KS 1739-2:2013

KENYA STANDARD

Mosquito nets

Part 2:Long lasting Insecticidal nets

KEBS 2013

First Edition 2013

TECHNICAL COMMITTEE REPRESENTATION

The following organizations were represented on the Technical Committee:
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Alpha Knits
Spinners and Spinners
KenKnit
Kenya Industrial Research and Development Institute
Sumitomo Chemical
Vector Solutions
Achelis
Vestergaard Frandsen
Kenya Textile and Training Institute
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In order to keep abreast of progress in industry, Kenya Standards shall be regularly reviewed. Suggestions for improvements to published standards, addressed to the Managing Director, Kenya Bureau of Standards, are welcome.

KENYA STANDARD

Mosquito nets

Part 2: Long lasting insecticidal nets-Specification (First Edition)

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Foreword

This Kenya Standard has been prepared by the Knitted Fabrics Technical committee under the guidance of the Projects Standards Committee and it is in accordance with the procedures of the Kenya Bureau of Standards

Malaria remains one of the most serious public health problem facing countries in the sub-saharan Africa as it accounts for 90 % of the 1.5 to 2.7 million deaths annually. Anopheles *gambiae* and anopheles *funestus* were found to be the principal vectors of malaria.

The long lasting insecticidal treated nets are as a result of new technology which aims to use pyrethroids incorporated in and/or coated on the fibre, to repeal or kill the malaria causing vector before getting in to contact with a sleeping person

In the preparation of this standard, reference was made to the following documents:

KS 1739-1 Mosquito nets

Part 1:Untreated mosquito nets-Specification

IS 9886-Mosquito nets-Specification

Acknowledgement is hereby made for the assistance received from these sources.

Mosquito nets, Part 2:Long lasting insecticidal nets — Specification (First Edition)

1 Scope

This draft Kenya Standard specifies the requirements for mosquito nets treated with insecticides

2 Normative References

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies

2.1 KS 1305-1:2000 Specification for mosquito netting Part 1 Round meshes
2.2 KS ISO 13938-1&2 Textiles- Bursting properties of fabrics.
Part 1:Hydraulic method for determination of bursting strength and bursting distension
Part 2: Pneumatic method for determination of bursting strength and distension
2.3 KS 479 -2 :1992 Sewing threads-Part 2: sewing threads made wholly or partly from synthetic fibres
2.4 KS ISO 3579 :2011 Textiles-Preparation, marking and measuring of fabric specimens and garments in tests for determination of dimensional change
2.5 KS ISO 6330:2012 Textiles-Domestic washing and drying procedures for textile testing
2.6 KS ISO 5077:2007 Textiles-Determination of dimensional change in washing and drying
2.7 KS ISO 3081 Textiles –Woven fabrics-Determination of mass per unit length and mass per unit area
2.8 KS 665:1998 Specification for textile labels
2.9 KS ISO 139 :2005 Textiles-Standard atmosphere for conditioning and testing
2.10 KS ISO 6941 Textile fabrics-Burning behaviour-Measurement of flame spread properties of vertically oriented specimens
2.11 16 CFR 1610 Standard for flammability of clothing textiles
2.12 KS ISO 3758:2012 Textiles-Care labeling code using symbols
2.13 KS ISO 2859 Sampling procedures for inspection by attributes. Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection
2.14 KS ISO 1833:2006 Textile s-Quantitative chemical analysis
Part 1 General principles of testing
Part 11 Mixtures of cellulose and Polyester fibres (method using sulphuric acid)
Part 16 Mixtures of Polypropylene fibres and certain other fibres (method using xylene)
Part 23 Mixtures of Polyethylene and Polypropylene (method using cyclohexanone)
2.15 KS ISO/TR 11827 Textiles-Composition testing-Identification of fibres
2.16 KS ISO 2076:2012 Textiles-Man-made fibres-generic names
2.17 KS 127;1979 Quantitative chemical analysis of binary fibre mixtures
2.18 KS ISO 8388 Knitted fabrics-Types-Vocabularly

3 Definitions

For the purpose of this standard the definitions given in KS 1305-1 shall apply along with the following:

3.1 long lasting insecticidal net

a net treated with insecticide manufactured to withstand at least 20 laboratory washes

3.2 active ingredients

World Health Organization recommended insecticides for use in mosquito nets

3.3 top ring

used in conical nets and made of suitable material ,fixed to the roof of the net (see Figure 2)

3.4 **height**

the dimension measured along a vertical seam from the top to the bottom edge of the net

3.5 **circumference**

the perimeter of the net at its bottom edge.

3.6 **length and width**

the dimension of a rectangular net measured from corner seam to corner seam

4 Requirements

4.1 Physical requirements

4.1.1 **Mesh size**

The nets shall have a minimum of 8 holes/cm².. The number of holes shall be tested in accordance with KS 1305 Part 1 Annex A

4.1.2 **Material**

The material shall be made from any of the polyolefin or polyester (See KS ISO 2706). The fibre composition of the netting fabric shall be tested in accordance with KS 127,KS ISO 1833 and KS ISO/TR 11827

4.1.3 **Construction**

The net shall be in any one of the warp knitted constructions for example raschel and tricot as specified in KS ISO 8388

4.1.4 **Bursting strength**

The bursting strength of the netting shall be a minimum of 220 Kpa when tested in accordance with KS ISO 13938-1&2 at test area of 7.3 cm².

