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Note

Microdetermination of *o*-phenylphenol in citrus fruits by gas–liquid chromatography

NORIHIDE NOSE, SUSUMU KOBAYASHI, AKIO TANAKA, AKIKO HIROSE and AKINOBU WATANABE

Saitama Institute of Public Health, 639-1, Kamiookubo-higashi, Urawa, Saitama (Japan)

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Biphenyl (BP), *o*-phenylphenol (OPP) and thiabendazole (TB) are used as preservatives for citrus fruits (or as fungicides) but only BP is allowed legally as a food additive in Japan.

Imported fruits sometimes contain OPP or TB, and about 10 ppm of OPP has been found in imported citrus fruits, grapefruits and lemons. OPP can be determined by gas–liquid chromatography (GLC)[1–4], thin-layer chromatography (TLC)[5,6], and high-performance liquid chromatography (HPLC)[7]. A flame-ionization detector (FID) is usually used in GLC but Hahn and Their[3] used an electron-capture detector (ECD) after brominating OPP. An FID is adequate for the determination of OPP at the level used as preservatives, but residual or impregnated OPP in fruits needs a more sensitive detector such as an ECD.

Phenols and benzoyl chloride react well in an aqueous medium and, in order to prepare phenol derivatives that would be detected with greater sensitivity by the ECD, PFB-Cl was reacted with OPP and optimal conditions were established.

EXPERIMENTAL

Reagents

Pentafluorobenzoyl chloride was obtained from Aldrich, (Milwaukee, Wisc., U.S.A.). All solvents and reagents were of analytical grade and were used as received. The extraction solvent contained heptachlor epoxide as the internal standard in *n*-heptane, accurately weighed to contain 1.6 μg/ml. The dialysis solution was prepared by dissolving 33 g of anhydrous sodium acetate and 200 g of sodium chloride in distilled water and making the volume up to 1000 ml.

Procedure

Weigh accurately 20 g of homogenized sample, add 50 ml of dialysis solution, add 80-ml of *n*-hexane and mix in an electric blender. After centrifugation, remove the hexane layer, add a further 80 ml of *n*-hexane extract the aqueous layer, combine the hexane layers and concentrate to 5 ml with a K-D concentrator. Extract the organic layer with two 5-ml portions of 0.1 *N* sodium hydroxide solution, combine the extracts, neutralize it with 1 *N* hydrochloric acid (2 ml) and add sodium hydrogen carbonate powder so that the final concentration becomes approximately 1%. Then add 10 μl of PFB-Cl, shake vigorously for 30 sec and allow to stand for 10 min at room temperature. Add exactly 5 ml of the extraction solution and shake vigorously for 1 min. The extract is subjected to GLC after dehydration.

Gas–liquid chromatography

A Shimadzu GC-4CM gas chromatograph equipped with an electron-capture detector was fitted with a U-shaped glass column (2 m × 3 mm O.D.), packed with 5% DEGS on Gas-Chrom Q, GC grade, pre-conditioned at 240° for 20 h. The temperatures used were: column, 190° (isothermal); injection and detector, 210°. The gas (nitrogen) flow-rate was 30 ml/min.

RESULTS AND DISCUSSION

Phenol benzoate is formed by the Schotten–Baumann reaction[8] from phenol and benzoyl chloride in the presence of sodium hydroxide. In the reaction of OPP and PFB-Cl, the pH conditions of the reaction media were examined. Sodium hydroxide, sodium carbonate and sodium hydrogen carbonate were used and the reactions were carried out between pH 8 and 14. The results are shown in Fig. 1. The reaction rate reached a maximum at pH 12 when sodium hydroxide was used, but the yield was inadequate (32%). When sodium carbonate was used, the yield of the product was 20% at pH 9, and increased to 97% at pH 10. However, at pH 11 the yield was lower. The reaction proceeded very well in sodium hydrogen carbonate solution and the reaction was therefore performed in a 1% solution.

