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## Note

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### Extraction and determination of *o*-phenylphenol and biphenyl in citrus fruits and apples

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The fungicides biphenyl and *o*-phenylphenol are widely used for the post-harvest protection of citrus fruits and apples. Dipping of citrus fruits in *o*-phenylphenol and the maintenance of a certain biphenyl concentration in the surrounding air, by means of wrappings saturated with biphenyl, for instance prevents spoilage during shipment and storage. Biphenyl and *o*-phenylphenol are commonly separated from citrus fruits and apples by steam distillation and subsequent extraction with an organic solvent. The final quantitative determinations are made by UV or visible-light spectrometry<sup>1-6</sup> or gas-liquid chromatography (GLC)<sup>4,7-10</sup>. A liquid chromatographic procedure has also been described<sup>11-13</sup>. Clean-up of the extract is often necessary before the spectrometric determinations and sometimes before the GLC determinations also.

In this paper we describe a simple and rapid procedure for the simultaneous steam distillation and extraction of *o*-phenylphenol and biphenyl with a modified Likens-Nickerson instrument<sup>14</sup>; the compounds are detected with high sensitivity by high-resolution glass capillary column GLC.

## EXPERIMENTAL

### *Reagents*

Cyclohexane was of pro analysi grade.<sup>3</sup> Biphenyl and *o*-phenylphenol were purchased from Fluka (Buchs, Switzerland).

### *Fruit materials*

Fruits free of *o*-phenylphenol and biphenyl were obtained from the Citrus Marketing Board of Israel and used within a few days of harvesting.

### *Steam distillation and extraction*

The Likens-Nickerson instrument (Fig. 1), which has been described in detail elsewhere<sup>14</sup>, was modified slightly: the inner diameter of the U-tube was 11 mm

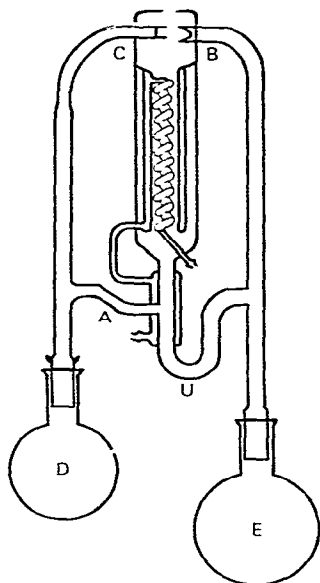


Fig. 1. The Likens-Nickerson instrument.

instead of 9 mm and there was a difference of 10 mm in the heights of the outlets of tube A and the U-tube. It is essential<sup>14</sup> that the outlets of tubes B and C are located so that the vapours are completely mixed.

For the extractions 100 ml of cyclohexane were placed in flask D and 50 g of fruit peel plus 250 ml of distilled water and 20 ml of orthophosphoric acid were added to flask E. A cold trap of dry-ice was used to prevent the loss of biphenyl and *o*-phenylphenol. The contents of the flasks were boiled, the boiling being adjusted so that 2–3 drops of water fell into the U-tube every 10 sec.

After the extraction, a few grams of dry sodium sulphate were added in order to dry the cyclohexane. The GLC determinations were carried out after drying for 15 min. When the concentrations of *o*-phenylphenol and biphenyl were very low, the cyclohexane was concentrated to 1 ml under vacuum.

#### Gas chromatography

The GLC glass capillary columns were constructed from soda-glass and had an inner diameter of 0.3 mm and lengths of 25–100 m. The inner surfaces of the columns were deactivated with Carbowax 20M as described by Blomberg<sup>15</sup> and the method of Schomburg *et al.*<sup>16</sup> was used for coating the columns with FFAP liquid phase. The non-polar liquid phases OV-101 and OV-1 and a semi-polar phase OV-17 were also used successfully to separate biphenyl and *o*-phenylphenol. The OV columns were constructed as described by Grob and co-workers<sup>17,18</sup>.

Carlo-Erba 2300 and Hewlett-Packard 5700 instruments were used. The injections were made by the splitless technique. Hydrogen or helium was used as the carrier gas and the chromatographs were programmed from 50 to 270° at 5–10°/min. A flame-ionization detector was used, but mass spectrometry was used to confirm that the compounds eluted from the column were undecomposed.

