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SIMULTANEOUS DETERMINATION OF FOUR ACTIVE INGREDIENTS OF DINOCAP IN CROPS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

A procedure for high-performance liquid chromatographic (HPLC) determination of four active ingredients of dinocap, 2,4-dinitro-6-octylphenyl crotonate, 2,6-dinitro-4-octylphenyl crotonate, 2,4-dinitro-6-octylphenol and 2,6-dinitro-4-octylphenol, in crops was examined. The four compounds were extracted with acetone and re-extracted into hexane. After clean-up successively by hexane–acetonitrile partition and silica gel column chromatography, the compounds were determined by HPLC with an UV detector (245 nm). The column was Cosmosil $5C_{18}$ (ODS, 5 μm) and the eluent was methanol–water–acetic acid (330:70:1). The limit of detection was 0.02 ppm and the recoveries from crops (spiked with 0.5 ppm) were 85–100%. The four compounds were determined simultaneously and the method was shown to be applicable to residue analysis of these compounds in crops sprayed with dinocap.

INTRODUCTION

Dinocap is used for the control of powdery mildews and several species of mites, *e.g.*, red spider, clover mite, European red and peach silver mite. Dinocap technical is a mixture of dinitrooctylphenyl cortonates (DNOPCs), dinitrooctylphenols (DNOPs), mononitrooctylphenols (MNOPs), which are considered active, and a number of inactive minor components[1,2].

For residue analysis of dinocap, spectrophotometric measurement has generally been employed using pyridine[3,4], tetraethylammonium hydroxide[5], dimethylformamide[4,6] or ethanolamine[7] as a colour-developing agent. These methods assay the total active ingredient contents and do not distinguish between the components

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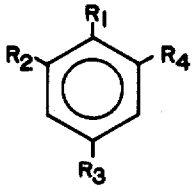
of dinocap. Gas chromatography can separate the components of dinocap and has been applied to the technical or formulation analysis of dinocap[1,2].

In the present study, four active ingredients, namely 2,4-dinitro-6-octylphenyl crotonate (2,4-DNOPC), 2,6-dinitro-4-octylphenyl crotonate (2,6-DNOPC), 2,4-dinitro-6-octylphenol (2,4-DNOP) and 2,6-dinitro-4-octylphenol (2,6-DNOP) (Fig. 1), which comprise approximately 78% of dinocap technical[1], were assayed simultaneously by high-performance liquid chromatography (HPLC). 2,4-DNOPC and 2,4-DNOP are considered effective as acaricides, 2,6-DNOPC and 2,6-DNOP as fungicides.



Compound	R_1	R_2	R_3	R_4
2,4–DNOPC	$OCO(CH)_2CH_3$	NO_2	NO_2	C_8H_{17}
2,6–DNOPC	$OCO(CH)_2CH_3$	NO_2	C_8H_{17}	NO_2
2,4–DNOP	OH	NO_2	NO_2	C_8H_{17}
2,6–DNOP	OH	NO_2	C_8H_{17}	NO_2

Fig. 1. Chemical structures for the compounds referred to in the text.

EXPERIMENTAL

Reagents and materials

The HPLC column, Cosmosil $5C_{18}$, was obtained from Nakarai, silica gel (Wako Gel C-100) from Wako. Pure standards, 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP, were obtained from Rohm & Haas via Sanyo. The organic solvents were of pesticide-residue-analytical grade (or their equivalent) and all the other reagents were of analytical-reagent grade.

Apparatus

A TRI-ROTAR chromatograph with a UVIDEC-100-V UV spectrophotometer (Japan Spectroscopic Co.) was used for HPLC.