4.1.5 **Dimensions**

4.1.5.1 The rectangular nets shall comply with the requirements given in Table 1.

Table 1 — Requirements for rectangular mosquito nets

Type	Width, cm (min)	Length, cm (min)	Height, cm (min)
Baby cot	?	?	?
Single	80	180	150
Double	120	180	150
Large size	160	180	150
Extra large size	180 cm	180 cm	150 cm
Test Method	Annex D		

4.1.5.2 The dimensions of circular nets shall comply with the requirements given in Table 2.

Table 2 — Requirements for circular mosquito nets

Size	Top ring diameter, min	Height, min	Circumference, min
Baby cot	?	?	?
Single	47 cm	180 cm	700 cm
Double	47 cm	180 cm	800 cm
Large	56 cm	180 cm	900 cm
Test Method	Annex D		

4.2 Active ingredients

The active ingredients of nets shall comply with requirements of table 3 and be when tested in accordance with annexes A, B and C

Table 3:Active ingredients of nets

SL NO	Ingredient	Dosage	
		g/Kg	Mg/m^2
1	Deltamethrin	1.35-2.25	55-68
2	Permethrin	17-23	510-1150
3	Alpha -cypermethrin	3.75-8.4	150-250

4.3 Seams and stitching

When visually examined the seams shall be of even tension and the loose ends shall be securely and neatly fastened off. Seams shall be free of twists, puckers, pleats and loose threads.
The net seams shall be made with lock stitch. The number of stitches per decimeter shall be 22 to 35 and shall be made from sewing thread complying with KS 479 Part 2.

4.4 Visual examination

When visually examined under an artificial light, the netting shall exhibit no noticeable defect arising out of knitting or making-up.

4.5 Flammability

The burning behaviour of nets shall be tested in accordance with KS ISO 6941 or 16 CFR Part 1610 be nonflammable or be expressed in terms of flame spread time (seconds)

4.6 Mass per metre square of net fabric

The mass per metre square of net fabrics shall be a minimum of 30 grams when tested in accordance with KS ISO 3801

4.7 Dimensional change of net fabric

The maximum dimensional change of net fabric shall 5 percent when tested in accordance with KS ISO 3579 ,6330 & KS ISO 5077

4.8 Retention index/Release index

The retention index shall be determined by the method prescribed in Annex D

5 Marking

The following information shall be indelibly and legibly marked on a label (complying with KS 665) and securely stitched at the top corner of the net.

i) manufacturer's name and or registered trade mark;

ii) size of the net, including top ring diameter, overall length (cm) x overall width (cm) x overall height (cm) or circumference;

iii) shape of net;

iv) care instructions in accordance with KS ISO 3758

v) recommended bed size;

vi) Date of manufacture;

vii) Country of origin (manufacture); and

viii) Active ingredient and dosage

6 Sampling

Sampling shall be done in accordance with KS ISO 2859

7 Packing

The mosquito nets shall be packed in visible, water-resistant packaging material to protect them from soiling while permitting visual examination.

Annex A

Determination of Deltamethrin content in Insecticide treated Mosquito nets by High performance Liquid Chromatography

A1 Sampling

A sample is defined as one finished bed net taken randomly from a batch of bed net. Sub sample by cutting 18 pieces of size 100cm^2 from a net either randomly from the whole net or at recommended position as in appendix 1. Cut 18 pieces into half, one portion is used for Deltamethrin content analysis and the other is used for washing test if required.

The portion used for Deltamethrin content analysis is cut into pieces of less than 2cm x 2cm each. Mix well. Sample shall be separately packed in aluminium foil and kept out of direct sunlight at room temperature or lower.

A2 Identification tests.

Use the HPLC method below. The retention time of Deltamethrin in the sample solution should not deviate by more than 15 s from that of the calibration solution if column oven is available.

A3 Active ingredient

A3.1 Outline of Method

The sample is extracted in a mixture of iso-octane and 1,4-dioxane.

The Deltamethrin content is determined by normal phase high performance liquid chromatography using dipropyl phthalate as internal standard and detection at 236 nm.

A3.2 **Scope**

This method is used for Deltamethrin determination in net sample, before and after washing.

A3.3 **Reagents**

A.3.3.1 Iso Octane, HPLC grade

A. 3.3.2 1,4 Dioxan, HPLC grade. Add 0.15% (v/v) water before use

A. 3.3.3 Deltamethrin, neat standard,

A. 3.3.4 Dipropyl Phthalate,

A.3.3.5 Water ,HPLC grade or high

A. 3.3.6 Extraction solvent (ES): iso octane + 1,4 dioxane=80+20

A. 3.4 Mobile Phase (MP):iso octan + 1,4 dioxane = 95 + 5

A. 3.5 Internal Standard solution (IS)0.5 mg/ml of dipropyl phthalate in extraction solvent.

A 3.6 Deltamethrin standard solution 0.6 mg/ml(DS)

Weight 0.03 g(to the nearest of 0.01` mg) of Deltamethrin neat standard, quantitatively transfer to 50 volumetric flask, dissolve completely with extraction solvent , keep regulated by water bath at 20^{o}C, fill up to mark with extraction solvent.

