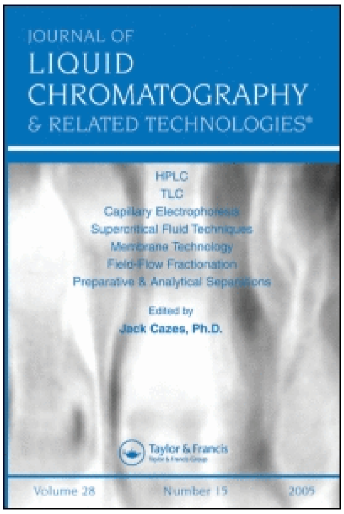

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Simultaneous Determination of Fungicide Residue in Citrus

Noboru Motohashi[a]; Hideo Nagashima[b]; Roger Meyer[c]
[a] Department of Medicinal Chemistry, Meiji College of Pharmacy, Tokyo, Japan [b] Setagaya-ku Research Laboratory of Public Health, Tokyo, Japan [c] Herbert Laboratories, Santa Ana, California

To cite this Article Motohashi, Noboru , Nagashima, Hideo and Meyer, Roger(1990) 'Simultaneous Determination of Fungicide Residue in Citrus', Journal of Liquid Chromatography & Related Technologies, 13: 2, 345 — 355

To link to this Article: DOI: 10.1080/01483919008049548

URL: http://dx.doi.org/10.1080/01483919008049548

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(2), 345-355 (1990)

SIMULTANEOUS DETERMINATION OF FUNGICIDE RESIDUE IN CITRUS

NOBORU MOTOHASHI[1], HIDEO NAGASHIMA[2],
AND ROGER MEYER[3]
[1]*Department of Medicinal Chemistry*
Meiji College of Pharmacy
1-22-1, Yato-cho, Tanashi-shi
Tokyo 188, Japan
[2]*Setagaya-ku Research Laboratory of Public Health*
3-11-1, Kitakarasuyama, Setagaya-ku
Tokyo 157, Japan
[3]*Herbert Laboratories*
1202 E. Wakeham
Santa Ana, California 92715

ABSTRACT

The selective determination of three common, yet toxic fungicides (diphenyl, o-phenylphenol and thiabendazole) was investigated with reversed-phase high-performance liquid chromatography (RP-HPLC). These fungicides were successfully chromatographed and quantitated by utilizing a reversed-phase Unisil Q C8 column. An acetonitrile-1%w/v H_3PO_4 (58:42) mobile phase was used. The fungicides at post-harvest were detected with fluorescence detection at typical residue levels on citrus. The practical quantitation limits of diphenyl, o-phenylphenol and thiabendazole were 0.1, 0.1 and 0.005 mg/kg, respectively. The recoveries were between 90.0 and 97.3%.

INTRODUCTION

Diphenyl (DP), o-phenylphenol (oPP), and thiabendazole (TBZ) (Figure 1) are used as post-harvest fungicides on citrus. Unfortunately, safety issues have been raised because of the use of these chemicals. DP has reported carcinogenicity problems (1-4). The same has been reported for oPP (5-6). Furthermore, TBZ has reported toxicological (7-13), mutagenicity (14-19), carcinogenicity (20-24) and teratogenicity problems (25). Therefore, the need to monitor these fungicides when used on citrus is evident.

Chromatographic techniques for simultaneous determination of DP, oPP, and TBZ have been reported by high-performance liquid chromatography (HPLC) (26-29). These separation methods have one difficulty in that there is poor sensitivity for the TBZ. To improve the sensitivity of TBZ, various mobile phases and fluorescence detection of TBZ were investigated by reversed-phase HPLC.