Sample preparation

About 1 kg of crops (apple, grape, Japanese pear) was homogenized with a mixer. A 20-g subsample was placed into a 300-ml flask, 5 ml of 1 *M* hydrochloric acid and 100 ml of acetone were added and the flask was shaken mechanically for 30 min. The mixture was filtered through a filter-paper under suction. The filter cake and flask were washed with 50 ml of acetone and the washing was filtered. Acetone in the filtrate was removed under reduced pressure in a water-bath at below 40°C. The residual aqueous solution was transferred to a 500-ml separating funnel. A 5-ml volume of 1 *M* hydrochloric acid, 10 g of sodium chloride and 100 ml of water were added, and 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP were extracted with 100 and 50 ml of hexane by shaking for 5 min. The hexane extracts were pooled, dried over anhydrous sodium sulphate, filtered through filter-paper and the filtrate was evaporated to dryness under reduced pressure in a water-bath at below 40°C. The dried residue was dissolved in 50 ml of hexane, transferred to a 200-ml separating

funnel and the four compounds were extracted with 50 and 30 ml of acetonitrile by shaking for 5 min. The acetonitrile layers were pooled, evaporated to dryness under reduced pressure and subjected to the following silica gel column chromatography.

A slurry of 10 g of Wako Gel C-100 in hexane was poured into a glass tube (300 mm × 15 mm I.D.) and 5 g of anhydrous sodium sulphate were added on top of the adsorbent. The dried residue was dissolved in two 5-ml portions of hexane and placed on top of the column. After discarding the eluate, 2,4-DNOPC, 2,6-DNOPC and 2,4-DNOP were eluted with 70 ml of ethyl acetate–hexane (2:8), then 2,6-DNOP with 50 ml of ethyl acetate–hexane (5:5). Each eluate was evaporated to dryness under reduced pressure. The dried residue was dissolved in 2.0 ml of methanol and a 5-μl aliquot was subjected to HPLC.

Samples for the study of the stability in frozen samples were prepared as follows: crop homogenate (20 g) was placed in a 300-ml flask and spiked separately with 10 μg of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP. The flask was stoppered, shaken thoroughly and then stored in a freezer at −20°C. After storage, the samples were thawed in a water-bath at 22°C for 10 min, then analyzed immediately.

HPLC

The conditions were: column, Cosmosil $5C_{18}$ (150 mm × 4.6 mm I.D.); eluent, methanol–water–acetic acid (330:70:1); flow-rate, 1.0 ml/min; column oven, 40°C; detection wavelength, 245 nm; sensitivity, 0.01 absorbance units full scale (a.u.f.s.).

Standard solutions of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP were prepared as follows: 50 mg of pure standard material were accurately weighed into a 100-ml volumetric flask and made up to the mark with methanol (500 μg/ml). This solution was diluted in methanol to provide standard solutions of concentrations 0.5, 1.0, 2.5, 5.0 and 8.0 μg/ml for each compound.

A 5-μl aliquot of each standard solution was subjected to HPLC. Standard curves were prepared by plotting the peak heights against the amounts of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP injected. The amounts of these compounds in the sample extract were determined by comparing the observed peak heights with each standard curve.

RESULTS AND DISCUSSION

HPLC conditions

Several columns were investigated as to their ability to separate the four compounds, including isomers and polar compounds. Normal-phase columns (Nucleosil $5NH_2$, 5CN and $5NO_2$) were unsuccessful; 2,4-DNOP and 2,6-DNOP were strongly retained, showing peak tailing and broadening. The reversed-phase column Cosmosil $5C_{18}$ (ODS, 5 μm) provided good peak shapes and responses for the four compounds. Baseline separation (resolution, $R_s > 1.5$) was obtained by using methanol–water–acetic acid (330:70:1) as an eluent. 2,4-DNOP and 2,6-DNOP, which are acidic, were co-eluted when methanol–water (330:70) was used as an eluent. Acidification of the eluent with acetic acid (2.5 ml/l) suppressed the ionization, which resulted in an increase in the capacity factors, k', and sufficient separation of the compounds (Fig. 2).

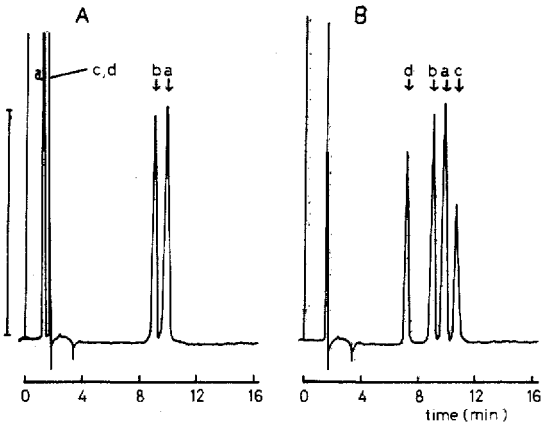


Fig. 2. Separation of the four compounds. Eluents: (A) methanol–water (330:70); (B) methanol–water–acetic acid (330:70:1). The other HPLC conditions are described in the text. Peaks: a = 2,4-DNOPC; b = 2,6-DNOPC; c = 2,4-DNOP; d = 2,6-DNOP. Amount injected: 25 ng for each compound (A and B).