A.3.4 Apparatus

A.3.4.1 Shaker

A.3.4.2 Ultrasonic bath

A.3.4.3 HPLC, equipment with pump, auto-injector , column oven (optional) and UV detector, Guard Column (Supelguard Si). Analytical Column Supelco Si 5 µm, 150 x 4.6 or Lichrosorb Si60, 5 µm, 150 x 4.6

A.4 Procedure

A.4.1 Operating conditions

A.4.1.1 Mobile Phase: iso octan + 1,4 dioxane =95 + 5

A.4.1.2 Flow rate 1.3ml/min, isocratic

A.4.1.3 Guard Column Superguard Si

A.4.1.4 Analytical Column Licherosorb Si60, 5µm, 150 x 4.6
40°C if column oven is available, or room temperature

A.4.1.5 Inject Volume 5µl

A.4.1.6 Wavelength 236 nm

A.4.1.7 Run time 6-8 minute
Temperature program for oven to be inserted

A.4.2 Preparation of calibration curve

A.4.2.1 Into a series of clean 20ml PTFE liner screw cap vials, add accordingly as table below. Filter through 0.45µm syringe filter before use.

Code	IS	DS	ES	Delta(mg)	Total Volume
C1	1ml	0.5ml	13.5 ml	0.30	15 ml
C2	1ml	0.7ml	13.2 ml	0.42	15 ml
C3	1ml	0.9ml	12.8 ml	0.54	15 ml
C4	1ml	1.1ml	12.4 ml	0.66	15 ml
C5	1ml	1.3ml	12.1 ml	0.78	15 ml

A.4.2.2 All standards should be kept in the refrigerator if not in use in WELL-TIGHTEN, paraffin film sealed cap.

A.4.2.3 **Calibration**

A.4.2.3.2Daily calibrate HPLC system with full series of 05 standards, beginning with the lowest level standard. The correlation coefficient should be less than 0.99 over this range.

A.4.3 **Preparation of sample**

A.4.3.1 Weigh (to the nearest 0.1 mg) into an Erlenmeyer or a screw cap neutral glass bottle (50ml) sufficient sample to contain about 0.5 mg of Deltamethrin. For 75D, 100D and 150D netting sample, suitable weight (w g) is 0.3 g, 0.4 g and 0.6 g respectively. Add by pipette 1.0 ml internal standard solution. Add 14 ml extract solvent. Replace the cap closely.

Put the bottles into the ultrasonic bath, setting temperature 80°C, running time 15 min. Vigorously shake the bottle using the shaker in 30 min at room temperature, speed of shaking is at level of 150-200 beats per minute.

Using a syringe membrane filter with pore size of 0.45 µm or finer, filter c.a 1ml of extract solution into clean amber. Sample shall be injected within 24 hours since extraction, for longer waiting time the vial should be kept in a refrigerator.

A.4.4 **Determination**

A.4.4.1 Inject sample with HPLC configured as in (a)

Deltamethrin content(g/kg)= $\frac{a}{w}$

a=Deltamethrin reading from the analysis in mg

w=mass of sample taken (g)

Note: Conventional unit of active ingredient content (deltamethrin) for netting is mg/m^2. The conversion from g/kg to mg/m^2 is made as follows:

Deltamethrin content (mg/m^2) = Deltamethrin content (g/kg) x D

D=weight per square meter
D of typical polyester netting
75 Denier netting, D=30g/m2
100 Denier netting, D=40g/m^2
150 Denier netting, D=60g/m^2

A.5 **Method Validation Summary**

A.5.1 Precision

Analyze 05 replicates RSD is 1.8%

A.5.2 Accuracy

Recovery of 04 Laboratory Synthetic samples is in the range of 97% to 101%
Averaged at 99.3%

A.5.3 Linearity

Over the range of Deltamethrin from 0.30 t0 0.78 mg, the correlation coefficient R^2 is observed not less than 0.995.

