# High-Performance Liquid Chromatography Method for the Determination of Dinoseb: Application to the Analysis of Residues in Raspberries

Sunny Y. Szeto* and Patricia M. Price

Agriculture Canada Research Station, 6660 Northwest Marine Drive, Vancouver, British Columbia, Canada V6T 1X2

Dinoseb residues were extracted from raspberries with a 3:2 mixture (v/v) of dichloromethane and acetone. After removal of acetone by washing with 2% NaCl solution, the crude extracts in dichloromethane were mixed with hexane and dinoseb residues were then partitioned into 0.025 M $NaHCO_3$. The aqueous extracts were acidified to about pH 1, and dinoseb residues were again extracted with dichloromethane and reconstituted in methanol for HPLC-UV (270 nm) analysis. Recoveries ranged from 78.6% to 85.5% for raspberries fortified with 0.1, 0.5, and 1.0 ppm of dinoseb. The performance of the HPLC method was compared with that of a GC-NPD method; the limits of detection were 0.025 and 0.01 ppm, respectively, for these two methods. Raspberry samples from seven farms were analyzed by the two methods, but no detectable dinoseb residues were found.

## INTRODUCTION

The lower Fraser Valley of southwestern British Columbia is one of the world's leading producers of red raspberries (*Rubus idaeus* L.). Production has increased in recent years to meet the demands of the domestic market and for export to the United States for food processing as well as fresh market berries. In vigorous raspberry plantings in British Columbia, dinoseb (2-*sec*-butyl-4,6-dinitrophenol) is used for primocane removal and thereby to increase yield. However, this herbicide is not registered in the United States for these purposes. Therefore, it is essential to ascertain that raspberries grown in British Columbia contain no dinoseb residues resulting from its use in production. In fact, dinoseb has never been detected in raspberries by the Health Protection Branch in the past. In the summer of 1990, a study was conducted to determine dinoseb residues resulting from all use patterns, including the worst case scenario, i.e., longest use history and most applications in the current growing season.

According to Gardner and McKellar (1980), the literature is deficient in extensive and well-validated methodology for dinoseb determination in crops. They published their GC method for determination of dinoseb in crops and soil (Gardner and McKellar, 1980) which had been practiced by them for 10 years on 16 different crops plus soil. However, their method requires extensive cleanup and the product is methylated with diazomethane prior to GC determination. Since diazomethane is highly toxic, mutagenic, carcinogenic, and potentially explosive, we developed an HPLC method as an alternative, allowing direct determination of dinoseb without derivatization with diazomethane. This paper describes our method and its application to the analysis of dinoseb residues in raspberries. In addition, dinoseb residues in those berries were also determined by GC after methylation, and residue data generated by both methods are compared.

## MATERIALS AND METHODS

**Sample Preparation and Fortification.** Stock solutions of dinoseb were prepared in HPLC grade methanol for sample fortification (100, 50, and 10 μg/mL) and HPLC analysis (2 μg/mL) as a reference standard. Raspberries from the Substation, Agriculture Canada, at Abbotsford, BC, that had never been treated with dinoseb were used for method development. They were macerated in a food processor, including seeds, and aliquots of 50 g of macerated berries were treated individually with 0.5 mL of an appropriate stock solution to give a concentration of 0.1, 0.5, or 1.0 ppm (fresh weight). They were allowed to stand at room temperature in a fume hood for about 1 h prior to extraction.

**Sample Extraction.** Prior to homogenization, 2 mL of 6 N HCl was added to each berry sample. Dinoseb residues were extracted twice with 150 and 100 mL of a 3:2 (v/v) mixture of dichloromethane and acetone in a Polytron homogenizer for 1 min each. The extracts were decanted and filtered through a Büchner funnel lined with glass fiber filter paper into a 500-mL separatory funnel. To the combined extracts were added 200 mL of 2% NaCl and 2 mL of 6 N HCl, and the separatory funnel was shaken vigorously for about 1 min. After phase separation, the dichloromethane phase (lower layer) was drained through a small funnel packed with anhydrous $Na_2SO_4$ into a 500-mL round-bottom flask. The aqueous phase was re-extracted with 25 mL of dichloromethane, the combined crude extracts were concentrated in a flash evaporator at 38 °C, and their final volumes were adjusted to 10 mL for cleanup.

