Determination of Propiconazole Residue in Boronia Extract (Concrete)

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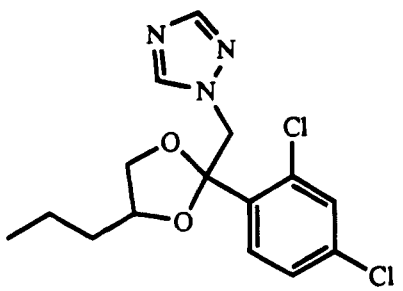
A method for detecting residues of the fungicide propiconazole in the petroleum ether extract (concrete) of *Boronia megastigma* Nees was developed. Gas chromatography/mass spectrometry (GC/MS) using low-resolution selected ion monitoring (LR SIM) had a 1 ppm detection limit in samples that had some preliminary cleanup. The use of GC/MS with high-resolution selected ion monitoring (HR SIM) had a minimum detection limit of 50 ppb for crude spiked samples of boronia concrete. This method was utilized to provide an automated assay for propiconazole residues in the concrete from boronia flowers and in extracts of vegetative material.

Keywords: *Boronia megastigma concrete; propiconazole residue; gas chromatography/mass spectrometry; high-resolution selected ion monitoring*

INTRODUCTION

Solvent extracts (concrete) of *Boronia megastigma* Nees are used in the food and fragrance industries (Arctander, 1960). As some of the resulting products are for human consumption, quality control of the concrete is very important, particularly with respect to pesticide residue levels.

The broad-spectrum systemic fungicide propiconazole (**I**) is used to control rust in a number of crops.



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Experiments were conducted to follow the fate of propiconazole during the growth of the boronia crop through the final extraction of concrete. Therefore, a reliable method for the investigation of propiconazole residues in boronia concrete was necessary.

In the past, analysis of pesticide residues in plant extracts has focused predominantly on the steam-distilled oils. Most research has been on citrus oils, such as lemon (Dugo et al., 1990), mandarin, orange, and bergamot (Leoni and D'Alessandro de Luca, 1978), and sweet orange (Di Bella et al., 1991). Other research has included peppermint (Inman et al., 1983), peppermint and monarda (Bélanger, 1989), and rose, lime, petitgrain, and rue (Stoffelsma and De Roos, 1973). However, there has been little work relating to pesticide residues in concretes. These solvent extracts have an abundance of compounds which have volatility, polarity, and molecular weight in the same range as many pesticides, making cleanup procedures and analyses difficult. In previous work, levels of propiconazole have been determined in a variety of matrices such as water, soil, and fresh plant material (Büttler, 1983) and must and wine (Lopez et al., 1989). Preconcentration of the fungicide from these matrices is achieved relatively easily.

Various techniques were investigated in an attempt to achieve a minimum detection limit in the parts per billion range so that quality assurance could be maintained at the highest level. It was also a requirement that the method be applicable to other potential pesticides which may be used on the boronia crop. GC/MS in the SIM mode and gas chromatography with electron capture detection (GC/ECD) were considered the most likely methods to fulfill these criteria. The most promising of these methods, GC/MS HR SIM, was fully developed to provide a semiautomated quantitative assay with low minimum detection limits.

EXPERIMENTAL PROCEDURES

Chemicals. Analytical grade propiconazole standard was supplied by Ciba-Geigy, Victoria, Australia. *n*-Octadecane (C_{18}) was purchased from Sigma Chemical Co. (St. Louis, MO). All chromatography solvents were HPLC grade, and extraction solvents were redistilled analytical grade. Silica Sep-Pak cartridges were purchased from Millipore-Waters (Australia). Boronia products, both concrete and flower samples, were supplied by Jocks Point Boronia (Australia).

Analysis of Propiconazole Standard. Preliminary investigations were undertaken on the propiconazole standard using GC/MS acquiring full mass spectra to determine its chromatographic behavior and suitable ions for SIM analyses.

Spiked Standard Preparation with Preconcentration. For preparation of spiked standards, 10 mg of propiconazole was dissolved in 50% hexane/ether and made up to 100 mL. This resulted in a 100 μg/mL stock solution from which, by volumetric serial dilution, 100, 10, 1, and 0.1 ng/mL propiconazole solutions in 50% hexane/ether were produced. One milliliter of each of these solutions was added to 10 mg samples of boronia concrete known to be pesticide free. This gave spiked standards of 10 ppm, 1 ppm, 100 ppb, and 10 ppb, respectively.

