KENYA STANDARD

3004:2024

FIRST EDITION

Pyrethrum Refined Extracts- Specification

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BASF EA LTD
Ecolab Kenya.
Agrochemicals company.
Competition Authority Kenya.
Consumer Information Centre.
Osho Chemical Industries Ltd
University of Nairobi
Pest Control Product Board.
Pyrethrum Processing company of Kenya.
Agrochemical Association of Kenya.
Agriculture and food Authority.
SC JOHNSON AND SON LTD.
National Public Health Laboratory.
Government Chemists
Kenya Bureau of Standards — Secretariat

REVISION OF KENYA STANDARDS

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.

DKS 3004:2024

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Pyrethrum Refined Extracts- Specification

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Foreword

This Draft Kenya Standard is being developed by the Technical Committee on pesticides under guidance of the Standard projects committee, and it is in accordance with the procedures of the Kenya Bureau of Standards.

This Draft Kenya Standard is the first edition of Pyrethrum refined Extracts – Specification.

This Draft Kenya Standard has been prepared with assistance drawn from the following documents:

IS 1051:1980 (Reaffirmed 2017) Specification for Pyrethrum Extracts, published by India Bureau of Standards.

TBS/CDC 8 (441) DTZS Pyrethrum Extracts- Specification.

WHO/SIT/7. R3 (AUG 2009)

The assistance obtained from the above source is hereby acknowledged with thanks.

Pyrethrum Refined Extracts –Specification

1. Scope

This Draft Kenya Standard specifies the requirements, sampling and test methods of pyrethrum refined extracts containing varying percentages of total pyrethrins.

2. Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ASTM D 92, Standard test method for flash and fire points by Cleveland open cup

ASTM D 445, Standard test method for kinematic viscosity of transparent and opaque liquids

ASTM D4052, Standard test method for density and relative density of liquids by digital density meter

ISO13736 Determination of flash point – Abel closed – cup method.

CIPAC MT 3.2.1 - Specific gravity, density and weight per millilitre.

KS ISO 760:1978 Determination of water — Karl Fischer method (General method).

KS EAS 123:2022 Distilled water — Specification.

3.Terms and definitions

For the purposes of this standard the following terms and definitions shall apply.

3.1 Pyrethrum

aromatic plant of the daisy family, typically having feathery foliage whose brightly coloured aromatic flower heads contain pyrethrins as the active compounds.

3.2 pyrethrum extracts

product in the form of solution prepared by extracting pyrethrum flowers (Chrysanthemum cinerariifolium) or (Chrysanthemum cinarariaefolium)

3.3 Pyrethrin

six naturally occurring isomers found in the flower of Chrysanthemum cinerariifolium as esters of pyrethric acid (pyrethrin-II, cinerin-II, and jasmolin-II) and chrysanthemic acid (pyrethrin-I, cinerin-1 and jasmolin-I) that have insecticidal property.

4. REQUIREMENTS.

4.1 General requirements

4.1.1 The active ingredients in pyrethrum extract shall be composed of six esters, namely, pyrethrin I &II, cinerin I &II and jasmolin I &II. (See Annex B)

4.1.2 The material shall be an extract of pyrethrum flowers (Chrysanthemum cinerariifolium) or

(Chrysanthemum cinarariaefolium).

4.1.3 The material shall be homogeneous viscous liquid.

4.1.4 Sediment and/or suspended matter shall be negligible.

4.1.5 It shall be free from synthetic pyrethroids.

4.1.6 It shall be free from other synthetic pesticide residues.

4.1.7 The material shall be greenish yellow to brown in colour and posses the characteristic odour of the commercial pyrethrum flowers.

