Journal of Chromatography, 77 (1973) 331–338

CHROM. 6495

DETERMINATION OF BIPHENYL AND ORTHOPHENYLPHENOL IN CITRUS FRUITS BY GAS CHROMATOGRAPHY

H. BEERNAERT
Institute of Hygiene and Epidemiology, Food Section, Brussels (Belgium)

(Received October 25th, 1972)

SUMMARY

A new gas chromatographic method has been developed for the rapid and accurate identification and determination of biphenyl and orthophenylphenol in citrus fruits.

Several illustrations are given of standard curves and recoveries.

A check on overlapping components has been carried out by using a combination of gas chromatography and mass spectrometry.

The analytical results on eighteen samples of citrus fruits are tabulated.

INTRODUCTION

Biphenyl and orthophenylphenol (OPP) are widely used fungicides that prevent the growth of moulds during storage and transport.

The tissue wrapping paper of the fruit or the paper pads that are placed between layers in boxes are usually impregnated with these substances. Part of the inhibitors is adsorbed by the fruit and is located in the peel.

A review of the toxicity of biphenyl has been given by VAN STRATUM[1] and articles on the toxicity of OPP have been published by SOUCI[2] and TRUHAUT[3].

Widely differing tolerances of residues have been legally established in various countries for biphenyl and OPP. In Great Britain, for instance, the tolerance is 100 p.p.m. for biphenyl and 70 p.p.m. for OPP. Belgium and other E.E.C. members, however, have established a tolerance of 70 p.p.m. for biphenyl and 12 p.p.m. for OPP.

Many papers have been published on the identification and determination of biphenyl[4–14] and OPP[15–23] in and on citrus fruits. In almost all instances, biphenyl has been isolated from the fruit by steam distillation and determined by conventional methods. After acidification, OPP has been isolated by steam distillation and generally determined by colorimetry.

Most of the identification methods previously used require a large amount of material and the isolation of OPP is often difficult and time consuming. The present paper describes the use of an internal standard in the determination of biphenyl and OPP by gas chromatography, which permits an analysis of a citrus fruit sample to be completed within 2 h.

EXPERIMENTAL

Principle of the method

The bulk of the citrus fruit is freed from biphenyl and OPP by steam distillation. After extraction of the distilled phase with light petroleum, the organic phase is reduced to a small volume. Both inhibitors are determined by gas chromatography, using benzophenone as the internal standard.

Reagents

The following reagents are used: an antifoaming agent; concentrated sulphuric acid; light petroleum, boiling range 40–60°; anhydrous sodium sulphate; standard solutions of biphenyl (0.8 mg/ml), OPP (0.2 mg/ml) and benzophenone (10 mg/ml).

Preparation of biphenyl and OPP standard curves

Place 5, 10, 15, 20, 25 and 30-ml volumes of the standard solutions of biphenyl and OPP into six 1-litre round-bottomed flasks and add 350 ml of distilled water, 2 ml of concentrated sulphuric acid and 3 or 4 carborundum chips.

Carry out steam distillation for 40 min and extract the aqueous phase twice with 100 ml of light petroleum. Separate the extract and filter it through a layer of anydrous sodium sulphate.

Add 1 ml of the standard solution of benzophenone and evaporate the light petroleum until a volume of *ca.* 1 ml remains.

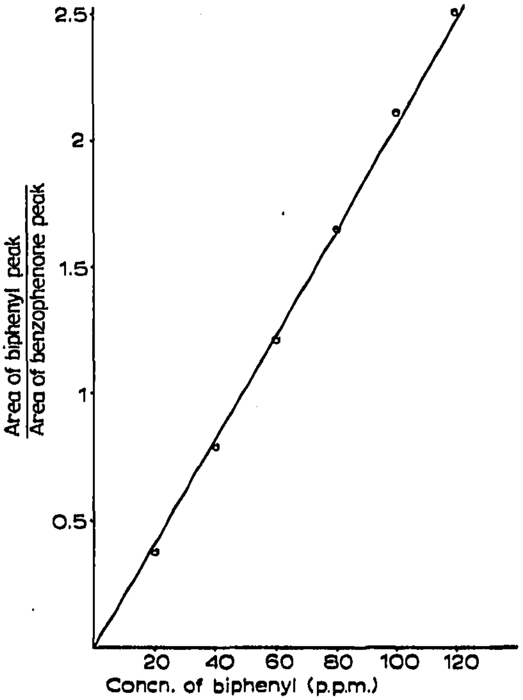
The preliminary experiments on the gas chromatography of the citrus extracts were performed with a Hewlett-Packard Model 5750 gas chromatograph equipped with a 15 ft. × 1/6 in. stainless-steel column packed with 1% SE-30 on Chromosorb Q, 80–100 mesh. The temperature programme comprised 15 min under isothermal conditions at 50°, a linear temperature increase from 50 to 300° at the rate of 8°/min, and a final period of 15 min under isothermal conditions at 300°. The detector was operated at 320°. The nitrogen carrier gas flow-rate was 40 ml/min and the hydrogen and air flow-rates for the detector were 50 and 500 ml/min, respectively. The injector port was operated at 300°. 1 μl of the extract was injected into the chromatographic column.