**Cleanup and HPLC Analysis.** Aliquots of 3-mL crude extracts equivalent to 15 g of raspberries were added to 125-mL separatory funnels containing 25 mL of hexane and then mixed thoroughly. Dinoseb was extracted from the organic phases by partitioning four times with 25 mL of 0.025 M aqueous $NaHCO_3$. The aqueous phases (lower layer) including the emulsion were drained into 250-mL separatory funnels. The combined aqueous phases were partitioned once with 25 mL of hexane, and about 9 g of NaCl was added to break the emulsion. After phase separation, the clear aqueous phases (lower layer) were drained into 250-mL separatory funnels and then acidified to about pH 1 with $H_3PO_4$. Dinoseb was extracted from the aqueous phase by partitioning twice with 25 mL of dichloromethane. The extracts were dried over anhydrous $Na_2SO_4$ and concentrated just to dryness in a flash evaporator at 38 °C, and the residues were dissolved in 2 mL of HPLC grade methanol for analysis.

Dinoseb residues were determined by HPLC with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard model 1040A high-speed spectrophotometric detector. The operating parameters were as follows: column, Supelcosil LC-8 (25.0 cm × 4.6 mm i.d., 5 μm); mobile solvent system, 80% methanol and 20% 0.0025 M $NaH_2PO_4$ adjusted to pH 2.4 with concentrated $H_3PO_4$, isocratic at 1.0 mL/min; UV detector wavelength, 270 ■ 2 nm. Aliquots of 20 μL of dinoseb standard or cleaned raspberry extracts were injected into the HPLC for measurement. Under the chromatographic conditions

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described, the absolute retention time of dinoseb was 4.53 min. Quantification of dinoseb was based on external standard. Detector response was calibrated for each analysis, and the calculation was based on average peak areas of these external standards, which were injected before and after each sample.

**Cleanup and GC Analysis.** Aliquots of 2-mL crude extracts equivalent to 10 g of raspberries were transferred to 125-mL round-bottom flasks. After the addition of 1 mL of keeper (1% hexane solution of OV-101, a methyl silicone liquid phase for coating packed column packing), they were evaporated just to dryness in a flash evaporator at 38 °C. Addition of keeper prevented the loss of analyte through volatilization. The residues were then dissolved in 2 mL of ether solution of diazomethane for methylation. By use of the procedures described by Szeto et al. (1989) for preparation of diazoethane, diazomethane was prepared from its precursor, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. A 300-mg sample of the precursor in 5 mL of glass-distilled ether reacted at room temperature with 2 mL of 5 N NaOH solution in a gas bubbler. One of the two side arms of the gas bubbler was connected to a gentle flow of nitrogen and the other arm connected by Tygon tubing to a Pasteur pipet for dispensing diazomethane into ether. The yield was high. To ensure high purity of the product, the glass-distilled ether must be fresh. **Since diazomethane is highly toxic, mutagenic, carcinogenic, and potentially explosive, all procedures involving diazomethane were carried out in an efficient fume hood and behind a safety shield.** The reaction was allowed to proceed for 30 min. After addition of 1 mL of keeper, the methylated extracts were solvent-exchanged three times with 5 mL of hexane and then purified by column chromatography.

Glass columns (30 cm × 1.1 cm i.d.) with Teflon stopcocks were packed from bottom to top with a glass wool plug, 1 cm of anhydrous $Na_2SO_4$, 2.5 cm of a 2:5 (w/w) mixture of Nuchar-activated charcoal/Whatman CF-11 cellulose, 1 cm of anhydrous $Na_2SO_4$, and another glass wool plug. The activated charcoal (Nuchar C, Kodak Laboratory Chemicals) was acid-washed prior to use (Brown, 1975). The packed columns were prewashed with 10 mL of acetone followed by 10 mL of hexane. The methylated extracts in hexane were quantitatively transferred from the round-bottom flasks to the cleanup columns by rinsing with 10 mL of hexane in 2-mL portions; the resulting eluates were discarded. Methylated dinoseb was eluted with 50 mL of 20% acetone in ethyl acetate. After addition of 1 mL of keeper, the cleaned extracts were evaporated just to dryness at 38 °C in a flash evaporator and the residues were dissolved in 2 mL of ethyl acetate for GC analysis.