4.1.8 Shall not contain traces of the solvents used during extraction.

4.2 Specific requirements

4.2.1 Pyrethrum extracts shall conform to the specific requirements given in Table 1

SL. No	Characteristic	Requirement	Test method
i)	Flash Point. min	32°C	KS ISO 13736
ii)	Volatiles level, %. max	1.0	ASTM D5191
iii)	Specific gravity at 15 ºC, min.	0.91	ASTM D 4052/IP 365 CIPAC MT 3.2.1
iv)	Total pyrethrin content, % min	25	Annex C
v)	Viscosity, cPs,at 23 °C, min/	4000	ASTMD 445
vi)	Moisture content % max	1.0	KS ISO 760
vii)	PH, %	1.0	Annex D
viii)	Optical density	0.9	Annex E
ix	Matter insoluble in dichloromethane	-	Annex F

5. Packaging and Marking

5.1. Packaging

5.1.1 The material shall be packed in clean, dry and leak proof containers made of mild steel or tinplate. Other non-corrosive/non-reactive, opaque containers may also be used. They shall be sealed air-tight after filling.

5.2 Marking and labelling.

5.2.1 The container shall be legibly and indelibly marked in accordance with the Pest Control Products Act Cap 346 Laws of Kenya (Herein "the Act"). The information on each package and/or on every label and leaflet shall be printed in both English and Kiswahili. The information shall be indelibly and legibly marked on a label. The following is the information that must appear and how it should appear on the display panels.

A) Primary display panel
   i) - Trade name of the products
      - Its physical form
      - Purpose of use

ii) Statement “READ THE LABEL BEFORE USING” Must appear prominently in capital letters.
iii) Statement “KEEP LOCKED OUT OF REACH OF CHILDREN” to appear prominently in capital letters.
iv) Class designation – must appear in capital letters as follows:
a. DOMESTIC CLASS
v) Nature and degree of hazard inherent in the pest control product and shown by a precautionary symbol and signal word.
vi) REGISTRATION NO. PCPB(CR) to appear prominently and distinctly.
vii) Net content
viii) Name and address of Registrant, Local Agent, Manufacturer, or Distributor
ix) Date of Manufacture, Batch No. & Expiry date that does not have to be printed.
x) Shelf life in an original unopened container. No Maximum or Minimum shelf-life period should appear
xi) Pictograms and Color Codes.

B) Secondary display panel
(i) A brief description of the product.
(ii) Directions of use that must include dosage, rate in the amount of formulation per unit area and the spray volume.
(iii) Significant hazards respecting handling, storage, and procedures to alleviate the hazards.
(iv) Decontamination procedures
(v) Disposal of product and empty containers
(vi) Significant hazards to the public health and environment and how to alleviate such hazards.
(vii) FIRST AID INSTRUCTIONS – in capital letters.
(viii) TOXICOLOGICAL INFORMATION/NOTICE TO DOCTOR.
(ix) NOTICE TO USER* - To appear as stated in the Act
(x) SELLERS GUARANTEE* – To appear as stated in the Act.
(xii) To be stored at room temperature.

6. Sampling

6.1. Representative samples of the material shall be drawn as prescribed in the Annex A.

6.2. Tests shall be carried out by the appropriate method referred to under 4.2.1.

6.3. Quality of Reagents

Unless specified otherwise, analytical grade chemicals and distilled water complying with KS EAS 123:2022 (see Clause 2) shall be used.

NOTE: Analytical grade chemicals shall mean chemicals that do not contain impurities which will affect the results of analysis.

Annex A

(Normative)

SAMPLING OF PYRETHRUM EXTRACTS

A.1 GENERAL PRECAUTIONS

A1.0 In drawing, preparing, storing and handling test samples, the following, precautions and directions shall be observed.

A1.1 Samples shall not be taken in exposed place.

A1.2 The sampling equipment shall be clean and dry when used.

A1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling equipment and the containers for samples from adventitious contamination.

A1.4 To draw a representative sample, the contents of each container selected for sampling shall be mixed as thoroughly as possible by shaking or stirring or both, by suitable means or by rolling so as to bring allportions into uniform distribution (See A. 3.3.1).

A1.5 The samples shall be placed in suitable, clean, dry and air-tight amber colored glass, containers, on which the material has no action.

