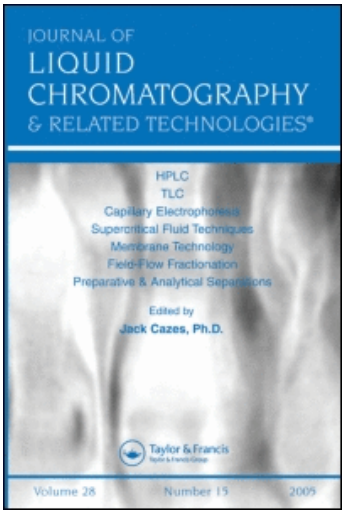

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Determination of Dinocap in Apples, Grapes, and Pears Using A Solid Phase Extraction Cleanup and HPLC-UV Detection

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DETERMINATION OF DINOCAP IN APPLES, GRAPES, AND PEARS USING A SOLID PHASE EXTRACTION CLEANUP AND HPLC-UV DETECTION

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ABSTRACT

A solid phase extraction (SPE) technique for the isolation and HPLC-UV determination of the pesticide dinocap in apples, grapes and pears is described. Samples are extracted with acetone; the acetone extract is subjected to cleanup on both C-18 and silica SPE columns. The residues are determined using HPLC with a UV detector. The average recovery from crop samples spiked at tolerance, 0.1 ppm dinocap, was 85.9%.

INTRODUCTION

Dinocap (Karathane) is a fungicide and acaricide used for the control of powdery mildew and several species of mites. Technical dinocap consists of a six-component mixture of 2,4-dinitro-6-(2-, 3-, and 4-octyl)phenyl crotonate (collectively 2,4-DNOPC), 2,6-dinitro-4-(2-, 3-, and 4-octyl)phenyl crotonate (collectively 2,6-DNOPC), together with mono- and dinitrooctylphenols (all

considered to be active agents) and a number of inactive minor components (1,2). Kurtz and Baum (1) assayed five lots of technical dinocap between 1966-1967 and found between 72.0 and 76.6% crotonate. The technical dinocap being manufactured presently contains ca. 90% crotonates (personal communication, Rohm and Haas Corp.). Tolerances have been established based on the two crotonate isomers as follows: 0.15 ppm total crotonates for caneberries and gooseberries and 0.10 ppm for apples, apricots, cantaloupes, cucumbers, grapes, honeydew melons, muskmelons, nectarines, peaches, pears, pumpkins, summer squash, watermelons and winter squash (3).

Spectrophotometric methods have been used for the determination of dinocap residues on crops (4-6); however, these methods do not distinguish between the various components. Johansson (7) used derivatization followed by GLC-electron capture for the determination of dinocap in fruits and vegetables. Liang et al. (8) used HPLC with UV detection for the determination of dinocap residues on various crops. All of these methods employ many time-consuming multiple extraction and solvent partition cleanup steps.

Solid phase extraction has been found to be a useful alternative to multiple solvent extractions for the cleanup of crop residue extracts. It utilizes disposable columns packed with small amounts of liquid chromatographic sorbent for the cleanup and requires much smaller volumes of solvent. Extracts are eluted through the columns, and the target analytes are retained on the stationary phase while the co-extracted interferences are eluted. Conversely, the target analyte may be eluted while the co-extractants are retained.

Reverse-phase SPE columns utilize silica chemically bonded with a polymeric lipophilic phase, e.g., C-18. Normal-phase SPE columns utilize polar sorbents such as alumina, Florisil or silica. This paper describes a reliable and rapid method for the determination of dinocap in apples, pears and grapes using SPE cleanup and HPLC determination.

Dinocap is extracted with acetone, and the cleanup of the extracts is performed using both reverse-phase (C-18) and normal-phase (silica) SPE columns. The elution solvents have been optimized so that the dinocap is eluted from the SPE columns while many of the interfering co-extractants are retained. Dinocap is then determined by HPLC with UV detection.

EXPERIMENTAL

Reagents and Materials

(a) Solvents- EM reagents glass-distilled quality. Acetonitrile- suitable for spectrophotometry and liquid chromatography.

