

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No.V4

BIPHENYL AND 2-HYDROXYBIPHENYL IN CITRUS FRUITS

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1. Scope and Field of Application

The method allows the examination of citrus fruits and their juices for trace residues of the fungicides biphenyl and 2-hydroxybiphenyl; it determines both analytes at levels corresponding to statutory limits (70 mg/kg and 12 mg/kg respectively) and will detect levels down to 1 mg/kg.

2. Definition

Biphenyl content: the content of biphenyl as determined by the method specified.

2-Hydroxybiphenyl content: the content of 2-hydroxybiphenyl as determined by the method specified.

3. Principle

Steam distillation using a modified Clevenger separator, extraction into organic solvent and quantitative gas-liquid chromatography.

4. Reagents

4.1 Anti-foaming Tablets

4.2 Anti-bumping granules

4.3 Sulphuric acid, 50% (V/V).

4.4 Cyclohexane, spectrosol grade.

4.5 Sodium sulphate, anhydrous

4.6 Heptadecane, 10 mg/ml: dissolve 500 mg AR grade heptadecane in cyclohexane and make up to 50 ml in a volumetric flask.

4.7 2-Hydroxybiphenyl, 10 mg/ml: dissolve 500 mg AR grade 2-hydroxybiphenyl in cyclohexane and make up to 50 ml in volumetric flask.

4.8 Biphenyl, 10 mg/ml: dissolve 500 mg AR grade biphenyl in cyclohexane and make up to 50 ml in a volumetric flask.

5. Apparatus

5.1 Food-mixer, Kenwood, or similar with liquidiser attachment.

5.2 Coffee grinder

5.3 Distillation apparatus: 1 litre round-bottom flask fitted with a modified Clevenger separator (see Fig. 1) and a water jacket condenser.

5.4 Heating mantle

5.5 Vacuum evaporator with 50 ml flasks.

5.6 Gas chromatograph

Becker model 420 fitted with a flame ionisation detector and connected to a Servoscribe chart recorder, model RE541. Column: glass, 2 m x 4.0 mm i.d., packed with 3% OV-17 on Gas Chrom. Q. Injector temperature: 210°C. Column temperature-programme: 130°C for 10 min., 130-160°C at 10°C/min, 160°C for 22 min. Detector temperature: 225°C. Carrier gas flow rate: 30 ml nitrogen per min. Detector gas pressures: hydrogen and air set according to manufacturer's instructions.

6. Procedure

6.1 Preparation of Extracts

Separate the flesh and the peel from at least 2 kg of fruit. Cut the peel into small pieces and grind in a coffee grinder. Homogenise the flesh in a liquidiser. Combine the portions and mix well. Take 300 g of

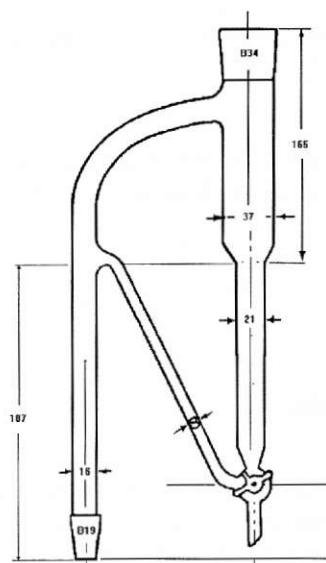


Fig. 1. Modified Clevenger Separator
Diagram reproduced from Statutory Instrument 1975 No.1487
Dimensions in millimeters

recombined fruit pulp and add 300 ml of water. Homogenise at low speed in a mixer and pour into the 1 litre round-bottom flask, rinsing the homogeniser with 50 ml water and adding this to the flask. Add an anti-foam tablet, a few anti-bumping granules and 15 ml of 50% sulphuric acid. Assemble the distillation apparatus and place 15 ml of water and 20 ml of cyclohexane in the side arm of the separator. Boil the mixture gently for 20 min. and then

more vigorously for the remainder of 2 hr. such that one drop distils every 3-5 sec. At the end of this time allow the mixture to cool.

