Simultaneous Determination of Diphenyl and *o*-Phenylphenol in Citrus Fruits

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A simple method for simultaneous determination of diphenyl and *o*-phenylphenol in citrus fruits was established. Fruits were distilled with a distillable oil analyzer. The citrus fruit extract was taken from this apparatus and anthracene was added as an internal standard. Gas chromatography was carried out with a column packed with 10% FFAP and a flame ionization detector. Column temperature was increased from 150 to 210°C. The retention times of diphenyl, *o*-phenylphenol and anthracene were 4.2, 14.0 and 15.4 min, respectively.

This method was completed within 2.5 hr and applied to samples of grapefruits, lemons, oranges, navel oranges, ponkan, iyokan, hassaku, unshu mikan and amanatsu mikan. In the recovery tests with these fruits, diphenyl was recovered in the range from 90.1 to 96.9% and *o*-phenylphenol was recovered in the range from 86.5 to 99.3%.

Diphenyl and *o*-phenylphenol are widely used to prevent citrus fruits from fungal spoilage during storage and transport. These substances are usually coated on the wrapping paper of the fruit or impregnated into the paper pads that are placed between layers in fruit boxes.^{1~3)} A lot of citrus fruits, mainly grapefruits, lemons and oranges, have been imported into Japan. From these imported citrus fruits diphenyl and *o*-phenylphenol were detected.^{4~6)} Tolerances of residues have been legally established in various countries. For instances, in the U.S.A. and Canada, tolerances are 110 ppm for diphenyl and 10 ppm for *o*-phenylphenol. E.E.C. members established tolerances of 70 ppm for diphenyl and 12 ppm for *o*-phenylphenol. In Japan diphenyl and *o*-phenylphenol are permitted at the levels of 70 ppm and 10 ppm, respectively.

Many papers have been published on the determination of diphenyl and *o*-phenylphenol in citrus fruits and a review has been written by Harada and Kawasaki.⁷⁾ In almost all instances, diphenyl and *o*-phenylphenol have been extracted from citrus fruits and separated from each other. And then each one is determined by colorimetry,^{8,9)} high speed liquid

chromatography¹⁰⁾ or gas chromatography.^{11,12)} Most of the identification methods previously used require a large amount of materials and the isolation of diphenyl and *o*-phenylphenol is often difficult and time-consuming.

The present paper describes a simple method of concurrent determination for diphenyl and *o*-phenylphenol in citrus fruits by gas chromatography.

MATERIALS AND METHODS

Samples. The following 9 citrus fruits were used; grapefruits, lemons, oranges, navel oranges, ponkan, iyokan, hassaku, unshu mikan and amanatsu mikan.

Reagents. Reagent grade diphenyl, *o*-phenylphenol and zone refined anthracene were purchased from Tokyo Chemical Industry Co., Ltd. Reagent grade cyclohexane, phosphoric acid and anhydrous sodium sulfate were obtained from Wako Pure Chemical Industry, Ltd. Cyclohexane was redistilled prior to use.

Equipment. A gas chromatograph of Shimadzu SAPFE type was used with Shimadzu TP-3A temperature programmer and Hitachi J211A digital integrator. Dual glass columns packed with 10% Free Fatty Acid Polyester (FFAP, 3 mm inner diameter × 1 m length) were attached. A combined gas chromatography-

mass spectrometry-computer system of Nihondenshi GC-20KP-MS-01SG2-COM-JEOL98A was used.

Preparation of calibration curves. The following amounts of each compound was dissolved with cyclohexane in a 1 liter volumetric flask; 700 mg of diphenyl, 100 mg of *o*-phenylphenol and 100 mg of anthracene as an internal standard. This solution was filled up to 1 liter with cyclohexane. Each concentration for gas chromatographic analyses was obtained by the dilution of this solution with the cyclohexane solution that contained 100 ppm of anthracene. Each height of diphenyl, *o*-phenylphenol and anthracene peak on gas chromatograms was measured and the ratios that were divided by peak height of anthracene were calculated. The ratios were plotted against their each concentration.

Preparation of citrus fruit extract. Samples were cut into some pieces and pulped up in an electric blender. The pulp (50 g) was transferred into a 1 liter round-bottomed flask together with the following substances; 200 ml of distilled water, 60 g of sodium sulfate, 1 ml of phosphoric acid, a few drops of silicone resin and some boiling tips. This flask was set as Fig. 1 with a distillable oil analyzer, and 3 ml of cyclohexane was added on the aqueous phase of the distillable oil analyzer. Distillation was carried out for 1.5 hr. The cyclohexane layer was taken out and dried with small amount of anhydrous sodium sulfate.

FIG. 1. Extraction Apparatus for Diphenyl and *o*-Phenylphenol from Citrus Fruits.