For determination of dinoseb as its methylated product, a Hewlett-Packard Model 5880 gas chromatograph equipped with a nitrogen/phosphorus detector and a Hewlett-Packard split/splitless inlet and a Hewlett-Packard cross-linked methyl silicone capillary column (25 m × 0.31 mm i.d.; 0.33 μm thick) were used. The operating parameters were as follows: detector temperature, 300 °C; inlet temperature, 220 °C; injection, 1 μL splitless. Column temperature was programmed as follows: initial, 80 °C for 0.5 min; first program rate 25 °C/min to 185 °C; second program rate, 5 °C/min to 225 °C and hold for 3 min. Helium was the carrier gas at 105 kPa. Detector gas consisted of hydrogen at 4 mL/min and air at 120 mL/min. Nitrogen was the makeup gas at 30 mL/min. Under the conditions described, the absolute retention time of methylated dinoseb was 6.63 min. Quantification was based on peak areas of the external standards injected before and after the sample.

**Residue Study.** Seven raspberry farms in the Abbotsford area were selected. These farms represent the typical use pattern of dinoseb in raspberry production in the region, including the worst case scenario, i.e., farms with the longest use history and farms with the most applications for the current season. The recommended application rate of dinoseb for primocane removal is 6.8 kg of ai/ha. The records of dinoseb applications are as follows: farm 1, three applications in 1990 only; farm 2, one application per year for the past 5 years; farm 3, two applications in 1990 only; farm 4, one application per year for the past 6 years; farm 5, one application in 1990 only; farm 6, one application per year for the past 3 years; farm 7, one application per year for the past 5 years. At harvest, about 1 kg of raspberries was randomly collected from each farm to give seven samples by farms for dinoseb residue determination. All samples were stored at 4 °C and extracted within 1 day. Each sample was homogenized in a food processor, and a 50-g subsample was processed and analyzed by both HPLC and GC according to the methods described in this paper.

**Table I. Percent Recovery of Dinoseb from Fortified Raspberries**

| fortification, ppm | % recovery ± SD ($n$ = 4) |
|---|---|
| HPLC Method | |
| 0.1 | 79.5 ± 2.6 |
| 0.5 | 78.6 ± 3.2 |
| 1.0 | 85.5 ± 2.0 |
| GC Method | |
| 0.1 | 99.7 ± 5.5 |
| 1.0 | 92.0 ± 1.0 |

## RESULTS AND DISCUSSION

**Residue Method Evaluation.** Samples of raspberries collected from the Abbotsford Substation, which had never been treated with dinoseb, were processed and analyzed according to the described methods with both HPLC and GC. There was no detector response that interfered with the analysis of dinoseb. Recoveries of dinoseb from fortified samples are given in Table I. The HPLC method was evaluated at three levels of fortification, namely 0.1, 0.5, and 1.0 ppm, whereas only two levels, namely 0.1 and 1.0 ppm, were evaluated according to the GC method.

To achieve stability, the HPLC must be equilibrated prior to analysis by pumping the mobile phase for about 30 min. At the end of daily operation, the HPLC system must be flushed first with water and then with methanol to prevent buildup of phosphate salt and growth of microorganisms. The UV absorption spectrum of dinoseb was pH dependent. At pH 2.4, the absorption maximum was at about 270 nm. Therefore, this particular wavelength was used for measurement of UV detector response for dinoseb. Under the chromatographic conditions described, our HPLC method was highly sensitive for the detection of dinoseb. A typical high-pressure liquid chromatogram of 40 ng of dinoseb is given in Figure 1. The cleanup procedures by solvent/solvent partitioning for HPLC determination were highly effective. Injection of cleaned extract equivalent to 500 mg of raspberries contained no significant response that interfered with dinoseb (Figure 1).

The mean recoveries of dinoseb by the HPLC method ranged from 78.6% to 85.5% (Table I). Most of the loss occurred at the solvent/solvent partition steps for cleanup. The composition of the organic phase is of primary importance for recovery of dinoseb. When 10 μg of dinoseb in 25 mL of dichloromethane or ethyl acetate was extracted four times with 25 mL of 0.025 M $NaHCO_3$, approximately 65% of the dinoseb was recovered from the combined aqueous extracts. The poor recovery was probably due to the relatively high solubility of dinoseb in dichloromethane and ethyl acetate. However, when 3 mL of dichloromethane containing 10 μg of dinoseb was first mixed with 25 mL of hexane before partitioning, the mean recovery and standard deviation were 87.4% ± 2.7% ($n$ = 4). The significant improvement in recovery with the addition of 25 mL of hexane in the organic phase was probably due to the lower solubility of dinoseb in hexane. On the basis of these findings, crude extracts in 3 mL of dichloromethane representing 15 g of raspberries were first mixed with 25 mL of hexane before partitioning. This particular step ensured improved recovery as well as effective cleanup. The recoveries by the HPLC method at each fortification level were highly reproducible as

