Gas Chromatographic−Mass Spectrometric Method for the Analysis of Dimethomorph Fungicide in Dried Hops

Matt J. Hengel* and Takayuki Shibamoto

Department of Environmental Toxicology, University of California at Davis, One Shields Avenue, Davis, California 95616

An analytical method for the determination of dimethomorph [(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine] residues in dried hops was developed utilizing liquid−liquid partitioning, automated gel permeation chromatography (GPC), Florisil and aminopropyl solid phase extraction (SPE) column cleanups, and gas chromatography (GC) with mass selective detection (MSD). Method validation recoveries from dried hops ranged from 79 to 103% over four levels of fortification (0.1, 1.0, 5.0, and 20 ppm). Control and dimethomorph-treated hop samples collected from three field sites had residue levels of <0.10 and 4.06−17.32 ppm, respectively. The method was validated to the limit of quantitation at 0.10 ppm. The limit of detection for this method was 0.045 ppm.

Keywords: *Dimethomorph; fungicide; gas chromatography; hops; mass spectrometry*

INTRODUCTION

In the production of a high-yield and high-quality agricultural product, the need for agrochemicals to control pests and diseases is paramount. One particular disease associated with the cultivation of hops, downy mildew (*Plasmopora viticola*), has resulted in large reductions in crop yield in many hop-producing regions in the United States, including New York, California, and western Washington. In the remaining hop-producing regions—eastern Washington, Idaho, and Oregon—the federally registered fungicide metalaxyl (Ridomil) has provided adequate control of downy mildew. However, growers in Oregon have reported resistance to metalaxyl (Schreiber and Ritchie, 1996). It is, therefore, important to identify viable alternatives for the control of downy mildew before resistance makes current control measures ineffective.

Dimethomorph [(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine] was developed by Shell Forschung GmbH (Tomlin, 1997). The effectiveness of dimethomorph as a fungicide was first reported in 1988 (Albert et al., 1988). This relatively new compound subsequently demonstrated good protectant, curative, and antisporulant activity, particularly against downy mildew (Wicks and Hall, 1990). Dimethomorph is currently produced by American Cyanamid (Princeton, NJ) and is being considered for registration in the United States to aid in the control of downy mildew on hops (D. Thompson, Rutgers University, personal communication, 1999).

Analysis of dimethomorph residues can be accomplished by high-pressure liquid chromatography (HPLC), gas chromatography (GC), or thin-layer chromatography (TLC). These analytical techniques are adequate for the analysis of matrices such as soil, grapes, tomatoes, and potatoes (Stout et al., 1998; Visi, 1994; Popova and Zaplishny, 1992). However, hop matrices are not always amenable to these analytical methods because they require extensive cleanup to remove resins and oils from the hop extract, which often complicate analysis by causing chromatographic interference.

In the present study, a rugged and sensitive method for the detection of dimethomorph in dried hop samples was developed to be used as an enforcement method to support pesticide registration. The new method uses liquid−liquid partitioning, automated gel permeation chromatography (GPC), Florisil column cleanup, aminopropyl solid phase extraction (SPE) cleanup, and detection using a capillary gas chromatograph (GC) equipped with a mass selective detector (MSD).

EXPERIMENTAL PROCEDURES

Pesticide. Dimethomorph (97.6%) was acquired from American Cyanamid Co., Agricultural Products Research Division, Princeton, NJ.

Materials. All solvents and reagents were of pesticide grade.

Stock and Fortification Solution Preparation. A stock solution (1.0 mg/mL) was prepared by adding 51.3 mg of pure dimethomorph analytical standard to a 50-mL volumetric flask. Acetone was added to the flask and mixed. A high-level fortification solution was prepared by taking a 5-mL aliquot of the 1.0 mg/mL stock solution and diluting the aliquot in a 50-mL volumetric flask with acetone, resulting in a 100.0 μg/mL solution. A low-level fortification standard was prepared by taking a 5-mL aliquot of the 100.0 μg/mL fortification solution and diluting the aliquot in a 50-mL volumetric flask with acetone, resulting in a 10.0 μg/mL solution. All stock and fortification solutions were stored at −20 °C in the dark until use.