A, cooler; B, distillable oil analyzer; C, 1 liter round-bottomed flask; D, cyclohexane layer; E, aqueous layer.

Addition of internal standard solution. To the dried extract, 1 ml of internal standard solution (500 ppm of anthracene in cyclohexane) was added. Finally the citrus fruit extract was made up to 5 ml with cyclohexane.

Gas chromatography. The chromatography of the citrus fruit extract was performed under the condition of Table I. The temperature program comprised 8 min under isothermal condition at 150°C, a linear increase from 150 to 210°C at the rate of 15°C/min, and final period of 6 min under isothermal condition at 210°C. At 6 min point from the start of gas chromatography, the range of the flame ionization detector was changed from 0.16V to 0.02V.

The peak height of diphenyl, *o*-phenylphenol and anthracene on the gas chromatograms were measured. Diphenyl and *o*-phenyl phenol were determined from the calibration curves by the internal standard method.

TABLE I. ANALYTICAL CONDITION OF GAS CHROMATOGRAPHY FOR DIPHENYL AND *o*-PHENYLPHENOL IN CITRUS FRUITS

Model	Shimadzu GC-5APFE
Column	10% FFAP
Support	Chromosorb W (AW. DMCS.)
Mesh	60~80
I. D. × Length	3 mm × 1 m
Carrier gas	Nitrogen
Flow rate	40 ml/min
Injection temp.	230°C
Column temp.	0~8 min; 150°C 8~12 min; Increased at the rate of 15°C/min 12~18 min; 210°C
Detector	FID
Sensitivity	100 Mohms
Range	0~6 min; 0.16 V 6~18 min; 0.02 V
Sample size	5 µl

Recovery tests. Diphenyl and *o*-phenylphenol were added to 9 kinds of citrus fruits and recovered from them. More than 200 g of citrus fruits was pulped. A blank test was carried out on 50 g of the pulp and the remaining three portions of 50 g each were used for the added recovery test. To each portion 70 ppm (3.5 mg) of diphenyl and 10 ppm (0.5 mg) of *o*-phenylphenol were added. Extraction and gas chromatography were performed as described above. The recovery was determined from the difference between the amount of compound found and that found in the blank test.

RESULTS AND DISCUSSION

Gas chromatography

Many kinds of gas chromatographic columns have been reported to analyze diphenyl and *o*-phenylphenol.^{2-7, 10-12} Most of the reported columns were not able to determine concurrently diphenyl and *o*-phenylphenol. Beerneart³⁾ reported that diphenyl and *o*-phenylphenol could be determined by 1% Silicone SE-30 column, but 1 hr was necessary to carry out one gas chromatographic analysis, and each peak was not completely separated. However, by 10% FFAP column, each peak of diphenyl, *o*-phenylphenol and an internal standard was completely separated from other peaks in citrus fruits extract.

Under isothermal conditions gas chromatography of these compounds required much times, so that it was carried out under a temperature increasing condition (Table I). As shown in Fig. 2, diphenyl was detected at 150°C and *o*-phenylphenol was detected at 210°C. Diphenyl, *o*-phenylphenol and anthracene were identified by their retention times which were 4.2, 14.0 and 15.4 min, respectively. In this method, the whole procedure was completed within 2.5 hr.

Tolerances of diphenyl and *o*-phenylphenol in citrus fruits are 70 ppm and 10 ppm, respectively,

in Japan. Other foreign countries have established tolerances almost as much as those of Japan. As there were the difference between the concentration of diphenyl and *o*-phenylphenol, the peak of diphenyl was too much higher than that of *o*-phenylphenol at the same detector range. When a digital integrator was used, peak calculation was effected in such a condition. Without a digital integrator, peak calculation was also performed when the detector range was changed from 0.16 V to 0.02 V after the appearance of diphenyl peak.

Calibration curves of diphenyl and *o*-phenylphenol are shown in Figs. 3 and 4. Figure 3 was obtained from peak area method with a digital integrator and Fig. 4 was obtained from peak height method without a digital integrator. To compare the accuracy of peak area method with that of peak height method, a sample solution which contained 70 ppm of diphenyl and 10 ppm of *o*-phenylphenol was analyzed by each method repeatedly 6 times. As shown in Table II, the coefficients of variation (C.V.) of peak area method were 0.72% for diphenyl and 1.04% for *o*-phenylphenol. The C.V. of the peak height method were 0.76% for diphenyl and 0.62% for *o*-phenylphenol. The accuracy of the peak height method was not

FIG. 2. Gas Chromatogram of Diphenyl, *o*-Phenylphenol and an Internal Standard under the Conditions of Table I.

DP, diphenyl; OPP, *o*-phenylphenol; IS, anthracene.

FIG. 3. Calibration Curves for Diphenyl and *o*-Phenylphenol by Peak Area Method.