A1.6 The sample container shall be of such as size that they are almost, but not completely, filled by the sample.

A1.7 Each container shall be closed tightly with a stopper after filling and marked with full details of sampling, the date of manufacture, name of the manufacturer and other particularities of the consignment.

A1.8 Sample shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature. It should preferably be refrigerated at $0^{O}C$ or below.

A.2 SAMPLING TUBE

A2.1 A pipette of convenient volume may be used for drawing the sample.

A.3 SCALING OF SAMPLING

A3.1 All the containers in a single consignment of the material drawn from the same batch of manufacture shall constitute a lot. If a consignment is declared or is known to consist of different batches these shall be grouped together and each such, group shall constitute a separate lot.

A3.2 Samples shall be tested for each lot for ascertaining the conformity of the material to the requirementof the specification.

A3.3 The number (n) of containers to be chosen from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 1.

A3.4 These containers shall be chosen at random from the lot and in order to ensure that randomness of selection, some random number table, as agreed to between the purchaser and the vendor, shall be used. In case such a table is not available, the following procedure shall be adopted.

A3.5 Starting from any container in the lot, count them as 1, 2, 3 . etc, up to r in a systematic manner, where r is equal to the integral part of the value of N/n. N being the total number of containers in thelot and n the number of containers to be chosen (see Table 1). Every *rth* container thus counted shall be separated until the requisite number of containers is obtained from the lot to give samples for test.

TABLE 1 Number of containers to be chosen for sampling (clauses A-3.3.1, A-3.2, A-4.1 and A-4.3)

Lot size	No. of containers to be chosen
N(1)	n(2)
3 to 15	3
16 to 40	4
41 to 65	5
66 to 110	7
Over 110	10

A.4 TEST SAMPLES AND REFERENCE SAMPLES

A4.1 Before drawing the test sample, mix thoroughly the contents of each container selected (see Table 1) by shaking or stirring by suitable means or by rolling. Draw, by inserting the pipette through the bung hole or any other convenient opening, small portions of the material from different parts of each container selected. The total quantity of the material drawn from each container shall be sufficient to conduct the tests for all the characteristics given in 4.1 and 4.2.

A4.2 Mix thoroughly all portions of the material drawn from the same container. Out of these portions, a small but equal quantity shall be taken for each selected container and shall be well mixed together so as toform a composite sample of not less than 200ml. This composite (sample shall be divided into three equal parts, one for the purchaser, another for the vendor and the third for the referee.

A4.3 Referee samples - Referee samples shall consist of the composite sample (see A-4.2) marked for this purpose and shall bear the identification mark of the purchaser and the vendor. These shall be kept at a place agreed to between the two.

A.5 NUMBER OF TESTS

A **A5.1** Tests for the determination of total pyrethrin content of the material shall be conducted on composite samples. (See A4.2).

B **A5.2** Test for the determination of the remaining characteristics namely, colour and odour and flash point shall be conducted on the composite sample as prepared under A-4.2

Annex B

(Normative)

IDENTITY TEST FOR PYRETHRINS

B.0 PRINCIPLE

B.0.1 HPLC Method of analysis for pyrethrins

B.1 APPARATUS

B.1.1 High pressure liquid chromatograph equipped with a C-8 reverse phase column (Agilent EclipseXDB, 5 micron), 4.6 mm id. x 150 mm length or (equivalent coloum) and a UV detector.

B.2 REAGENTS

B.2.1 Acetone – UV Grade

B.2.2 Methanol – UV Grade

B.3 HPLC Conditions

B.3.1 Mobile phase: 85 % Methanol/ 15 % water

B.3.2 Flow rate: 1.0 ml/min

B.3.3 Wavelength: 225 nm

B. 4 STANDARD PREPARATION

B.4.1 weigh out the following standard amount into a 100 ml volumetric flask.

B.4.2 add 5.0 mls acetone, mix and bring to volume with methanol.

