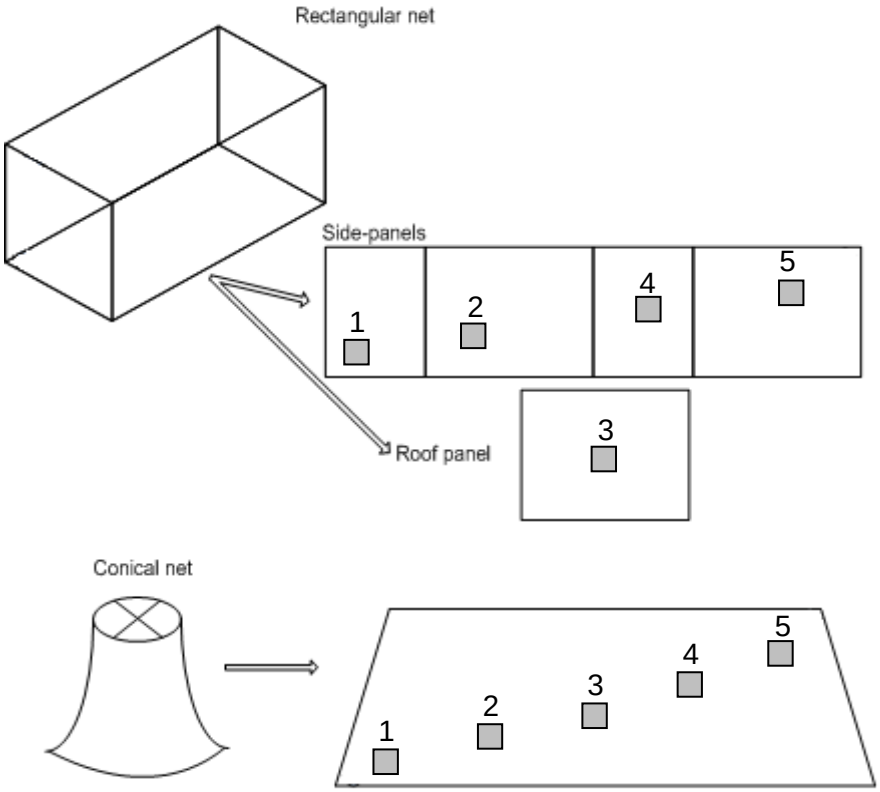
The sample is extracted with heptane using dicyclohexyl phthalate as internal standard. Alpha-cypermethrin and/or chlorfenapyr contents are determined by capillary gas chromatography using flame ionization detection (GC-FID).

1 Sampling:

This sampling procedure is suitable for net samples taken from either new or used LNs.

Samples of at least 25 x 25 cm from LNs are taken following the sampling method described in the specification template for long-lasting insecticidal nets or netting (LN) of the Manual on Development and Use of FAO and WHO Specifications for Pesticides, third revision of the first edition, Rome, 2016.

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



In total 5 samples are taken.

The samples are cut in small pieces (max. 5 x 5 mm) and shall be carefully mixed. The samples 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 sample allows getting information about the spatial distribution of the active ingredient(s) besides the mean of content of active ingredient(s) in net.

A sufficient amount of sample could be taken further for the determination of the active ingredients content.

2 Identity test

2.1 Chlorfenapyr **GC.** As for *alpha-cypermethrin technical 454/TC/(M)/2.1, but replace alpha-cypermethrin by chlorfenapyr.*

2.2 Alpha-Cypermethrin **GC.** As for *alpha-cypermethrin technical 454/TC/(M)/2.1.*

3 Chlorfenapyr and Alpha-cypermethrin

REAGENTS

Chlorfenapyr (CFP), standard of known purity
Alpha-cypermethrin (ACY), standard of known purity
Dicyclohexyl phthalate, internal standard (ISTD) of known purity
n-Heptane, analytical reagent grade
Acetone, analytical reagent grade
Citric acid, analytical reagent grade
Eventually *Tetrahydrofuran (THF),* analytical reagent grade, if heptane or acetone are not available.