The spiked standards were flushed onto 2 mL silica Sep-Pak cartridges with 15 mL of 50% hexane/ether solution and

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then sequentially eluted with 5 mL of methylene chloride, 4 mL of 1% methanol in methylene chloride, and 3 mL of 2% methanol in methylene chloride. Initially, 1 mL fractions were collected separately and screened for the fungicide using GC/MS LR SIM. It was determined that the propiconazole eluted in the 3 mL of 2% methanol in methylene chloride. In subsequent extractions, only the final 3 × 1 mL was collected separately. Ten microliters of 0.16 mg/mL *n*-octadecane (C_{18}) standard was added to each sample and the solvent evaporated. Samples were made up in minimum volume for automatic injections. These samples were analyzed by GC/MS LR SIM. The minimum detection limit was determined, a standard curve calculated, and the viability of the method assessed.

Spiked Standard Preparation without Preconcentration. For preparation of standards, 10 mg of boronia concrete was spiked with various volumes of 1 μg/mL and 100 ng/mL propiconazole stock solutions to produce a range of eight standards from 20 ppm to 10 ppb. To each of these was added 2 μg of C_{18} internal standard. They were made up to volume with chloroform, depending on the level of spiking: 10 ppb, for instance, was made up to 200 μL and 10 ppm made up to 2 mL. These samples were analyzed by GC/MS HR SIM, a minimum detection limit was determined, a standard curve was calculated, and the method was assessed for viability as an assay. Variation between injections was determined by reinjecting the same 1 ppm spiked standard 10 times over 30 h.

Solvent Extraction of Experimental Flower Samples. Duplicate extracts were made from each of three samples of flowers: A [clone 17 (1 year old, untreated)]; B [clone 17 (~3 years old, treated at time zero and again at 2 weeks)]; and C [clone 250 (~3 years old, treated at time zero)]. Propiconazole was applied at a rate of 125 g/ha. The flowers were extracted at 43 weeks, using petroleum ether according to the method of Roberts and Menary (1994). Ten milligram samples of each of these extracts were dissolved in 200 μL of chloroform, and 20 μL of 0.2 mg/mL C_{18} was added. The samples were then analyzed for propiconazole using GC/MS HR SIM.

Solvent Extraction of Experimental Vegetative Samples. Samples of leaf and stem were also collected from boronia clone 250 (as per sample C above). Further samples were collected at 16, 19, and 40 weeks. These were blended with petroleum ether with further extraction as for the flowers. The resulting samples were made up to 200 μL in chloroform, 20 μL of 0.2 mg/mL C_{18} was added, and then the samples were analyzed for propiconazole using GC/MS HR SIM.

Analysis by GC/MS LR SIM. Mass spectrometric analyses were performed on 1 μL splitless injections of samples using a HP 5890 GC coupled via an open split interface to a HP 5970B mass selective detector (MSD). The GC was equipped with a HP1 fused silica capillary column (25 m × 0.32 mm i.d., 0.17 μm film thickness) and run with an injection temperature of 250 °C, a detection temperature of 290 °C, and a head pressure of 15 psi. The oven was programmed from 50 °C (held for 1 min), ramped to 220 °C at 30 °C/min and then to 290 °C at 10 °C/min (held for 5 min). Electron ionization was undertaken with a source temperature of 200 °C and an electron energy of 70 eV. The MSD was operated in the SIM mode with nominal mass resolution. The propiconazole ions monitored were m/z 259.05 and 261.05, and the C_{18} ion m/z 85.05 was monitored. The dwell time was 200 ms.