Experimental

A. Apparatus

A Shimadzu LC-3A liquid chromatograph (Shimadzu, Kyoto, Japan) with a syringe pump was used. Sample injection was performed by a Rheodyne injector (Model 7125 with a 20microliter

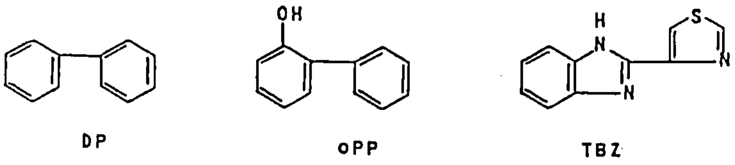

Figure 1. Diphenyl, o-phenylphenol, and thiabendazole.

loop connected directly to the column). The detector was a Hitachi fluorescence spectrophotometer (Model F-3000 with a 150 watt xenon lamp and LC micro flow cell unit with a cell volume of 18 microliters). The following additional equipment was also used: juicer (Mitsubishi Model JM-1200), autohomogenizer with stainless steel cup (EXCEL, Nihon Seiki, Ltd, Tokyo, Japan), and rotary vacuum evaporator (Tokyo Rikakikai Model N-2, Tokyo, Japan).

HPLC conditions: Column-Unisil Q C8 (5 micron, Gasukuro Kogyo, Tokyo, Japan), 250 x 4.0 mm i.d. Mobile phase-acetonitrile-1%w/v H_3PO_4 (58:42). Column temperature-40°C. Flow rate-1.0ml/min. Fluorescence wavelengths-excitation (Ex) 245 nm-emission (Em) 330 nm. When low levels of TBZ were expected (less than 1 mg/kg citrus), the Ex and Em were set at 302 and 350 nm, respectively, to improve sensitivity. Sample size-5 microliters.

B. Reagents

All solvents and chemicals were reagent grade. DP, oPP, phosphoric acid and anhydrous disodium hydrogen phosphate were from Wako Pure Chemical (Osaka, Japan). Acetonitrile, 2-ethoxyethyl acetate and anhydrous sodium sulfate were from Kanto Chemical (Tokyo, Japan). TBZ, sodium 1-pentanesulfonate, and sodium 1-hexanesulfonate were from Tokyo Kasei (Tokyo, Japan). Sodium 1-dodecanesulfonate and sodium laurylsulfate were from Nakarai Chemical (Osaka, Japan).

Standard solutions: In one 100ml volumetric flask weigh 0.100 g each of DP, oPP, and TBZ. Dissolve and q.s. in a solution of n-hexane-99.5% ethanol-methanol (1:1:1) (Hexane-EtOH-MeOH). Dilute the primary standard solution (with Hexane-EtOH-MeOH) to prepare solutions containing 0.5, 1.0, 2.0, 5.0, and 10.0 micrograms DP, oPP, and TBZ/ml.

C. Preparation of sample extracts

Obtain a representative sample containing 5-10 individual fruit. Slice each of them to 16 parts. Randomly select 20 to 40 parts and homogenize with the juicer. Weigh 10 g of homogenated mixture into a blender cup, and add 3 g of anhydrous Na_2HPO_4, 10g of anhydrous Na_2SO_4, and 80 ml of ethyl acetate. Extract by homogenizing for 10 min at 7000 rpm, then filter supernatant through filter paper (Toyo Filter paper No. 5B, Tokyo, Japan) into a 300 ml round bottom flask. Homogenize precipitate twice with additional 80 ml aliquots of ethyl acetate and then filter. Add 5 ml of 2-ethoxyethyl acetate to the combined ethyl acetate extracts, and concentrate to about 5 ml on the rotary vacuum evaporator (40°C water bath). Quantitatively transfer the concentrated solution to a 20 ml volumetric tube and q.s. with Hexane-EtOH-MeOH. Mix and then filter through a 0.45 micron filter.

RESULTS AND DISCUSSION

Figure 2 presents the capacity factors (*k'*) of the three fungicides as a function of the varied mobile solvent. Optimazation of eluent composition within ten minutes gives a solvent ratio of 58:42 (acetonitrile-1% w/v H_3PO_4).

Burzi, et al. (30) have extracted with 0.1N HCl as a clean-up procedure for TBZ and chromatographed it on an RP-8 column. Mourot. et al. (31) have separated TBZ by reversed-phase elution with acetonitrile-1% H_2SO_4. Fluorescence spectroscopy was used for the sensitive detection of TBZ (32). Figure 3 shows the correlation between the pH of the mobile phase and peak height with the use of fluorescence detection. The pH of the mobile phase solutions was adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 with 30% NaOH. Although the retention times of DP, oPP and TBZ were almost constant within the pH range of 2.3 to 6.0, the peak height of TBZ itself changed greatly in this range. This demonstrates that a mobile solvent of around pH 2.3 to 3.0 maximizes the sensitivity for the determination of TBZ.