A.6 Determination of release /Retention Index

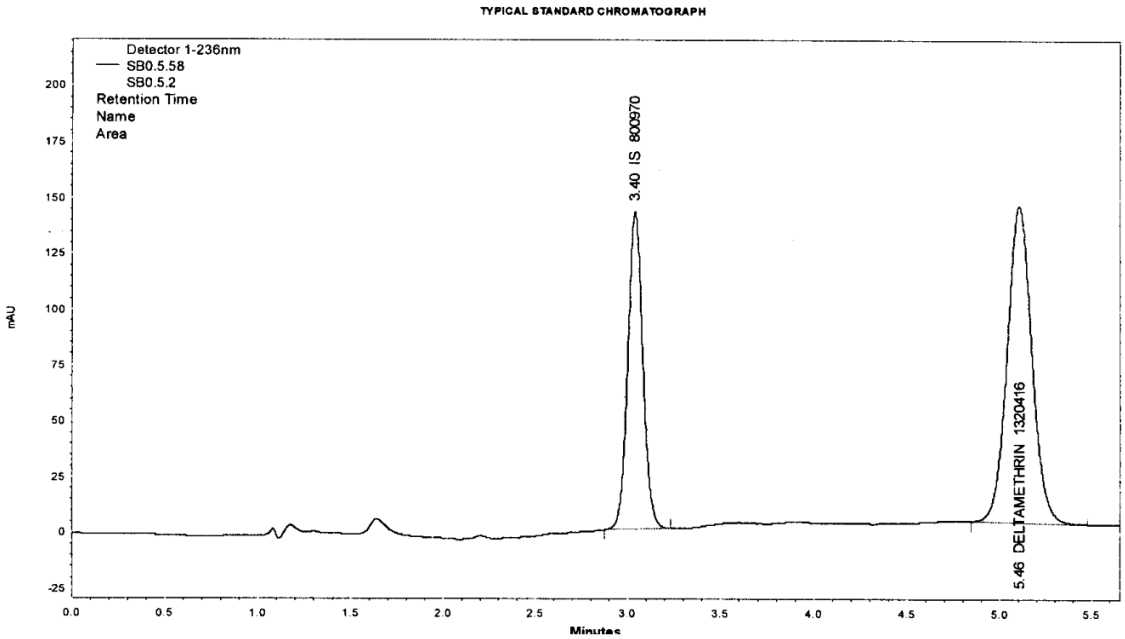

Figure 1:Chromatogram of calibration solution

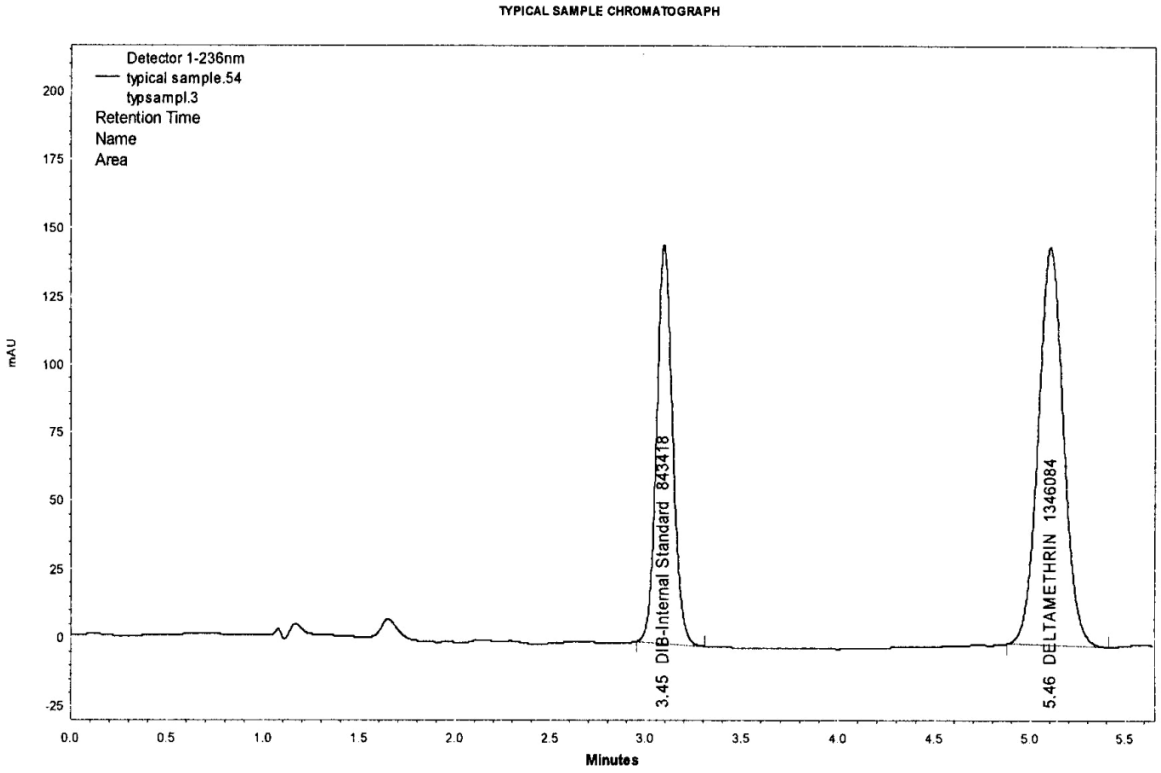

Figure 2: Chromatogram of sample

Annex B

B.1 Determination of Permethrin content in Insecticide treated mosquito nets

B.1.1 Sampling : Take at least 100g

B.1.2 Identity tests

B.1.3 GLC. Equipped with a split/splittless and a flame ionization detector. Capillary column fused silica, 30mx0.25 (i.d) mm, film thickness:0.25 µm, coated with cross linked dimethyl (DB-1 or equivalent) and electric integrator or data system

B.1.4 Infrared. Extract the sample with suitable solvent. Filter and evaporate the solvent. Proceed as for 331/TC/m/2.2

B.1.5 Permethrin . The content of permethrin in test samples are determined by capillary GC using flame ionization detection and triphenyl phosphate as internal standard, and trans-isomer ratio is calculated from the chromatogram obtained

B.1.6 Reagents

B.1.6.1 Heptane analytical grade

B.1.7 **Internal standard solution**. Dissolve triphenyl phosphate (1.0g) in heptane (150 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.

B.1.8 **Calibration solution**. Homogenise the permethrin working standard. When the permethrin working standard is waxy solid or partly waxy solid homogenize it by warming it to melting and stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 72 to 88 mg(s mg) of permethrin working standards into a vial or stoppered flask (200 ml). Add by pipette internal standard solution (10 ml) and dissolve completely. Add by measuring cylinder heptanes (90 ml) and mix well (Solutions C_A and C_B)

B.1.8 **Procedure**

B.1.8..1 **Preparation of sample solution**.