Biphenyl, OPP and benzophenone are identified by their retention times, R_T, which are 21 min 40 sec for biphenyl, 25 min for OPP and 28 min for benzophenone.

The areas of the gas chromatographic peaks are measured with an Infotronics CR 405 integrator.

The concentrations of biphenyl and OPP are plotted on the abscissa, and the ratios of the peak areas of biphenyl to benzophenone and OPP to benzophenone, corresponding to the concentrations of biphenyl and OPP, are plotted on the ordinate. By connecting the different points, standard curves for biphenyl and OPP are obtained (Figs. 1 and 2).

The experimental error in the measured areas with the integrator system is ± 2%.



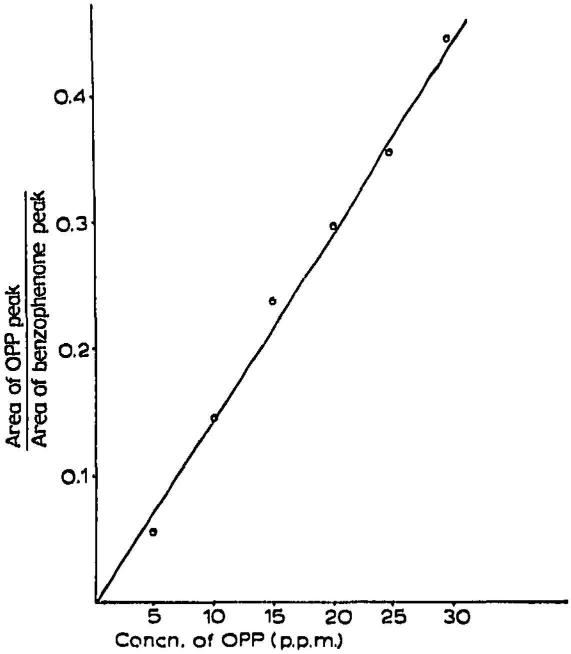


Fig. 1. Standard curve for biphenyl.

Fig. 2. Standard curve for orthophenylphenol (OPP).

Preparation of citrus extract

Cut some fruits into very small pieces and pulp them in an electric blender. Transfer 200g of the pulp to a 1-litre two-necked round-bottomed 29/42 flask. Add 150 ml of distilled water, 2 ml of concentrated sulphuric acid, antifoam agent and some carborundum chips. Steam distillation, extraction and gas chromatographic

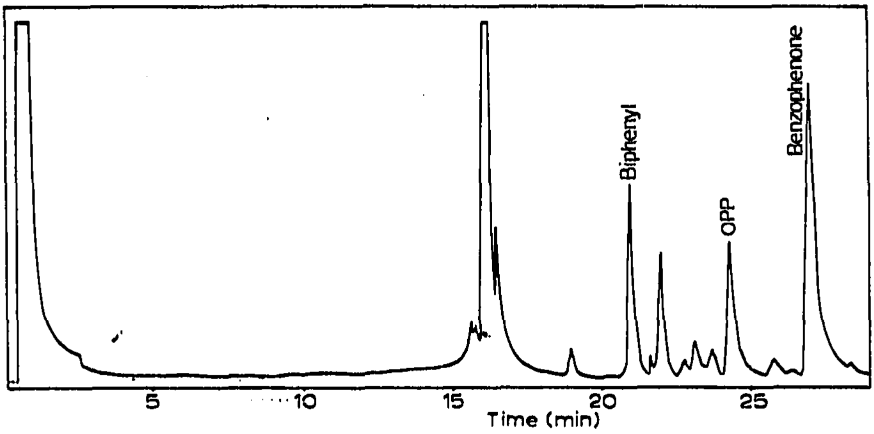


Fig. 3. Chromatogram of the extract of grapefruit.

