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Determination of ortho-phenylphenol, diphenyl and diphenylamine in apples and oranges using HPLC with fluorescence detection

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Abstract

A reversed-phase high performance liquid chromatographic (RP-HPLC) method for the determination of the fungicides ortho-phenylphenol (OPP), diphenyl (DP) and diphenylamine (DPA) is developed. A mobile phase consisting of methanol:water (70:30) and fluorescence detection (E_x and E_m set at 285 and 340 nm, respectively) was used. The fungicide residues were extracted with ethyl acetate, and concentrated prior to the HPLC analysis. The proposed HPLC method was clearly superior as compared to an earlier report that was based on a mobile phase of acetonitrile:methanol:water (47:13:40 v/v) for the determination of DP, OPP and thiabendazole, especially with respect to lower detection limits and faster analysis times. Limits of detection were 0.005, 0.100 and 0.02 ppm for OPP, DP and DPA, respectively. Recovery studies that were done by fortifying apples and oranges at 0.25 and 0.50 ppm levels respectively, were satisfactory (average percent recoveries of 108.8 and 84.0%, respectively for apples and oranges). Results on the determination of these fungicides in several apple and orange samples will also be discussed.

Keywords: Fungicides; Ortho-phenylphenol; Diphenyl; Diphenylamine; Apples; Oranges; HPLC

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1. Introduction

A number of pre- and post-harvest fungicides are heavily used in modern agricultural practices. Diphenyl (DP) and ortho-phenylphenol (OPP) have found wide applications on citrus fruits, while diphenylamine (DPA) is commonly used to control scald in apples during storage (Allen & Hall 1980; Garrido, Alba, Jimenez, Cadado, & Folgeiras, 1998) (Fig. 1). Other major use of OPP is in the disinfection of storage material and also as fungistatic wax for coating vegetables (Appel, 2000). As consumers are becoming more and more concerned about food contaminants, and pesticide residue feature highly on their worry list, it is important that appropriate analytical methods are readily available for reliable monitoring of common food items.

HPLC with fluorescence detection has been reported for the analysis of DP and OPP (Nakazato et al., 1995; Yamazaki & Ninomiya, 1999). GC–MS have been used for the analysis of DPA and OPP (Johnson, Harsy, Geronimo, & Wise, 2001; Yu, Schoen, Dunkin, Firman, Cushman, & Fontanilla, 1997). More recently, the versatility of micellar electrokinetic chromatography has been demonstrated for the separation and determination of a few common fungicides, including OPP (Rodriguez, Pico, Font, & Manes, 2001). DPA can also be analysed by gas chromatography (GC) directly using nitrogen–phosphorus detection; or alternatively, is derivatized first before the electron capture (ECD) detection (Allen & Hall 1980; Garrido et al., 1998).

Nakazato et al. (1995) has described a HPLC method for the determination of imazalil, DP, OPP and thiabendazole in citrus fruits using fluorescence detection. The mobile phase consisted of acetonitrile/methanol/water (47:13:40) adjusted to pH 2.4 containing 0.01 M sodium dodecyl sulphate. This method has since been adopted as a routine procedure in many laboratories.

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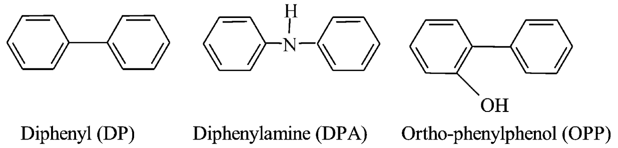

Fig. 1. Chemical structures of fungicides discussed.

However, the separation of DPA was not described in that procedure. Thus the present work describe an HPLC methodology that would be suitable for the routine determination of DP, DPA and OPP. A different mobile phase consisting of methanol and water was used instead, and as will be shown later, is capable of separating OPP, DP and DPA effectively to be used for the analysis of these fungicides in apples and oranges.

2. Materials and methods

2.1. Reagents and materials

Standard DP, DPA and OPP were obtained from Wako, Japan. HPLC grade methanol, ethyl acetate, anhydrous sodium sulphate and 1-butanol was obtained from Merck, Germany. Stock solutions of the fungicides were dissolved in methanol. Samples were homogenized using a Warring blender while a Buchi Rotavapor R-200 unit was used for the preconcentration of solvent.