(b) Water- Filtered and deionized, Millipore Milli-Q water treatment system (Waters Corp., Milford MA).

(c) Sodium sulfate- ACS reagent grade, granular, anhydrous.

(d) Solid phase extraction columns- Mega Bond Elut C-18, 6-mL size, 1.0 g; Bond Elut silica, 3-mL size, 500 mg (Varian Sample Preparation Products, Harbor City, CA).

(e) Bond Elut column adapters and 60 mL reservoirs (Varian).

(f) SPE eluant- ethyl ether in petroleum ether (10/90, v/v)- prepared fresh daily.

(g) Dinocap reference standard- Reference standard #2560, Lot No. A03N, U.S. Environmental Protection Agency (Research Triangle Park, NC).

(h) Stock standard solution (312 μg/mL) was prepared by dissolving dinocap reference standard in methanol.

(i) Intermediate standard solution (5.0 μg/mL) in methanol was prepared from the stock standard solution.

(j) Working standard solutions (0.25, 0.35 and 0.45 μg/mL) in mobile phase were prepared from the intermediate standard solution.

(k) 2,4--DNOPC [mixture of -6(2-, 3-, and 4-octyl)phenyl isomers]-analytical standard- Lot #RP09304FP, purity 97.5% (Rohm and Haas,
Philadelphia, PA).

(l) 2,6-DNOPC [mixture of -4(2-, 3-, and 4-octyl)phenyl isomers]- analytical standard- Lot #RP09306FP, purity 98.3% (Rohm and Haas, Philadelphia, PA).

Apparatus

(a) HPLC system- Series 410 LC pump and ISS-100 autosampler (injection volume 200 μL) (Perkin-Elmer Corp, Norwalk, CT); Model 383A UV-visible detector (ABI-Kratos Inc, Ramsey, NJ) set at 245 nm and 0.005 AUFS; Octadecylsilyl (ODS)-derivatized silica column, 3 μm, 15.0 cm x 4.6 mm, Econosphere (Alltech Associates, Deerfield, IL) with a 15 mm Brownlee Newguard guard column cartridge (Applied Biosystems Inc.,Ramsay, NJ); solvent flow rate 1.0 mL/min; HP-3396 integrator (Hewlett-Packard, Palo Alto, CA).

(b) Solid phase extraction vacuum manifold (Supelco Inc., Bellefonte, PA).

(c) High speed blender.

(d) Culture tubes- borosilicate glass, 16 x 125 mm (Corning Glass, Corning, NY).

HPLC mobile phase composition

Mobile phase I- 60% acetonitrile and 40% water. Adjust acetonitrile/water ratio so that the retention time of the first crotonate peak is ca. 22 minutes. Total run time for sample extracts should be at least 45 minutes.

Mobile phase II- 56% acetonitrile and 44% water. Adjust the acetonitrile/water ratio so that the retention time of the first crotonate peak is ca. 32 minutes. Total run time for sample extracts should be 75 minutes.

Sample preparation

Stems were removed and discarded from apples, pears and grapes. Fruit was then comminuted in a Hobart Cutter-Mixer to obtain a homogeneous composite.

Extraction and Cleanup of Sample

Blend 100 grams of sample composite with 200 mL of acetone in a high speed blender for two minutes and filter. Transfer 10.0 mL of the filtrate to a 50 mL glass stoppered graduated cylinder. Dilute to volume with water and mix by inverting gently.

Prepare a C-18 SPE column by pre-washing with 5 mL petroleum ether, 5 mL acetone, two 5-mL portions of methanol and two 5-mL portions of water. (Do not allow column to dry after the last water wash; leave ca. ½" of water above the sorbent.) Attach a solvent reservoir to the C-18 column and install the column on an SPE vacuum manifold. Transfer the contents of the graduated cylinder to the reservoir and apply vacuum to elute the sample at a rate of 2-3 drops per second. Discard the eluate. Rinse the graduated cylinder with 5 mL of acetone/water (25/75) and also elute it through the column. Discard the eluate. Remove the reservoir and wash the column with two 5-mL portions of water. When all the water is eluted increase the vacuum to maximum to draw air through the column for at least 15 minutes.