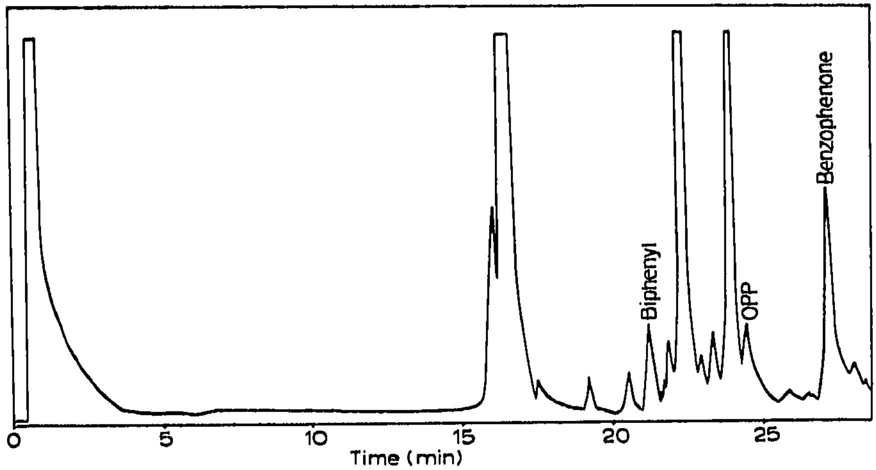


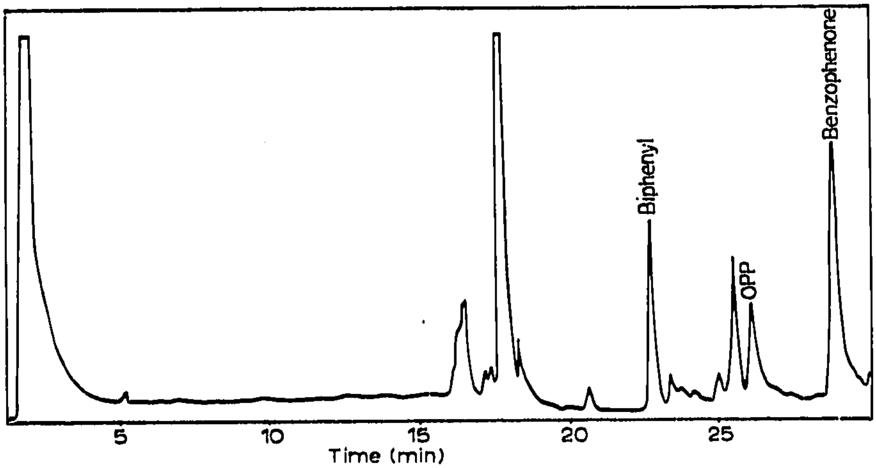
Fig. 4. Chromatogram of the extract of lemons.

analysis of the samples are carried out as described for preparing the standard curve.

Gas chromatograms of the extracts of grapefruits, lemons and oranges are illustrated in Figs 3, 4 and 5.

A Varian Mat CH 5 mass spectrometer with a two-stage Frite separator[23] is used to check the overlapping components. Conditions for the combined gas chromatograph–mass spectrometer were as follows:

Column: 4.5 m × 4 mm I.D. glass column
Stationary phase: 60–80 mesh Chromosorb W-AW
Liquid phase: 5% SE-52
Injected amount: 0.5 μl
Temperature of column: programmed at 7°/min from 50 to 210°
Temperature of Frite separator: 210°
Carrier gas: helium, flow-rate 45 ml/min



[illegible]

Source temperature: 250°
Emission current: 30 or 100 μA
Electron energy: 70 eV
Scan speed: 10 sec in the mass range m/e 12–250.