GC Calibration Solution Preparation. GC calibration solutions were prepared by adding 200 μL of the 10.0 μg/mL solution to 0.1% corn oil/ethyl acetate in 25-, 50-, 100-, and 200-mL volumetric flasks to make 80, 40, 20, and 10 pg/μL solutions, respectively. All calibration solutions were stored at −20 °C in the dark until use.

Collection of Field Samples. A total of 12 hop samples (6 treated and 6 untreated controls) were collected from IR-4

* Author to whom correspondence should be addressed [telephone (530) 752-2402; fax (530) 754-8556; e-mail mjhengel@ucdavis.edu].

10.1021/jf000620j CCC: $19.00
Published on Web 10/27/2000

Table 1. Field Application Methods and Conditions during First Treatment, as well as Harvest Conditions

field location	application method	air temp (°C)	RH[a] (%)	wind speed (km/h)	sky cloudy (%)
Washington	Pak Blast[b]	18,[c] 21[d]	49	0	50
Oregon	Pack Sprayer[e]	32, 26	35	4−9	27
Idaho	Mist Blower[f]	18, 20	80	0	100

[a] RH, relative humidity. [b] Back pack, pump-driven sprayer. [c] Temperature during first application. [d] Average temperature during harvest. [e] Back pack, compressed CO_2 sprayer. [f] Back pack, motorized sprayer.

(Interregional Research Project 4) field trial sites in Oregon, Idaho, and Washington. Treated samples received six dimethomorph applications at a rate of 0.45 kg (active ingredient)/ha. See Table 1 for application methods and conditions, as well as harvest conditions. The final application was 7 ± 1 days prior to harvest. Harvesting dates for the fields in Hubbard, OR; Parma, ID; and Prosser, WA, were September 2−4, 1998; September 21, 1998; and September 8, 1998, respectively. Following collection, the hop samples were dried in a manner consistent with commercial drying methods (heated air kilns) and transferred, frozen, to our facility.

Sample Preparation. Hop samples (∼300 g each) were chopped with equal amounts of dry ice using a Hobart food chopper (Hobart Corp., Troy, OH). Each chopped sample was stored in an ∼1-L jar, and a lined lid was loosely closed on top to allow the dry ice to dissipate during storage at −20 °C.

A 5-g aliquot of dried hops was weighed into a 250-mL Erlenmeyer flask. A known level (20, 5, 1, or 0.1 ppm) of dimethomorph standard was fortified, via syringe (minimum volume of 100 μL), for recovery tests at this point. Acetone (120 mL) was added, and the sample was blended using an Ultra-Turrax T-25 (Janke & Kunkel) for 2 min at 13500 rpm. The homogenized sample was then filtered, with mild vacuum, through a Büchner funnel fitted with a Whatman GF/F (5.5 cm) filter, backed by a Whatman No. 1 (5.5 cm) filter. The resulting filter cake was transferred back to the blending flask and homogenized for another 2 min with 80 mL of acetone and refiltered. The blending flask was rinsed with 20 mL of acetone, and this was added to the filter cake.

After filtration, the entire sample was transferred to a 1000-mL separatory funnel that contained 500 mL of water and 50 mL of saturated sodium chloride solution. The filtration flask was rinsed with 100 mL of dichloromethane, and this was added to the separatory funnel, which was then shaken for 2 min. The phases were then allowed to separate, and the lower organic layer was drained through a funnel plugged with glass wool and sodium sulfate into a 500-mL round-bottom flask. The remaining aqueous layer was re-extracted with another 100 mL of dichloromethane for an additional 2 min and pooled with the first partition of dichloromethane. The sodium sulfate was rinsed with 20 mL of dichloromethane, and this was added into the 500-mL round-bottom flask. The sample was next concentrated to dryness on a rotary evaporator under vacuum (water bath at 35 °C).