The addition of various ion-pairing reagents to the mobile phase was tried to improve the chromatography. Figure 4 shows the effects of these reagents on the three fungicides. As can be

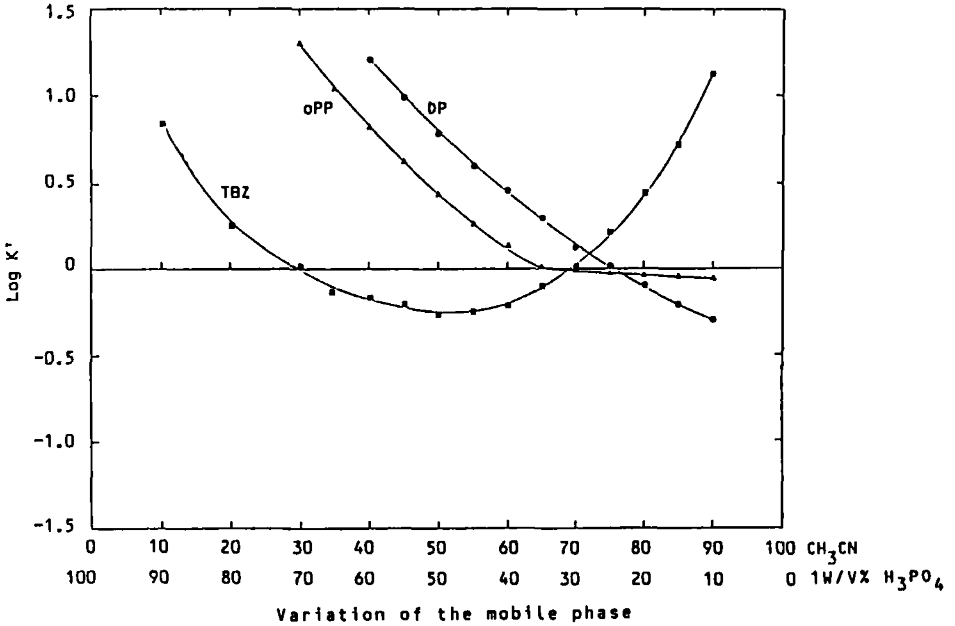

Figure 2. Variation of logarithm of capacity factor *k'* for DP, oPP and TBZ as a function of the mobile phase.

seen, very little change in retention times was noted. Consequently, this idea was not continued.

From the above experiments, practical applications were studied for the separation of fungicide mixtures on Hassaku oranges. Figure 5 (A) is a chromatogram of 5 micrograms each of DP, oPP and TBZ/ml. Figure 5 (B) is a chromatogram of extracted , fungicide-free Hassaku oranges. Other untreated fruits (i.e., Satsuma mandarin kunquat and lemon) were analysed to check the background interference from natural substances. No peaks were noted to interfere with the determination of the fungicides. Figure 5 (C) is a chromatogram of 10 mg/kg of the fungicides added to Hassaku oranges.

