SCHEDULE 5

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

PART I

QUALITATIVE ANALYSIS FOR RESIDUES OF BIPHENYL, 2-HYDROXYBIPHENYL AND SODIUM BIPHENYL-2-YL OXIDE IN CITRUS FRUIT

Purpose and scope

1. The method described below enables the presence of residues of biphenyl, 2-hydroxybiphenyl (orthophenylphenol) or sodium biphenyl-2-yl oxide (sodium orthophenylphenate) in the peel of citrus fruit to be detected. The sensitivity limit of this method, in absolute terms, is approximately 5 µg. for biphenyl and 1 µg. for 2-hydroxybiphenyl or sodium biphenyl-2-yl oxide, which is the equivalent of 5 mg. of biphenyl and 1 mg. of 2-hydroxybiphenyl respectively in the peel of 1 kg. of citrus fruit.

Principle

2. An extract is prepared from the peel using dichloromethane in an acid medium. The extract is concentrated and separated by thin layer chromatography using silica gel. The presence of biphenyl, 2-hydroxybiphenyl or sodium biphenyl-2-yl oxide is shown by fluorescence and colour tests.

Reagents

3. The following reagents shall be used–

(a) cyclohexane (analytical reagent grade);

(b) dicholoromethane (analytical reagent grade);

(c) hydrochloric acid 25 per centum (weight/volume);

(d) silica gel GF 254 (Merck or equivalent);

(e) 0.5 per centum (weight/volume) solution of 2,4,7-trinitrofluorenone (TNF) (Fluka, BDH or equivalent) in acetone;

(f) 0.1 per centum (weight/volume) solution of 2,6-dibromo—*p*-benzoquinone-chlorimine in ethanol (stable for up to one week if kept in the refrigerator);

(g) concentrated solution of ammonia, specific gravity: 0.9;

(h) standard 1 per centum (weight/volume) solution of pure biphenyl in cyclohexane;

(j) standard 1 per centum (weight/volume) solution of pure 2-hydroxybiphenyl in cyclohexane.

Apparatus

4. The following apparatus shall be used–

(a) a mixer;

(b) a 250 ml. flask with ground glass joint and with a reflux condenser;

(c) a reduced pressure evaporator;

(d) micropipettes;

(e) a thin layer chromatographic apparatus with plates measuring 20×20 cm.;

(f) an ultra-violet lamp (254 nm.), the intensity of which should be such that a spot of 5 µg. of biphenyl is visible;

(g) equipment for pulversing reagents;

(h) an oven.

Method of Analysis

5. The analysis shall be carried out as follows–

(a) Preparation and extraction. All the fruit in the sample for analysis is cut in half. Half of each piece of fruit is kept for quantitative determination of the residue of any biphenyl or 2-hydroxybiphenyl present. Pieces of peel are taken from the other halves to give a sample of about 80 g. These pieces are chopped, crushed in the mixer and placed in the 250 ml. flask; to this is added 1 ml. of 25 per centum hydrochloric acid and 100 ml. dichloromethane. The mixture is heated under reflux for 10 minutes. After cooling and rinsing of the condenser with about 5 ml. of dichloromethane, the mixture is filtered through a fluted filter. The solution is transferred to the evaporator and some anti-bumping granules are added. The solution is concentrated at reduced pressure at a temperature of 60°C. to a final volume of about 10 ml. If a rotary evaporator is used, the flask should be kept in a fixed position to avoid loss of biphenyl through the formation of a film of the product on the upper wall of the flask.

(b) Chromatography: 30 g. of silica gel and 60 ml. of water are placed in a mixer and mixed for one minute. The mixture is then spread on to 5 chromatographic plates to form a layer approximately 0.25 mm. thick. The plates covered with this layer are subjected to a stream of hot air for 15 minutes and then placed in an oven where they are kept for 30 minutes at a temperature of 110°C.

After cooling, the surface layer of each plate is divided into lanes, 2 cm. wide, by parallel lines penetrating the silica gel down to the surface of the glass plate. 50 µl. of the extract to be analysed are applied to each lane as a narrow band of contiguous spots approximately 1.5 cm. from the lower edge of the plate. At least one lane is kept for the controls consisting of a spot of 1 µl. (that is, 10 µg.) of the standard solutions of biphenyl and 2-hydroxybiphenyl, one standard per lane. The chromatographic plates are developed in a mixture of cyclohexane and dichloromethane (25 : 95) in tanks previously lined with filter paper.

(c) Detection and identification: The presence of biphenyl and 2-hydroxybiphenyl is shown by the appearance of spots in ultra-violet light (254 nm.). The sodium biphenyl-2-yl oxide will have been converted to 2-hydroxybiphenyl during the extraction in an acid medium, and its presence cannot therefore be distinguished from that of 2-hydroxybiphenyl. The products are identified in the following manner–

(i) biphenyl gives a yellow spot in daylight when sprayed with the TNF solution;

(ii) 2-hydroxybiphenyl gives a blue spot when sprayed with the solution of 2,6-dibromo—*p*-benzoquinonechlorimine, followed by rapid passage through a stream of hot air and exposure to an ammonia-saturated atmosphere.