Once the sample had reached dryness, 30 mL of acetonitrile was added to the round-bottom flask to facilitate sample transfer to a 125-mL separatory funnel. The 500-mL round-bottom flask was rinsed with 30 mL of hexane, which was added to the separatory funnel that was then shaken for 2 min. The lower acetonitrile layer was drained and repartitioned twice more with 30 mL of hexane (2 min each). All hexane washes were discarded. The remaining acetonitrile fraction was transferred to a 100-mL round-bottom flask and rotary-evaporated under vacuum to near dryness (water bath at ∼35 °C). After concentration, the sample was dissolved into 10 mL of 1:1 (v/v) dichloromethane/cyclohexane for cleanup via GPC.

Gel Permeation Chromatography. The GPC system consisted of a Kontes Chromaflex gel permeation column (Kontes, Vineland, NJ), a Foxy 200 X-Y fraction collector (Isco, Inc., Lincoln, NE), and a Benchmate II Workstation (Zymark Corp., Hopkinton, MA). The Benchmate was programmed to automatically weigh, vortex, and filter (PTFE, 0.45-μm Millipore filter disk, Millipore Corp., Bedford, MA) each sample prior to injection (5-mL sample loop is equal to 2.5 g of sample on column) onto the GPC column. The column was 62 cm × 2.5 cm i.d. packed with 200/400 mesh S-X3 resin (Bio-Rad, Richmond, CA) to a bed length of 50 cm. The GPC mobile phase consisted of dichloromethane/cyclohexane (1:1, v/v), with a flow rate of 5 mL/min. Once the sample was loaded onto the column, the fraction collector was programmed to discard the first 120 mL (24 min) of eluate and then collect the next 45 mL (9 min) of eluate into a 250-mL TurboVap tube (Zymark Corp.). The GPC column was regenerated with 250 mL (50 min) of mobile phase prior to the next sample injection.

After GPC cleanup, the samples were placed into a TurboVap II concentration workstation and were concentrated to dryness with dry nitrogen (water bath at 35 °C). The sample residues were then redissolved in 5 mL of 40% ethyl acetate in hexane.

Florisil Column Cleanup. A glass chromatography column (1.0 cm i.d. × 10 cm column with a 150-mL reservoir) was plugged with glass wool and 9.5 mL of activated Florisil (Floridin Co., Berkeley Spring, WV). Activated Florisil was stored in a 105 °C oven prior to use. After the Florisil had been added, the column was gently tapped to facilitate packing and topped off with ∼1.0 cm of sodium sulfate. Prior to the loading of the sample, the Florisil column was rinsed with 20 mL of hexane. When solvent reached the top of the packing, the sample was loaded to the column. Once the sample was loaded onto the column, the TurboVap tube was rinsed with 5 mL of 40% ethyl acetate/hexane, and the rinsate was added to the column. Following the 5-mL rinsate, the column was washed with 25 mL of 40% ethyl acetate in hexane, and all of the eluate up to this point was discarded. Dimethomorph residues were eluted with 50 mL of acetone into a 100-mL round-bottom flask. Samples were rotary-evaporated to dryness and redissolved in 5 mL of 40% ethyl acetate in hexane.

Solid Phase Extraction (SPE). Isolute aminopropyl SPE columns (1-g packing/6-mL tube, International Sorbent Technology, Glamorgan, U.K.) were preconditioned with 2 column volumes (CV) of 40% ethyl acetate in hexane. When the solvent reached the top of the aminopropyl packing, the sample was loaded to the SPE and the eluate was collected in a 100-mL round-bottom flask. Flasks used for sample concentration, prior to SPE cleanup, were rinsed with 5 mL of 40% ethyl acetate in hexane, and the rinsate was added to the SPE. Following the addition of the rinsate, the SPE was fitted with a 75-mL reservoir and dimethomorph was eluted with 40 mL of 40% ethyl acetate in hexane. Solvents were allowed to pass through the SPE by gravity. The eluted sample was rotary-evaporated to dryness and redissolved with an appropriate amount of 0.1% corn oil in ethyl acetate for determination by GC-MSD (typically 12.5-mL final volume for method sensitivity).