Remark : expected concentrations of CFP and ACY in the sample solutions are widely lower than the solubility limit in heptane. So, this solvent is convenient for this application.

Remark : THF is <u>more toxic</u> for operators than heptane. However, when heptane is not available, THF can be used as an alternative solvent to prepare internal standard, calibration and sample solutions.

<u>Internal standard solution</u>. Weigh, accurately to the nearest 0.1 mg, about 400 to 500 mg of dicyclohexyl phthalate into a 250 ml volumetric flask. Add heptane and place the flask in an ultrasonic bath for 2 min or mix until complete dissolution. Allow the solution to cool to room temperature and fill to the mark with heptane (solution C_{ISTD}). Ensure sufficient quantity of this solution is prepared for all the samples and calibration solutions to be analyzed.

<u>Chlorfenapyr calibration stock solutions</u>. Weigh in duplicate, accurately to the nearest 0.1 mg, about 20 to 25 mg of chlorfenapyr (*s* mg) into two separate 25 ml volumetric flasks, add heptane and place the flasks in an ultrasonic bath for 2 min or mix until complete dissolution. Allow the solution to cool to room temperature and fill to the mark with heptane (Solutions C_{CFP} and C^*_{CFP}).

<u>Alpha-Cypermethrin calibration stock solutions</u>. Weigh in duplicate, accurately to the nearest 0.1 mg, about 20 to 25 mg of alpha-cypermethrin (*s* mg) into two separate 25 ml volumetric flasks, add heptane and place the flasks in an ultrasonic bath for 2 min or mix until complete

dissolution. Allow the solution to cool to room temperature and fill to the mark with heptane (Solutions C_{ACY} and C^*_{ACY}).

Citric acid solution. Weigh accurately 10.0 g citric acid into a 100 ml glass bottle (e.g. "Schott®" type), add 100 ml acetone and place the flask in an ultrasonic bath for 2 min or mix until complete dissolution (10% citric acid solution).

Remark : Citric acid is widely soluble in acetone. As this solvent do not induce epimerization of alpha-cypermethrin during analysis, it is convenient for this application.

Remark : When acetone is not available, THF can be used to solubilize citric acid although it is more toxic for operators than acetone.

Chlorfenapyr and alpha-cypermethrin calibration working solutions (*for determination of relative response factors of chlorfenapyr and alpha-cypermethrin against internal standard*) Transfer precisely 2 ml of C_{CFP}, 1 ml of C_{ACY} and 1 ml of the internal standard solution (C_{ISTD}) into a laboratory glassware or disposable flask (e.g. 50 ml conical flask). Add about 21 ml of heptane. Mix well the diluted calibration solutions (= **calibration solution C_A**).

Into another laboratory glassware or disposable flask, transfer precisely 4 ml of C^*_{CFP}, 3 ml of C^*_{ACY} and 1 ml of the internal standard solution (C_{ISTD}). Add about 17 ml heptane. Mix well the diluted calibration solutions (= **calibration solution C_B**).

Transfer an aliquot of C_A and of C_B into GC vials. Add about 50 µl of the citric acid solution, to avoid epimerization of alpha-cypermethrin during analysis. Seal and shake the vials.

C_A concentration: +/- 64-80 µg/ml of chlorfenapyr ;
+/- 32-40 µg/ml of alpha-cypermethrin ;
+/- 64-80 µg/ml of internal standard.

C_B concentration: +/- 128-160 µg/ml of chlorfenapyr ;
+/- 96-120 µg/ml of alpha-cypermethrin ;
+/- 64-80 µg/ml of internal standard.

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

APPARATUS

Gas chromatograph capable to operate with temperature rate, equipped with flame ionization detector (FID), split / splitless injection and automatic sampler.

Remark : to avoid epimerization of alpha-cypermethrin, use a clean (new) de-activated inlet.

Remark : pulsed-split injection mode can be useful to reduce epimerization of alpha-cypermethrin when injection inlet becomes slightly dirty.