Clean scissors with acetone before use. Cut the sample with the scissors into 5-10mm square. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1mg) sufficient sample to contain 36 to 44mg (w mg) of permethrin into a vial or stoppered flask (100ml). 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(85-90ºC) 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)

B.1.9 **Calculation of permethrin content.**

B.9.1Calculate 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.

$$fi = \frac{lrxsxP}{Hsx2}$$

Content of permethrin $= \frac{fxHw}{lqxw} g / kg$

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)

B.1.10 **Precision**

B.1.10.1 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

B.1.10.2 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

B.2Determination of Surface concentration and release index

B.2.1 Reagents

Internal standard solution for calibration solution.
Dissolve triphenyl phosphate (0.1 g) in acetone (200ml) to prepare a stock solution. Transfer by pipette the stock solution (5 ml) to a volumetric flask (50ml). Make up to volume with acetone and mix well. Ensure that a sufficient quantity of this solution is prepared for all calibration standard to be analysed.
Calibration solution. Homogenise the permethrin working standard. When the permethrin working standard is always solid or partly 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 (Solution C_A and C_B).

B.2.2 Apparatus as for 331/TC/m/3 except:
Constant temperature oven capable of controlling temperature with the range of ±2^{0}C is recommended.
Rotary evaporator

B.2.3 Procedure as for 331/TC/m/3 except:
B.2.3.1 Gas chromatographic conditions (typical):
B.2.3.2 Injection system
B.2.3.3 Slit flow approximately 10ml/min

B.2.4 Linearity check.

Check the linearity of the detector response by injecting 1 µl of solution s with permethrin concentrations 0.1, 1 and 2.5 times that of the calibration solution before conducting analysis.

B.2.5 **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.

B.2.6 **Preparation of sample solution**

Clean scissors and tweezers with acetone before use. Prepare sample solutions in tripricate 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 ht netting with the tweezers and discard the solution.
Let the netting dry at room temperature for ca. 10 minutes. Transfer it into a vial (20ml) with the tweezers. Cap the vial and place it in a temperature -controlled oven set at 70Oc. 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 (10ml) into the vial. Cap the vial tweezers. Let the netting dry at room temperature for ca. 10 minutes. Transfer the sample solution from the vial into the same round-bottom flask. Evaporate the solution in vacuo to dryness. Add by pipette acetone (2ml) into the flask to dissolve the residue (Solutions for “surface concentration at pot-wash 1”, $S_{A1,}S_{BI}$ and S_{C1}).
Transfer the dried netting into a vial (20ml) with the tweezers. Repeat heating, internal standard solution adding, evaporation 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 (20ml) with the tweezers. Repeat heating, internal standard solution adding, evaporation 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 streams of warm air.

B.2.6 Determination .

Inject in duplicate 1 µl portions of each sample solution bracketing them by injections if the calibration solutions as follows; calibration solution CA, sample solution SA1, sample solution SA1, calibration solution CB, sample solution SB1, sample solution SB1, calibration solution CA and so on measure the relevant peak areas.

B.2.7 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 concentration of the bracketed sample injections.

$$fi = \frac{lrxsxP}{Hs}$$

Surface concentration= $\frac{fxHw}{lqxwx2}$ µg/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 [ermethrin (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 permenthrin working standard (g/kg)

B.2.8 Calculation of release index.

Calculate the mean value of the two injections of sample solutions SA3, SB3, and SC3 by the equations described in (f) , and the release index for each piece of the netting.

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)