Sample Analysis. Sample analysis was conducted with a Hewlett-Packard (HP) 6890-5973 GC-MSD (Hewlett-Packard, Avondale, PA) equipped with a 15 m × 0.25 mm i.d. ($d_f = 0.25$ μm) DB-XLB column (J&W Scientific, Folsom, CA). The MSD source (held at 230 °C) was operated in positive electron ionization mode, while the mass filter quadrupole (held at 150 °C) was operated in selective ion monitoring (SIM) mode for *m*/*z* 387.1 (quantification) and *m*/*z* 301.0 (confirmation). See Figures 1 and 2 for a representative mass spectrum of dimethomorph (both *E*- and *Z*-isomers have very similar spectra) and total ion chromatogram of calibration standard, respectively. The injector and GC-MSD transfer line were operated at 250 and 280 °C, respectively. An HP 6890 series autoinjector was used to inject 3 μL of sample in pulsed splitless mode (50 psi for 1 min). The oven temperature started at 150 °C and was then programmed at 20 °C/min to 280 °C and held for 5 min. The carrier gas was helium at a flow rate of 2.0 mL/min, which resulted in retention times of 8.3 and 8.6 min for *Z*- and *E*-dimethomorph, respectively. For sample quantitation, the peak areas for both *Z*- and *E*-isomers were summed.

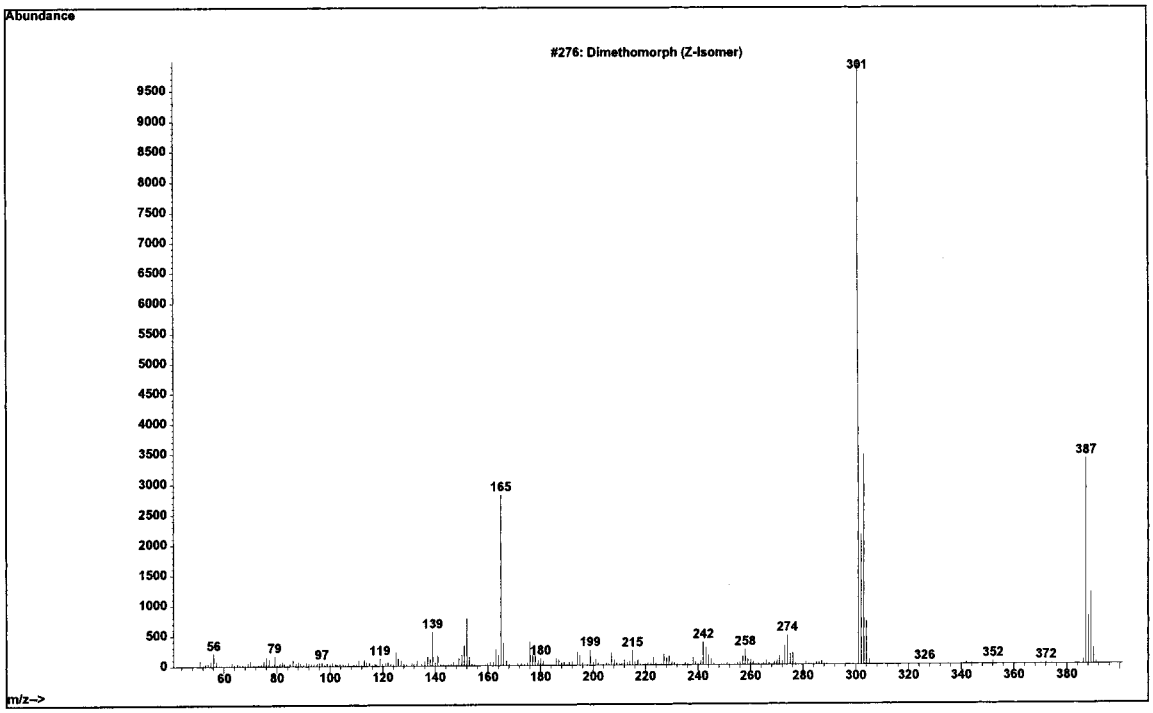

Figure 1. 70 eV electron ionization mass spectrum of *Z*-dimethomorph.

