

CHROM. 9631

Note

Determination of thiabendazole by electron-capture gas-liquid chromatography after reaction with pentafluorobenzoyl chloride

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(Received July 13th, 1976)

Thiabendazole [2-(4'-thiazolyl)benzimidazole; TBZ] is used as an antifungal agent for oranges and bananas, in a similar manner to biphenyl and *o*-phenylphenol. It is also used as an agricultural chemical.

Ultraviolet (UV) spectrometric¹⁻³, colorimetric⁵, fluorimetric⁶⁻⁸ and gas-liquid chromatographic (GLC)^{3,9,10} methods have been used for the determination of TBZ.

In this work, the GLC of the derivative formed by reaction of TBZ with pentafluorobenzoyl chloride (PFB-Cl; Aldrich, Milwaukee, Wisc., U.S.A.) using an electron-capture detector (ECD) was studied.

EXPERIMENTAL

Procedure

Weigh approximately 20 g of a sample, add 80 ml of ethyl acetate and 50 ml of sodium acetate-sodium chloride solution (33 g of anhydrous sodium acetate, 200 g of sodium chloride and water to 1000 ml), homogenize the sample well, centrifuge the mixture for 5 min and separate the organic layer. Repeat the procedure, combine the organic layers and wash with 50 ml of water. Then extract twice with 50 ml of 0.1 *N* hydrochloric acid and adjust the pH of the extract to 9-10 with *ca.* 20 ml of 1 *N* sodium hydroxide solution. Transfer the aqueous solution into a separating funnel and extract twice with 40-ml portions of ethyl acetate. Dry the organic layer with anhydrous sodium sulphate and remove the solvent with a Kuderna-Danish (KD) concentrator equipped with 5-ml KD test-tubes. Add 0.5 ml of benzene, 10 μ l of PFB-Cl and *ca.* 2 mg of anhydrous sodium carbonate to the residue, close the tube with a tightly fitting ground-glass joint and then seal the joint with Parafilm (American Can Co.). Immerse the half of the test-tubes in a paraffin oil bath at 120° and allow the contents to react for 30 min with occasional shaking.

After the reaction, remove the solvent by means of a water aspirator at 50°. If any odour of PFB-Cl is detected, blow air through the sample. Add 0.4 ppm of decachlorobiphenyl (internal standard) in 1 ml of acetone, then subject the sample to GLC.

Gas-liquid chromatography

A Shimadzu GC-4CM gas chromatograph was used, equipped with an electron-capture detector (^{63}Ni , 10 mCi) and a 1.5 m \times 3 mm I.D. glass column packed with 5% OV-101 on Gas-Chrom G(HP) (80-100 mesh). The temperature of the injection port and detector was 270° and that of the column 230°. Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min.

Mass spectrometry (GC-MS)

An LKB-9000 combined gas chromatograph-mass spectrometer was used, with a glass column (1 m \times 3 mm I.D.) packed 5% OV-101 on Gas-Chrom G(HP) (80-100 mesh); the column temperature was at 230°. The carrier gas (helium) flow-rate was 30 ml/min, separator temperature 260°, ion source temperature 290°, electron energy 70 eV, accelerating voltage 3.5 kV and flash heater temperature 270°.

Synthesis of *N*-pentafluorobenzoylthiabenzazole (PFB-TBZ)

Dissolve 1.2 g of TBZ in 10 ml of acetonitrile, add 1.5 g of PFB-Cl and reflux the mixture on a boiling water-bath for 4 h. After cooling, filter off the deposited crystals, mostly unreacted TBZ, and wash with benzene. The combined filtrate and benzene washings are extracted with ethyl acetate after adding twice the volume of water acidified with hydrochloric acid. Dry the extract with anhydrous sodium sulphate and remove the solvent with a rotary evaporator. Then add 20 ml of benzene-methanol (18:3) to dissolve the residue and pass the solution through a column of activated alumina (10 cm long \times 10 mm I.D.). Concentrate the eluted solution and apply the concentrate to a silica gel HF₂₅₄ (Merck, Darmstadt, G.F.R.) thin-layer chromatographic (TLC) plate. Carry out TLC with benzene-methanol (18:3), and after developing, scrape off the silica gel corresponding to $R_f = 0.86$, extract it with ethanol and recrystallize the crystals obtained after evaporation of the solvent to give PFB-TBZ.

RESULTS AND DISCUSSION

The sample size is an important factor in microanalysis: when a micro-determination is not needed, the sample size may be less because the amount of interfering material may not be sufficient to give erroneous results, but in microanalysis, the sample size must be larger, and a 20-g sample was used in this study.

The extraction of TBZ from the sample was followed by Mihara *et al.*'s method⁹. A benzene solution (0.5 ml) containing 1 ppm of TBZ and 10 μl of PFB-Cl were added to a 5-ml KD test-tube, the reaction was carried out in range 25-150° for 30 min and the formation of the product was examined. Anhydrous sodium carbonate (*ca.* 2 mg) was added to the reaction mixture of PFB-Cl and TBZ, as it resulted in a 15% increase in the yield of PFB-TBZ. The reaction proceeded well up to 120° and then reached maximum yield. Reaction at 150° in a paraffin oil bath did not give better results and therefore it was decided to use 120° for the reaction.

A benzene solution (0.5 ml) of TBZ was placed in a 5-ml KD test-tube, and the reaction was performed for 5-60 minutes. The reaction reached a maximum yield in 15 min and became constant, so the reaction time was set at 30 min.

After heating, the solvent was removed by distillation under reduced pressure.

When the solvent was removed gradually, the sample became concentrated and further reaction might have occurred, and therefore the evaporation should be carried out rapidly and carefully. A reduced pressure of 12 mm Hg gave the best results.