2.2. HPLC

Samples were separated using a Hewlett Packard Series 1050 HPLC unit fitted with a C18 150×4.6 mm column from SGE. The mobile phase was methanol/water (70:30 v/v) and operated at 1 ml min^{-1}. The fluorescence detector was set at E_x and E_m 285 and 340 nm, respectively. Quantification of the fungicides was achieved by measuring their respective peak areas.

2.3. Samples

Apple and orange samples were bought from regular retail outlets and supermarkets around Penang, Malaysia. Five pieces of each type of fruits were randomly purchased. Each fruit was sliced into eight pieces from top to bottom. One slice from each fruit was collected, homogenized and 25 g of each type of fruit was analysed. Five grams of sodium acetate, 20 g sodium sulphate and 40 ml ethyl acetate were added to it, and homogenized for 1 min using a Warring blender. Samples were subjected to a liquid–liquid extraction using the method of Nakazato et al. (1995). The ethyl acetate phase was then filtered. Fresh ethyl acetate was then added and homogenized for a second time. Sulphuric acid (40 ml) was next added twice and was shaken for 3 mins each time, and the layers were allowed to separate. Sodium sulphate was next added to the organic phase, and was then filtered. Three millilitres of 1-butanol was added to the extract, and concentrated to 3 ml using a rotary evaporator. The concentrated extract was topped to 10 ml with methanol. Details of the entire procedure is shown in Fig. 2.

3. Results and discussion

In the work of Nakazato et al. (1995), a mobile phase consisting of acetonitrile, methanol and water (47:13:40 v/v) and fluorescence detector operated at $E_x = 285$ nm and $E_m = 325$ nm was used. In the present work, we focussed on mobile phases that eliminated the use of acetonitrile. From our investigations, it was found that the use of methanol/water (70:30 v/v) with the detector set at $E_x = 285$ nm and $E_m = 340$ nm yielded the most satisfactory separation of DP, DPA and OPP. This mobile phase is considered more advantageous as it is not only cheaper but also more environmental friendly as the use of acetonitrile and sodium dodecyl sulphate have been avoided. Furthermore, the method is more rapid as complete separation can be achieved within 11 min, while the earlier report required about 25 min. Another distinct advantage of the proposed HPLC method is the ability to separate an additional fungicide DPA from DP and OPP, a feature which was not described in any of the earlier work. Due to these seemingly favourable features of the technique, other analytical characterization of the proposed system was investigated.

Key analytical characteristics of the HPLC system when operated at the optimum conditions are summarized in Table 1. The detection limits (signal-to-noise 3) of the presently developed method is lower than that reported earlier (Nakazato et al., 1995) for both OP and OPP. Recoveries studies were done by spiking 0.25–5 ppm each of the fungicides to apples, while for oranges, the fortification level was at least 0.5 ppm. Average recoveries for apples and oranges were 108.6 and 84.0%, respectively (Table 2). It cannot be explained conclusively, at the moment, about the higher recoveries for apples, but lower values for oranges. The accuracy of the method was established by determining ($n = 16$)

Table 1
Analytical characteristics of the HPLC method

Fungicide	Detection limit, ppm	Accuracy, % error	Relative S.D., %
OPP	0.005 (0.05)[a]	8.4	0.29
DPA	0.02	3.6	0.92
DP	0.10 (0.50)[a]	3.2	3.20

[a] From Nakazato et al. (1995).

the concentrations of the fungicides in a standard mixture containing 0.5 ppm OPP, 0.5 ppm DPA and 5.0 ppm DP. The relative standard deviation was also calculated from the data. The method offers good reproducibililty and acceptable accuracy (Table 1).