Quantitative analyses were based on the peak areas on the chromatograms obtained from the samples and from a reference solution containing known concentrations of *o*-phenylphenol and biphenyl. For very high accuracy an internal standard can be added to the cyclohexane after the extraction procedure. *m*-Phenylphenol is suitable for this purpose, although the different GLC responses have to be taken into account.

## RESULTS AND DISCUSSION

Fig. 2 shows the recoveries obtained when 70 or 20 mg/kg of biphenyl and 10 or 2.5 mg/kg of *o*-phenylphenol were added to fresh oranges (A), grapefruit (B), lemons (C) and apples (D) and allowed to stand for 2 days before the extraction procedure.

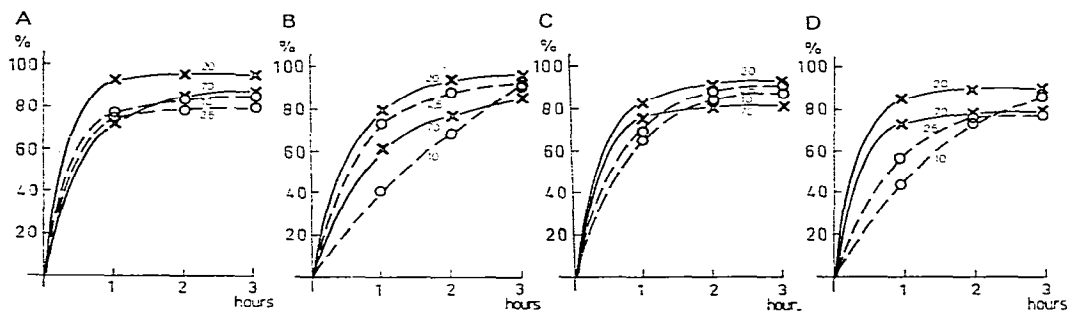


Fig. 2. Recoveries obtained in extracting biphenyl (solid lines) and *o*-phenylphenol (broken lines) from oranges (A), grapefruit (B), lemons (C) and apples (D). The numbers on the curves refer to concentrations of biphenyl and *o*-phenylphenol in parts per million.

Similar experiments were also carried out with 40 ppm of biphenyl and 5 ppm of *o*-phenylphenol and the results were analogous. In most instances 1 h is sufficient for the extraction of biphenyl with a high recovery, whereas 2–3 h are normally needed for the extraction of *o*-phenylphenol.

Fig. 3 shows gas chromatograms obtained from the cyclohexane extracts, without concentration, from grapefruit containing 5 ppm of biphenyl and 5 ppm of *o*-phenylphenol (A), oranges containing 5 ppm of *o*-phenylphenol and 0.1 ppm of biphenyl (B) and apples containing 5 ppm of *o*-phenylphenol and 50 ppm of biphenyl. The samples were steam distilled and extracted for 60 min.

There are a number of methods for determining *o*-phenylphenol and biphenyl in citrus fruits<sup>1-7</sup>. However, the method described here has several advantages, as follows.

The use of the Likens–Nickerson instrument permits the simultaneous steam distillation and extraction of *o*-phenylphenol and biphenyl in a procedure that takes only a few hours. In practice, after treatment with *o*-phenylphenol the citrus fruits are sprayed with wax. This makes it difficult to extract *o*-phenylphenol with a high recovery. It seems, however, that the Likens–Nickerson instrument provides conditions that are severe enough for recoveries of more than 90% to be obtained, even