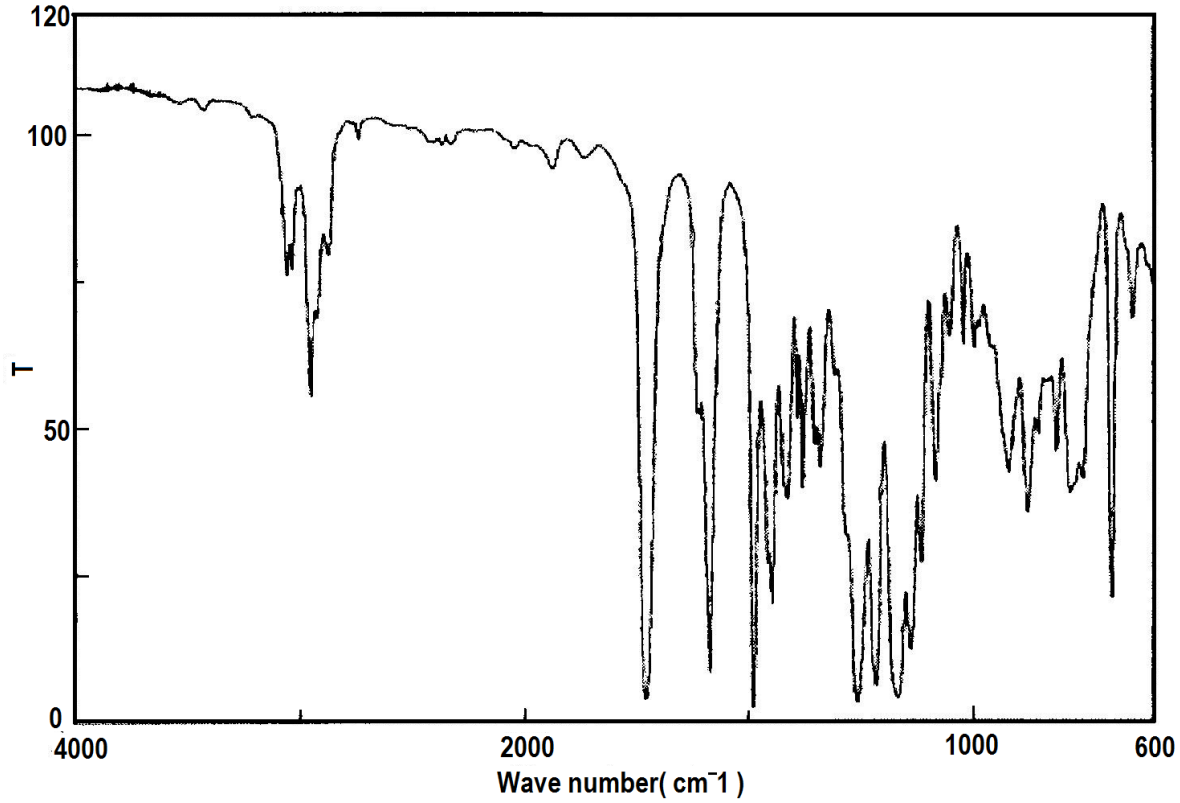

Fig. 1 Infrared Spectrum of permethrin

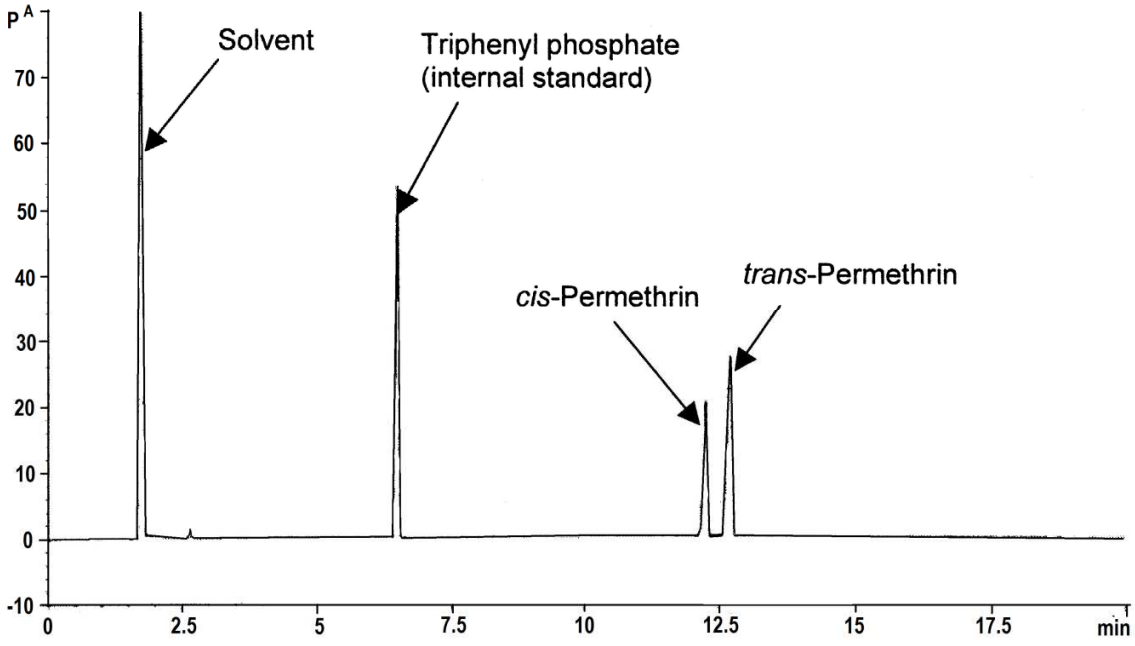

Fig 2 Example of gas chromatogram of permethrin TC

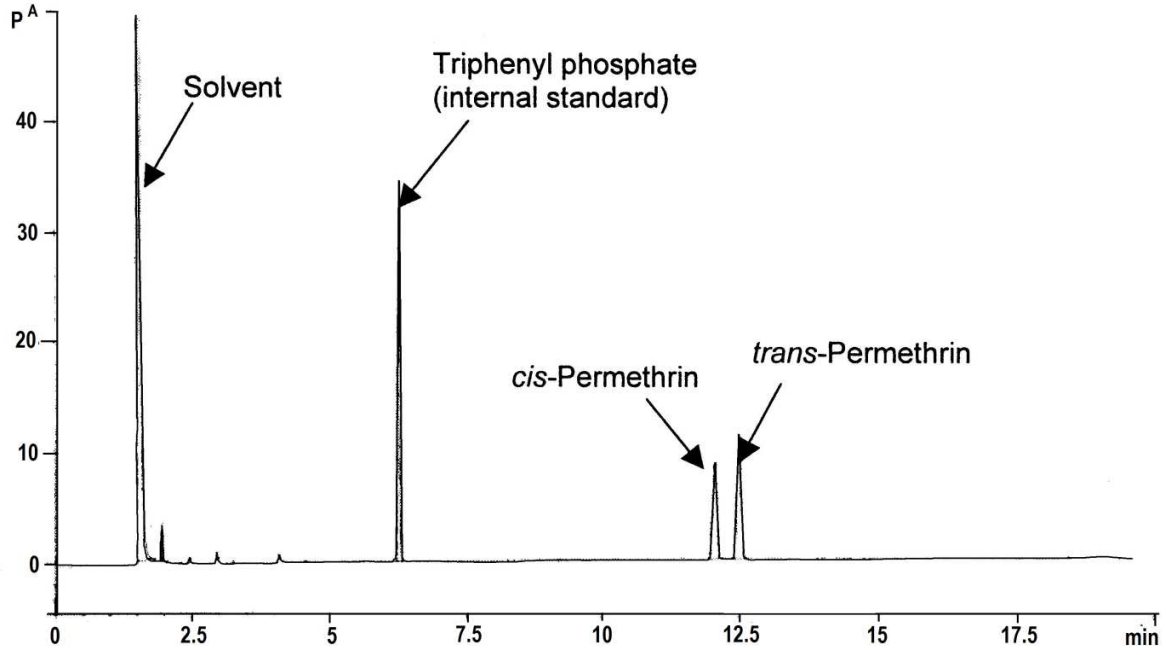

Fig 3 Example of gas chromatogram of permethrin LN

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Annex C

Determination of Alphacypermethrin content in Insecticide treated mosquito nets

C.1 Sampling

Take net specimens

C.2 Identity test

C.2.1 GLC capable of operating over a range 100 to 300ºC, fitted with a flame ionization detet ector