The method was finally applied for the determination of OPP, DPA and DP on imported apples and oranges that are popularly consumed in Malaysia. It was found that the levels of OPP and DPA are very much lower than the legal limits in Malaysia (25 and 5 ppm, respectively) (Table 3). However, DP, which is not presently regulated in apples, ranging from 0.16 to 0.71 ppm was detected in the apple samples tested. The levels of OPP

Table 2
Recoveries of fungicides fortified to apples and oranges

Fungicide	Fortified level, ppm	Recovery, %	
		Apple	Orange
OPP	0.25	96.5	–
	0.5	119.7	84.0
DP	2.5	109.5	–
	5.0	97.7	83.0
DPA	0.25	105.0	–
	0.5	123.0	86.0

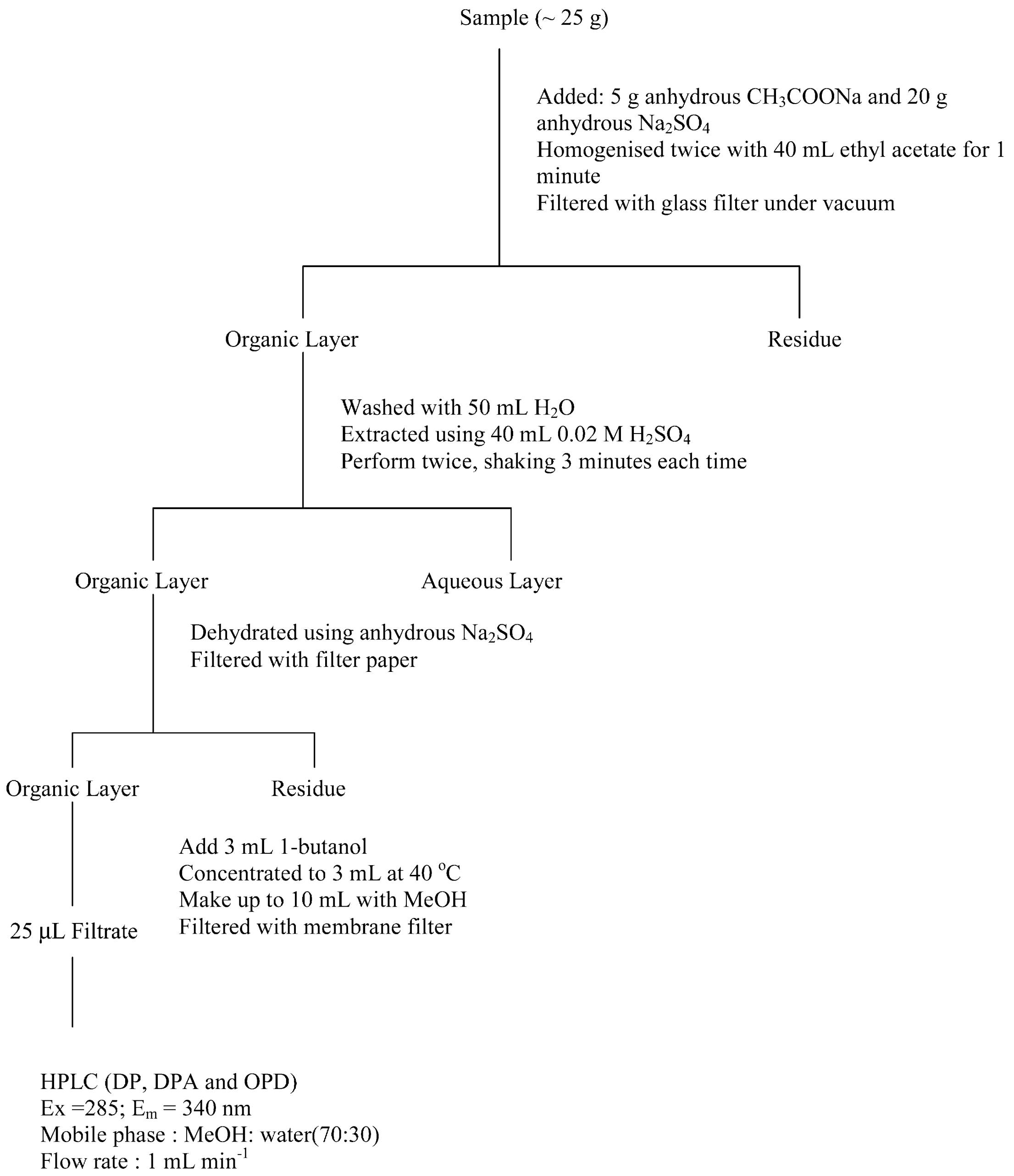

Fig. 2. Analytical scheme for the sample pretreatment and HPLC separation of the fungicides (adopted from Nakazato et al., 1995).