Capillary column fused silica, coated with dimethylpolysiloxane (e.g. DB-1)

- 30 m x 0.32 mm i.d., 0.25 µm film thickness, or

- 30 m x 0.25 mm i.d., 0.25 µm film thickness, or
- 20 m x 0.18 mm i.d., 0.18 µm film thickness, for faster chromatographic analysis, better resolution and lower carrier gas flow.

Ultrasonic bath

Usual laboratory equipment, e.g. analytical balance, standard laboratory glassware and eventually automatic solvent dispenser.

2 ml GC vials for auto sampler.

PROCEDURE

(a) <u>*Preparation of sample (TC)*</u> Weigh in duplicate, accurately to the nearest 0.1 mg, a sufficient amount of technical material containing about 45 to 55 mg of active substance and transfer into separate 50 or 100 ml glass bottles (e.g. "Schott®" type) or disposable flasks (e.g. 50 ml conical tubes), add precisely 25 ml of the internal standard solution C_{ISTD}. Place the flasks in an ultrasonic bath for 2 min or mix until complete dissolution. Allow the flasks to cool to room temperature and transfer about 1 ml of the extracts into separate glass or disposable laboratory flasks (e.g. 50 ml test tubes) and add about 24 ml heptane*. Mix well the diluted solutions and transfer portions of each solution into separate GC injection vials. Add about 50 µl of the 10% citric acid solution, to avoid epimerization of alpha-cypermethrin during analysis. Seal and shake the vials (= **TC sample solutions S_A and S_B**).

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

<u>*Preparation of sample (LN)*</u> Weigh in duplicate, accurately to the nearest 0.1 mg, a sufficient amount of sample containing about 1.2 to 3.4 mg alpha-cypermethrin and about 2.4 to 3.2 mg chlorfenapyr (corresponding to about 500 mg of sample of a treated net such as Interceptor® and Interceptor® G2) into separate 50 or 100 ml glass bottles (e.g. "Schott®" type) or disposable flasks (e.g. 50 ml conical tubes). Add precisely 1 ml of the internal standard solution C_{ISTD} and about 24 ml of heptane. Place the flasks in an ultrasonic bath for 30 minutes. Allow the flasks to cool to room temperature and filter an aliquot of each solution through a 0.45 µm pore size filter into a GC injection vial*. Add about 50 µl of the citric acid solution to avoid epimerization of alpha-cypermethrin during analysis. Seal and shake the vials (= **LN sample solutions S_A and S_B**).

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

> ***Remark : after addition of citric acid solution in the injection vial, heptanic extract becomes slightly cloudy. Nevertheless, it can be directly analyzed. Some hours later, turbidity decreases and citric acid crystals can appear (excess of acid) on vial walls without any influence on further analysis.***
>
> ***Remark : do not keep heptanic 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.***

(b) Operating conditions (typical)

Column :	DB-1 (100% dimethylpolysiloxane), or equivalent		
	30 m x 0.32 mm i.d., 0.25 µm film thickness	*30 m x 0.25 mm i.d., 0.25 µm film thickness*	*20 m x 0.18 mm i.d., 0.18 µm film thickness*
Injection system			
Injector	Split injection	Split injection	Split injection
Injector temperature	*260°C*	*260°C*	*260°C*
Split ratio	*10:1*	*10:1*	*10:1*
Injection volume	*1 µl*	*1 µl*	*0.5 µl*
Detector system			
Type	Flame ionization (FID)	Flame ionization (FID)	Flame ionization (FID)
Detector temperature	300°C	300°C	300°C
Oven			
Oven temperature rate:	*180°C for 0.5 minute 20°C/min to 280°C 280°C for 12 min.*	*180°C for 0.5 minute 20°C/min to 280°C 280°C for 5 min.*	*180°C for 0.5 minute 20°C/min to 280°C 280°C for 2.5 min.*
Gas			
Carrier:	*1.5 ml/min helium*	*1 ml/min helium*	*0.7 ml/min helium*
Make up:	*30 ml/min helium*	*30 ml/min helium*	*30 ml/min helium*
FID:	*30 ml/min hydrogen 400 ml/min air (clean)*	*30 ml/min hydrogen 400 ml/min air (clean)*	*30 ml/min hydrogen 400 ml/min air (clean)*
Retention times			
Chlorfenapyr	*ca. 7 min*	*ca. 5.9 min*	*ca. 4.9min*
Dicyclohexylphthalate	*ca. 9 min*	*ca. 7.2 min*	*ca. 5.9 min*
Alpha-Cypermethrin	*ca. 13 min (cis 1) ca. 13 min (cis 2)*	*ca. 9.8 min (cis 1) ca. 9.9 min (cis 2)*	*ca. 7.0 min(cis 1) ca. 7.1 min (cis 2)*