C.3 Alphacypermethrin

C.3.1 As for alphacypermethrin 454/TC/(M)3 (please provide the specifications)
C.3.2 Outline of method: As for alphacypermethrin is dissolved in tetrahydrofuran and determined by capillary gas chromatography in split injection mode using flame ionization and internal standardization
C.3.3 Reagents:
C.3.3.1 Tetrahydrofuran
C.3.3.2 Aphacypermethrin standard of known purity,Di(2-ethylhexyl)phthalate(dioctyl phthalate, DOP), internal standard,purity atleast 980 g/kg and giving no peaks with similar retention times to alphcypermethrin
C,3.3.3 Citric acid 5% solution. Dissolve citric acid(25g) in water (500ml)
C.3.3.4 Internal standard solution. Disolve dioctyl phthalate (5.0g) intetrahydrofuran (500ml). Ensure sufficient quantity of this solution is prepared for all samples and calibration solutions to be analysed

C.4.0 **Calibration Solutions**

4.1 Weigh 50 mg alpha cypermethrin (to the nearest 0.1 mg) in a volumetric flask (25ml). Fill to the mark with THF. Place the flask in an ultrasonic bath for 10 min. After temperature equilibrium pipette 1.50 ml, 4.50ml of this solution into three volumetric flaks (50 ml). Add 0.5 ml of internal standard solution (dioctyl phthalate, 1% in acetone) and fill up each to the mark with THF.

These solutions are used as calibration solutions A (C_A)' B (C_B) and C (C_C). Transfer 200 µl out of each flask into separate GC vials. Add one drop of citric acid in each case and seal the vials. Place the vials into the sample tray (cooled down to 15º C) of the GC apparatus.
Note: Citric acid is added to stop epimerization of alphacypermethrin in solution
4.1 Description of the calibration solutions:
C_A :concentration of approximately 3.0 mg alphacypermethrin in 50ml THF
C_B:concentration of approximately 6.0 mg alphacypermethrin in 50 ml THF
C_C:concentration of approximately 9.0 mg alphacypermethrin in 50 ml THF

C.5.0 **Apparatus**
C.5.1 Gas chromatograph with flame ionization detector (e.g. HP6890 Plus)
C.5.2 Automatic sample injector (e.g. HP series 7683) equipped with a sample which is cooled down to 15ºC
C.5.3 Capillary column fused silica (e.g. DB-1) 30mx 0.32 mm, film thickness of 0.25µm.
C.5.4 Electronic data evaluation system (e.g. HP ChemStation)
C.5.5 Ultrasonic bath
C.5.6 Electronic balance
C.5.7 Laboratory glassware

C.6.0 **Procedure**
C.6.1 Operating conditions. As per alphacypermethrin 454/TC/(M)3(please provide the specification)
C.6.2 Preparation of the samples
C.6.2.1 Determination via the water/detergent extraction process

C.6.2.1.1 **First step:** Quantification of the adherent contents of alphacypermethrin on surface fibers

a) Procedure done by manufacturer
Place 2 g impregnated net in a beaker (1l).Add soap (commercially available bar soap or as flakes, 2g/l) and water (500ml). The washing is done for 10 min. at 30º C on a shaker with 155 movements/min. Directly after the washing, the wash liquor is acidified with 10 ml/l acetic acid 30% in order to prevent hydrolysis of the extracted alphacypermethrin (note:there is no difference in hydrolysis in neutral water compared to wash liquor after 10 min. at 30 ºC). Transfer 150 ml of the washing liquid into a separation funnel (500 ml). The extraction procedure is done twice with 100ml ethyl acetate each by shaking twice for 30 seconds. Both portions of ethyl acetate are combined together in a flask (250 ml). Ethyl acetate is evaporated in a rotary evaporator. The flask is stoppered and sent to the analytical laboratory.

b) Procedure done by analytical laboratory
Fill up the flask with THF(50ml). Add 0.5 ml of internal standard solution (dioctyl phthalate, 1% in acetone). Transfer 200 µl of sample out of the flask into a separate GC vial. Add 1 drop of citric acid into the vial.

C.6.2.1.2 Second step: Quantification of the total contents of alphacypermethrin

a) Weigh (to the nearest 0.1 mg) 1 g of the impregnated net into a volumetric flask (100 ml) and add THF (50 ml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90º C (oil bath temperature). Sampling will be done after 5 min. refluxing. Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone). Transfer 200 µl of the sample into separate GC vial. Add 1 drop of citric acid into the vial.

C.6.2.2 **Determination via the n-hexane extraction process**

C.6.2.2.1 First step: Quantification of the adherent contents of alphacypermethrin on the surface of the fibers

a) Weigh (to the nearest adherent drops of n-hexane) out of the flask into another volumetric flask (100 ml) and add THF (50 ml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90º C. Sampling will be done after 5 min refluxing. Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone) . Transfer 200 µl of the sample into a separate GC vial. Add I drop of citric acid into the vial.