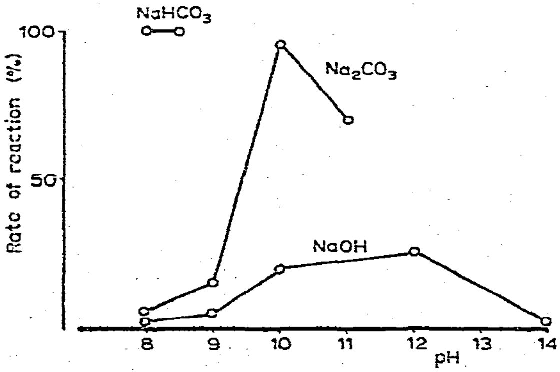

Fig. 1. Effect of pH on rate of reaction.

The yields of the product at various temperatures were compared; when the temperature exceeded about 50° the yield decreased, and good yields were obtained at room temperature and below. The reaction time was also varied at 5, 10, 20 and 30 min, and it was found that the reaction proceeded to completion within 5 min and the yield had not altered at the end of the 30-min period. The time of reaction was therefore set at 10 min.

The amount of PFB-Cl required for reaction with OPP was examined. To 100 and 1 μg of OPP, various amounts of PFB-Cl were added separately (2, 5 and 10 μl). The results are shown in Fig. 2. No undesirable effect was observed when excess of PFB-Cl was used, and 2 μl of PFB-Cl were found to be sufficient for use with both 1 and 100 μg of OPP. The permitted limit of OPP within the E.E.C. is 12 ppm, so that 10 μl of PFB-Cl is considered to be sufficient for most samples.

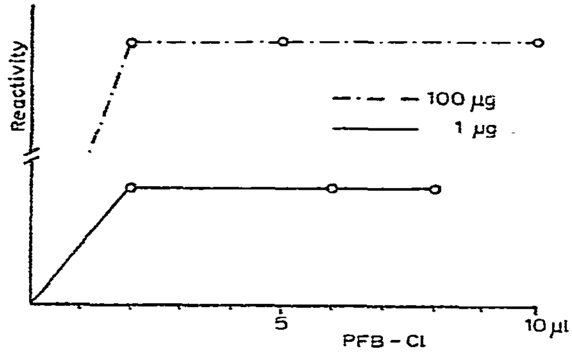

Fig. 2. Effect of amount of PFB-Cl on reactivity.

Solvents for the extraction of the reaction product were examined, and it was found that cyclohexane, *n*-hexane, benzene, *n*-heptane and ethyl acetate all had the same extraction efficiency. No disturbance to the gas chromatogram was found with any of these solvents, but a non-polar solvent was preferable because certain essential oils in fruits are not extracted. *n*-Heptane was used as it separates from water rapidly, and it is not miscible with water; shaking for 1 min was sufficient for extraction.

Unstable reaction products may affect on the accuracy of the assay, so that the stability of the products was examined. No change in the reaction products was observed when they were examined after 5, 15, 30 and 60 min and 10 h.

The reaction product was assumed to be the benzoate formed between OPP and PFB-Cl, but it is possible that the benzoate might have been decomposed during the GLC process, so the identity of the product was therefore confirmed by GC–MS. The mass spectrum is shown in Fig. 3. The molecular ion occurs at *m/e* 364 and the fragment of OPP ($C_{12}H_9O$–) is *m/e* 169 (M — 195) which corresponds to the loss of *m/e* 195 (C_6F_5CO–), indicating that the reaction product is *o*-phenylphenol pentafluorobenzoylester.

DEGS, QF-1, OV-17 and SE-30 were compared as the GLC column packing material. It was found that DEGS shown good separation from contaminating substances, while QF-1, OV-17 and SE-30 gave overlapped peak of OPP and interfering substances. DEGS was therefore selected.