Run the water from the Clevenger separator into a 100 ml separating funnel and the cyclohexane into a 25 ml volumetric flask. Rinse the Clevenger separator with 4 ml of cyclohexane and add this to the water in

the separating funnel. Shake well, allow the layers to separate, discard the water and add the cyclohexane to that in the 25 ml volumetric flask. Make up to volume with cyclohexane, add a little anhydrous sodium sulphate and mix well (this is extract "a"). Set up the separator again with a fresh portion of 20 ml of cyclohexane and repeat the two-hour distillation and subsequent separation as above (this is extract "b").

6.2 Preparation of standard graphs

Prepare five standard solutions (A-E) containing the indicated amounts of fungicide and internal standard.

	A	B	C	D	E
Volume (ml) of biphenyl solution 10mg/ml	0.4	0.8	1.2	1.6	2.0
Volume (ml) of 2-hydroxybiphenyl 10mg/ml	0.1	0.2	0.3	0.4	0.5
Volume (ml) of heptadecane 10mg/ml	1.0	1.0	1.0	1.0	1.0

Make each up to 5 ml with cyclohexane.

Inject 1.5 μ l of each standard and record the chromatogram. Measure the areas (height x width at half height) of the peaks. Plot the area ratio of fungicide to internal standard against mg fungicide per 10 mg of heptadecane for each compound.

6.3 Determination of biphenyl

To 10 ml of extract "a" add 0.4 ml of heptadecane solution, 10 mg/ml. Inject 1.5 μ l and record the chromatogram. Measure the peak areas and calculate the ratio of peak area of heptadecane. From the standard graph read off the biphenyl content, allowing for the amount of heptadecane added.

If necessary the solution may be reduced in volume under vacuum but because of the volatility of biphenyl the volume should not be taken below 2 ml. A typical chromatogram is shown in fig. 2A.

6.4 Determination of 2-hydroxybiphenyl

Combine 10ml of extract "a" and 10ml of extract "b" and add 0.1 ml of heptadecane solution, 10 mg/ml. Evaporate the solution under vacuum but without rotating the flask until the volume is reduced to a few ml. Inject 1.5 μ l and record the chromatogram. Measure the peak areas and calculate the ratio of peak area of 2-hydroxybiphenyl to peak area of heptadecane. From the standard graph read off the 2-hydroxybiphenyl content, allowing for the amount of heptadecane added. The attenuation of the instrument should be adjusted so that the heptadecane peak is at least 40% of maximum. The biphenyl peak may then be off scale.

Care should be taken to identify correctly the 2-hydroxybiphenyl peak particularly if it is small; natural substances present in citrus fruit sometimes give peaks corresponding to 0.5-1.0 mg/kg of 2-hydroxybiphenyl at the same retention time. A typical chromatogram is shown in fig. 2B.

Fig. 2A

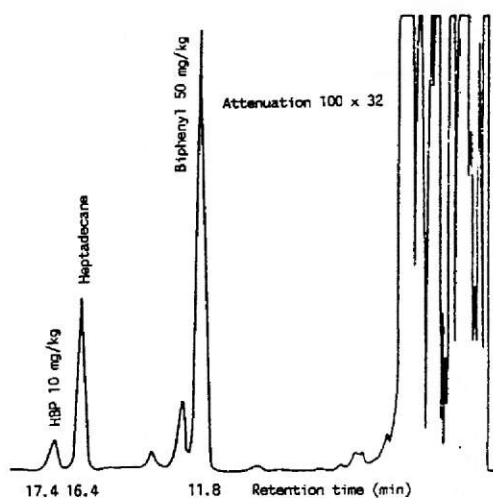


Fig. 2a Chromatogram for the determination of: biphenyl

Fig. 2B

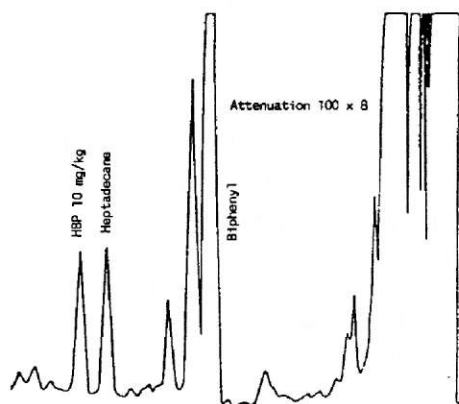


Fig. 2b Chromatograms for the determination of 2-hydroxybiphenyl

Two 300 g portions from the same sample of oranges spiked with 15 mg biphenyl (50 mg/kg) and 3 mg 2-hydroxybiphenyl (10 mg/kg) were treated by the distillation, extraction and glc procedures described in the text.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