Elute the C-18 SPE column with 1 mL petroleum ether followed by 5 mL chloroform, combining the eluates in a glass culture tube. Evaporate the petroleum ether-chloroform eluate to dryness under a stream of nitrogen at 50°C. Dissolve the residue remaining in the culture tube in ca. 1 mL petroleum ether.

Prepare a silica SPE column by adding one-half inch of anhydrous sodium sulfate to the top of the column and wash the column with ca. 2 mL petroleum ether. Transfer the residue mixture in the culture tube to the silica column using a Pasteur

pipette. Elute the petroleum ether residue mixture through the silica column at a rate of ca. one drop per second. Rinse the culture tube two times with one mL petroleum ether, eluting the successive rinsings through the silica column. Discard the petroleum ether eluates. Elute the silica SPE column with three-2 mL portions of ethyl ether/petroleum ether (10/90), collecting and combining the eluates in a glass culture tube. Evaporate to dryness under a stream of nitrogen at 25° C, and dissolve the residue in 1.0 mL of mobile phase.

Handling of Chromatographic Data and Calculations

Inject 200 μL of each standard and sample solutions into the HPLC. Add the peak areas of the two crotonate composite peaks for each of the standard and sample solutions. Derive the concentration of dinocap in the sample extract solution by comparing its total crotonate peak area to the least squares regression of the total crotonate peak areas of standard curve responses.

Calculate the grams of sample taken through the SPE cleanup (G) as follows:

$$G = \frac{100g \times 10\ mL}{T\ mL}$$

where: 100 = The number of grams of sample extracted.
10 = The number of mL of the sample extract taken through the SPE cleanup.
T = The total volume of the sample extract (200 mL acetone + mL of water present in 100 g of sample - 10 mL acetone/water contraction volume). (If the moisture content of the product is unknown, use 0.85 X 100 for the number of mL of water in the sample.)

Calculate the dinocap content in the sample as follows:

$$\text{dinocap, ppm} = C \times (V/G)$$

where: C = concentration of dinocap (μg/mL) in the sample extract as determined from the HPLC standard curve.
G = grams of sample taken through the SPE cleanup.
V = the final volume (mL) of the sample extract.

RESULTS and DISCUSSION

This method uses the same acetone extract as the method of Luke et al. (9). The Luke method is widely used for the multi-residue screening of raw agricultural products for pesticide residues. The SPE method can be used for the cleanup of a large number of extracts using minimal amounts of solvent and time. We were able to take ten acetone extracts through the SPE cleanup in ca. 2 hours.

Isomer Composition of Dinocap

The two major components of technical dinocap, 2,4-DNOPC and 2,6-DNOPC are present in a 2:1 ratio. Each of these components is actually a mixture of three isomers because the phenyl group can be attached to the octyl chain at the carbon-2, -3 or -4 position. Kurtz and Baum (1) determined the relative gas chromatographic retention times of all six isomers. They found that some of the isomers co-eluted as composite peaks while other isomers were resolved into discrete peaks.

HPLC Chromatography of Dinocap Standard

Figure 1 (A) shows a representative chromatogram of dinocap reference standard. Two composite peaks centered at ca. 24 and 27 minutes are present. In order to establish the identity of these peaks, analytical standards of the two crotonate isomers, 2,4-DNOPC and 2,6-DNOPC, each of which consists of a mixture of three octyl-substituted isomers (*vide supra*), were chromatographed (figures 1B and 1C, respectively). Comparison of the three chromatograms shows that each of the two major composite peaks in the dinocap reference standard consists of octyl isomers of both 2,4-dinitrophenyl crotonate and 2,6-dinitrophenyl crotonate.