DP, diphenyl; OPP, *o*-phenylphenol; IS, anthracene.

FIG. 4. Calibration Curves for Diphenyl and *o*-Phenylphenol by Peak Height Method.

DP, diphenyl; OPP, *o*-phenylphenol; IS, anthracene.

TABLE II. COMPARISON OF METHODS FOR PEAK CALCULATION IN GAS CHROMATOGRAPHY

The sample solution contained 70 ppm of diphenyl and 10 ppm of *o*-phenylphenol was analyzed repeatedly 6 times.

Method		Diphenyl	<i>o</i> -Phenylphenol
Peak height	Ratio ^a	1.18	0.838
	S. D.	0.0089	0.0052
	C. V. (%)	0.76	0.62
Peak area	Ratio ^b	8.11	0.761
	S. D.	0.0585	0.0079
	C. V. (%)	0.72	1.04

^a Peak height/peak height of an internal standard. Average of 6 trials.

^b Peak area/peak area of an internal standard. Average of 6 trials.

S. D.: standard deviation.

C. V.: coefficient of variation.

inferior to that of the peak area method, so that only the peak height method was used thereafter. A digital integrator was no longer necessary to use.

Recovery tests

Some gas chromatograms of citrus fruit extracts at the recovery tests are shown in Fig. 5. Table III shows the average recovery and standard deviation for three times of recovery tests. Diphenyl was recovered in the range from 90.1 to 96.9%. The recovery of *o*-

FIG. 5. Gas Chromatograms of Citrus Fruit Extracts at the Recovery Tests of Diphenyl and *o*-Phenylphenol.

Gas chromatography was performed under the conditions of Table I. Diphenyl and *o*-phenylphenol were added at the levels of 70 and 10 ppm, respectively.

DP, diphenyl; OPP, *o*-phenylphenol; IS, anthracene.

TABLE III. RECOVERIES OF DIPHENYL AND *o*-PHENYLPHENOL FROM CITRUS FRUITS

Diphenyl and *o*-phenylphenol were added at the levels of 70 ppm and 10 ppm respectively.

Citrus fruit	Recovery (%)	
	Diphenyl	<i>o</i> -Phenylphenol
Grapefruit	96.9 ± 1.2 ^a	93.0 ± 2.2 ^a
Lemon	95.3 ± 2.1	97.0 ± 5.4
Orange	94.5 ± 7.0	95.3 ± 4.9
Navel orange	91.5 ± 3.1	95.4 ± 5.1
Ponkan	93.6 ± 2.7	86.5 ± 5.4
Iyokan	90.3 ± 2.2	90.2 ± 7.7
Hassaku	90.7 ± 4.9	99.3 ± 6.7
Unshu mikan	90.1 ± 1.3	97.2 ± 2.5
Amanatsu mikan	92.7 ± 3.2	90.1 ± 5.4

^a Average recovery ± standard deviation for three times.

phenylphenol was in the range from 86.5 to 99.3%.

Diphenyl and o-phenylphenol residues in citrus fruits

Table IV shows diphenyl and *o*-phenylphenol residues found in 54 citrus fruits of 9 kinds. Diphenyl and *o*-phenylphenol were detected in grapefruits, lemons and oranges. These compounds were not detected in navel oranges,

TABLE IV. DIPHENYL AND *o*-PHENYLPHENOL RESIDUES FOUND IN CITRUS FRUITS

Citrus fruit	Number	Diphenyl (ppm)	<i>o</i> -Phenylphenol (ppm)
Grapefruit	1	3.5	3.0
	1	10	ND
	1	13	ND
	1	23	ND
	1	24	ND
	1	30	ND
	1	39	4.8
	1	50	ND
Lemon	3	ND	ND
	1	4.0	4.4
	1	5.2	2.8
	1	11	4.7
	1	12	3.9
	1	17	2.0
	1	30	4.0
Orange	1	6.4	4.2
	1	10	3.7
	1	15	5.4
	1	16	3.0
	1	24	ND
	1	37	3.0
	1	58	ND
Navel orange	5	ND	ND
Ponkan	5	ND	ND
Iyokan	5	ND	ND
Hassaku	5	ND	ND
Unshu mikan	5	ND	ND
Amanatsu mikan	5	ND	ND

ND: not detected. Both detection limits of diphenyl and *o*-phenylphenol were 1 ppm.

ponkan, iyokan, hassaku, unshu mikan and amanatsu mikan at the both detection limits of 1 ppm. There was no sample that exceeded the Japanese tolerances. But there were 2 grapefruits, 6 lemons and 5 oranges from which both diphenyl and *o*-phenylphenol were detected.

GC-MS study

The comparison of standard spectra of diphenyl, *o*-phenylphenol and anthracene with the spectra of those taken from citrus fruit extracts showed that the interference of overlapping peaks could be neglected.

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