SCHEDULE 5

ANALYSIS OF CITRUS FRUIT TREATED WITH BIPHENYL, 2-HYDROXYBIPHENYL OR SODIUM BIPHENYL-2-YL OXIDE

PART III

QUANTITATIVE ANALYSIS OF THE RESIDUES OF 2-HYDROXYBIPHENYL AND SODIUM BIPHENYL-2-YL OXIDE IN CITRUS FRUIT

Purpose and scope

1. The method described below enables a quantitative analysis of the residues of 2-hydroxybiphenyl and sodium biphenyl-2-yl oxide in whole citrus fruit to be made. The method gives results which for a 2-hydroxybiphenyl or sodium biphenyl-2-yl oxide content of the order of 12 mg. per kg. are low by an average value of between 10 per centum and 20 per centum.

Principle

2. After distillation in an acid medium and extraction by di-isopentyl ether, the extract is purified and treated with a solution of 4-aminophenazone. A red colour develops, the intensity of which is measured spectrophotometrically at 510 nm.

Reagents

3. The following reagents shall be used–

(a) 70 per centum (weight/weight) orthophosphoric acid;

(b) silicone-based anti-foaming emulsion;

(c) di-isopentyl ether (analytical reagent grade);

(d) purified cyclohexane: shake 3 times with a 4 per cent (weight/volume) solution of sodium hydroxide, wash 3 times with distilled water;

(e) 4 per centum (weight/volume) sodium hydroxide solution;

(f) buffer solution at pH 10.4: into a 2 litre graduated flask put 6.64 g. of boric acid, 8.00 g. of potassium chloride and 93.1 ml. of N sodium hydroxide solution; mix and bring up to calibration mark with distilled water;

(g) reagent I: dissolve 1.0 g. of 4-aminophenazone (4-amino-2, 3-dimethyl-1-phenyl-5-pyrazolone; 4-aminoantipyrin) in 100 ml. of distilled water;

(h) reagent II: dissolve 2.0 g. of potassium ferricyanide in 100 ml. of distilled water. Reagents I and II must be kept in brown glass flasks and are only stable for approximately 14 days;

(j) silica gel;

(k) standard solution: dissolve 10 mg. of pure 2-hydroxybiphenyl in 1 ml. of 0.1 N NaOH; dilute to 100 ml. with a 0.2 M sodium borate solution (1 ml.=100 µg. 2-hydroxybiphenyl). For the standard curve, dilute 1 ml. to 10 ml. with the buffer solution.

Apparatus

4. The following apparatus shall be used–

(a) a shredding or crushing mill;

(b) a mixer;

(c) a 1 litre distillation flask with a modified Clevenger-type separator as shown in the diagram in Schedule 6 and a reflux condenser;

(d) an electrically controlled heating mantle;

(e) a 200 ml. separating funnel;

(f) graduated cylinders of 25 and 100 ml.;

(g) graduated flasks of 25 and 100 ml.;

(h) 1 to 10 ml. pipettes;

(j) 0.5 ml. graduated pipettes;

(k) a spectrophotometer with 4 or 5 cm. cells.

Method of Analysis

5. All the fruit in the sample for analysis is cut in half. Half of each piece of fruit is kept for qualitative analysis for residues of biphenyl, 2-hydroxybiphenyl or sodium biphenyl-2-yl oxide. The other halves are put all together and shredded in a mill or crushed until a homogeneous mixture is obtained. From this at least two sub-samples of 250 g. are taken for analysis in the following manner–

Each sub-sample is placed in a mixer with 500 ml. of water and mixed until a very fine homogeneous mixture is obtained in which the oily cells are no longer perceptible. A sample of 150 to 300 g. of the purée is taken, depending on the presumed 2-hydroxybiphenyl content and placed in the 1 litre distillation flask with a quantity of water sufficient to dilute the mixture to 500 g. in the flask. After the addition of 10 ml. of 70 per centum orthophosphoric acid, several anti-bumping granules and 0.5 ml. of anti-foaming emulsion, the separator and the reflux condenser are fitted on to the flask. 10 ml. of di-isopentyl ether are placed in the separator and the flask is heated gently in the electrically controlled heating mantle until the mixture boils. Emulsion formation is minimised if the mixture is boiled gently for the first 10 to 20 minutes. The rate of heating is then gradually increased until the mixture boils steadily and one drop of water reaches the trapping solvent every 3 to 5 seconds. After distilling for 6 hours, the contents of the separator are poured into the 200 ml. separating funnel, and the separator and the condenser are rinsed with 60 ml. of cyclohexane and then with 60 ml. of water. The rinsings are added to the contents of the separating funnel. The mixture is shaken vigorously and when the phases have separated the aqueous phase is discarded.

To extract the 2-hydroxybiphenyl, the organic phase is shaken vigorously 5 times, each time for 3 minutes, with 10 ml. of 4 per centum sodium hydroxide. The alkaline solutions are combined, adjusted to pH 9–10 with orthophosphoric acid in the presence of phenolophthalein paper, and diluted to 100 ml. with distilled water. A pinch of silica gel is added in order to clarify the solution which will have a slightly cloudy appearance. The solution is then shaken and filtered through a dry, fine-grain filter. Since colouring is developed with the maximum of accuracy and precision using quantities of 2-hydroxybiphenyl of between 10 and 70 µg. an aliquot sample of between 0.5 and 10 ml. of solution is taken with a pipette, taking into account the quantities of 2-hydroxybiphenyl which might be expected to be found. The sample is placed in a 25 ml. graduated flask; to this are added 0.5 ml. of reagent I, 10 ml. of the buffer solution and then 0.5 ml. of reagent II. The mixture is made up to the calibration mark with the buffer solution and shaken vigorously.

After 5 minutes the absorption of the red colouring at 510 nm. is measured spectrophotometrically against a control containing no extract. The colour does not lose intensity within 30 minutes. Evaluation is made by reference to a standard curve drawn from determinations using the standard 2-hydroxybiphenyl solution under the same conditions.

Observations

6. For each analysis it is recommended that the spectrophotometric determination be made with two different volumes of the neutralised alkaline extract.

Untreated citrus fruit give by this method a “blank” reading of up to 0.5 mg. per Kg. for oranges and 0.8 mg. per Kg. for lemons.