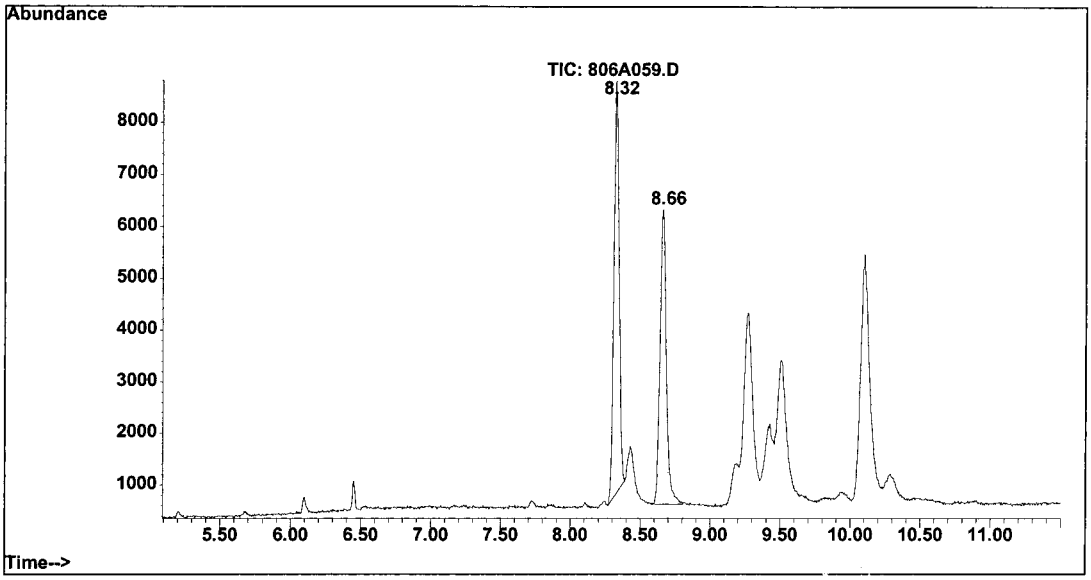

Figure 2. Total ion chromatogram of 10 pg/μL calibration standard. Dimethomorph *Z*-isomer and *E*-isomer are at 8.32 and at 8.66 min, respectively.

Storage Stability Study. A storage stability study on dimethomorph was conducted by preparing six untreated samples of dried hops. The storage samples were fortified at 5 ppm and stored at −20 °C until analysis. Three stability samples were analyzed after all of the field samples had been analyzed. The three remaining samples were retained for long-term storage.

RESULTS AND DISCUSSION

The method sensitivity (lowest fortification level with satisfactory recoveries, between 70 and 120% recovery) and the limit of detection (based on 10% below the lowest calibration standard in the standard curve) obtained in the present study were 0.1 and 0.045 ppm, respectively. Visi (1994), who utilized solvent extraction and liquid chromatography with ultraviolet (LC-UV) detection for dimethomorph analysis in grapes, obtained 0.1 ppm sensitivity. Weitzel (1995), who used HPLC followed by gel permeation and silica gel column clean-ups for analysis of dimethomorph in grapes (fruits, fruit waste, resin, fruit juice, and wine), tomatoes, and potatoes, achieved 0.01 ppm method sensitivity. This HPLC-UV method provided satisfactory results for dimethomorph analysis in relatively high-water-content crops such as tomatoes (94%), grapes (81%), and potatoes (79%) (McMahon and Hardin, 1994). However, the HPLC-UV method would not provide adequate results for dimethomorph analysis in low-water-content crops (8−12%) or high-lipophilic-content crops such as dried hops (Daniels, 1997). Therefore, application of improved