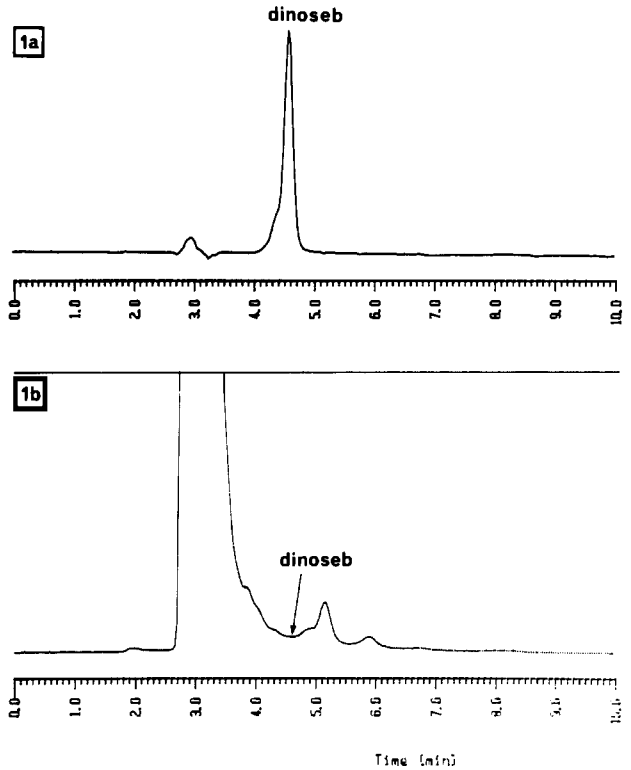


**Figure 1.** High-pressure liquid chromatograms of UV absorption at 270 nm: (a) 40 ng of dinoseb; (b) cleaned extracts representing 500 mg of untreated raspberries.

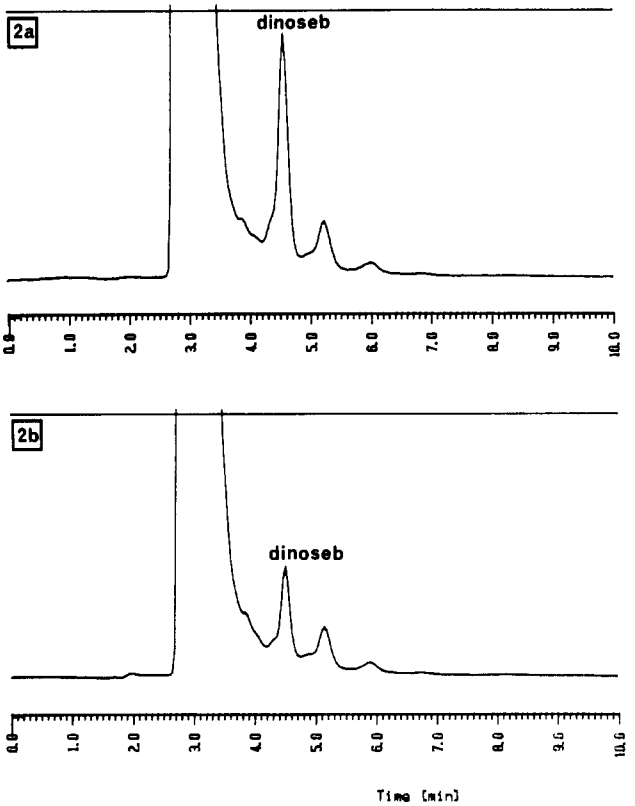


**Figure 2.** High-pressure liquid chromatograms of UV absorption at 270 nm: (a) 500 mg of raspberries fortified with 0.5 ppm of dinoseb; (b) 500 mg of raspberries fortified with 0.1 ppm of dinoseb.

indicated by the small standard deviations (Table I). High-pressure liquid chromatograms of raspberries fortified with 0.1 and 0.5 ppm of dinoseb are given in Figure 2. Based on the sensitivity of detector response and the effectiveness