C.6.2.2.2 Second step:Quantification of the total contents of alpha-cypermethrin

Transfer the net(without adherent drops of n-hexane) out of the flask in to another volumetric flask (100ml) and add THF (5Oml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90ºC. Sampling will be done after v5 min refluxing. Add 0.5ml of internal standard solution (dioctyl phthalate, 1% in actone). Transfer 200µl of the same into a separate GC vial. Add 1 drop of citric acid into the vial

C.6.2.3 **System calibration**

As for alphacypermethrin 454/TC/(M3)

C.6.2.4 Determination

Each calibration solution C_i and each sample solution S_J is injected twice. The following sequence is advised:

C_A , $C_{A,,}$ C_B, C_B,C_C, $C_{C,}$ S_A, S_A,S_B, S_B,S_C,$S_{C,...}$

C.6.2.5 **Calculation**

As for alphacypermethrin 454/TC/(M)3 and see test of linearity

Concentration = $\frac{fxHw}{lqxw}$

Where: f=response factor

Hw=total peak area of alphacypermethrin (cis +ll) in the sample solution

Lq=peak area of internal standard in the sample solution

w=mass of sample taken

C.6.2.6 **Surface concentration and Release index**

a) Determination

The determination of alphacypermethrin content is done via the n-hexane extraction process from fibers

Each calibration solution C_j and each sample solution S_j ai injected twice. The following sequence is advised: $C_{A,}$ C_A,C_B,C_B,C_B,C_C,C_C,S_{A1},S_{A1},S_{A2},S_{A2},S_{A3},S_{A3},S_{B1},$S_{B1,...}$

S_A=refers to first rinse; S_A mean value of $S_{A1,}$ SA_2,S_{A3}

S_B=refers to second rinse; S_B mean value of $S_{B1,}$$S_{B2,}$$S_{B3}$

S_c=refers to third rinse;S_C mean value of $S_{C1,}$ $S_{C2,}$$S_{C3}$

Where $_{1,2,3}$ are net samples

b) Calculation of the release index

Calculate the mean value of sample solutions S_C and S_B by the equations described in 6.2.5 and the release index for each piece of netting.

Release index=$1-(S_{C/S}S_B)$, where

S_B =refers to second rinse; S_B mean $S_{B1,}$$S_{B2,}$$S_{B3}$

S_C=refers to third rinse; S_C mean value of $S_{C1,}$ $S_{C2,}$$S_{C3}$

Annex D

Measurement of net dimensions

D1 Measurement of net dimensions for rectangular nets

1.1 Length , Width and height

1.1.1 Apparatus
1.1.1.1 Flat table
1.1.1.2 Measuring tape or steel rule
1.1.2 Conditioning
Condition the net samples in accordance with KS ISO139
1.1.3 Procedure
Lay the conditioned net sample on a flat table(1.1.1.1), and take measurements of height, width and diameter
1.1.4 Calculation
If more than one net sample is tested, take the average measurement for each dimension
1.1.5 Report
Report the value of the net dimension as the average calculated in 1.1.4 in centimetres

D **2 Measurement of net dimensions for circular nets**

D2.1 Measurement of top circumference and bottom circumference
D.2.1 .1 Apparatus
D.2.1.1.1 Hook supported at a vertical distance at least more than the height of the net sample to be tested
D.2.1..1.2 Measuring tape or steel rule
D.2.1.1.3 Twine of measuring at least 10 metres
D.2.1.1.4 Felt pen marker

D.2..1.2 **Conditioning**
Condition the net sample in accordance with KS ISO 139

D2.1.**3 Procedure**

D.2.1.3.1 **Top circumference**

Place the top portion of the net sample on a flat table (D.1.1.1.1) and put the twine(see D.1.1.1.3) around the circumference of the top ring of the net sample, indentifying the two ends with a marker (see D 2.1.1.1.4) which represent the dimension of the top ring. Using a measuring tape(see D.2.1.1.2) determine the top ring circumference (S) of the net as the distance between the two points marked on the twines.
Repeat the test on each of the other net sample

D.2.1.4 **Calculation**
Take the average of the individual measurements as the top circumference of the conical nets

D.2.1.5 Report
Report the top ring circumference as the value(S) calculated in D.2.1.5 in centimetres

D.2.1.3.2 **Bottom circumference**

D.2.1.3.2.1 **Procedure**
Lay the bottom part of the net on a flat table , removing any curls and take the measurement(*N*) from one end of the flattened net to the other using a twine. Repeat the procedure for other net samples

D.2.1.3.2.2 **Calculation**
Take the average of the measurements taken in D.2.1.3.2.1(*N.*) Calculate the bottom circumference as *N*X2

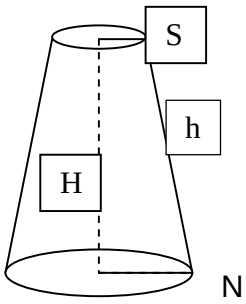
D.2.1.3.2.3 **Report**
Report the value of bottom circumference of the net as the value(Nx2) calculated in D.2.1.3.2.2 in centimetres

D.2.1.3.3 **Conical Net Height**

D.2.1.3.3.1 Hang the net with the loop from a hook. Take measurement of the height h with tape measure. Calculate the height H from the following expression:

$$H=[h^{2-}(NX2-S)^{2}]^{1/2}$$

Height of conical(H)



D.2.1.3.3.2 Report the value of conical height calculated in D.2.1.3.3.1 in centimetres