Active	MG.(actual)
Pyrethrum 20% standard	100 (20 mg PY's)

B.4.3 a well-established standard quantitated using official AOAC Mercury reduction methodology should be used qualified standards solution may be supplied as needed by the manufacturers.

B.5 SAMPLE PREPARTION

B.5.1 weigh out sample equivalent of 20 mgs of pyrethrins into a 100 ml volumetric.

B.5.2 add 5.0 mls acetone, mix and dilute to 100 mls with methanol.

B.6 HPLC INJECTION PROCEDURE

B.6.1 inject 5 µ l of standard solution into HPLC instrument until acceptable reproducibility is obtained. Inject sample solution in the same manner. Use peak area or height for calculation. The pyrethrin II's are calculated using the sum of the two peaks at 2.9 and 3.4 minutes. The pyrethrin I's are calculated using the sum of the two peaks at 4.8 and 5.9 minutes.

Annex C

(Normative)

DETERMINATION OF TOTAL PYRETHRIN CONTENT

C.0 PRINCIPLE

C.0.1 The pyrethrins after extraction are hydrolysed to chrysanthemum mono- and dicarboxylic acids. Monocarboxylic acid from 'Pyrethrin I, after extraction is reacted with Deniges reagent and mercuroussulphate formed is titrated with potassium iodate. Dicarboxylic acid from Pyrethrin II, remaining after the extraction of monocarboxylic acid is extracted and titrated with standard sodium hydroxide.

C.1 REAGENTS

C.1.1 Deniges Reagents - Mix 5 g yellow mercuric oxide with 40 ml water, and while stirring, slowly add 20 ml sulphuric acid, then add additional 40 ml water and stir until all dissolves. Test for the absence of mercurous mercury by adding a few drops of iodine monochloride solution to 10 ml and titrating with potassium iodate standard solution as in B.2.2.

C.1.2 Iodine Monochloride Solution - Dissolve 10 g potassium iodide and .6.44 g potassium iodate in 75 ml water in glass stoppered bottle, add 75 ml hydrochloric acid and 5 ml chloroform and adjust to faint iodine colour (in chloroform) by adding dilute potassium iodide or potassium iodate solution. If much iodine is liberated, use stronger solution of potassium iodate than 0.01 M at first, making final adjustment with 0.01 M solution. Keep in dark and readjust when necessary. It should not be stored in refrigerator.

C.1.3 Potassium Iodate Standard Solution - 0.01 M, Dissolve 2.14 g pure potassium iodate, previously dried at 105°C, in water and dilute to one litre. One ml of solution = 0.0057 g pyrethrin I.

C.1.4 Alcoholic Sodium Hydroxide Solution -1 Nand *0.5* N

C.1.5 Petroleum Ether - aromatic free, boiling range 40-60°C or 60-80°C

C.1.6 Ethyl Ether -peroxide free

C.1.7 Sodium Hydroxide Standard Solution – 0.02 N

C.1.8 Filter Cel

C.1.9 Barium Chloride Solution -10 percent *(m/v).*

C.1.10 Dilute Sulphuric Acid -1: 4 (vlv*)*.

C.1.11 Ethyl Alcohol -95 percent *(vlv)* and 99 percent *(vlv),* anhydrous

C.1.12 Sodium Chloride - solid as well as saturated solution,

C.1.13 Chloroform

C.1.14 Dilute Hydrochloric Acid-3: 2 (v *Iv),*

C.1.15 Phenolphthalein Indicator Solution - one percent *(m/v)* in ethyl alcohol.

C.2 PROCEDURE

C.2.0 Weigh sample containing 40-150 mg total pyrethrins, add 50 ml petroleum ether and I g filter cell, and place in refrigerator at 0 ± 0.5°C overnight. Filter through Gooch filter into 300 ml Erlenmeyer flask and wash with three 15 ml portions of cold petroleum ether, evaporate filtrate and washings on water bath, using air current until the petroleum ether is almost entirely removed.