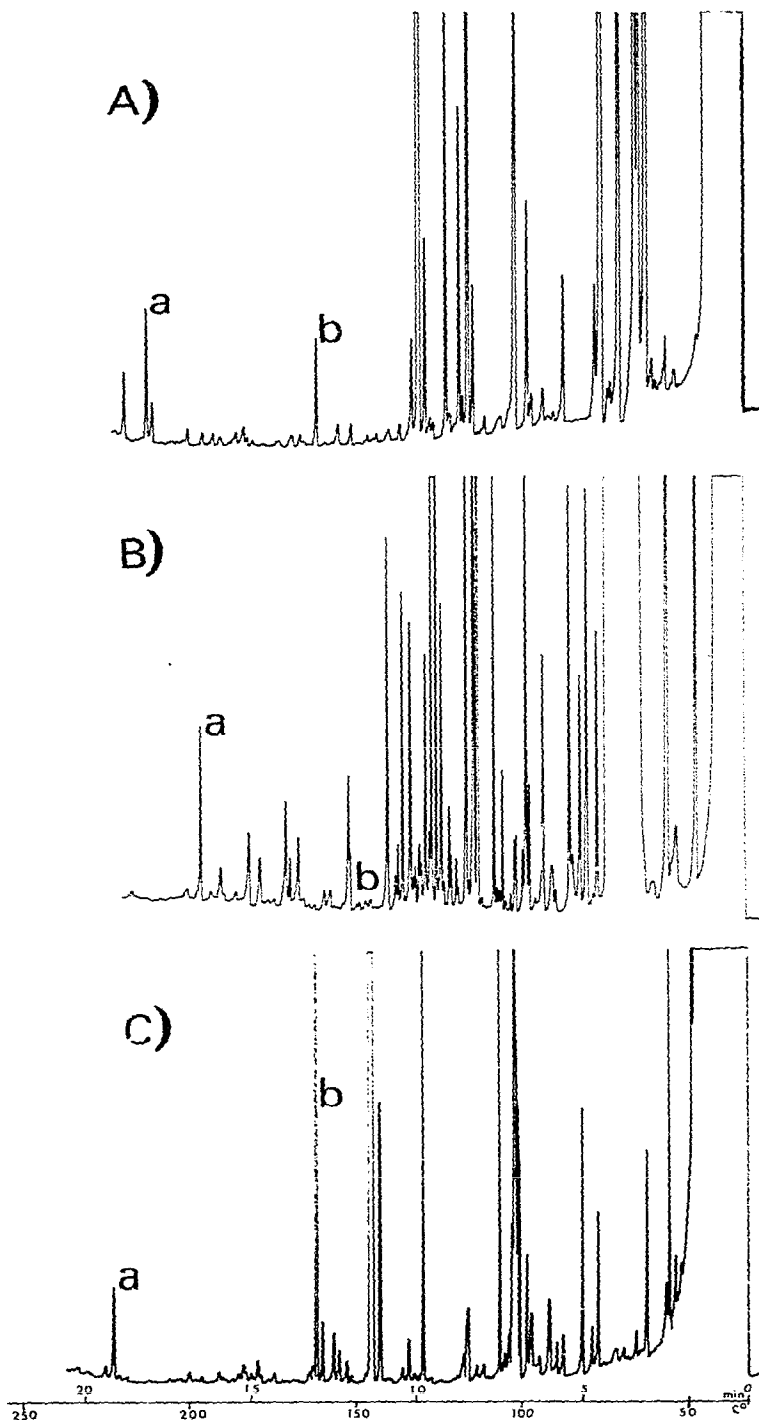


Fig. 3. Gas chromatograms obtained from (A) grapefruit containing 5 ppm of biphenyl and 5 ppm of *o*-phenylphenol, (B) oranges containing 5 ppm of *o*-phenylphenol and 0.1 ppm of biphenyl and (C) apples containing 5 ppm of *o*-phenylphenol and 50 ppm of biphenyl. Column: 30-m FFAF glass capillary. Carrier gas (hydrogen) flow-rate: 2 ml/min.

from samples that have been stored for several months. Thus, when classical steam distillation and extraction with cyclohexane were first used and the samples were then re-treated by the present method, we regularly found an additional 20–100% of *o*-phenylphenol.

The Likens–Nickerson instrument permits vacuum conditions to be used and hence a low temperature, which can be considered advantageous when working with unstable compounds.

With high-resolution glass capillary-column GLC, no concentration of the cyclohexane extract is needed. Conventional GLC with packed columns can also be used, but the risk of error increases with the lower separation ability of such columns. Moreover, compared with glass capillary columns the packed columns give a considerably lower sensitivity and greater concentration of the cyclohexane extract may be needed.

Using the method described here, a trained technician can work with several instruments at the same time, and with the proper equipment ten or more analyses can be carried out per day.

#### ACKNOWLEDGEMENT

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