GC/MS Analysis by HR SIM. One microliter splitless injections of samples were analyzed using a HP 5890 GC directly coupled to a Kratos Concept ISQ mass spectrometer. The GC was equipped with a BP1 fused silica capillary column (25 m, 0.22 mm i.d., 0.25 μm film thickness) and run with an injection temperature of 260 °C. The oven program of 60–290 °C at 20 °C/min ensured rapid processing of samples. A carrier gas flow program was also utilized and run at 30 psi/min from 25 to 40 psi, held for 0.1 min, then run at 30 psi/min to 25 psi, and then run at 1 psi/min to 35 psi. Ions monitored by SIM were m/z 259.0293, 172.9561, and 174.9531 for propiconazole and m/z 254.2973 for the C_{18} standard, all with a dwell time of 200 ms/ion and 50 ppm voltage sweep. These analyses were undertaken at 10 000 resolution (10% valley definition) using the ions at m/z 254.9856 and 168.9888 from perfluorokerosene as lock masses for the C_{18} and propiconazole, respectively. Electron ionization was undertaken at a source temperature of 240 °C, an electron energy of 70 eV, and an accelerating voltage of 8 kV.

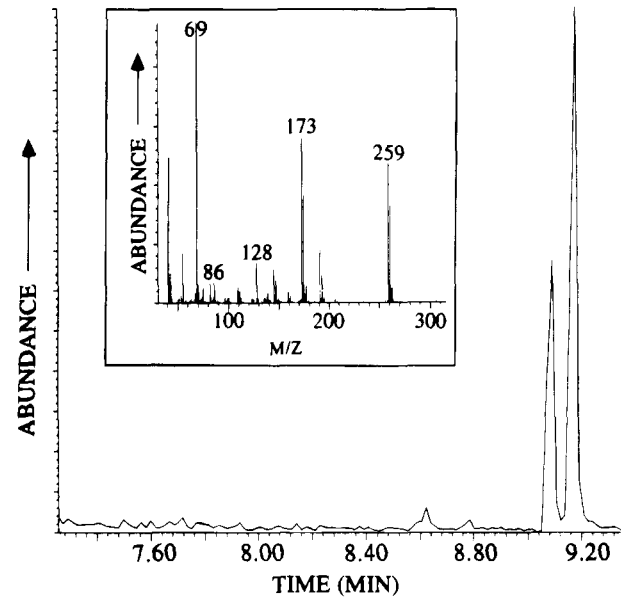

Figure 1. LR GC/MS total ion chromatogram of propiconazole with mass spectrum inset.

RESULTS AND DISCUSSION

The gas chromatogram and mass spectrum obtained for the propiconazole standard are presented in Figure 1. The two almost fully resolved peaks correspond to two diastereomers of propiconazole, the mass spectra of which were essentially indistinguishable. The ions selected for monitoring were the chlorine clusters at m/z 259/261, corresponding to a loss of the methylene triazine group (leaving $C_{12}H_{13}Cl_2O_2$), and at m/z 173/175, which was due to a further loss of $C_5H_{10}O$ (leaving $C_7H_3Cl_2O$). These ions were confirmed experimentally by HR MS to be m/z 172.95608 (calculated for $C_7H_3Cl_2O$ 172.95609) and m/z 259.02916 (calculated for $C_{12}H_{13}Cl_2O_2$ 259.02926). The two chlorine isotopes were monitored for confirmation of identification, while only the ^{35}Cl-containing ion was used for quantification.

The preconcentration procedure for spiked standards resulted in a 13-fold concentration of the propiconazole. Although the 173/175 cluster was slightly more intense than the m/z 259/261 cluster, the latter had less interference from other compounds in the concrete and was therefore monitored. The minimum detection limit by GC/MS LR SIM was 1 ppm after the preconcentration procedure. The standard curve of [peak area propiconazole(173):peak area C_{18}(80)] vs concentration of propiconazole (ppm) gave the line defined by $y = 0.230x + 0.062$, which had a correlation coefficient of 0.997. A lower minimum detection limit was required, so this method was developed only to the semiquantitative stage with the deviation between samples and between runs not examined. Preliminary experiments with GC/ECD showed a similar lack of specificity, and this technique was not developed further either.