C.2.1 Add 20 ml 1 N alcoholic sodium hydroxide, or more, if necessary, to extract pyrethrins, connect to reflux condenser, and boil gently for 60 to 90 minutes. Transfer to 600-ml beaker and add enough waterto make aqueous layer 200 ml. If more than 20 ml alcoholic sodium hydroxide solution was used, add enough water so that all alcohol is removed when volume is reduced to 150 ml. Add few glass beads andboil aqueous layer down to 150 ml. Transfer to 500-ml separator and drain aqueous layer into 250-ml volumetric flask. Wash oil layer once with water and add washings to aqueous portion. If slight emulsionstill persists after draining aqueous layer and washings, add two to three ml 10 percent barium chloride solution but do not shake vigorously after adding barium chloride, because reversed emulsion difficult toseparate may form. To aqueous solution in 250-ml flask add I g filter cel and approximately 10 ml of the barium chloride solution, swirl gently and let stand for 30 minutes. Dilute to volume; mix thoroughly and filter off 200 ml. Test filtrate with barium chloride solution to see if enough has been added to obtain clear solution. Neutralize with sulphuric acid (1:4), using 1 drop phenolphthalein and add 1 ml excess. (If necessary to hold solution overnight at this point, leave in alkaline condition.)

C.2.2 Determination of Pyrethrin I - Filter acid solution from C.2.1 through 7 cm paper, coated lightly with suspension of filter cell in water, on buchner, and wash with three 15 ml portions of water. Transfer to 500-ml glass stoppered separator and extract with two 50 ml portions petroleum ether. Shake each extract approximately for one minute, releasing pressure, if necessary, by inverting separator and carefully venting through stopcock. Let layers separate for approximately five minutes or until aqueous layer is clear before draining and re-extraction, Reserve aqueous layer for pyrethrin II determination. Do not combine petroleum ether extracts but wash each in sequence with same three 10 ml portions water, and filter petroleum ether extracts through small cotton plug, into a clean 250-ml separator. Wash separators and cotton in sequence with 5 ml petroleum ether. Extract combined petroleum ether solutions with 5 ml 0.1 N sodium hydroxide, shaking vigorously for approximately one minute. Let layers separate for approximately 5 minutes before draining aqueous layer into I00-ml beaker. Wash petroleum ether with additional 5-ml portion 0.1 N sodium hydroxide and with 5 ml water, adding washings to beaker. Add 10 ml Deniges reagent and let stand in complete darkness for one hour at 25 ± 2°C. Add 20 ml alcohol and precipitate mercurous chloride with 3 ml saturated sodium chloride solution. Warm to approximately 60°C and let stand several minutes until precipitate coagulates and settles. Filter through small paper, transferring all precipitate to paper, and wash with approximately 10 ml hot alcohol. Wash with 2 or more 10 ml portions hot chloroform and place paper and contents in 250-ml glass stoppered conical flask. Add 50 ml cooled dilute hydrochloric acid (3: 2). Add 5 ml chloroform and 1 ml freshly adjusted iodine monochloride solution and titrate with standard potassium iodate solution, shaking vigorously approximately 30 second after each addition, until no iodine colour remains in chloroform layer. Take as end point when red colour disappears from solvent layer and does not return within three minutes. From standard potassium iodate solution used in titration and blank on Deniges reagent, calculate percent pyrethrin I.

C.2.3 Calculation -1 ml of 0.01 M KIO_3= 0.005 7 g pyrethrin I.

NOTE - Chrysanthemum monocarboxylic acid reacts with Deniges reagent to form series of colours beginning with phenolphthalein red, which gradually changes to purple, then to blue and finally to bluish green. Colour reaction is very distinct with 5 mg monocarboxylic acid and amounts as low as 1 mg can usually be detected. Therefore, no pyrethrin I should be reported if colour reaction is negative. With samples containing much perfume or other saponifiable ingredients, it may be. necessary to use as much as 50 ml 1 N alcoholic sodium hydroxide. When lethanes are present, after washing mercurous chloride precipitate with alcohol and chloroform, wash once more with alcohol and then several times with hot water.