RESULTS AND DISCUSSION

Accuracy of the method

A 1-kg amount of a citrus fruit was pulped. A blank test was carried out on 200 g of the pulp and the remaining 800 g was divided into four portions of 200 g each. To each portion, 40 p.p.m. (8 mg) of biphenyl and 20 p.p.m. (4 mg) of OPP were added.

The procedures of steam distillation, extraction and gas chromatographic analysis of the sample were carried out as described above.

TABLE I

RECOVERY OF BIPHENYL AND OPP FROM CITRUS FRUITS BY STEAM DISTILLATION

Citrus fruit (200 g)	*Recovery of biphenyl (%)*	*Recovery of OPP (%)*
Orange, Jaffa	97.5	101
	100	100
	109	96
	100	95
	95	98.7
	100	100
Lemon, Jaffa	97.5	95
Grapefruit, Jaffa	96	101
	94	100
	99	92.5
	102.5	104
$\bar{x}$ (average)	99.1	98.1
Standard deviation, S[a]	4.1	3.4

[a] $S = \sqrt{\frac{\Sigma \Delta^2}{n-1}}$

The recoveries of added biphenyl and OPP were determined as the differences between the total amounts of compounds found on the citrus fruits and the amounts found on the citrus fruits in the blank test.

Analysis of the extracts gave a recovery of 100% of the amounts added. The standard deviations, S, of the recovery results for biphenyl (4.1) and OPP (3.4) remain within the limits of experimental error.

Table I shows the results for the recoveries of added biphenyl and OPP by steam distillation.

Interpretation of mass spectra

Comparison of standard spectra of biphenyl, OPP and benzophenone with the spectra of biphenyl, OPP and benzophenone taken from citrus extracts shows that the interference of overlapping peaks can be neglected.

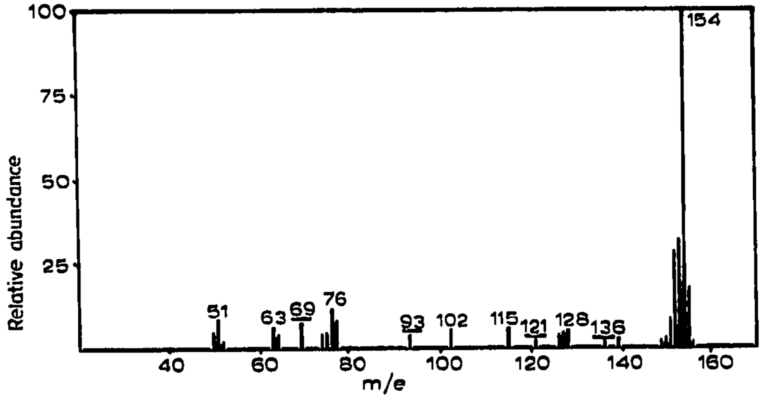


Fig. 6. Mass spectrum of biphenyl from the extract of lemons; 69 = interfering fragment peaks.

The mass spectrum of biphenyl is characterized by the molecular ion peak at *m/e* 154 and the fragment peaks at *m/e* 153, 152, 76, 155, 51, 63, 115, 125, 102 and 139. The mass spectra of biphenyl taken from the extracts of oranges and grapefruits are identical with the standard spectrum. However, the extract of lemons gives a spectrum that also contains fragment peaks at *m/e* 136, 121, 93 and 69 (Fig. 6), which correspond to the mass spectrum of lavandulyl acetate. The area of the overlapping peak of lavandulyl acetate is less than 2% of that of the biphenyl peak and can therefore be neglected.

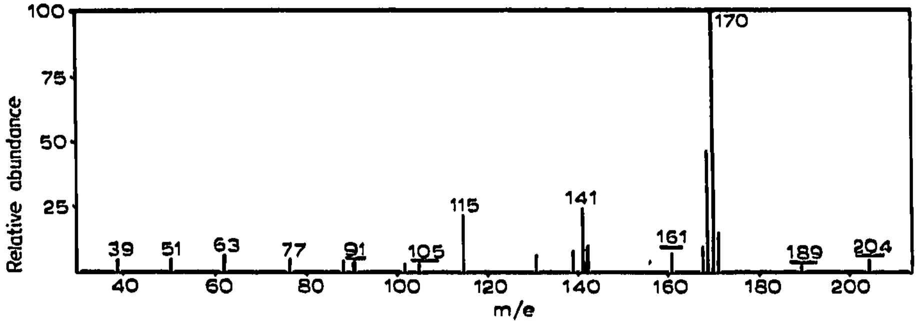


Fig. 7. Mass spectrum of OPP from the extract of oranges; 91 = interfering fragment peaks.