(c) System equilibration Inject 1 µl or 0.5 µl (see above table for the injection volume) of a calibration working solution (C_A or C_B) until the response factor (f_i) obtained for two consecutive injections differs by less than 1.0%. Then inject 1 µl or 0.5 µl (see above table for the injection volume) of the other calibration working solution (C_B or C_A). For each active ingredient, the two response factors, obtained using C_A and using C_B, should not deviate by more than 1.0 %. Otherwise, prepare new calibration solutions. If the peak retention times differ significantly from the values given, then adjust the flow rate accordingly.

(d) Determination Inject blank solutions and calibration working solutions (C_A and C_B) first. The following sequence is advised: solvent, blank ISTD, C_A in duplicate and C_B in duplicate. Then, inject the sample extracts in duplicate. Each 2 to 4 sample extracts are bracketed with a calibration solution (C_A or C_B, alternatively), as follows: calibration solution C_A, sample solution $S1_A$, sample solution $S1_B$, sample solution $S2_A$, sample solution $S2_B$, calibration solution C_B, sample solution $S3_A$, sample solution $S3_B$, sample solution $S4_A$, sample solution $S4_B$, calibration solution C_A and so on for further samples. Measure the relevant peak areas.

Remark : During an analytical sequence the ACY relative response factor (f_{ACY}) calculated with a calibration solution (C_A or C_B) can decrease, indicating the fouling of injector inlet. This change is not

only due to epimerization but also to degradation of alpha-cypermethrin in the injector inlet. When f ACY deviates by more than 5% from the initial value, it is advised to change the inlet injector.

(e) <u>*Calculation*</u> calculate the mean value of each pair of response factors bracketing the injections of 2 to 4 samples extracts (*f*) and use this value for calculating the active ingredient content of the bracketed sample injections.

$$f_{i\ ACY\ or\ CFP} = \frac{I_r \times s_{\ ACY\ or\ CFP} \times P_{ACY\ or\ CFP} \times V_{ACY\ or\ CFP}}{H_{s\ ACY\ or\ CFP} \times V_{stock\ ACY\ or\ CFP}}$$

Where :

$f_{i\ ACY\ or\ CFP}$ = individual response factor, for alpha-cypermethrin or chlorfenapyr

$H_{s\ ACY\ or\ CFP}$ = peak area of alpha-cypermethrin or chlorfenapyr in the calibration solution (C_A or C_B)

I_r = peak area of internal standard in the calibration solution (C_A or C_B)

$s_{\ ACY\ or\ CFP}$ = mass of alpha-cypermethrin or chlorfenapyr reference standard in the calibration stock solution $C_{\ ACY\ or\ CFP}$ or $C^*_{\ ACY\ or\ CFP}$, in mg

$P_{ACY\ or\ CFP}$ = purity of alpha-cypermethrin or chlorfenapyr reference standard used to prepare the calibration stock solution ($C_{\ ACY\ or\ CFP}$ or $C^*_{\ ACY\ or\ CFP}$), in g/kg

$V_{stock\ ACY\ or\ CFP}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{\ ACY\ or\ CFP}$ or $C^*_{\ ACY\ or\ CFP}$), in ml (= 25 mL).