C-2.4 Determination of Pyrethrin II - If necessary, filter aqueous residue reserved in **C.2.2** from petroleum ether extract through Gooch. Concentrate filtrate to approximately 50 ml and transfer to 500-ml glass stoppered separator. Wash beaker with three 15 ml portions water. Acidify with 10 ml hydrochloric acid and saturate with sodium chloride. (Acidified aqueous layer must contain visible sodium chloride crystals throughout following extractions.)

Extract with 50-ml ether, drain aqueous layer into second separator and extract again with 50 ml ether. Continue extracting and draining aqueous layer, using 35 ml for third and fourth extractions. Shake each extract for approximately one minute, releasing pressure, if necessary, by inverting separator and carefully venting through stopcock. Let layers separate for approximately five minutes or until aqueous layer is clear before subsequent draining and extraction. Combine ether extracts, drain and wash with three 10 ml portions saturated sodium chloride solution. Filter ether extracts through cotton plug into 500-ml conical flask and wash separator and cotton with additional 10 ml ether. Evaporate ether on water bath and remove any fumes of hydrochloric acid with air current and continue heating for approximatelyfive minutes. Dry for 10 minutes at 100°C. Add 2 ml neutral alcohol and 20 ml water and heat to dissolveacid. Cool, filter through Gooch, if necessary, add two drops phenolphthalein and titrate with 0'02 N sodium hydroxide. Check normality of 0'02 N sodium hydroxide on the same day, as the sample is titrated.

C.2.5 Calculation

1 ml of 0.02 N NaOH = 0.00374 g pyrethrin **II.**

Total pyrethrin shall be obtained by adding pyrethrin I and pyrethrin II

Note: Total pyrethrin should be in percentage (w/w)

Annex D

(Normative)

DETERMINATION OF PH

D.1 Apparatus

A pH Electrode System such as a single or dual glass electrode system conditioned and maintained according to the manufacturer's instructions.

D.2 Reagents

Freshly distilled/deionized water free from accumulation of CO2 from the atmosphere.

D.3 Procedure

Prepare 1 % solution of the extract by weighing 1.0 g of sample into a calibrated beaker. Add 50 mL

Distilled water or equivalent neutral organic solvent such as ethanol. Top up reagent water to 100 mL, mix vigorously using magnetic stirring rod until the formulation is completely.

Mixed or dispersed.

Prior to pH measurement, calibrate the pH-meter using a standard buffer 4, 7 and 10 but ensure that the temperature of the solution does not differ from the reference solutions used for calibration. Rinse the cell several times with a small amount of the solution. Immerse the electrode into the solution and measure the pH without stirring. Record the pH value after a stable figure is obtained.

Annex E

(Normative)

DETERMINATION OF OPTICAL DENSITY

E.1 General

The natural colouring matter content of pyrethrum extract is extracted with mineral turpentine oil. The absorbance of the solution obtained is measured by using a UV-Visible Spectrometer at a wavelength of 435 nm.

E.2 Apparatus

A UC-visible Spectrophotometer suitable for measuring at a wavelength of 435 nm and having a band width of 2 nm.

A 20 mL volumetric flask

Measuring Cylinder

A pair of cuvettes

E.3 Reagents

Mineral turpentine oil of known purity.

E.4 Procedure

Measure and transfer exactly 1 mL of the pyrethrum extract in the 20 mL volumetric flask. Add to it mineral turpentine oil and make up the volume up to the mark. Shake the flask to mix the content thoroughly.

Take a pair of clean cuvettes. Switch on the Spectrophotometer and set the wavelength at 435 nm. In one cuvette take the solvent and place this cuvette in the reference cell of the spectrophotometer. Take the diluted sample from the volumetric flask in the other cuvette and place the cuvette in the sample cell of the spectrophotometer. Close the chamber and measure the absorbance at 435 nm.