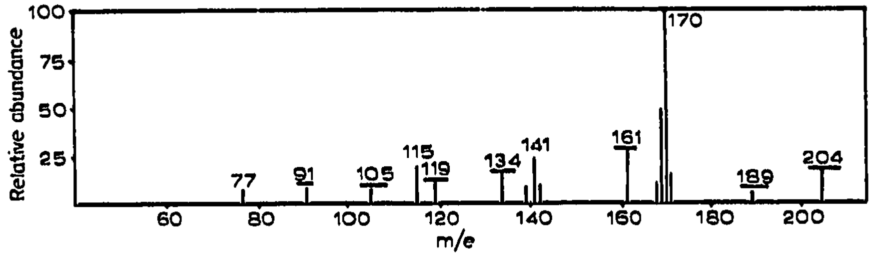


Fig. 8. Mass spectrum of OPP from the extract of grapefruits; 91 = interfering fragment peaks.

The molecular ion peak at *m/e* 170 and the fragment peaks at *m/e* 169, 141, 115, 63, 39 and 51 are the most characteristic of the mass spectrum of OPP.

The extract of lemons gives an identical spectrum. However, mass spectral fragments at *m/e* 204, 189, 161, 134, 119, 105 and 102 (Figs. 7 and 8) indicate that δ-cadinene may be part of the OPP peak taken from the extracts of oranges and grapefruits. Several experiments showed that the overlapping compound δ-cadinene slightly influenced the area of the OPP peak.

The most intense peaks in the mass spectrum of benzophenone are the molecular ion peak at *m/e* 182 and the fragment peaks at *m/e* 105, 77 and 51. The benzophenone peak in the gas chromatograms of the extracts of oranges, grapefruits and lemons show no interfering peaks.

Biphenyl and OPP residues in citrus fruits

The results in Table II show that in most instances the E.E.C. tolerances of 70 p.p.m. for biphenyl and 12 p.p.m. for OPP are not exceeded. Exceptions are the biphenyl residue found in the grapefruit sample from Cyprus (85 p.p.m.) and the OPP residues found in the sample of Californian lemon (15 p.p.m.) and Sanginelli orange (16.8 p.p.m.). Furthermore, the amounts of biphenyl and OPP residues determined differ from one piece of fruit to another and from brand to brand, and also depend on the kind of citrus fruit. For instance, the OPP residues detected in Hamilin orange (sample 6) and in Sanginelli orange (sample 11) are 0 and 16.8 p.p.m., respectively, while the biphenyl residues in the Moroccan orange (sample 14) and in the Greek orange (sample 15) are 30 and 70 p.p.m. The biphenyl residues found in lemons from California (samples 9 and 10) are 4 and 33 p.p.m., while the amounts of OPP are 1.8 and 15 p.p.m., respectively. From the results, it can be concluded that only a small percentage of the treated citrus fruit exceeded the E.E.C. tolerances of 70 p.p.m. for biphenyl and 12 p.p.m. for OPP.

TABLE II

BIPHENYL AND OPP RESIDUES FOUND IN CITRUS FRUITS

Sample No.	*Product to be analyzed*	*Biphenyl found (p.p.m.)*	*OPP found (p.p.m.)*
1	Grapefruit, Jaffa	26	6.5
2	Lemon, Jaffa	39	3
3	Orange, Jaffa	47	4
4	Orange, Jaffa	30	4
5	Orange, Jaffa	34	0
6	Orange, Hamilin	0	0
7	Orange, Jaffa	48	3
8	Grapefruit, Jaffa	35	5.8
9	Lemon, California	4	1.8
10	Lemon, California	33	15
11	Grapefruit, Sanginelli	0	16.8
12	Grapefruit, Cyprus	85	0
13	Clementine, Morocco	0	0
14	Orange, Morocco	30	5.8
15	Orange, Greek	70	5.4
16	Orange, Sanginelli	0	5.2
17	Grapefruit, Jaffa	40	9.6
18	Lemon, Morocco	28	0

CONCLUSION

The UV spectrometric method[24] for the determination of biphenyl and the colorimetric method[20] for the determination of OPP in and on citrus fruits are in general the most used techniques in European countries.