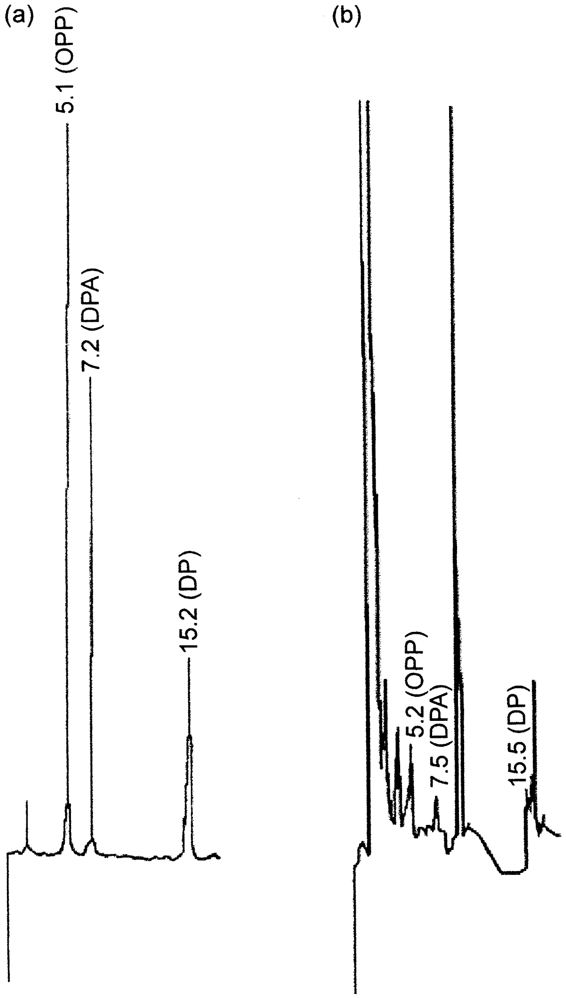

Fig. 3. Typical chromatograms of (a) standard DP (5 ppm), DPA (0.5 ppm), OPP (0.5 ppm) and (b) extract of an apple sample. Please refer to text for chromatographic conditions.

Table 3
Level of fungicides found in apples ($n = 3$) using the proposed HPLC method

Sample no.	Variety	Country of origin	Concentration, ppm		
			OPP	DPA	DP
1	Granny Smith	New Zealand	0.02	0.34	0.55
2	Braeburn	New Zealand	0.01	0.03	0.49
3	Pacific Rose	New Zealand	0.02	0.01	0.57
4	Hi Early	Australia	0.01	0.004	0.62
5	Red Delicious	Australia	0.02	N.D.	0.45
6	Red Delicious	South Africa	N.D	0.004	0.16
7	Granny Smith	South Africa	N.D.	0.43	0.71
8	Golden Delicious	America	0.003	0.08	0.49
9	Red Delicious	America	0.01	1.46	0.68
10	Sun Moon	China	N.D.	0.03	0.31
Maximum legal limits			25	5	–

Table 4
Levels of fungicides found in oranges ($n = 3$) using the proposed HPLC method

Sample no.	Variety	Country of origin	Concentration, ppm		
			OPP	DPA	DP
11	Honey Murcott	China	0.02	0.004	0.44
12	Thai Orange	Thailand	0.02	0.01	1.65
13	Velencia	Australia	0.02	0.01	0.43
14	Late Lane Navel	Australia	0.01	0.02	0.35
15	Mountain Duck	Australia	0.01	0.01	1.02
Malaysian Legal Limit			10	–	110

and DP detected in oranges were also both very much below the legal limits (10 and 110 ppm, respectively) (Table 4). Low levels of DPA, ranging 0.004–0.015 ppm, which is not presently regulated in oranges was also found. Typical chromatograms are shown in Fig. 3.

4. Conclusions

The proposed HPLC method offers clear advantages such as lower detection limits and faster analysis times as compared with the report of Nakazato et al. (1995). Another favourable feature of the method is the complete elimination of acetonitrile from the mobile phase. It is indeed reassuring to note that results from the application of the method indicate that the levels of OPP and DPA in apples and OPP and DP in oranges are all below the Malaysian legal limits. However, traces of DP in apples and DPA in oranges were detected. The use of such fungicides in these food items are not legally permitted in Malaysia.

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