Analyses of the crude spiked boronia standards by GC/MS HR SIM demonstrated a detection limit of 50 ppb. The ion pair monitored was m/z 173/175. From analysis, the equation for the standard curve of [peak area propiconazole(173):peak area C_{18}(254)] vs concentration of propiconazole (ppm) was defined by $y = 0.100x$

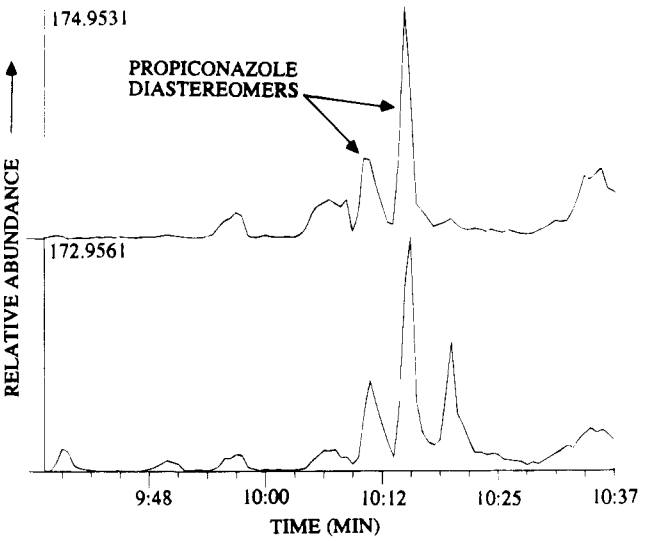

Figure 2. GC/MS HR SIM of boronia concrete spiked with 200 ppb propiconazole

Table 1. Detection of Propiconazole in Concrete Extracted from Boronia Field Samples

boronia sample	av propiconazole content (ppm)
A	not detected
B	9.2 ± 0.7
C	4.3 ± 0.3

+ 0.007, with a correlation coefficient of 0.998. A representative chromatogram at the 200 ppb level is presented in Figure 2. The two diastereomers were clearly present at this level and were confirmed by monitoring both ions in the cluster. Ten replicate injections at the 1 ppm level gave the very low relative standard deviation of 4% over 30 h.

Initially, the deviation at the 1 ppm level was considerably higher than 4%. This error was found to be associated with the slightly different accelerating voltages. These were used to focus the C_{18} and propiconazole ions when default values for the "start" mass at full accelerating voltage were used in each window. When start masses were chosen such that the ratio of start mass to target ion mass was the same for both internal standard and propiconazole channels, this source of variation was removed. The start masses were 240.00 for C_{18} and 163.23 for propiconazole, resulting in 94.4% of full accelerating voltage required in each case to focus the target ions for quantification.

The GC/MS HR SIM assay was used to detect the concentration of propiconazole in the three floral extracts, and the results are presented in Table 1. This table shows that there are small amounts of propiconazole contaminating the concrete of the flowers of the experimentally treated plants.

The vegetative sample extracts, which were from the same trial as flower extract C (Table 1), showed a decrease in propiconazole from 350 ppm at 16 weeks to ~85 ppm at 40 weeks (Figure 3). This was probably due to a dilution effect as a result of growth. The flowers used in sample C were harvested just 3 weeks after the 40 week sample of vegetative material and had a propiconazole concentration of approximately 4 ppm. Thus, if this program of propiconazole treatment is adhered to, the contamination of the boronia concrete extracted from the flowers will be low. It may be necessary to adopt a new system to minimize residue concentration further.

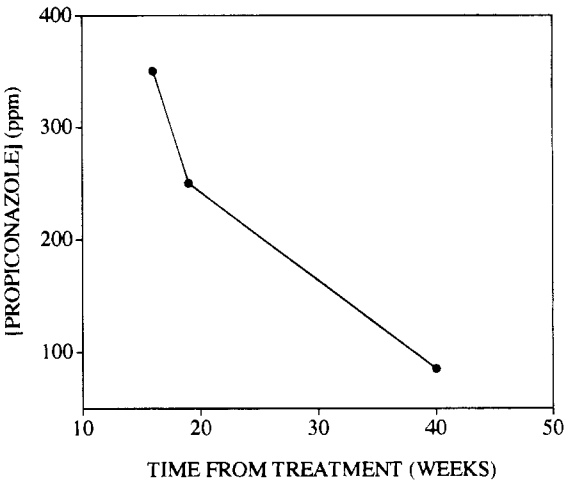

Figure 3. Decreasing propiconazole concentration in vegetative extracts of *B. megastigma* Nees.

ACKNOWLEDGMENT

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