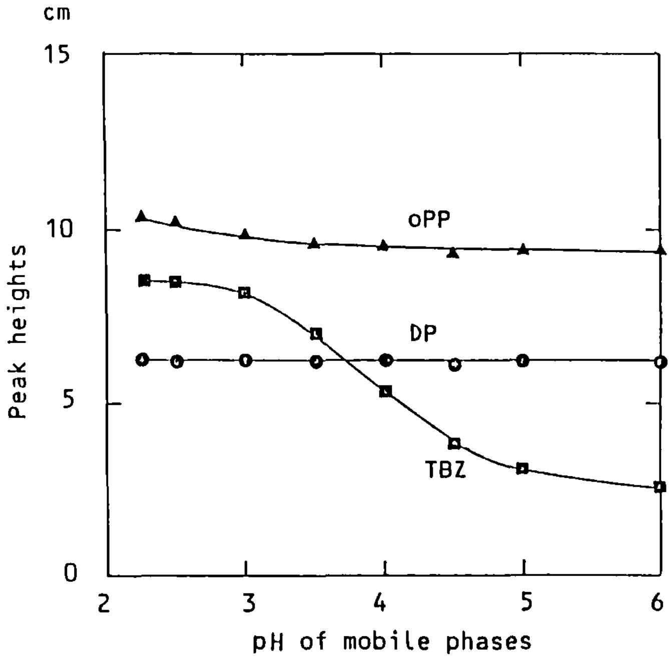

Figure 3. Change in peak height as a function of pH in the mobile phase, acetonitrile-1%w/v H_3PO_4 (58:42).

TABLE 1
Recovery and Coefficient of Variation (c.v.) of DP, oPP and TBZ from Hassaku Orange.

Added	TBZ		oPP		DP	
mg/kg	Recovery[a] (%)	c.v. (%)	Recovery[a] (%)	c.v. (%)	Recovery[a] (%)	c.v. (%)
1.0	97.3	1.0	93.6	1.6	93.5	1.8
5.0	93.2	1.2	92.0	1.2	91.5	2.2
10.0	91.0	2.5	92.4	2.9	94.1	2.6
20.0	92.4	2.3	90.0	1.8	90.5	1.6
50.0	96.0	0.7	93.3	1.2	94.6	2.6
100.0	95.5	2.4	91.7	2.0	93.2	2.6

[a] Average of five trials.

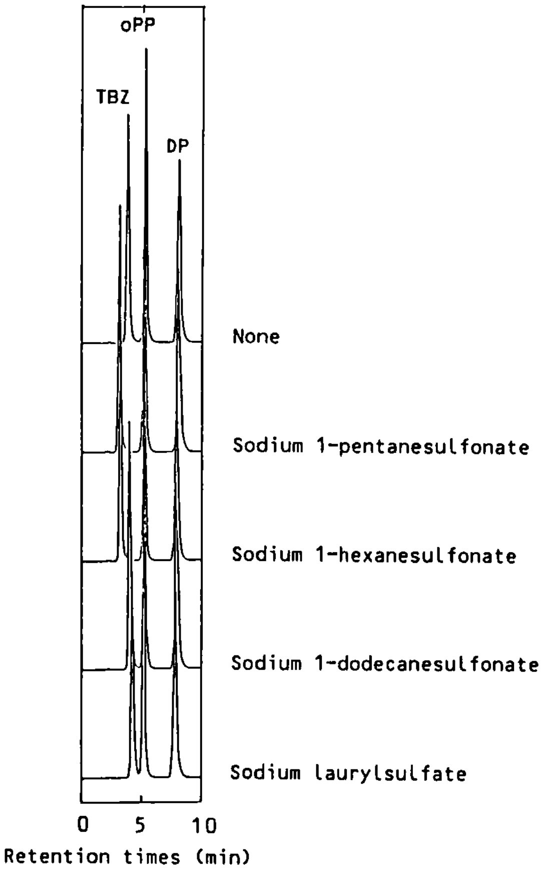

Figure 4. The effect of ion-pair reagents on retention time (min).

Percent recoveries were determined when various concentrations of fungicides were added to the Hassaku orange. (Note that all Hassaku oranges used as a control were initially free from these fungicides). The spiked levels were 1.0, 5.0, 10.0, 20.0, 50.0, and 100.0 mg/kg each of DP, oPP, and TBZ (Table 1). Table 1 presents the recovery data.