The four compounds have different maximum absorptions, λ_{max}, in the UV region (Fig. 3). By investigating the sensitivity at various wavelengths, detection at 245 nm was found to provide better and almost the same sensitivities of the four compounds.

The proposed HPLC method gave adequate separation of the four compounds from each other and from other co-extractives, and also provided adequate sensitivity. The retention times of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP were 10.0, 9.2, 10.9 and 7.3 min, respectively, and the minimum detectable quantity

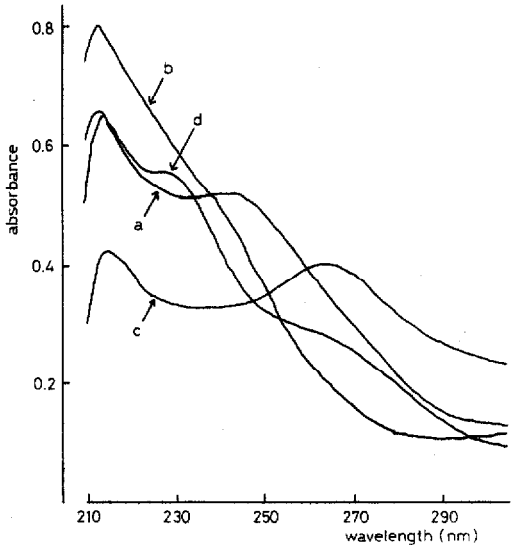


Fig. 3. UV absorption spectra of 10 μg/ml solutions of 2,4-DNOPC (a), 2,6-DNOPC (b), 2,4-DNOP (c) and 2,6-DNOP (d) in methanol.

for each compound was 1 ng (signal-to-noise ratio 3). The relationship between the amount and the peak height was linear over the range 2.5–40 ng with correlation coefficients of greater than 0.999 for each compound. The reproducibility of the determination was also good: coefficients of variation for four analyses of each standard solution were less than 1.5% for the peak height and less than 0.4% for the retention time.

Clean-up

The adsorbents Florisil and silica gel for column chromatography were evaluated for removal of co-extracted sample constituents. The former gave poor results; 2,4-DNOP and 2,6-DNOP, which are polar compounds, were tightly adsorbed and could not be eluted from the column. Silica gel proved to be effective. In order to resolve the interferences still remaining in the eluate, the eluate from silica gel column chromatography was collected in two separate fractions; the first comprised ethyl acetate–hexane (2:8) containing 2,4-DNOPC, 2,6-DNOPC and 2,4-DNOP; the second comprised ethyl acetate–hexane (5:5), containing 2,6-DNOP. It was found that lipophilic co-extractives in the extract should be eliminated prior to silica gel column chromatography because they disturbed the elution of the four compounds. To resolve this problem, clean-up with hexane–acetonitrile partition and coagulation with ammonium chloride were evaluated. The former gave satisfactory results, while the latter caused some losses of 2,4-DNOP and 2,6-DNOP during the operation.

Recovery and limit of detection

Known amounts of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP were added to the crops and determined by the proposed procedure. The recoveries were between 85 and 100% (Table I) and the limit of detection was 0.02 ppm for each compound (Table II). The method was also reproducible; the coefficient of

TABLE I

RECOVERIES OF THE COMPOUNDS FROM CROPS

Crop	*Compound*	*Fortification (ppm)*	*Recovery (%) (mean ± S.D., n=4)*	*Coefficient of variation (%)*
Apple	2,4-DNOPC	0.5	99 ± 0.8	0.8
	2,6-DNOPC	0.5	99 ± 0.8	0.8
	2,4-DNOP	0.5	95 ± 0.6	0.6
	2,6-DNOP	0.5	92 ± 2.7	2.9
Grape	2,4-DNOPC	0.5	97 ± 1.9	2.0
	2,6-DNOPC	0.5	98 ± 2.5	2.6
	2,4-DNOP	0.5	95 ± 3.2	3.4
	2,6-DNOP	0.5	85 ± 1.8	2.1
Japanese pear	2,4-DNOPC	0.5	96 ± 1.3	1.4
	2,6-DNOPC	0.5	100 ± 1.5	1.5
	2,4-DNOP	0.5	97 ± 1.4	1.4
	2,6-DNOP	0.5	85 ± 2.5	2.9

TABLE II

LIMITS OF DETECTION

The minimum detectable quantity was 1 ng for 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP.

Crop	*Sample weight (g)*	*Final volume (ml)*	*Injection volume (μl)*	*Limit of detection* (ppm)*
Apple	20	2	5	0.02
Grape	20	2	5	0.02
Japanese pear	20	2	5	0.02

* For 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP.

variation was less than 3.4% for all compounds (Table I). Typical chromatograms are shown in Fig. 4. The peaks of the four compounds were completely separated from interfering peaks.

Stability of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP in crop homogenates during storage

The stabilities of pesticide chemicals in environmental samples during storage have become of interest in recent years[8–15] because in residue analysis, samples often

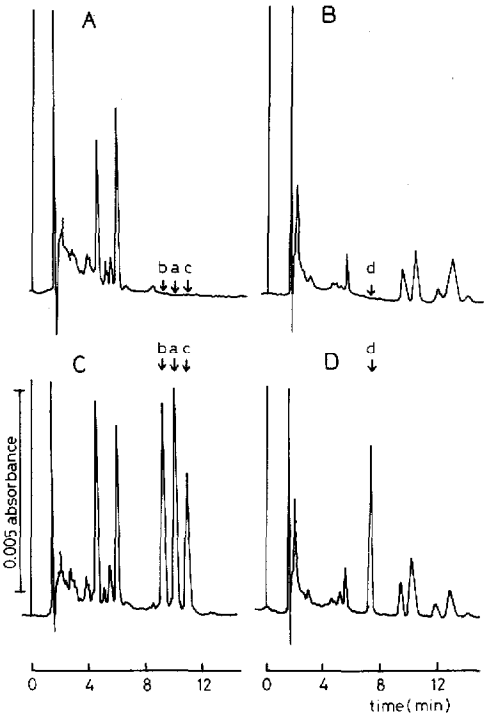


Fig. 4. Chromatograms of apple extracts: (A) and (B), controls; (C) and (D), spiked at 0.5 ppm. Sample: 20 g. Final volume: 2 ml. Injection volume: 5 μl. Peaks as in Fig. 2. For operating conditions see text.

cannot be analyzed immediately after sampling. They have to be stored under cold conditions in an intact state or after homogenization. The stabilities of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP were, therefore, examined when crop homogenates were stored at −20°C.

Table III indicates that the four compounds were fairly stable in homogenates of apple and grape when frozen during storage. in Japanese pear, slight losses (14–15%) of 2,6-DNOPC and 2,4-DNOP were observed, but the values were negligible. The hydrolysis of 2,4-DNOPC and 2,6-DNOPC, which was observed in environmental samples[16], did not occur in crop homogenates stored frozen. For analysis of the four compounds in crops, it was concluded that samples could be homogenized and stored in a freezer before analysis.

TABLE III

STABILITIES OF THE COMPOUNDS IN CROP HOMOGENATES DURING STORAGE

The fortification level of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP was 0.5 ppm.

Crop homogenate	*Storage period* (day)*	*Decomposition** (%)*			
		2,4-DNOPC	*2,6-DNOPC*	*2,4-DNOP*	*2,6-DNOP*
Apple	49	<5	5	<5	<5
Grape	30	<5	<5	<5	<5
Japanese pear	28	<5	14	15	8

* In a freezer at −20°C.
** Mean of duplicate experiments.

The proposed method permitted simultaneous determination of the four active ingredients of dinocap, including isomers and compounds of different polarities. The method is simple, precise and selective, and may be applicable not only to the residue analysis of 2,4-DNOPC, 2,6-DNOPC, 2,4-DNOP and 2,6-DNOP in crops sprayed with dinocap in fileds, but also to the study of their behaviour in the environment.

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