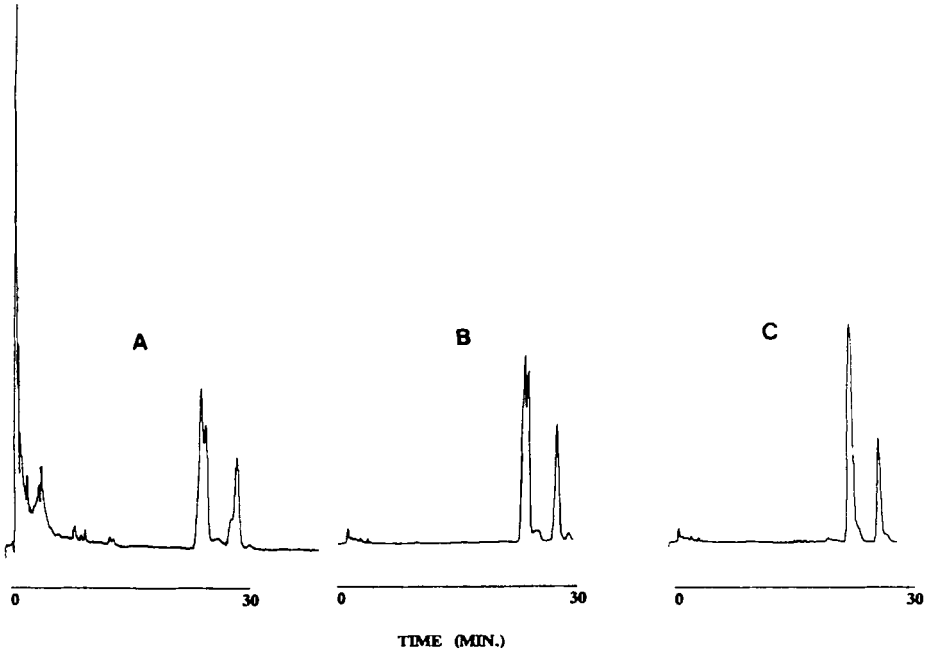

FIGURE 1 HPLC chromatograms of dinocap reference standard (A); 2,4-dinitro-6-octylphenyl crotonate analytical standard (3-isomer mixture) (B); and 2,6-dinitro-4-octylphenyl crotonate analytical standard (C), using mobile phase I.

Extraction and SPE Cleanup

After extraction of dinocap from the sample matrix with acetone, water is added to the extract and dinocap is partitioned from the aqueous phase onto the C-18 sorbent. Dinocap is then eluted from the C-18 with petroleum ether and chloroform. This eluate still contains many co-extracted plant constituents which would interfere with the HPLC determination.

The solvent is evaporated and the residue is dissolved in petroleum ether and eluted through a silica SPE column. After evaluating various elution solvents for the silica column, a 10% (v/v) mixture of ethyl ether in petroleum ether was found to consistently elute the dinocap while minimizing the number of

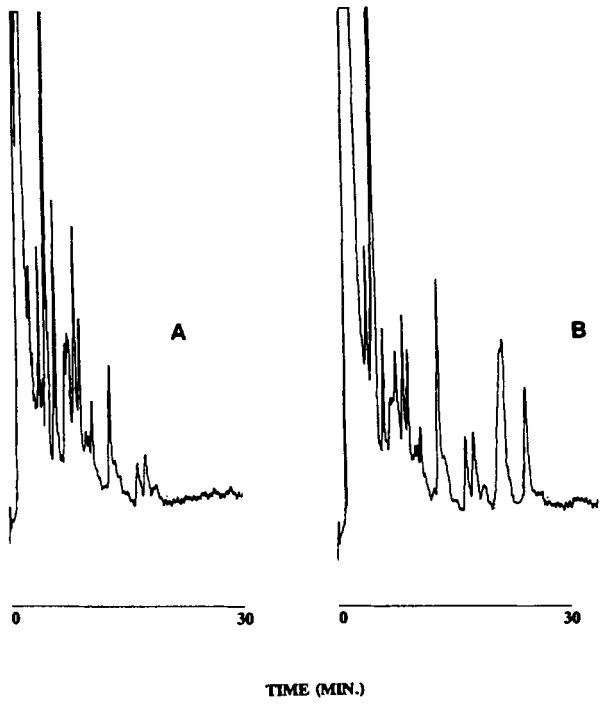

FIGURE 2 HPLC chromatograms of a control pear sample (A), and a pear sample spiked with 0.10 ppm dinocap (B), using mobile phase I.

eluted interfering co-extractants. A slightly more polar elution solvent, a 15% (v/v) mixture of ethyl ether in petroleum ether eluted numerous plant tissue co-extractants which interfered with the HPLC determination of dinocap.