$V_{ACY\ or\ CFP}$ = volume of the calibration stock solution ($C_{\ ACY\ or\ CFP}$ or $C^*_{\ ACY\ or\ CFP}$) transferred to prepare the working calibration solution (C_A or C_B), in ml (for alpha-cypermethrin: to prepare C_A, using $C_{\ ACY}$ = 1 mL ;
to prepare C_B, using $C^*_{\ ACY}$ = 3 mL ;
for chlorfenapyr: to prepare C_A, using $C_{\ CFP}$ = 2 mL ;
to prepare C_B, using $C^*_{\ CFP}$ = 4 mL).

$$\text{Content of alpha} - \text{cypermethrin or chlorfenapyr} = \frac{f_{ACY\ or\ CFP} \times H_w \times V}{I_q \times w}\ g/kg$$

Where :

$f_{ACY\ or\ CFP}$ = mean response factor, for alpha-cypermethrin or chlorfenapyr

$H_{w\ ACY\ or\ CFP}$ = peak area of alpha-cypermethrin or chlorfenapyr in the sample solution

I_q = peak area of internal standard in the sample solution

V = *dilution factor of the sample solution (=1 for LN*
=25 for TC)

w = mass of the sample taken (mg).

Figure 1: Typical chromatographic profile of Calibration solution (e.g. C_A) (with a 20 m x 0.18 mm i.d. column)

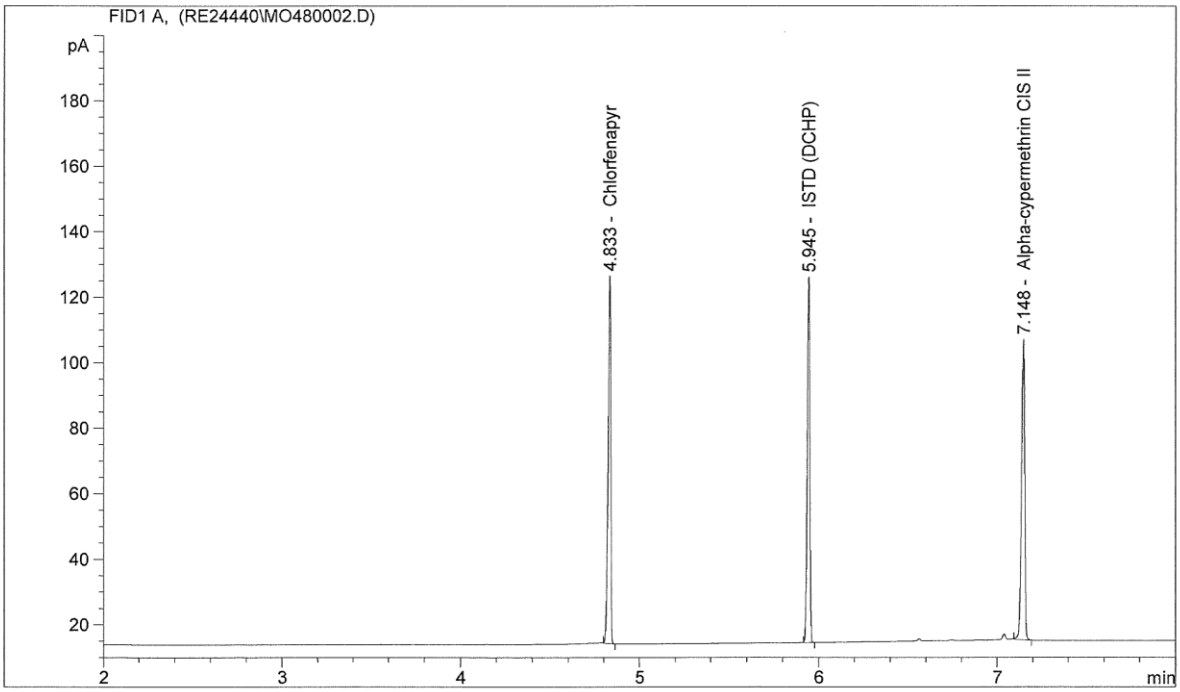


Figure 2: Typical chromatographic profile of Calibration solution (e.g. C_A) with epimerization (with a 20 m x 0.18 mm i.d. column)