

PYRETHRUM

Specification WHO/SIT/7.R1
Approved 25 October 1965

1. Specification

1.1 Material

The material shall be extracts or concentrates of substances occurring naturally in pyrethrum flowers and shall be free from extraneous impurities or added modifying agents.

1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

1.2.1 *Pyrethrins¹ content*

The pyrethrins¹ content shall be declared and shall be not less than 190 g/kg when determined by the method described in section 2.1.

1.2.2 *Material insoluble in dichlorodifluoromethane²*

The material insoluble in dichlorodifluoromethane, determined by the method described in WHO/M/9, shall not be higher than 15 g/kg.

1.3 Packing and marking of packages

The pyrethrum shall be packed in suitable, clean containers, as specified in the order³.

All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Pyrethrum to specification WHO/SIT/7.R1
"Pyrethrins", ... g/kg
Batch or reference number, and date of test
Net weight of contents

¹ Pyrethrin I and cinerin I are analysed together and called "pyrethrin I"; similarly pyrethrin II and cinerin II are called "pyrethrin II". Collectively these four esters are called the "pyrethrins".

² If the pyrethrum is for other than aerosol use, this requirement may be omitted.

³ Pyrethrum is sensitive to bright light and should therefore be kept in perfectly lacquered drums or dark-coloured bottles, the latter preferably stored in the dark.

2. Determination of chemical properties

2.1 "Pyrethrin" content

2.1.1 *Outline of method*⁴

The sample is diluted with light petroleum and hydrolysed with alkali to chrysanthemummonocarboxylic and dicarboxylic acids. These acids are converted to their water-soluble barium salts, the aqueous solution filtered to remove insoluble materials, and the acids liberated from the salts by treatment with sulfuric acid.

Chrysanthemummonocarboxylic acid, which represents "pyrethrin I", is extracted with light petroleum and reacted with Denigès reagent to form mercury (I) chloride (Hg_2Cl_2), which is then titrated with potassium iodate.

Chrysanthemumdicarboxylic acid, which represents "pyrethrin II", is extracted with ether from the water layer remaining from the monocarboxylic acid extraction and is then determined by titration with standard alkali.

2.1.2 *Special reagents*

Potassium iodate 0.01 mol/l. Dissolve 2.14 g of pure potassium iodate, previously dried at 105°C in distilled water and dilute to 1 litre; 1 ml of this solution is equivalent to 0.0057 g of pyrethrin I, without further standardization.

Iodine monochloride solution. Dissolve 10.00 g of potassium iodide and 6.44 g of potassium iodate in 75 ml of distilled water in a glass-stoppered bottle. Add 75 ml of concentrated hydrochloric acid and 5 ml of chloroform, and adjust to a faint iodine colour (in chloroform) by adding diluted potassium iodide or iodate solution. If much iodine is set free, use a stronger solution of potassium iodate than 0.01 mol/l at first, making a final adjustment with the 0.01 mol/l solution. Keep in a dark cupboard and readjust when necessary.

Denigès reagent. Mix 5 g of yellow mercury (II) oxide with 40 ml of distilled water and, while stirring, add slowly 20 ml of concentrated sulfuric acid; then add another 40 ml portion of distilled water and stir until solution is complete. Test as follows for the possible presence of mercury (I). To 10 ml of the reagent add a few drops of the iodine monochloride solution, 30 ml of concentrated hydrochloric acid and 20 ml of distilled water. Cool the flask, add 6 ml of chloroform (or carbon tetrachloride), and titrate with

⁴ This method is based on methods described in (1) Association of Agricultural Chemists. *Official methods of analysis of the Association*, 11th ed., Washington DC, 1970, pp. 88-79 and (2) Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on the Methods of Assay of Crude Drugs. *Analyst*, 89: 689, 1964.

the 0.01 mol/l potassium iodate, shaking vigorously after each addition, until disappearance of the red colour from the chloroform (or carbon tetrachloride) layer. Reject the reagent if the titre exceeds 0.2 ml of 0.01 mol/l potassium iodate solution.