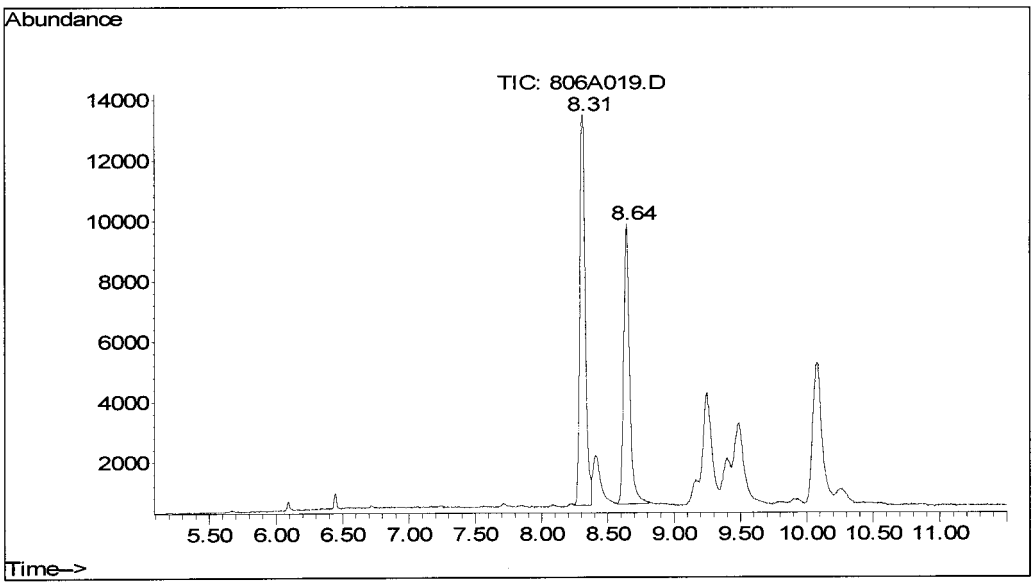

Figure 3. Total ion chromatogram of a sample (12.24 ppm) obtained from the Oregon field site. Dimethomorph *Z*-isomer and *E*-isomer are at 8.31 and at 8.64 min, respectively.

sample cleanup processes and high-resolution capillary gas chromatography (GC) for the analysis of dimethomorph present in a lipophilic matrix may give a satisfactory result.

Weitzel (1996) achieved a method sensitivity of 2.02 ppm in the analysis of dimethomorph in dried hop cones using a GC with a nitrogen/phosphorus detector (NPD). Later, Weeren and Pelz (1997), who validated this GC method, obtained a method sensitivity of 0.202 ppm using both GC-NPD and GC-MSD.

In the present study, the fully automated GPC method was utilized to separate the dimethomorph from unwanted waxes, resins, oils, and pigments in hops. Further purification of sample extracts was conducted using Florisil column chromatography followed by aminopropyl SPE. However, recovery efficiencies of dimethomorph from low-level fortification samples (0.1 ppm) ranged from 160 to 180%. This recovery enhancement (>100%) has been a problem in the analysis of complex matrices, including food crops, using GC-MSD (Wylie and Uchiyama, 1996; C. J. Stafford, U.S. Environmental Protection Agency, Fort Meade, MD, personal communication, 2000). In the present study recovery enhancement was greatly improved by the addition of corn oil to both standards and analytical samples. It is hypothesized that the enhancement stems from a real time deactivation of the injection port liner by the sample matrix (Wylie and Uchiyama 1996). Thus, by adding corn oil to mimic matrix, the chromatographic response was improved.

The results of recovery tests on dimethomorph in the present study were 98 ± 4% for 0.10 ppm, 95 ± 9% for 1 ppm, 98 ± 8% for 5 ppm, and 100 ± 2% for 20 ppm (mean ± standard deviation, $n = 5$). The storage stability test resulted in a recovery efficiency of 94 ± 6% ($n = 3$). The results of the storage stability test suggest that dimethomorph does not degrade significantly during long-term storage (321 days) at −20 °C.