Recoveries were 90.0% to 97.3% of added fungicides.This paper presents a rapid method to separate and determine three

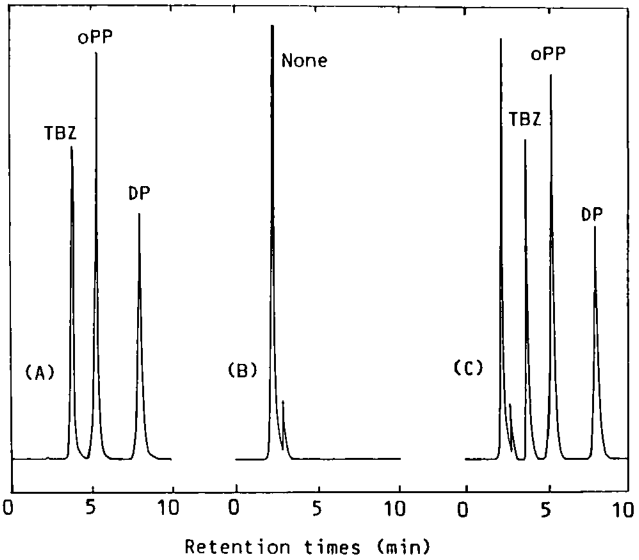

Figure 5. Chromatographic result of DP, oPP and TBZ.
(A) Chromatogram of 5 micrograms each of DP, oPP and TBZ/ml standard solution. (B) Chromatogram of the extract of Hassaku orange (fungicide-free). (C) Chromatogram of the extract of Hassaku orange in which 10 mg/kg DP, oPP and TBZ was added, respectively. RP-HPLC conditions: Type of column-Unisil Q C8 (5 micrometer, Gasukuro Kogyo) 250 x 4.0 mm i.d.. Mobile phase-acetonitrile-1%w/v H_3PO_4 (58:42). Flow rate-1.0 ml/min. Column temperature-40°C Detection-Fluorescence, Ex-245 nm, Em-330 nm.

commonly used fungicides on citrus (DP, oPP, TBZ). This reversed-phase HPLC method uses a Unisil Q C8 column and a mobile phase of acetonitrile-1%w/v H_3PO_4 (58:42). Detection is with a fluorescence detector to improve sensitivity of the method.

REFERENCES

1. Moracci, E. and Bracale, U., Hyperestrinism and uterine fibroids. An experimenral study. Arch. Ostet. ginecol., 53, 308,1948.

2. Miller, O.J. and Gardner, W.U., The role of thyroid function and food intake in experimental ovarian tumorigenesis in mice. Cancer Res., 14, 220, 1954.

3. Pecchiai, L., Saffiotti, U., Toxicity of biphenyl, diphenyl oxide, and their mixture.(Dowtherm). Med. lavoro, 48, 247, 1957.

4. Li, M.H. and Ts'ai, H.Y., Influence of hormones on carcinogenesis of the uterine cervix of mice induced by methylcholanthrene. Acta Unio. Intern. Contra Concrum., 20, 1514, 1964.

5. Boutwell, R.K. and Bosch, D.K., The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res., 19, 413, 1959.

6. Truhaut, R. and de Clercq, M., The role of nicotine and its pyrogenation derivatives in carcinogenesis. Compt. rend., 253, 1506, 1961.

7. Harry, J.R., Herbert, C.S. and Otto, E.G., The toxicologic and pharmacologic properties of thiabendazole. Toxicol. Appl. Pharmacol., 7, 53, 1965.

8. Robinson, H.J., Silber, R.H. and Graessle, O.E., Thiabendazole: toxicological pharmacological, and antifungal properties. Tex.. Rep Biol. Med., 27 (Suppl. 2), 537, 1969.

9. Danilevicius, E. and Girnius, B., Efficacy and toxicity of thiabendazole. Farmakol. Toksikol., 34 (1), 67, 1971.

10. Gladenko, V.I., Thiabendazole toxicity. Farmakol. Toksikol., 34 (4), 483, 1971.

11. Dilov, P., Chaleva, E., Dzhurov, A., Stoyanov, P., Iotsev, M., Dimtrov, K. and Mikhalkov, I., Toxicology studies of thiabendazole in mammals and birds. Vet.-Med. Nauki., 19 (3), 92, 1982.

12. Baicu, T., Toxicity of some pesticides to Trichoderma viride Pers. Crop Prot., 1, 349, 1982.

13. Elgebaly, S.A., Forouhar, F. and Dore-Duffy, P., Thiabendazole-induced suppression of renal damage in a murine model of autoimmune disease. Am. J. Pathol., 115, 204, 1984.