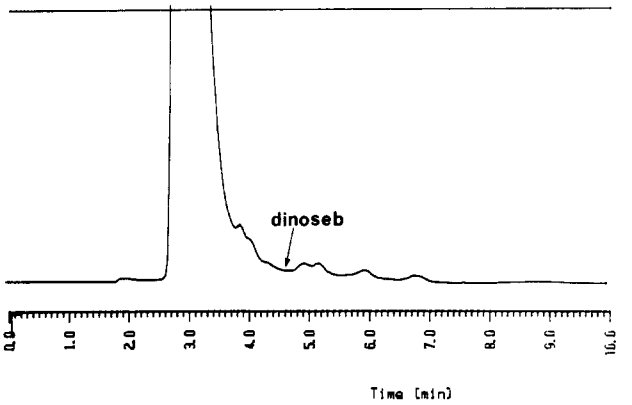


**Figure 3.** High-pressure liquid chromatogram of UV absorption at 270 nm of 500 mg of raspberries from farm 1, which were treated three times with dinoseb in 1990.

of sample cleanup, the calculated limit of detection of the HPLC method was 0.025 ppm. However, the recovery at this level was not evaluated.

A GC method for the determination of dinoseb was also described in this paper for comparison with the HPLC method and for confirmation in the residues study. As in the methods of Yip et al. (1968) and Gardner and McKellar (1980), dinoseb was methylated with diazomethane for determination. However, our method differed from theirs in extraction and cleanup, and we analyzed methylated dinoseb with a nitrogen/phosphorus detector instead of an electron capture detector. We evaluated for cleanup, gel permeation with Bio-Beads S-X12, and several adsorbents, including Florisil, acid alumina, and a 2:5 (w/w) mixture of Nuchar-activated charcoal/Whatman CF-11 cellulose. Regardless of the eluting solvent used, the recoveries of methylated dinoseb were all over 85%, but no recovery was complete. The activated charcoal mixture was most effective in cleanup and, therefore, incorporated in our GC method. Raspberry extracts contain coextractives that are highly detrimental to the retention gap used in the cool on-column inlet and to the cross-linked methyl silicone capillary column. Most of these coextractives were removed by discarding the first 10 mL of hexane eluates from the column cleanup steps. To ensure GC peak integrity and stable detector response, a split/splitless inlet should be used instead of a cool on-column inlet. The GC method was evaluated at two fortification levels, 0.1 and 1.0 ppm, and the recoveries were better than 90% (Table I). Based on the sensitivity of detector response and the effectiveness of sample cleanup, the calculated limit of detection of the GC method was 0.01 ppm. However, the recovery at this level was not evaluated.

By comparison, the HPLC method is slightly less sensitive than the GC method and its detection limit is somewhat higher (Table I). However, the simple procedures used for cleanup and the direct measurement of dinoseb by UV absorption without derivatization with diazomethane make the HPLC method preferable on balance, considering efficiency and safety.

**Residue Study.** Raspberry samples collected from seven farms at the Abbotsford area were analyzed according to both the HPLC and the GC method for dinoseb residues. None of these samples contained detectable residues, and the calculated limits of detection were 0.025 ppm by HPLC and 0.01 ppm by GC. A representative high-pressure liquid chromatogram of the raspberries from farm 1 is given in Figure 3. At this farm dinoseb was applied three times in the 1990 growing season for primocane removal, but no residues were detected in the

berries at harvest. When use history and number of applications for the current season as represented by the seven farms are taken into consideration, it is evident that no residues would be accumulated in the berries resulting from the use of dinoseb for primocane removal in vigorous raspberry plantings. These findings confirm the unpublished data, generated by the Health Protection Branch of Health and Welfare Canada and the British Columbia Ministry of Agriculture and Fisheries, that no dinoseb residues have ever been detected in British Columbia grown raspberries in their residue monitoring programs.

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LITERATURE CITED

Brown, M. J. Improved Determination of Residues of Phorate and Its Principal Metabolites. *J. Agric. Food Chem.* **1975**, *23*, 334.

Gardner, R. C.; McKellar, R. L. A Method to Determine Dinoseb Residues in Crops and Soil by Gas Chromatography. *J. Agric. Food Chem.* **1980**, *28*, 258–261.

Szeto, S. Y.; Burlinson, N. E.; Rettig, S. J.; Trotter, J. Identification of Hydrolysis Products of the Fungicide Vinclozolin by Spectroscopic and X-ray Crystallographic Methods. *J. Agric. Food Chem.* **1989**, *37*, 1103–1108.

Yip, G.; Howard, S. F. Extraction and Cleanup Procedure for the Gas Chromatographic Determination of Four Dinitrophenolic Pesticides. *J. Assoc. Off. Anal. Chem.* **1968**, *51*, 24–28.

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