2.1.3 *Initial treatment of sample*

*Pyrethrum extract and concentrates*⁵. Weigh (to the nearest 0.1 mg) an amount of the sample containing 0.10-0.15 g of "pyrethrins" into a 250 ml conical flask, add 50 ml of light petroleum, mix, add 1 g of diatomaceous silica filtering aid, mix, stopper the flask, and set aside in a refrigerator at 0°C ± 0.5°C for at least 2 hours (preferably overnight). Filter quantitatively through asbestos in a Gooch crucible into a 250 ml conical flask, washing with three 15-ml portions of cold light petroleum, and combine the filtrate and washings.

Add several glass beads and evaporate on a water-bath, without attempting to heat the residue long enough to remove the last traces of solvent. Add 20 ml (or more if necessary) of 1 mol/l ethanolic sodium hydroxide, connect to a reflux condenser, and boil gently for 45 minutes. Transfer to a 600 ml beaker and add distilled water to make an aqueous layer of 200 ml. (If more than 20 ml of 1 mol/l ethanolic sodium hydroxide has been used, add sufficient distilled water so that all the ethanol will be removed when the volume has been reduced to 150 ml). Add a few glass beads, or preferably use a boiling-rod, and boil until the volume of the aqueous layer is reduced to 150 ml, using an air stream if necessary to depress frothing. Transfer to a 500 ml separating funnel and draw off the aqueous layer into a 250 ml volumetric flask. Wash the oily layer with distilled water and add the washings to the aqueous layer.

To the aqueous solution in the volumetric flask add 1 g of diatomaceous silica filtering aid and 10 ml (or more) of 100 g/l barium chloride solution, without shaking. Make up to the mark with distilled water, mix thoroughly, and filter off 200 ml. Test the filtrate with 100 g/l barium chloride solution to see if a sufficient quantity has been added to obtain a clear solution. Add 1 drop of phenolphthalein indicator solution, neutralize with 200 g/l sulfuric acid, and add 1 ml in excess.

2.1.4 *Determination of "pyrethrin I"*

By means of a Buchner funnel, filter the solution obtained as described in section 2.1.3 through a 7 cm filter paper that has been coated lightly with a suspension of diatomaceous silica filtering aid in distilled water and wash with three successive 15-ml portions of

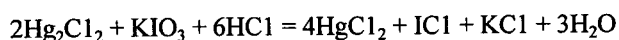
⁵ If purified extracts are being analysed, test for the presence of oxidized "pyrethrins" by dissolving an amount of sample containing 0.10-0.15 g of "pyrethrins" in 50 ml of light petroleum. If the solution is clear or only slightly opalescent, omit the treatment with the diatomaceous filtering aid given above, i.e. add 20 ml of 1 mol/l ethanolic sodium hydroxide to the aliquot taken and proceed with the hydrolysis.

water. Transfer to a 500 ml separating funnel and extract with two 50-ml portions of light petroleum. Wash the extracts successively with two 10 ml portions of distilled water and filter the petroleum extract through a cotton plug into a clean 250 ml separating funnel. Wash the cotton plug with 5 ml of light petroleum. Reserve the aqueous phase from these extracts for the determination of "pyrethrin II" (section 2.1.5). Extract the petroleum layer with 5 ml of 0.1 mol/l sodium hydroxide, shaking vigorously. Draw off the aqueous layer into a 100 ml conical flask, wash the petroleum layer with a second 5-ml portion of 0.1 mol/l sodium hydroxide, and add the extract to the flask. Add 10.0 ml of the Denigès reagent and allow to stand for 60 minutes in the dark at $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ (see note, page 84). Add 20 ml of ethanol and precipitate the mercury (I) chloride with 2 ml of saturated sodium chloride solution. Warm to about 60°C and set aside for several minutes, until the precipitated mercury (I) chloride has coagulated and settled. Decant the supernatant liquid through a 9 cm filter paper in a small glass funnel (4.5 cm diameter) and drain carefully, retaining most of the precipitate in the flask. Wash the precipitate in the flask with 10 ml or more of hot anhydrous ethanol, decanting the ethanol through the same filter paper as before. Wash the precipitate with three successive 10 ml portions of hot chloroform, decanting each washing through the same filter paper, and transfer the filter paper and any precipitate to the flask containing the bulk of the precipitate. Add 30 ml of concentrated hydrochloric acid and 20 ml of distilled water to the flask and cool. Add 6 ml of chloroform (or carbon tetrachloride) and 1 ml of the iodine monochloride and titrate with the 0.01 mol/l potassium iodate, shaking vigorously after each addition, until disappearance of the red colour from the chloroform (or carbon tetrachloride) layer.⁶ Carry out a blank determination by repeating the whole procedure but omitting the "pyrethrins".