Different solvents for the reaction were examined. Pyridine, acetonitrile, ethanol, acetone, ethyl acetate, *n*-hexane and benzene were used to prepare 1 ppm TBZ solutions, and 0.5 ml of each solution was used in the reactions for comparison. It was found that reaction in benzene was the best, followed by pyridine. Reaction in pyridine, however, yielded considerable amounts of side-products, so that its use was excluded. On the other hand, TBZ was not very soluble in benzene, but as no better solvents were available benzene was selected as the solvent for the reaction. The low solubility of TBZ in benzene was partially overcome by occasional shaking of the reaction mixture during the reaction.

If the odour of PFB-Cl was detectable after the solvent had been removed, air was blown through the sample. No loss of the product resulted if this process was performed below 50°.

The amount of reagent used was decided as follows. The amount of PFB-Cl necessary for the reaction with TBZ was examined, varying it from 1 to 20 μ l for 0.5 and 20 μ g of TBZ. The results are shown in Fig. 1. The yield of the products was maximal when 5 μ l of PFB-Cl was used with either 0.5 or 20 μ g of TBZ. An excess of PFB-Cl might affect the sensitivity of detection in GLC, and 10 μ l of PFB-Cl was therefore used in this work. Impurities in the sample will not affect the analytical results under the conditions used.

The reaction products were analyzed by elemental analysis and GC-MS. Separately synthesized PFB-TBZ ($C_{17}H_6N_3O_1S_1F_3$) showed the following composition: calculated, C 51.56%, H 1.31%, N 10.49%; found, C 51.64%, H 1.52%, N 10.63%.

Fig. 2 shows the mass spectrum of the substance with the direct inlet system. The GC-MS spectrum of the reaction product obtained agreed completely with the above compound. The product was stable for more than 5 h in acetone.

A linear calibration graph was obtained with 0.05–0.5 ppm of TBZ, as shown in Fig. 3. The limit of detection was 0.01 ppm. By altering the sensitivity selecting, 0.5–5 ppm of TBZ was linearly calibrated. Various internal standards were tried, but most of them disturbed the PFB-TBZ peak; decachlorobiphenyl was eventually selected.

DEGS, QF-1, DC-200, SE-30, OV-1, OV-17 and OV-101 were tried as GLC

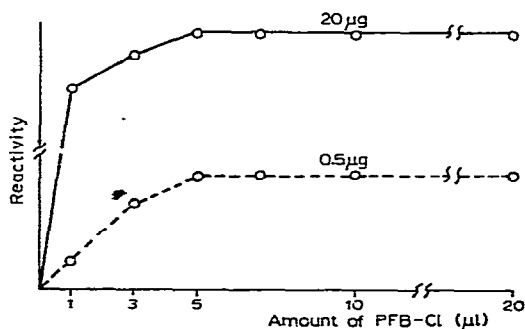


Fig. 1. Effect of the amount of PFB-Cl used with 0.5 and 20 μ g of TBZ.

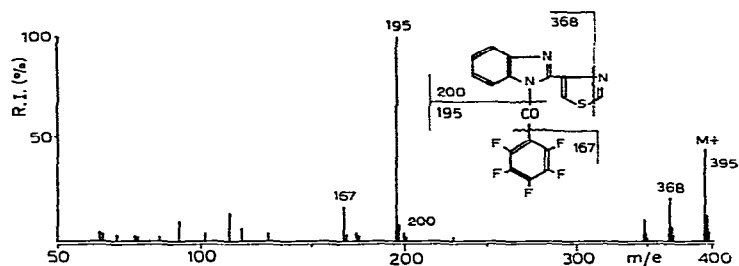


Fig. 2. Mass spectrum of synthesized pentafluorobenzoylthiabenzazole with the direct inlet system.

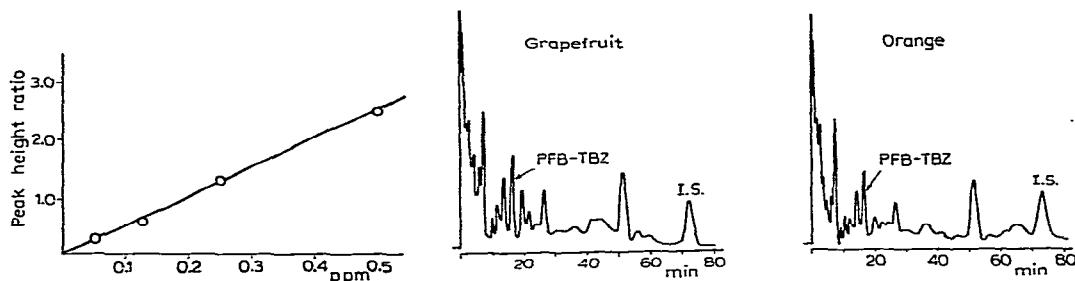


Fig. 3. Calibration graph of thiabendazole.

Fig. 4. Gas chromatograms of reaction products of extracts from grapefruit and orange. I.S. = internal standard.

column packings. The sensitivity and separation were found to be good with OV-101 and OV-17; DC-200 gave a good separation but poor sensitivity. OV-101 was eventually selected as the column packing. A gas chromatogram of extracts from oranges and grapefruits obtained on this column is shown in Fig. 4.

CONCLUSION

Benzene was the best solvent for the derivatization reaction. A volume of PFB-Cl of $10 \mu\text{l}$ in 0.5 ml of benzene was found to be sufficient. A linear calibration graph was obtained from 0.05 to 0.5 ppm of TBZ. The minimum detectable amount was 0.01 ppm of TBZ.

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