Calculate the levels of biphenyl and 2-hydroxybiphenyl and express as mg/kg sample.

9. References

- 9.1 E Lord, NG Bunton and NT Crosby, J. Assoc. Publ. Analysts, 1978, **16**, 25-32.
9.2 RB Player and R Wood, J. Assoc. Publ. Analysts, 1980, **18**, 109-117.

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V0 of the series.

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r , deduced from the collaborative trial data summarised below (Table 1). When biphenyl is the analyte at levels of about 85 mg/kg, r may be taken as 8.9 mg/kg; with 2-hydroxybiphenyl at about 15 mg/kg, the corresponding value of r is 1.6 mg/kg.

This precision corresponds to a relative standard deviations of repeatability (Coefficient of variance of repeatability), RSD_r , of about 5.4% and 7.9% respectively.

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R , deduced from the collaborative trial data summarised below (Paragraph 6, Table 1) in the same way as repeatability above. The recommended values of R are 21 mg/kg at 85 mg biphenyl/kg and 9 mg/kg at 15 mg 2-hydroxybiphenyl/kg. This precision corresponds to a relative standard deviations of reproducibility (Coefficient of variance of reproducibility), RSD_R , of about 13% and 40% respectively. It should be noted that close quantitative agreement between laboratories analysing

very low levels of 2-hydroxybiphenyl cannot be expected, but the method is still recommended until improvements can be demonstrated.

A3 Trueness (Bias)

The collaborative trial was based on the analysis of a sample of comminuted orange (including juice) before and after spiking with 84 mg biphenyl/kg, 14.4 mg 2-hydroxybiphenyl/kg and 12.0 mg thiabendazole/kg. The untreated sample was shown to contain about 1 mg of each analyte/kg. The results summarised in paragraph 5 demonstrated that only 70% of the biphenyl and 48% of the 2-hydroxybiphenyl were recovered. While these recoveries are lower than would be normally regarded as satisfactory, they are acceptable for general control purposes, since alternative methods, requiring 50% more time to undertake, have similar recoveries.

A4 Limit of Detection

This limit has not been established. However, the analysis of the unspiked sample during the collaborative trial indicated that these control levels were at or close to the limit of detection. The true levels were unknown; the observed levels were subject to considerable error, but indicated a limit of detection of roughly 1 mg of either analyte/kg.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Twenty-two laboratories agreed to analyse two samples of a natural comminuted orange product, one of which had been spiked with known levels of biphenyl, 2-hydroxybiphenyl and thiabendazole. While other methods were also tested, the data summarised in Table 1 result from the application of the method described in this booklet to the spiked sample only. The results of one laboratory were omitted because the prescribed method was not followed. The levels of each analyte were expressed as mg/kg sample.

TABLE 1
Statistical Analysis of Biphenyl and 2-Hydroxybiphenyl in Natural Comminuted Orange Product Samples

Sample	biphenyl	2-hydroxybiphenyl
Number of laboratories retained after eliminating outliers	21	21
Number of laboratories eliminated as outliers	0	0
Number of results accepted	42	40
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	59.6	7.33
Accepted "true" value	85	15.4
REPEATABILITY		
Standard Deviation S_r	3.19	0.58
Relative Standard Deviation $RSD_r(\%)$	5.4	7.9
Repeatability $r [2.8 \times S_r]$	8.94	1.62
REPRODUCIBILITY		
Standard Deviation S_R	7.46	2.91
Relative Standard Deviation $RSD_R(\%)$	12.5	39.7
Reproducibility $R [2.8 \times S_R]$	20.9	8.16

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility