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Note

Determination and confirmation of binapacryl and dinobuton residues on apples and cucumbers by high-performance liquid chromatography

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Binapacryl (2-*sec.*-butyl-4,6-dinitrophenyl-3′,3′-dimethylacrylate) and dinobuton (isopropyl-2-*sec.*-butyl-4,6-dinitrophenylcarbonate) are used as fungicides and acaricides on apples, cucumbers and melons. Various methods for their analysis have been described in the literature[1–3]. Most of these methods are based on a de-esterification of binapacryl and dinobuton to 2-*sec.*-butyl-4,6-dinitrophenol (dinoseb) which is then determined spectrophotometrically after a clean-up by means of column chromatography or steam distillation.

Often, high blank values are obtained using these methods. Therefore, it seemed appropriate to use a gas chromatographic method which has been described for binapacryl[2].

The problem of high blank values can also be solved by determining dinoseb by gas chromatography[4,5], but with that method no distinction can be made between dinobuton and binapacryl. For this reason it was necessary to develop a method for the determination of dinobuton and binapacryl. Because of the rather polar nature of these compounds high-performance liquid chromatography (HPLC) was chosen for the final determination.

EXPERIMENTAL

Reagents

Chloroform and light petroleum (b.p. 40–60°C) were obtained from Brocacef (Maarssen, The Netherlands) and distilled prior to use. Sodium sulphate (AnalaR) was obtained from BDH (Poole, Great Britain) and dried for 5 h at 300°C. Aluminium oxide (W200, neutral) was obtained from Pleuger (Amstelveen, The Netherlands) and deactivated by adding 7.5 ml of water to 100 g of aluminium oxide. Tetramethylammonium hydroxide (TMAH) was obtained as a 10% (w/v) solution in methanol from Merck (Darmstadt, G.F.R.) and diluted with water and methanol (zur Analyse, Merck) to give a 0.1 *M* solution in methanol–water (80 : 20, v/v).

Procedures

Extraction. Fifty grams of the homogenized sample were macerated with 100 ml of methanol–light petroleum (25 : 75, v/v). After centrifugation the extract was

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transferred into a separatory funnel and the extraction was repeated twice with 100 ml of light petroleum. The combined extracts were extracted twice with distilled water in order to remove the methanol and some interfering polar substances. After drying over sodium sulphate the extract was concentrated to 10 ml in a Kuderna–Danish apparatus.

Column clean-up. In a chromatographic tube (10 cm × 6 mm I.D.) 2 g of deactivated alumina was put on top of a small plug of glass wool and 1 ml of the concentrated extract was put on top of this column. The column was then washed twice with 2 ml of light petroleum and the eluent was discarded. The compounds of interest were then eluted with a mixture of 3 ml of light petroleum and 2 ml of chloroform. This eluate was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 1 ml of mobile phase. From this solution 10–50 μl were injected onto the HPLC column for the determination of dinobuton and binapacryl.

HPLC. Analyses were conducted with a component system, consisting of a Varian 8500 pump, a Valco loop injector and a Pye-Unicam LC–UV detector, operated at 230 nm. The column was 15 cm × 4.6 mm I.D., stainless-steel packed with LiChrosorb 5 RP-18. The solvent was methanol–water (80 : 20, v/v) in the determination of dinobuton and binapacryl. The flow-rate was 60 ml/h and chromatography was carried out at ambient temperature. For the determination of dinoseb the same conditions were used, except that 1 % (v/v) glacial acetic acid was added to the mobile phase, and that the detection wavelength was set at 365 nm.

De-esterification. The eluate of the column clean-up was evaporated to dryness and the residue dissolved in the 0.1 *M* TMAH solution. This solution was heated to about 80°C on a water bath for 30 min. After cooling down and making up the volume to 1.0 ml, 10–50 μl of this solution were injected onto the HPLC column for the determination of dinoseb.

RESULTS AND DISCUSSION

Extraction of binapacryl and dinobuton with hexane or light petroleum has been shown to give good recoveries in most cases, while a minimum of interfering substances is co-extracted. However, the recoveries and also the blank values, tend to vary with variety and maturity of the crop, especially apples. With the addition of methanol to the extracting solvent the recoveries were reproducibly better than 90 %.