2.1.5 *Determination of "pyrethrin II"*

If necessary, filter the aqueous residue from the light petroleum extraction (see section 2.1.4) through a Gooch crucible. Concentrate the filtrate to a volume of about 50 ml and transfer to a 500 ml separating funnel. Acidify with 10 ml of concentrated hydrochloric acid and saturate with sodium chloride⁷. Extract with 50 ml of ether, draw off the aqueous layer into a second separating funnel, and extract again with 50 ml of ether. Run off the aqueous phase into a 100 ml conical flask and transfer the ether extract to the first separating funnel. Return the aqueous phase to the second separating funnel and extract in a similar manner with two 35 ml portions of ether, combining all ether extracts in the

⁶ KIO_3 reacts with mercury (I) to form mercury (II) and iodine; further addition of KIO_3 in presence of HCl oxidizes the iodine to iodine chloride (ICl):



Addition of ICl does not change the volume relationship between mercury (I) and KIO_3 solution and aids in determining the end-point in the titration of small quantities of mercury.

⁷ Take care that the acidified aqueous layer is saturated with sodium chloride throughout the subsequent extraction.

first separating funnel. Discard the aqueous phase and any aqueous liquid that separates from the combined ether extracts. Wash the ether extract with three successive 10ml portions of saturated sodium chloride solution. Filter the ether extract through a cotton plug into a 500 ml conical flask and wash the cotton plug with 10 ml of fresh ether. Evaporate the ether on a water-bath, removing the last traces of vapour with a current of air. Dry the residue in an oven at 100°C for 10 minutes.

Treat with 75 ml of boiling water and filter the solution through a high-grade filter paper, washing the flask and filter paper with five successive 20 ml portions of boiling water or until the washings are neutral to litmus. Add 1 or 2 drops of phenolphthalein indicator solution to the combined filtrate and titrate with 0.02 mol/l sodium hydroxide; 1 ml of 0.02 mol/l sodium hydroxide is equivalent to 0.00374 g of "pyrethrin II". Carry out a blank determination by repeating the whole procedure on the aqueous liquid set aside in the blank determination for "pyrethrin I".

2.1.6 Calculation

$$1. \quad \text{Content of "pyrethrin I" (g/kg)} = \frac{(v_1 - v_2) \times 7.125}{m}$$

Where: v_1 = volume (ml) of 0.01 mol/l potassium iodate required for titration of "pyrethrin I"
 v_2 = volume (ml) of 0.01 mol/l potassium iodate required for titration of the blank solution
 m = mass (g) of initial sample

$$2. \quad \text{Content of "pyrethrin II" (g/kg)} = \frac{(v_3 - v_4) \times 4.675}{m}$$

v_3 = volume (ml) of 0.02 mol/l sodium hydroxide required for titration of "pyrethrin II"

v_4 = volume (ml) of 0.02 mol/l sodium hydroxide required for titration of the blank solution.

m = mass (g) of initial sample.

Note: Chrysanthemum monocarboxylic acid reacts with the Denigès reagent to form a series of colours beginning with red, which gradually changes to purple, then blue, and finally bluish-green. The colour reaction is very distinct with 5 mg of the acid, and quantities as low as 1 mg can usually be detected. Therefore, no "pyrethrin I" should be reported if the colour reaction is negative.