14. Hastie, A.C. and Georgopoulos, S.G., Mutational resistance to fungitoxic benzimidazole derivatives in Aspergillus nidulans. J. Gen. Microbiol., 67 (Pt. 3), 371, 1971.

15. Vanfleteren, J.R. and Roets, D.E., Influence of some anthelmintic drugs on the population growth of the free-living nemtodes Caenorhabditis briggsae and Turbatrix aceti (nematoda: Rhabditidia). Nematologica, 18, 325, 1972.

16. Polach, F.J. and Molin, W.T., Benzimidazole-resistant mutant derived from a single ascospore culture of Botryotinia fuckeliana. Phytopathology, 65, 902, 1975.

17. Vashishat, R.K. Vasudeva, M., and Kakar, S.N., Testing carboxin, thiabendazole and hinosan for genotoxicity on Saccharomyces cerevisiae. Haryana Agric. Univ. J. Res., 12 (2), 214, 1982.

18. Papavizas, G.C. and Lewis, J.A., Physiological and biocontrol characteristics of stable mutants of Trichoderma viride resistant to MBC fungicides. Phytopathology, 73, 407, 1983.

19. Kochman, J.K., and Langdon, P.W., Fungicide treatment of sunflower seed to inhibit germination of admixed sclerotia of Sclerotina sclerotiorum. Aust. J. Exp. Agric., 26, 489, 1986.

20. Korolkovas, A. and Tamashiro, K., Charge transfer as a mechanism of action of some chemotherapeutic agents. Rev. Farm. Bioquim, Univ. Sao Paulo, 12 (2), 109, 1974.

21. Wegman, R.C.C., Northolt, M.D. and Greve, P.A., Optimization of the determination of fungicides by bio-assay. Meded. Fac. Landbouwwet., Rijksuniv. Gent, 40 (2, Pt. 2), 1077, 1975.

22. Lovett, E.J. and Lundy, J., The effect of thiabendazole in a mixed leukocyte culture. Transplantation, 24 (2), 93, 1977.

23. Lundy, J., Lovett, E.J., Hinchliffe, D. and Schor, M., Chemoimmunotherapy of a murine fibrosarcoma: critical factors for success of combined modality therapy. J. Surg. Oncol., 9 (4), 339, 1977.

24. Layton, D.W., Mallon, B.J., Rosenblatt, D.H. and Small, M.J, Deriving allowable daily intakes for systemic toxicants lacking chronic toxicity data, Regul. Toxicol. Pharmacol., 7, 96, 1987.

25. Khera, K.S., Whalen, C., Trivett, G. and Angers, G., Teratological assessment of maleic hydrazide and daminozide, and formulations of ethoxyquin, thiabendazole and naled in rats. J. Environ. Sci. Health, Part B, B14 (6), 563, 1979.

26. Farrow, J.E., Hoodless, R.A., Sargent, M. and Sidwell, J.A., Determination of residues of post-harvest fungicides on citrus fruit by high-performance liquid chromatography. Analyst, 102, 752, 1977.

27. Gieger, U., Determination of residues of fungicide preservatives and antioxidants on fresh fruit by HPLC and automatic column control. Lebensmittelchem. Gerichtl. Chem., 40, 25, 1986.

28. Ohlin, B., A high performance liquid chromatography multiresidue method for determination of pesticides in fruits and vegetables. Vaar Foeda, 38 (Supple. 2), 111, 1986.

29. Luckas, B., A method for the determination of residues of fungicides in fruits and citrus fruits by HPLC and selective detection. Z. Lebensm.-Unters. Forsch., 184, 195, 1987.

30. Burzi, F.F., Berri, A. and Zacchetti, M., High-pressure liquid chromatographic determination of MBC and thiabendazole residues in fruit. Riv. Soc. Ital. Sci. Aliment., 10, 19, 1981.

31. Mourot, D., Boisseau, J. and Gayot, G., Separation of benzimidazole anthelmintics by high-pressure liquid chromatography. Anal. Chim. Acta, 99, 371, 1978.

32. Shim, J.S.K., Tolan, J.W. and Fink, D.W., Fluorometric determination of thiazole-containing compounds. J. Pharm. Sci., 69, 275, 1980.