The column clean-up on neutral aluminium oxide proved to be very effective in removing interfering substances, as can be seen in Fig. 1. Chromatograms of blank samples are completely clean at the retention time of dinobuton and binapacryl; without the clean-up many interferences were present. Care should be taken not to overload the column with too much material. When the equivalent of more than 10 g of apple was put on the column, the clean-up did not give satisfactory results, and in many cases the residue of the clean-up could not be dissolved completely in 1 ml of the mobile phase. This often resulted in low recoveries and in plugging of the analytical column.

In the liquid chromatographic system used, dinobuton and binapacryl gave well-separated, symmetrical peaks. At the detection wavelength used, the sensitivity is very good, the minimum detectable quantity being 1 ng per injection. When detection is carried out at 254 nm, the minimum detectable quantity is *ca.* 2 ng.

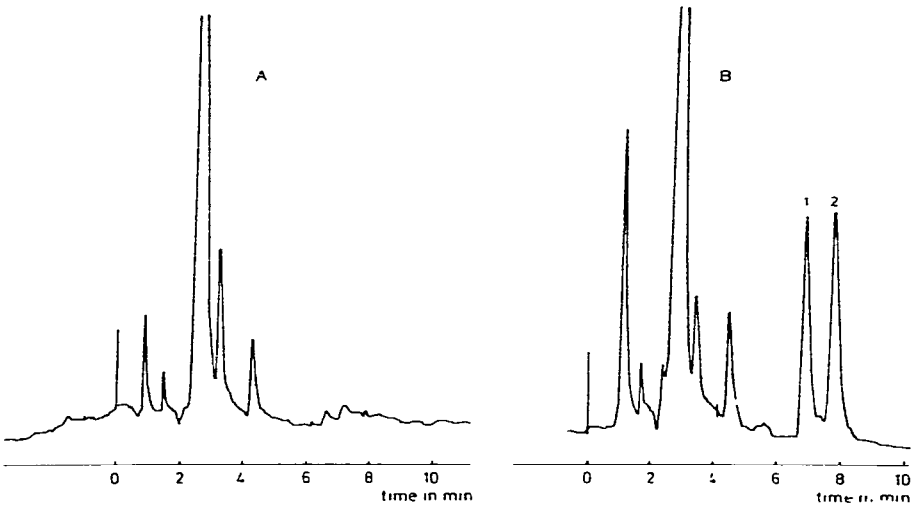

Fig. 1. Chromatograms of apple samples, for conditions see text. A, Blank; B, sample spiked with dinobuton (1) and binapacryl (2), both to a concentration of 0.5 mg/kg.

The results of recovery studies carried out with apples, spiked to various concentrations, are given in Table I. From this table it can be concluded that the recoveries are quite good, and that the reproducibility is satisfactory; for binapacryl (maximum residue limit 0.3 mg/kg) the coefficient of variation is 5% at the three levels studied; for dinobuton (maximum residue limit 1 mg/kg) the coefficient of variation is also 5% at 1.2 mg/kg and 0.6 mg/kg; at 0.1 mg/kg the coefficient of variation is 13%.

On cucumbers the recoveries are somewhat lower; for both compounds a recovery of 84% was found with a coefficient of variation of 5% when blank samples were spiked to concentrations near the maximum residue limit and analysed as described.

The de-esterification of dinobuton and binapacryl has been used as the basis for a quantitative determination of these compounds[1,3] and also as a confirmation of binapacryl residues[2]. The time course of the de-esterification was followed by determining dinoseb after heating a binapacryl and dinobuton solution during 15, 30

TABLE I
RECOVERIES OF BINAPACRYL AND DINOBUTON FROM APPLES AT VARIOUS CONCENTRATIONS

Concentration (mg/kg)	*Recovery (%)**	
	Binapacryl	*Dinobuton*
1.2	–	89
0.7	97	–
0.6	–	94
0.3	93	–
0.1	91	102

* Each value is the mean of four determinations.

and 60 min. After 15 min the recovery was 92%; after 30 min, 95% and after 60 min also 95%. Therefore, a reaction time of 30 min seems to be sufficient.

Detection of dinoseb at 365 nm is much more specific than detection at 230 nm. Detection at this wavelength is so specific for nitrophenols that they probably can be determined in various crops without clean-up. These studies are presently being conducted and will be reported separately.

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