However, the aim was to develop an analytical procedure by which the identification and determination of both fungicides could be ascertained simultaneously. After several modifications of the steam distillation and extraction procedures, biphenyl and OPP were identified by a new, rapid and accurate gas chromatographic method.

The combination of gas chromatography and mass spectrometry has proved that biphenyl, OPP and benzophenone are completely separated by gas chromatography and are slightly influenced by the overlapping peaks of the terpenes lavandulyl acetate and δ-cadinene. The advantages of speed, precision and reproducibility, together with more than adequate sensitivity, have resulted in a system that is designed to be applied as a method for routine determinations of biphenyl and OPP in and on citrus fruits.

ACKNOWLEDGMENTS

The author thanks Mr. M. C. Ten Noever de Brauw of the Central Institute for Nutrition and Food Research TNO of Zeist for assistance in obtaining the mass spectra, and Mr. J. Gosselé, Director of the Food Section of the Institute of Hygiene and Epidemiology, who assisted in much of the work reported.

REFERENCES

1 P. G. C. Van Stratum, *CIVO TNO Rep. R-1838*, Zeist, 1964.
2 S. W. Souci, *Deut. Forschungsanst. Lebensm. Chem., München*, 1959.
3 R. Truhaut, *Bull. Soc. Sci. Hyg. Aliment.*, 50 (1962) 77.
4 A. Rajzman, *Analyst (London)* 85 (1960) 116.
5 A. Rajzman, *Trav. Chim. Aliment. Hyg.*, 58 (1967) 195.
6 A. P. Steyn and F. Rosselet, *Analyst (London)*, 74 (1949) 89.
7 W. F. Newhall, E. J. Elvin and L. R. Knodel, *Anal. Chem.*, 26 (1954) 1234.
8 E. E. Dickey and J. W. Green, *Foudrinier Kraft Board Inst., Appleton/Wisc. Project 1108-7-4*, 1955.
9 R. B. Bruce and J. W. Howard, *Anal. Chem.*, 28 (1956) 1973.
10 J. Vogel and J. Deshusses, *Trav. Chim. Aliment. Hyg.*, 55 (1964) 84.
11 P. E. Corneliussen, *J. Ass. Offic. Anal. Chem.*, 50 (1967) 934.
12 R. Mesters, C. Chave and M. Dudieuzere-Priu, *Ann. Fals. Exp. Chim.*, 674 (1967) 73.
13 W. L. Stanley, S. H. Vannier and B. Gentili, *J. Ass. Offic. Anal. Chem.*, 40 (1957) 282.
14 G. Westöo, *Analyst (London)*, 94 (1969) 406.
15 R. De Vos, *Proc. 1st Int. Citrus Symp.*, Vol. 2, 1969, p. 1081.
16 A. Rajzman and A. Appelbraum, *J. Sci. Food Agr.*, 19 (1968) 740.
17 E. Kröller, *Deut. Lebensm.-Rundsch.*, 63 (1967) 242.
18 T. S. Hermann and A. A. Post, *Anal. Chem.*, 40 (1968) 1573.
19 H. Benger, *Arch. Hyg. Bakt.*, 152 (1968) 225.
20 G. d'Amore and G. Calabro, *Alti Conv. Reg. Aliment., 1st, Conv. Naz. Qual., 4th, Trieste, 1965*, p. 528; *C.A.*, 68 (1968) 48435b.
21 A. Rajzman, *Prelim. Rep. Nat. Univ. Inst. Agric., Rehovat*, No. 334, May, 1961.
22 P. L. Schuller, *R. I. V. Rep. LCLO 20*, Utrecht, 1967.
23 J. Vogel and J. Deshusses, *Trav. Chim. Aliment. Hyg.*, 54 (1963) 330.
24 M. C. ten Noever de Brauw and C. Z. Brunnée, *Z. Anal. Chem.*, 229 (1967) 321.
25 F. A. Gunther, R. C. Blinn and J. H. Barrley, *Analyst (London)*, 88 (1963) 36.