Figure 1 shows a typical total ion chromatogram of dimethomorph isolated from an Oregon sample dissolved in ethyl acetate containing 0.1% corn oil. The amounts of dimethomorph found in the treated samples from Idaho, Oregon, and Washington were 16.19, 11.96, and 4.04 ppm, respectively. The values were averages of two samples from each site. The samples from Oregon contained the highest amount of dimethomorph residue. All untreated control samples had no residues above the method sensitivity (0.10 ppm).

In the wake of the Food Quality Protection Act of 1996, analytical methodologies for the detection of pesticides have been actively modified and redeveloped to lower sensitivities and improve compound confirmation. The new method developed in the present study provides for the simultaneous quantitation and confirmation of dimethomorph with increased sensitivity and selectivity.

ACKNOWLEDGMENT

We thank Dr. David Thompson for his advice and assistance in this project.

LITERATURE CITED

Albert, G.; Curtze, J.; Drandarevski, C. H. A. Dimethomorph (CME 151), a novel curative fungicide. *Brighton Crop Prot. Conf.—Pests Dis.* **1988**, *1*, 17−21.

Daniels, R. Hop physiology and chemistry. *Zymergy* **1997**, *20*, 41−47.

McMahon, M., Hardin, N. F., Eds. *Pesticide Analytical Manual, Vol. I: Multiresidue Methods*; Food and Drug Administration, U.S. Department of Health and Human Services: Washington, DC, 1994; pp 201-1−201-16.

Popova, T. V.; Zaplishny, V. N. Determination of dimethomorph residues in potatoes and tomatoes using the technique of thin-layer chromatography. *Izv. Timiryazevsk. Skh. Akad.* **1992**, *6*, 159−161.

Schreiber, A.; Ritchie, L. *Washington Minor Crops*; Washington State University: Richland, WA, 1996; pp 158−160.

Stout, S. J.; Babbitt, B. W.; DaCunha, A. R.; Safarpour, M. M. Microwave-assisted extraction coupled with gas chromatography with nitrogen−phosphorus detection or electron capture negative chemical ionization mass spectrometry for determination of dimethomorph residues in soil. *J. AOAC Int.* **1998**, *5*, 1054−1059.

Tomlin, C. D. S., Ed. *The Pesticide Manual*; British Crop Protection Council: Surrey, U.K., 1997.

Visi, E. Determination of dimethomorph residues by liquid chromatography. *Novenyvedelem (Budapest)* **1994**, *30*, 555−556.

Weeren, R. D.; Pelz, S. Independent laboratory validation (ILV) of Cyanamid analytical method FAMS 073-03 for the determination of residues of dimethomorph (CL 336379) in dried hops cones; analytical report for American Cyanamid Co., Agricultural Products Research Division, Princeton, NJ, 1997.

Weitzel, R. Dimethomorph (CL 183776, CL 336379): Method for the determination of residues of dimethomorph in potatoes, tomatoes, and grapes (including grape waste material, raisins, fruit juice, wine); analytical method (FAMS 002-04) for Cyanamid Forschung GmbH, Schwabenheim, Germany, 1995.

Weitzel, R. Dimethomorph (CL 336379): GC-NPD determinative and GC-MSD confirmatory method for the determination of CL 336379 residues in dried hops. Analytical method (FAMS 073-03) for Cyanamid Forschung GmbH, Schwabenheim, Germany, 1996.

Wicks, T.; Hall, B. Efficacy of dimethomorph (CME151) against downy mildew of grapevines. *Plant Dis.* **1990**, *74*, 114−116.

Wylie, P. L.; Uchiyama, K. Improved gas chromatographic analysis of organophosphorus pesticides with pulsed splitless injection. *J. AOAC Int.* **1996**, *79*, 571−577.

Received for review May 18, 2000. Revised manuscript received September 8, 2000. Accepted September 11, 2000. The work was supported, in part, by USDA IR-4, U.S. Hop Commission, and the Washington State Commission on Pesticide Registration.

JF000620J