The sensitivity of detection was extremely good as an ECD was used for the detection of OPP, and picogram amounts of OPP could be detected. A calibration graph for the determination of OPP was constructed with five points (from 0.1 to 1 ppm), and for the determination of the residual level the detector sensitivity was set back to the normal condition and five points (from 0.01 to 0.1 ppm) were used for the calibration graph (in this instance, the extraction solvent was diluted 8-fold with *n*-

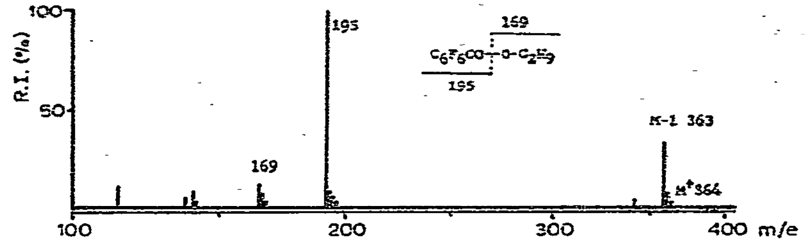

Fig. 3. GC–MS spectrum of reaction product.

heptane). The linear ranges of the calibration graph were from 0.1 to 1.2 ppm in the former and from 0.01 to 0.12 ppm in the latter instance. The results are shown in Fig. 4.

Quantitative determinations by GLC were made by using an internal standard (heptachlor epoxide) and comparing relative peak heights. The internal standard is excluded from the sample during alkaline extraction, even if it is present in sample.

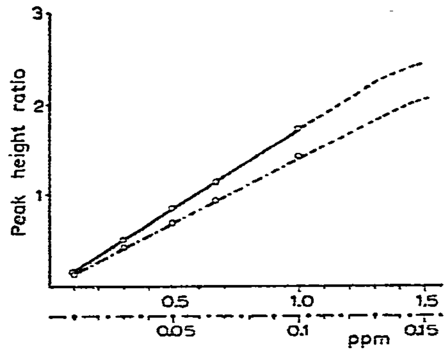

Fig. 4. Calibration graph for *o*-phenylphenol.

If the amount of hydrochloric acid exceeds that required for the neutralization of the reaction medium in the reaction of OPP and PFB-Cl, the subsequent reaction does not proceed well, so that precise neutralization is required.

The recovery of the sample was examined. Tonogai and co-workers[9,10] used ethyl acetate as the extraction solvent when BP and TB were determined simultaneously with good recovery. In our work *n*-hexane was used in order to exclude interfering substances such as certain essential oils, and good results were obtained, as shown in Table I.

TABLE I

RECOVERY OF OPP ADDED TO LEMON FRUITS

Results are mean values of five experiments.

Amount added (μg)	*Amount found (μg)*	*Recovery (%)*
0	2.12	—
1.0	3.02	90.0
5.0	6.88	95.2
10	11.9	97.8

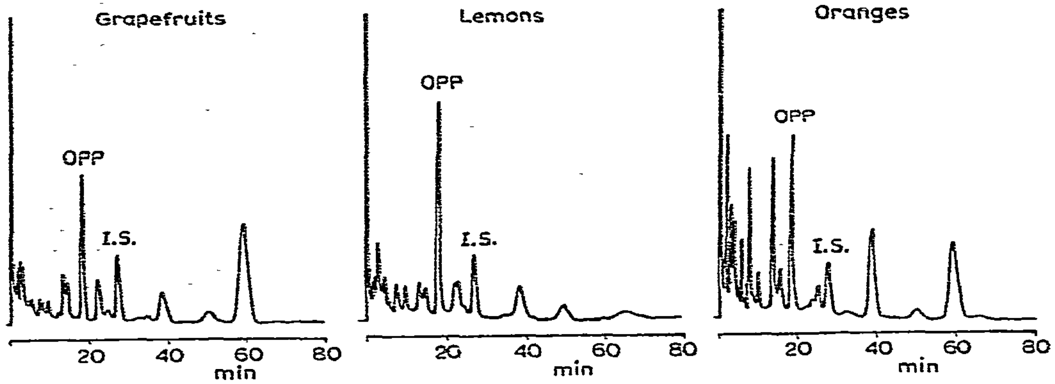

Fig. 5. Gas chromatograms of reaction products of extracts from citrus fruits. I.S. = internal standard.

OPP in lemon, orange and grapefruit was determined, and no interfering materials were found. Much more contaminating materials are present in orange than in lemon and grapefruit. The gas chromatograms are shown in Fig. 5.

It can be concluded from the results that the micro-determination of OPP in citrus fruits can be achieved by forming a derivative with PFB-Cl and using a sensitive ECD, to the extent of as low as 0.005 ppm OPP in solution.

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