HPLC Chromatography of Sample Extracts

Representative HPLC chromatograms, resulting from the analysis of a pear sample and a pear sample spiked with 0.10 ppm dinocap, using HPLC mobile phase I are shown in figures 2(A) and 2(B), respectively. The two crotonate composite peaks eluted at

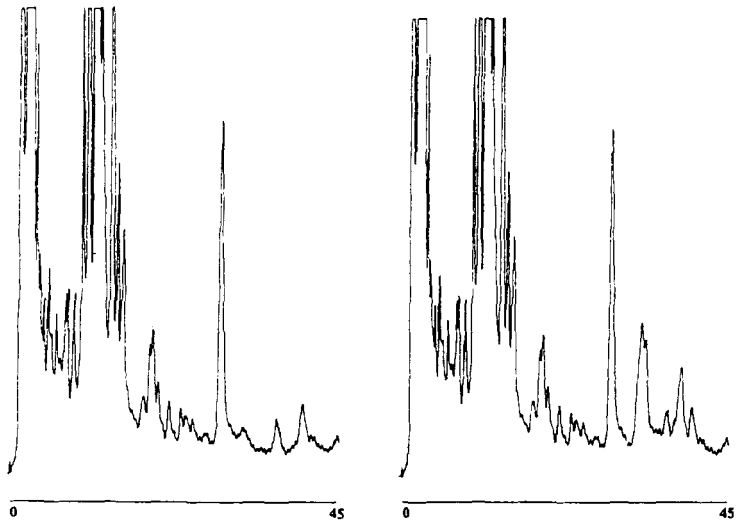

FIGURE 3 HPLC chromatograms of a control apple sample (A), and an apple sample spiked with 0.10 ppm dinocap (B) using mobile phase II.

ca. 21 and 24 minutes and were resolved from the crop matrix peaks. Twelve pear and ten grape samples were analyzed using mobile phase I. None of these chromatograms exhibited matrix peaks that would have interfered with the chromatography of the two crotonate peaks.

When mobile phase I was used for the analysis of apple samples, approximately 25% of the sample chromatograms displayed matrix peaks that could interfere with one of the two crotonate composite peaks. When these sample extracts were chromatographed using HPLC mobile phase II, none of the matrix peaks were eluted at the retention time of the two crotonate composite peaks. Representative HPLC chromatograms, resulting from the analysis of a control apple sample and a control apple sample spiked with 0.10 ppm dinocap using HPLC mobile phase II are shown in figures 3(A) and 3(B) respectively. The two crotonate peaks eluted at ca. 32 and 38 minutes and were resolved from all the crop matrix peaks.

TABLE 1

Recovery of Dinocap from Spiked Samples.

Sample	Spiked (μg/g)	% Recovered (% c.v.)*
Apples	0.05	95.4 (4.3)
	0.10	88.5 (3.7)
	0.50	87.7 (4.5)
Grapes	0.05	94.7 (5.5)
	0.10	85.0 (4.0)
	0.50	82.8 (5.1)
Pears	0.05	87.4 (9.4)
	0.10	84.3 (6.0)
	0.50	85.9 (6.8)

* n=3

Recoveries

Table 1 lists per cent recoveries and coefficients of variation of dinocap isolated from spiked fruit samples. The percent recoveries for samples spiked at 0.05 ppm were slightly higher than for samples spiked at 0.10 and 0.50 ppm. This might be due to a greater relative contribution of matrix co-extractants to the analyte peak areas for the lowest level of spiking.

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